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Nutrition and Disease

Prenatal and Postnatal Maternal Psychological Distress and the Metabolomic Profile of Human Milk

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A B S T R A C T

Background: Human milk is the gold standard of infant nutrition, providing nutrients and bioactive metabolites that shape infant immune, cognitive, and metabolic development. Maternal psychological distress, including stress, anxiety, and depression, is prevalent during the perinatal period and may influence milk composition. Emerging evidence suggests that these alter metabolite classes, with potential long-term consequences for infant health.

Objectives: This study aims to identify whether maternal stress, depressive, and anxiety symptoms are associated with differential metabolomic signatures in human milk from 2.5 to 24 mo postpartum.

Methods: Human milk samples were collected longitudinally at multiple postpartum time points from 424 mothers in the FinnBrain Birth Cohort study, yielding 718 samples. Human milk metabolite concentrations were quantified using ^{1}H nuclear magnetic resonance–based metabolomics. Maternal depressive, anxiety, and stress symptoms were assessed using validated self-report scales prenatally and postnatally. Simple linear regression and linear mixed models examined associations between maternal symptom scores and human milk metabolite concentrations across lactation (false discovery rate <0.05).

Results: Human milk oligosaccharides such as Lacto-N-tetraose (LNT), lacto-N-fucopentaose I (LNFP I), and lacto-N-difucohexaose I/II (LNDFH I/II) showed negative associations with depressive and stress symptoms, whereas 3′-fucosyllactose (3′FL), 3′-sialyllactose (3′SL), and lacto-N-neotetraose (LNnT) were positively linked, particularly with prenatal symptoms.. Among amino acids, threonine concentrations declined with higher maternal symptom scores, whereas glutamate, glutamine, and taurine exhibited time-dependent associations across lactation. Succinate and hippurate showed positive associations with prenatal maternal symptom scores that shifted to negative associations later in lactation, whereas lactose and caprylate were negatively associated with postnatal symptoms.

Conclusions: Both prenatal and postnatal maternal distress are associated with human milk metabolites. Human milk may buffer infants from some effects of maternal psychological distress because increases in key metabolites suggest potential compensatory roles in immune modulation and healthy gut microbiota. However, reductions in metabolites with established prebiotic and immune regulatory functions reinforce the importance of interventions that support maternal mental health during the perinatal period.

Keywords: maternal stress, anxiety, depression, human milk composition, metabolome

Abbreviations: EPDS, Edinburgh Postnatal Depression Scale; FAA, free amino acid; HMO, human milk oligosaccharide; NMR, nuclear magnetic resonance; PRAQ, Pregnancy-Related Anxiety Questionnaire; SCL-90, Symptom Checklist-90.

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Introduction

Human milk is the gold standard for infant nutrition and is rich in lipids, proteins, oligosaccharides, and other bioactive metabolites essential for infant health [1–5]. Evidence suggests that human milk is a dynamically regulated secretion, capable of programming infants' immune, cognitive, and metabolic development [2,5,6]. The composition of human milk is influenced by infants' gestational and lactational age, as well as many maternal factors, such as maternal genotype, BMI, and lifestyle factors such as diet and mental health [2,7,8].

Recent global evidence reports perinatal depression and anxiety prevalence rates of ≤29%–31%, respectively [9–12], with ≤84% experiencing psychosocial stress [13,14]. There is building evidence of infant exposure to such maternal psychopathologies during the first 1000 days being linked to inherited risk of metabolic, immunologic, and neurobehavioral disorders that extend into adulthood [15–20]. Given this high prevalence, understanding how maternal risk is transmitted to the infant is crucial for preventing long-term health implications. Recent studies have linked changes in human milk composition to perinatal psychopathologies, focusing on macronutrients (e.g., lipids and carbohydrates) and bioactive components like oligosaccharides that make up the human milk metabolome [8, 21–29].

Human milk components are derived either from de novo synthesis within mammary epithelial cells or from the maternal circulation, with each class of metabolites differing in its mode of production and benefits for the infant, and individual metabolites within the same classes varying in function and origin [5, 30]. Given the diverse functions of macronutrients and bioactive components across metabolite classes and the changing nature of human milk composition [1,5,30–33], it is critical to investigate a wide range of milk metabolites over multiple time points. Although prior research has addressed maternal psychological symptoms during the prenatal or early postpartum periods [8,21, 22,24,34,35], no studies have investigated their impact on the human milk metabolome throughout the perinatal period and extended lactation. This approach will help determine how maternal psychological distress may differentially affect human milk composition throughout lactation.

This study is exploratory, and no a priori hypotheses were specified because of limited evidence across metabolite classes. Therefore, this study aims to identify whether maternal stress, depressive, and anxiety symptoms are associated with differential metabolomic signatures in human milk from 2.5 to 24 mo postpartum. Our objectives are *1*) to identify if prenatal maternal psychological distress measured at gestational weeks 14, 24, and 34 is associated with a differential metabolomic signature in human milk sampled at 2.5 mo, 6 mo, 12–14 mo, and 18–24 mo postpartum; *2*) to determine if postnatal maternal psychological distress measured at 3–24 mo postpartum is associated with altered human milk metabolites at 6 mo, 12–14 mo, and 18–24 mo postpartum; and *3*) to determine whether the associations between prenatal or postnatal maternal psychological distress and human milk metabolite concentrations differ between early (0–6 mo) and later (6–24 mo) stages of lactation. This timing is based on the WHO (2023) recommendations of the introduction of solid foods to the diet at 6 mo.

Methods

Study participants

This study sample consists of 424 mothers who provided human milk sample(s) and were recruited from the FinnBrain Birth Cohort study, described by Karlsson et al. [36]. Milk samples were collected at 2 wk ($n = 404$), 6 wk ($n = 177$), 14 wk ($n = 80$), 18 wk ($n = 40$), and 24 wk ($n = 17$) postpartum. This ongoing prospective observational study is conducted at the University of Turku in Finland, with participants for the pregnancy cohort initially recruited from Southwestern Finland between December 2011 and April 2015. Inclusion criteria required that families have sufficient knowledge of Finnish or Swedish and a normal first-trimester ultrasound result; no additional inclusion or exclusion criteria were applied for this subpopulation. The study has been approved by the Ethics Committee of the Hospital District of Southwest Finland, with all participants giving written informed consent for participation, data, and sample use.

The final sample for analysis included mothers and milk samples with complete data on key covariates, including breastfeeding status, birth mode, infant age, and sex. Of these, 389 dyads also had at least half of the maternal prenatal questionnaires completed, with an average response rate of 90.4% (Table 1). At gestational week 14, maternal background information, including education level, use of selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors, was reported [36]. Breastfeeding information was self-reported with questionnaires during study visits and at 3, 6, 12 mo postpartum and at 2 y, and categorized as never breastfed, breastfeeding ceased, partial breastfeeding, and exclusive breastfeeding. Maternal prepregnancy BMI (kg/m^2), gestational duration (preterm <37 wk), infant birth weight (g), length (cm), and delivery mode (cesarean section compared with vaginal delivery) were collected from hospital records (Wellbeing Services of the County of Southwest Finland). Such key covariates identified within the literature [1,23] as potential influencers of human milk metabolites were extracted from the data and presented along with other maternal and infant characteristics in Table 2.

Maternal prenatal and postnatal psychological distress questionnaires

Prenatal maternal symptoms of depression and anxiety were self-reported at gestational weeks 14, 24, and 34 using the validated 10-item Edinburgh Postnatal Depression Scale (EPDS) [37,38], along with the Symptom Checklist-90 (SCL-90) anxiety subscale [39]. Daily life stressors were measured prenatally with the Daily Hassles scale [40], using the negative scale only, relating to worries with work finances, household matters, news, media, and substance use. The Pregnancy-Related Anxiety Questionnaire (PRAQ-R2) [41] was used to capture fears about appearance, birth, and infant health. Postnatal depression was assessed using the EPDS at 3 mo, 6 mo, 1 y, and 2 y, and anxiety was assessed using the SCL-90 anxiety subscale at 3 mo, 6 mo, and 2 y. Mean scores across the time points, along with the missing data summary, are presented in Table 1.

TABLE 1
Mean questionnaire scores at time point and missing data % for each questionnaire at that time point

Time point		EPDS	SCL-90	PRAQ	Daily hassles
14 wk of gestation	Mean ± SD (missing data)	4.9 ± 3.6 (11%)	2.8 ± 3.1 (10%)	22.2 ± 7.4 (68%)	5.6 ± 2.7 (1%)
24 wk of gestation	Mean ± SD (missing data)	3.3 ± 0 (0%)	3.4 ± 4.1 (9%)	21.6 ± 6.4 (9%)	5.4 ± 2.3 (1%)
32 wk of gestation	Mean ± SD (missing data)	4.4 ± 3.9 (11 %)	2.7 ± 3.3 (11.8%)	22.5 ± 6.6 (12%)	5 ± 2.4 (1%)
3 mo postnatal	Mean ± SD (missing data)	3.8 ± 3.1 (21%)	2.4 ± 2.9 (20%)	—	—
6 mo postnatal	Mean ± SD (missing data)	4.8 ± 4.9 (31%)	2.8 ± 3.9 (30%)	—	—
1 y postnatal	Mean ± SD (missing data)	4.9 ± 4.2 (41%)	—	—	—
2 y postnatal	Mean ± SD (missing data)	5.1 ± 4.3 (46%)	3.3 ± 3.8 (46%)	—	—

Abbreviations: EPDS, Edinburgh Postnatal Depression Scale; PRAQ, Pregnancy-Related Anxiety Questionnaire; SCL-90, Symptom Checklist—90.

TABLE 2
Maternal and infant characteristics that had complete background information (n = 434)

Maternal/infant characteristic	Mean ± SD/level	Range/percentage
Maternal age	31.6 ± 4.3	19–45
Maternal BMI	24.3 ± 3.9	17–44
Exclusive breastfeeding (mo)	4.9 ± 1.3	0–7
Partial breastfeeding (mo)	14.3 ± 6	0–25
Education level	Secondary (high school or vocational education)	49
	Vocational university (university of applied sciences)	34
	University (bachelor, master, or doctoral degree)	17
Monthly income (euros)	>500	35.5
	501–1000	52.3
	1001–1500	10.3
	1501–2000	1.9
Maternal obesity	Normal	68.3
	Overweight	24.2
	Obese	7.4
Gestational hypertension	No	81
	Yes	19
Parity	Multipara	35.6
	Primipara	64.4
Prenatal SSRI use	No	97.2
	Yes	2.8
Postnatal SSRI use	No	96.2
	Yes	3.8
Delivery mode	C-section	12.7
	Vaginal	87.3
Prematurity	No	96.1
	Yes	3.9
Introduction to solid foods	4.6 ± 0.85 mo	2–6

Abbreviation: SSRI, selective serotonin reuptake inhibitor.

Human milk samples

Human milk samples were collected at 2 (n = 404), 6 (n = 177), 14 (n =80), 18 (n = 40), and 24 (n = 17) mo after birth during study visits at the research facilities in the presence of a study nurse. The study visits took place during the daytime, with sample collection occurring between 08:30 and 19:00. Mean and median collection times converted to clock times were 2 mo, 13:17 (median 13:00); 6 mo, 12:00 (median 11:53); 14 mo, 11:34 (median 11:00); and 18 mo, 10:46 (median 10:00). Mothers were instructed to feed from the right breast 1.5–2 h before the visit and then refrain from feeding from it, whereas feeding from the left breast was unrestricted. Foremilk samples were collected to preferentially capture water-soluble metabolites, consistent with the study's metabolomic focus. Using latex gloves, mothers manually expressed 10 mL of foremilk from the right breast into a sterile cup. Samples were stirred, divided into aliquots, and frozen at −80 °C. If the aliquots were not instantly frozen, they were stored at 4 °C before freezing to −80 °C on the collection day (maximum 6 h of 4°C storage).

Milk metabolomics

Metabolomic analysis of human milk was conducted with ^{1}H nuclear magnetic resonance (NMR), as previously described [4]. The milk samples were analyzed at the Department of Food Sciences at Aarhus University. The samples intended for ^{1}H NMR-based metabolomics were processed in a random order as follows, using the standard protocol for milk-based metabolomics [4]. Human milk samples were skimmed by centrifugation, followed by centrifugal filtration to remove residual macromolecules.The resulting filtrate (400 μL) was transferred to an individual 5-mm NMR tube and mixed with 200 μL of deuterium oxide (D_2O) containing sodium 3-(trimethylsilyl) propionate (TSP) as an internal chemical shift reference. The samples were analyzed by ^{1}H NMR spectroscopy using a 600 MHz Bruker spectrometer with a 5-mm ^{1}H proton observe triple-resonance inverse (TXI) probe (Bruker BioSpin), with water suppression as described by Sundekilde et al. [4]. Spectra were acquired at 298 K with a ^{1}H frequency of 600.13 MHz. A 1-dimensional experiment was conducted using a single 90°

pulse (pulse sequence: *noesypr1d*) with a relaxation delay of 5 s, during which water suppression was applied. Each spectrum consisted of 64 scans, with 32,768 data points and a spectral width of 12.15 ppm. All spectra were referenced to the TSP signal at 0 ppm. A line-broadening factor of 0.3 Hz was applied before the Fourier transformation. Preprocessing—including automatic and manual phase and baseline corrections—was performed using TopSpin 3.2 (Bruker BioSpin). Metabolites were identified and quantified using Chenomx NMR Suite, supported by an in-house milk metabolite library, resulting in the quantification of 44 metabolites.

To account for variability in milk volume and concentration, metabolite levels were normalized to lactose, a relatively stable component of mature human milk (S File 11 with means and IQRs of each metabolite). Data were log2-transformed before statistical analysis.

Confounders

Previous research has established that variation in the milk composition between individuals is associated with factors such as delivery mode, parity, maternal BMI, and maternal health and nutrition [5,8,30,33,42–44]. These variables were listed as potential confounders within this study. A directed acyclic graph was then constructed using the web-based tool DAGitty (www.dagitty.net) to inform covariate selection and identify a minimally sufficient adjustment set for controlling confounding (Supplemental File 1). The final set of covariates included delivery mode, parity, maternal prepregnancy BMI, maternal age, gestational age, and presence of gestational hypertension.

Modeling of maternal psychological distress

There is currently no evidence to support time-specific effects of stress on the milk metabolome [23]. Given this, we decided to use a mean prenatal score across each of the 4 symptom scales. Using a mean score as the prenatal predictor reduces data loss from relying on a single prenatal time point, helps manage the volume of analyses, and provides an estimate of overall symptom exposure measured by 4 scales (EPDS, SCL-90, PRAQ-R2, and Daily Hassles) across 3 prenatal assessments (14, 24, and 34 wk). Descriptive statistics, including mean and SD for the maternal distress scores, are presented in Table 1. The mean prenatal symptom scores were then standardized using *z*-scores to allow comparisons of the differences in distress measurements. Given the repeated collection of milk samples, maternal symptom scores were aligned to the timing of each sample for postnatal analysis. There was no report of SCL-90 at 18 mo, but the report at 12 mo postpartum was used instead.

Statistical analyses

All statistical analyses were performed using R statistical software (R version 4.4.1) [45]. Libraries included were tidyverse, dyplr, lme4, lmerTest, lpsline, broom.Mixed, and ggplot2 [45–49]. Coefficients (*B*) and 95% confidence intervals were calculated, and results were summarized using heatmaps. Statistical significance was defined as an adjusted P value <0.05, using the Benjamini–Hochberg procedure to control the false discovery rate for all results.

Multivariable linear regression and linear mixed models (LMMs) were used to examine associations between maternal symptom scale scores and the human milk metabolite concentrations. Model 1 is a cross-sectional multiple linear regression model applied using the prenatal mean predictor at each time point (2, 6, 18, 24 mo) to examine the associations between maternal symptoms and milk metabolites:

Metabolite concentration ~intercept + Predictor + Prepregnancy BMI + Mother's age + parity + Gestational weeks at birth + Hypertensive disorders or proteinuria + Delivery mode.

This analysis was repeated for postnatal data using the same formula, applied to matched cross-sectional symptom scores (EPDS and SCL-90) at the relevant time points.

Model 2 is a mixed-effects model used to examine associations between maternal psychological symptoms and predicted trajectories of milk metabolite concentrations over time. It also tested whether these associations differed between early (0–6 mo) and later (6–24 mo) lactation:

*Metabolite concentration ~intercept + Predictor * TimeTerms + Prepregnancy BMI + Mother's age + parity + Gestational weeks at birth + Hypertensive disorders or proteinuria + Delivery mode + (1 | subject identity).*

TimeTerms refer to terms of the piecewise linear function that was used to model the continuous milk metabolite sampling age [48–51]. The breakpoint, that is, where the line was allowed to turn, was set at 6 mo to reflect the recommended weaning age [52], allowing linear trends for the 0–6 and 6–24-mo intervals. This is best illustrated by Figures 1–5 in the main text. This completes 4 sets of analysis in total with prenatal and postnatal predictors run for both the simple regression model (model 1) and linear spline mixed-effects model (model 2).

Results

Maternal psychological symptom scores were generally in the low-to-moderate range across the perinatal period (Table 1). Mean EPDS scores ranged from 3.3 to 5.1; only a small proportion of mothers scored above commonly used thresholds for possible clinical depression. Among the 424 mothers with data at each of the 6 EPDS time points, the number scoring above 10 at any single time point ranged from 15 to 38. Overall, 99 mothers (23%) had an EPDS score above 10 at least once across the 6 assessments, indicating that elevated potentially clinically depressive symptoms were relatively infrequent. Similarly, mean SCL-90 anxiety scores were low (2.4–3.4), whereas PRAQ and Daily Hassles scores indicated the presence of ongoing, albeit modest, stress exposure. Overall, the sample reflects a population with generally low symptom levels, while still capturing variability in maternal psychological distress. The results from pre- and postnatal analysis from models 1 and 2 were aggregated in a table detailed in a metasummary file (Supplemental File 2) and are organized by metabolite class, including human milk oligosaccharides (HMOs), energy metabolism, amino acids and protective nutrients, bacterial fermentation, and lipids and fatty acids. Heat maps are used to provide a visual summary of the direction and relative strength of cross-sectional associations between maternal psychological symptoms and milk metabolites at each sampling time point. Longitudinal spline plots are presented to illustrate model-predicted trajectories across lactation, highlighting how the timing and direction of associations change over time according to maternal symptom levels. Together, these visualizations contextualize the statistical estimates reported in

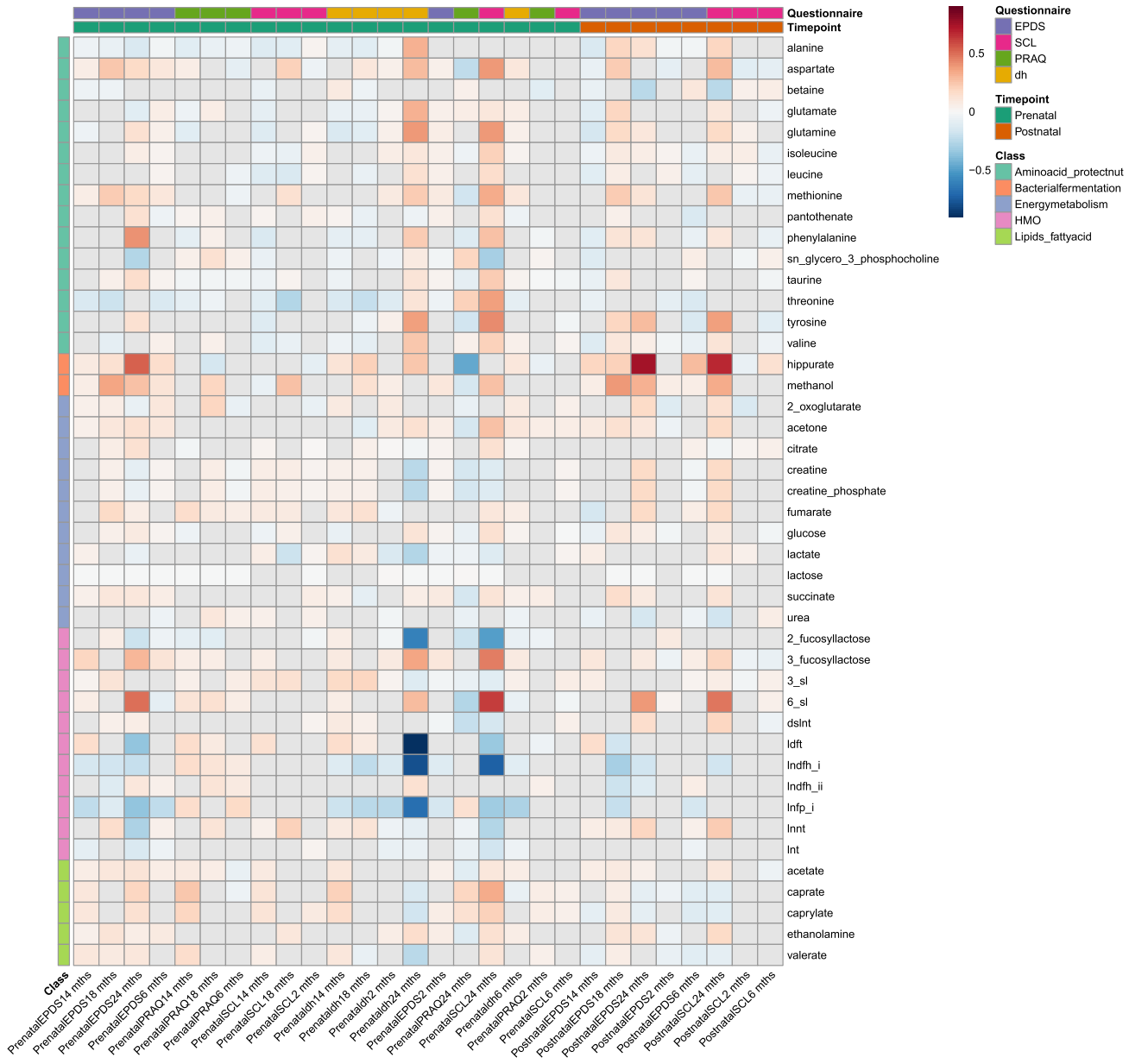


FIGURE 1. Heatmap of all significant results from simple linear regression models (model 1) (adj $P < 0.05$). Horizontal rows match the metabolites. The vertical annotation colors describe the metabolite class. Horizontal columns are questionnaires and milk sampling time points. Colors in the horizontal panel describe questionnaires and prenatal or postnatal predictors. Negative associations (regression estimates $\beta < 0$) are shown in light to dark blue, whereas positive associations ($\beta > 0$) are shown in light to dark red. EPDS, Edinburgh Postnatal Depression Scale; HMO, human milk oligosaccharide; PRAQ, Pregnancy-Related Anxiety Questionnaire; SCL, Symptom Checklist.

the metasummary table (Supplemental File 2) and mixed-effects models.

HMOs association with maternal symptoms

Overall, the association between maternal symptoms and HMOs was heterogeneous, with both positive and negative associations observed across different HMOs, predictors, and time points. Both prenatally and postnatally, LNT, LNFP I, LNDFH I, and LNDFH II were consistently negatively associated with EPDS and Daily Hassles scores across time points. In contrast, 3′-fucosyllactose (3′FL), 3′-sialyllactose (3′SL), 6′-sialyllactose (6′SL), lacto-N-neotetraose (LNnT), and disialyllacto-N-tetraose (DSLNT) showed more positive associations, particularly with prenatal symptoms (EPDS, PRAQ, and SCL) (Figure 6, Supplemental Files 3 and 4).

In addition, the longitudinal analysis showed a positive association between increased prenatal EPDS and 3'FL concentrations, with levels increasing over time across both sampling intervals (Figure 1A), whereas postnatal EPDS is observed to have a negative association with 3'FL; however, it had a positive association 2'FL in both time intervals (Figure 1B and C). Also, in support of the cross-sectional models, LNnT displays positive associations with prenatal PRAQ in both time intervals (Figure 1D) and Daily Hassles scale (significant only in 0–6 mo) (Figure 1E); however, the concentrations decline ≤6 mo.

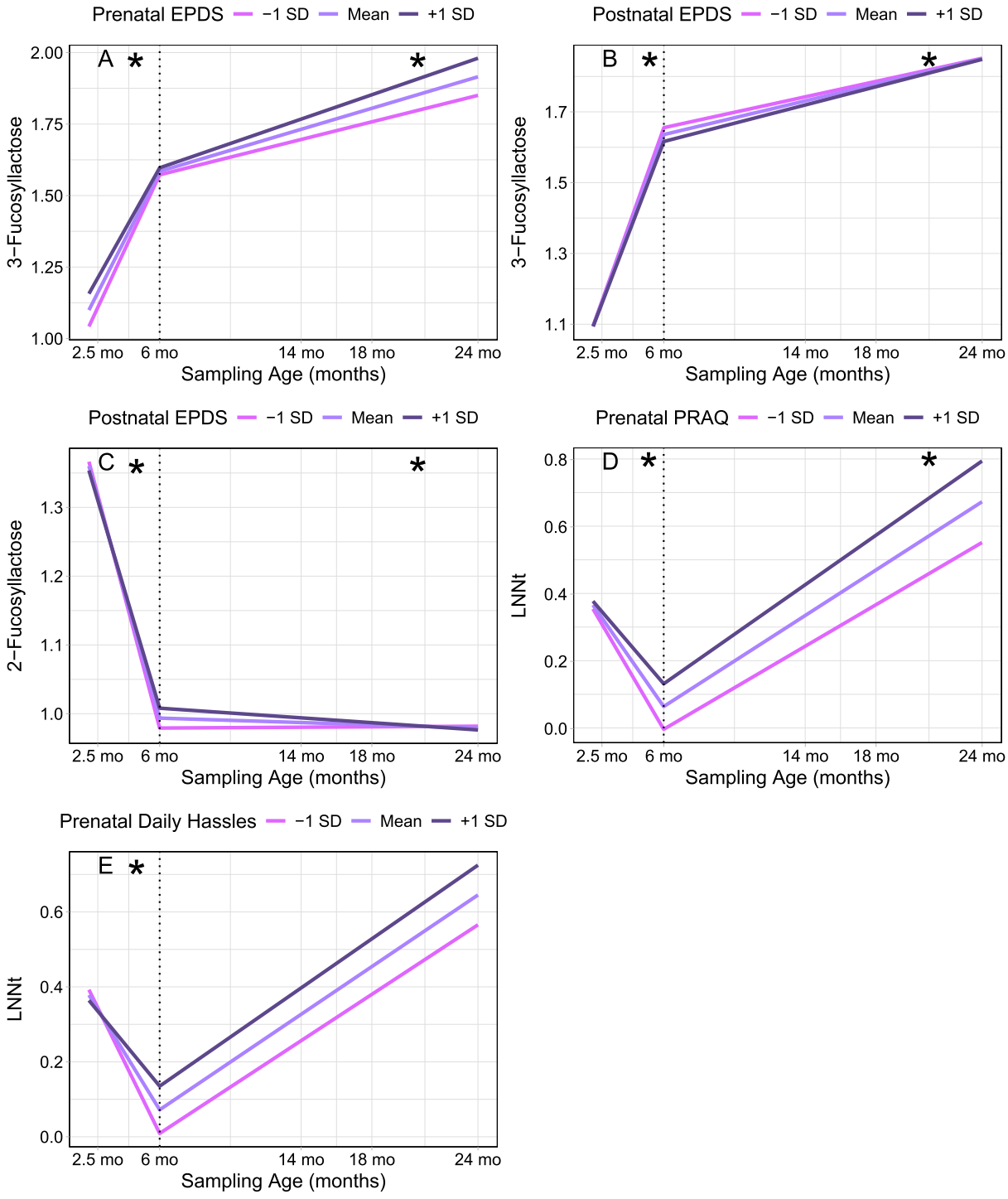


FIGURE 2. Predicted trajectories of log-transformed human milk oligosaccharide concentrations across lactation at selected levels of maternal psychological symptoms. Lines represent predicted mean log-transformed metabolite concentrations (±SD) derived from linear spline mixedeffects models (model 2) with a breakpoint at 6 mo separating early (0–6 mo) and later (6–24 mo) lactation. Maternal symptoms are modeled as a continuous variable, but only 3 selected values are shown to make the visualization possible. The other covariates are fixed at their mean/mode values. An asterisk (*) indicates a statistically significant association between maternal symptom score and metabolite trajectory within the corresponding lactation interval, based on the symptom × time interaction term (FDR-adjusted $P < 0.05$). Axes are scaled to the observed concentration range for each metabolite. (A) Prenatal EPDS ~3'FL. (B) Postnatal EPDS ~ 3'FL. (C) Postnatal EPDS ~ 2'FL. (D) Prenatal PRAQ ~ LNnT. (E) Prenatal Daily Hassles ~ LNnT. EPDS, Edinburgh Postnatal Depression Scale; FDR, false discovery rate; PRAQ, PregnancyRelated Anxiety Questionnaire; SCL, Symptom Checklist.

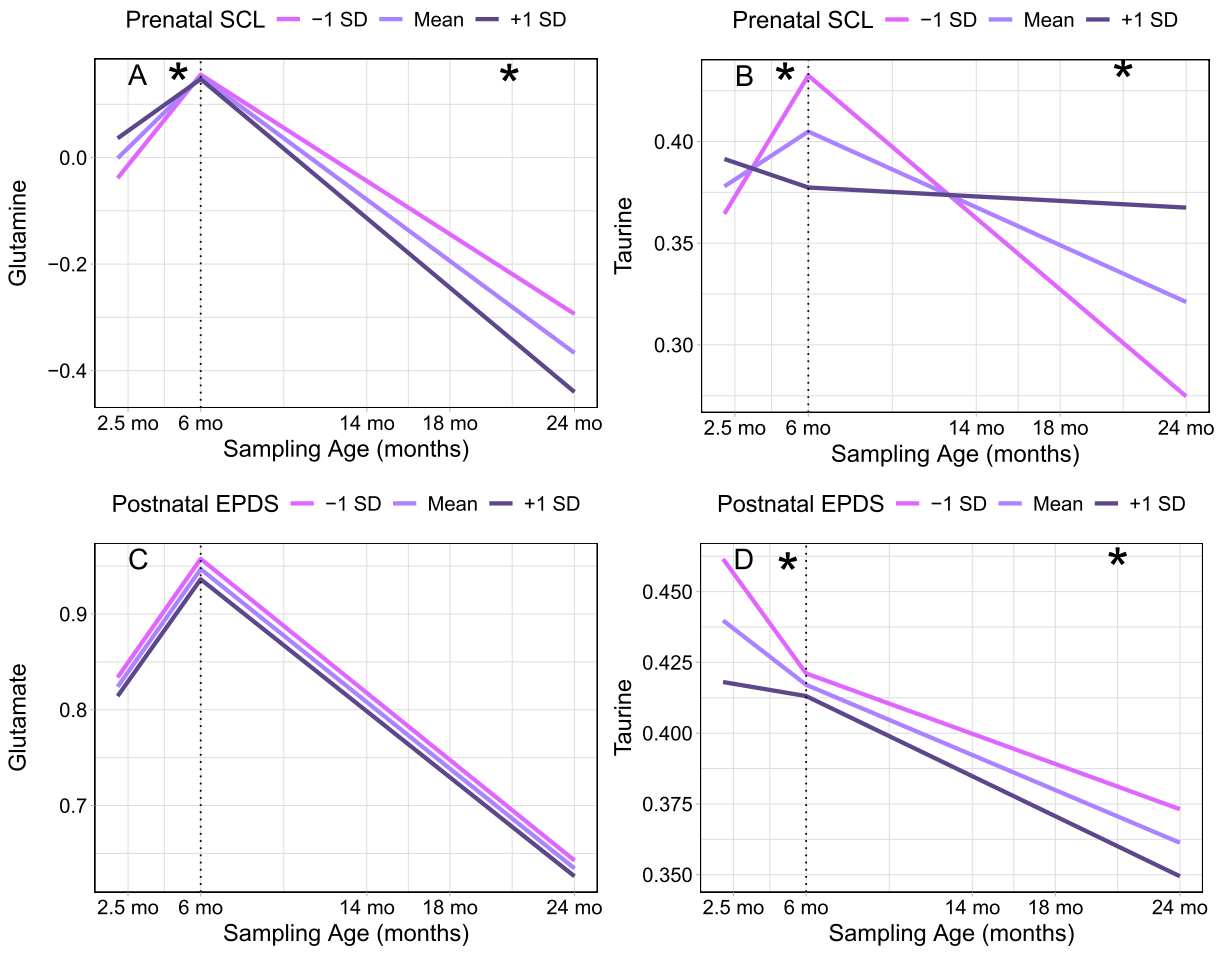


FIGURE 3. Predicted trajectories of log-transformed amino acid concentrations across lactation at selected levels of maternal psychological symptoms. Lines represent predicted mean log-transformed metabolite concentrations (±SD) derived from linear spline mixed-effects models (model 2), with a breakpoint at 6 mo separating early (0–6 mo) and later (6–24 mo) lactation. Maternal symptoms are modeled as a continuous variable, but only 3 selected values are shown to make the visualization possible. The other covariates are fixed at their mean/mode values. An asterisk (*) indicates a statistically significant association between maternal symptom score and metabolite trajectory within the corresponding lactation interval, based on the symptom × time interaction term (FDR-adjusted $P < 0.05$). Axes are scaled to the observed concentration range for each metabolite. (A) Prenatal SCL ~ glutamine. (B) Prenatal SCL ~ taurine. (C) Postnatal EPDS ~ glutamate. (D) Postnatal EPDS ~ taurine. EPDS, Edinburgh Postnatal Depression Scale; FDR, false discovery rate; SCL, Symptom Checklist.

Amino acids and protective nutrients association with maternal symptoms

The amino acid threonine was consistently lower in association with higher EPDS and Daily Hassles scores, showing a negative correlation, whereas glutamate, glutamine, and taurine were generally positively associated, especially in the later time points. A change in association patterns is evident at 14 mo between glutamine and glutamate concentrations and EPDS, SCL-90, and Daily Hassles symptoms (Figure 6, Supplemental Files 3 and 4). These findings were supported by longitudinal analysis (model 2) revealing dynamic associations over the lactation period. Glutamine exhibited a time-dependent association with prenatal SCL, shifting from positive in early lactation to negative in later stages (Figure 2A). In contrast to this, taurine showed a negative association with prenatal SCL scores reverting to a positive association by 14 mo (Figure 2B). In contrast, consistent negative associations with postnatal EPDS were observed for both glutamate and taurine across all time points (Figure 2C and D).

Energy and metabolism metabolites association with maternal symptoms

Succinate consistently showed positive associations with EPDS and SCL symptoms both prenatally and postnatally, as well as with prenatal daily hassles symptoms across the milk sampling time points (Figure 6, Supplemental Files 3 and 4). Succinate's association with PRAQ changed from positive in earlier milk samples to negative by 24 mo. Conversely, lactose exhibited predominantly negative associations with EPDS, SCL, PRAQ, and Daily Hassles symptoms at several cross-sectional time points, though with some positive associations emerging at 2 and 24 mo with Daily Hassles and PRAQ, respectively (Figure 6, Supplemental Files 3 and 4). Longitudinal analysis displayed prenatal EPDS with variable associations with succinate concentrations; however, prenatal PRAQ and SCL were linked to positive associations in early lactation stages and changed to negative associations at later lactation stages (Figure 3A, D, and E). Negative associations were also observed between postnatal EPDS and succinate consistently across both

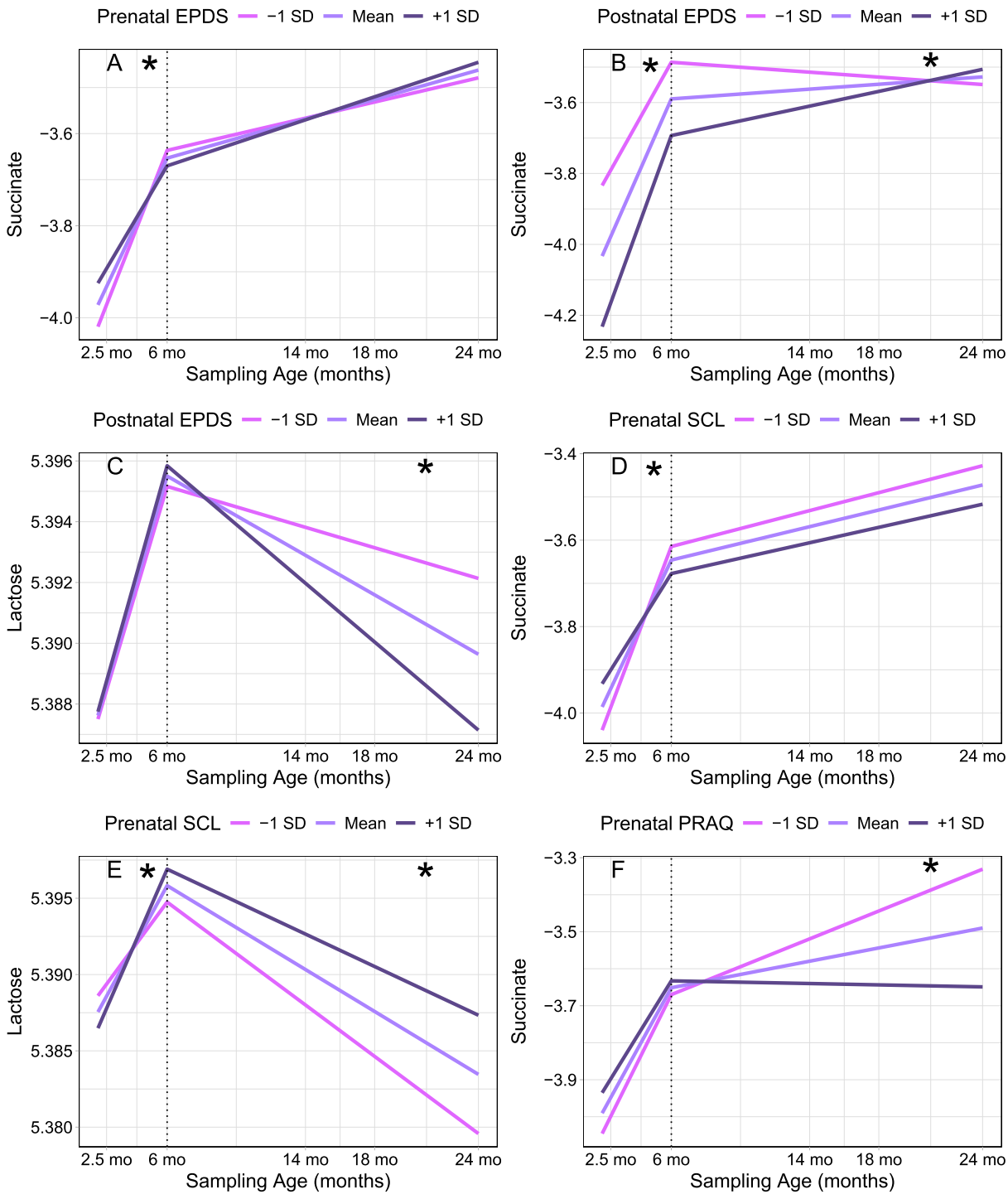


FIGURE 4. Predicted trajectories of log-transformed energy metabolite concentrations across lactation at selected levels of maternal psychological symptoms. Lines represent predicted mean log-transformed metabolite concentrations (±SD) derived from linear spline mixed-effects models (model 2), with a breakpoint at 6 mo separating early (0–6 mo) and later (6–24 mo) lactation. Maternal symptoms are modeled as a continuous variable, but only 3 selected values are shown to make the visualization possible. The other covariates are fixed at their mean/mode values. An asterisk (*) indicates a statistically significant association between maternal symptom score and metabolite trajectory within the corresponding lactation interval, based on the symptom × time interaction term (FDR-adjusted $P < 0.05$). Axes are scaled to the observed concentration range for each metabolite. (A) Prenatal EPDS ~ succinate. (B) Postnatal EPDS ~ succinate. (C) Postnatal EPDS ~ lactose. (D) Prenatal SCL ~ succinate. (E) Prenatal SCL ~ lactose. (F) Prenatal PRAQ ~ succinate. EPDS, Edinburgh Postnatal Depression Scale; FDR, false discovery rate; PRAQ, Pregnancy-Related Anxiety Questionnaire; SCL, Symptom Checklist.

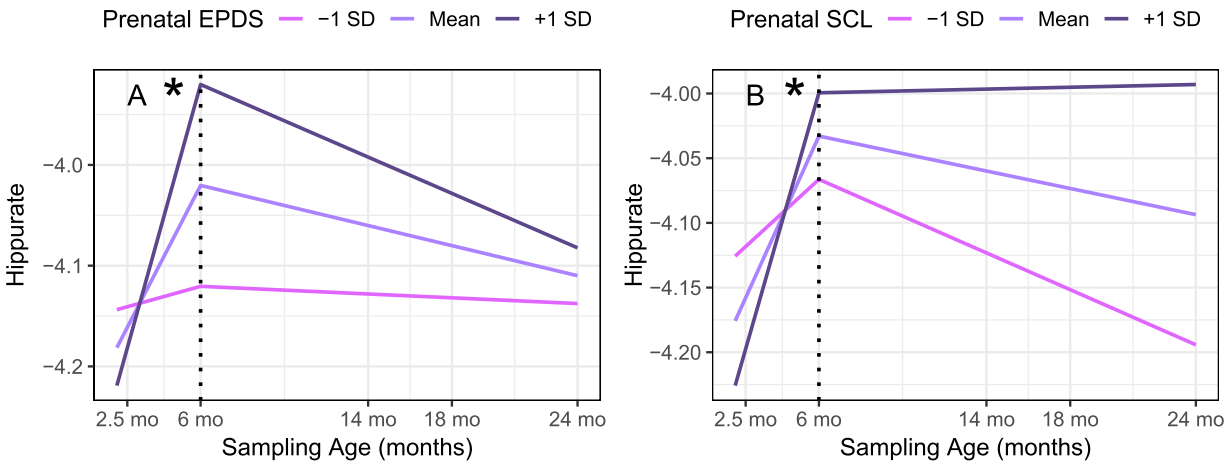


FIGURE 5. The y-axis presents predicted trajectories of log-transformed hippurate concentrations across lactation at selected levels of maternal psychological symptoms. Lines represent predicted mean log-transformed metabolite concentrations (±SD) derived from linear spline mixedeffects models (model 2), with a breakpoint at 6 mo separating early (0–6 mo) and later (6–24 mo) lactation. Maternal symptoms are modeled as a continuous variable, but only 3 selected values are shown to make the visualization possible. The other covariates are fixed at their mean/mode values. An asterisk (*) indicates a statistically significant association between maternal symptom score and metabolite trajectory within the corresponding lactation interval, based on the symptom × time interaction term (FDR-adjusted $P < 0.05$). Axes are scaled to the observed concentration range for hippurate. (A) Prenatal EPDS ~ hippurate. (B) Prenatal SCL ~ hippurate. EPDS, Edinburgh Postnatal Depression Scale; FDR, false discovery rate; SCL, Symptom Checklist.

early and late lactation stages (Figure 3B). Over time, negative associations between lactose and later postnatal EPDS were observed. However, longitudinally, prenatal SCL did display a positive association with lactose (Figure 3C and E)

Metabolites from microbial fermentation, lipids, and fatty acids

Multiple metabolites originating from microbial fermentation and lipids and fatty acids or their derivatives were associated with maternal psychological distress (Supplemental File 2). Here, hippurate from the bacterial fermentation class and caprylate from the fatty acids of lipids were identified as metabolites of interest within these results due to their consistent patterns in the results.

Cross-sectional analyses identified that hippurate presented a consistent pattern of positive associations with EPDS both prenatally and postnatally, as well as with Daily Hassles and SCL symptoms (Figure 6, Supplemental Files 3 and 4). Exceptions were observed with PRAQ, which displayed negative associations at 2, 18, and 24 mo. Prenatal EPDS, SCL, and PRAQ-R2 were positively associated with caprylate at 2, 6, 14, and 24 mo. However, postnatal EPDS associated negatively with caprylate at 2, 6, and 24 mo milk samples (Figure 6, Supplemental Files 3 and 4). Longitudinal analysis supported the cross-sectional findings for hippurates, revealing predominantly positive associations with prenatal EPDS and SCL and hippurate (significant in the first time interval only) (Figure 4A and B).

Further longitudinal models revealed negative associations with postnatal EPDS and caprylate and later negative associations between prenatal SCL, but earlier negative associations with PRAQ (Figure 5A–C).

Sensitivity and attrition analysis

Because of the low sample size at later time points (Supplemental File 2), we assessed the robustness of the LMM estimates by omitting the final time point from the prenatal mean analyses. This resulted in the loss of significance for 6 metabolites (Supplemental File 2). Repeating the sensitivity analysis with postnatal EPDS scores as predictors led to 3 metabolites losing significance, whereas use of the postnatal SCL score resulted in a larger loss of significant associations (Supplemental File 2). Consequently, postnatal SCL LMM findings were excluded from the results.

To assess potential attrition bias, independent samples t-tests compared questionnaire scores between mothers who did and did not provide milk samples at 6, 14, and 24 mo. Few significant differences were observed: mothers with higher anxiety at 6 mo postpartum were less likely to provide samples at 18 mo ($P = 0.023$), and those reporting lower pregnancy-related anxiety at 24 wk of gestation were more likely to provide samples at 14 and 18 mo ($P = 0.024$). No other significant prenatal or postnatal differences were detected across the 2–24 mo period (Supplemental File 2).

Discussion

To our knowledge, this is the first study to examine associations between prenatal and postnatal maternal psychological distress and a broad range of human milk metabolites. In our sample, maternal symptom scores were generally low to moderate, reflecting predominantly subclinical levels of anxiety and depression. Nevertheless, subclinical maternal psychological distress symptoms have been linked in prior literature to physiological changes, including inflammatory marker alterations, hypothalamic pituitaryadrenal (HPA)-axis shifts, and immune modulation [53–55]. These maternal physiological changes provide a plausible context for interpreting our observed associations between psychological distress and human milk metabolites, which are explored further in this section. In our study, we observed both positive and negative associations

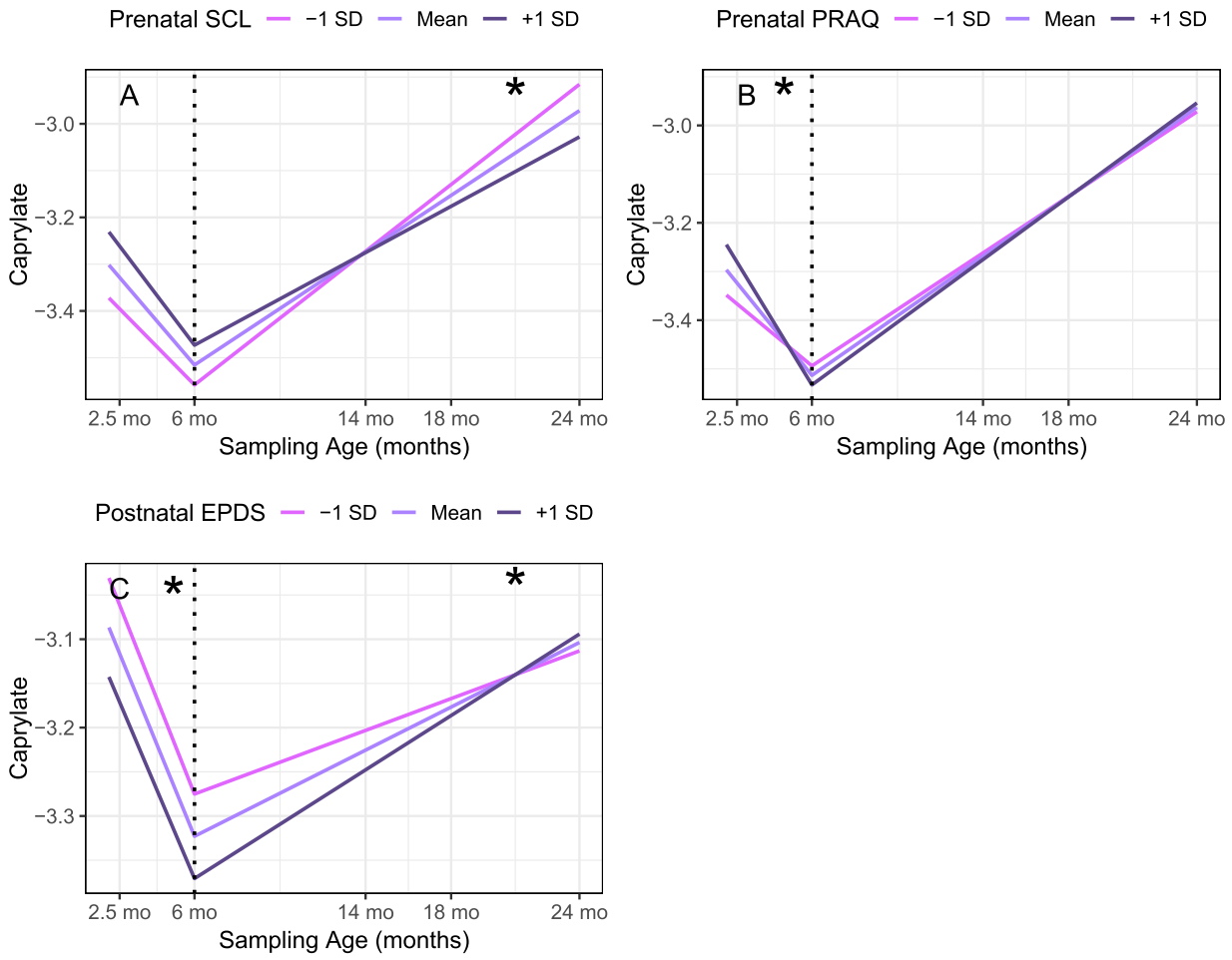


FIGURE 6. The y-axis is the predicted trajectories of log-transformed caprylate concentrations across lactation at selected levels of maternal psychological symptoms. Lines represent predicted mean log-transformed metabolite concentrations (±SD) derived from linear spline mixedeffects models (model 2), with a breakpoint at 6 mo separating early (0–6 mo) and later (6–24 mo) lactation. Maternal symptoms are modeled as a continuous variable, but only 3 selected values are shown to make the visualization possible. The other covariates are fixed at their mean/mode values. An asterisk (*) indicates a statistically significant association between maternal symptom score and metabolite trajectory within the corresponding lactation interval, based on the symptom × time interaction term (FDR-adjusted $P < 0.05$). Axes are scaled to the observed concentration range for caprylate. (A) Prenatal SCL ~ caprylate. (B) Prenatal PRAQ ~ caprylate. (C) Postnatal EPDS~ caprylate. EPDS, Edinburgh Postnatal Depression Scale; FDR, false discovery rate; PRAQ, Pregnancy-Related Anxiety Questionnaire; SCL, Symptom Checklist.

between maternal psychological distress symptoms and milk metabolites. Longitudinal analyses showed that prenatal psychological distress symptoms were associated with variable metabolite changes, often exhibiting increased concentrations, particularly in early lactation. Postnatal depressive symptoms were also linked to changes across lactation; however, decreased metabolite concentrations were more frequently observed. Consistent with Laketic et al. [54], maternal depressive symptoms, as opposed to stress or anxiety symptoms, were associated with the largest number of significant alterations in the human milk metabolic signature [56].

HMOs

This is the first study to identify positive associations between maternal prenatal depressive and anxiety symptoms and HMOs. Specifically, higher prenatal symptom scores were positively associated with acidic sialylated HMOs (3'SL, 6'SL, DSLNNT) as well as neutral HMOs (3'FL, LNnT). In contrast, prenatal depressive symptoms and daily hassle stressors were negatively associated with neutral fucosylated and non-fucosylated HMOs (LNT, LNFP I, LNDFH I, and LNDFH II). HMO concentrations generally decline across lactation except for 3'FL, which increases and is negatively correlated with 2'FL, possibly due to coregulation between enzymes responsible for their synthesis [57,58]. In human milk, sialic acid (mainly Neu5Ac) is found in α2-3- or α2-6-linked oligosaccharides synthesized by monosialyltransferases in the mammary gland, forming HMOs like 3′-SL, 6′-SL, and DSLNT [59–61]. Around 70%–83% of milk sialic acid is bound to HMOs [62]. A growing body of evidence links sialylated HMOs in milk to infant health outcomes, particularly brain development [59,60,63–65]. Sialic acid, a key component of gangliosides, plays essential roles in brain maturation, synapse structure, and function, as well as learning, memory, and cognitive development [55,56,59,61,62]. Although influenced by maternal genotype, sialic acid levels also vary with blood group, geography, lactation stage, gestation, and diet; 3 of these variables are controlled in this

study [57,59–61,66,67]. Increased α2-3/α2-6 sialylation is associated with inflammatory states and immune dysregulation [45,59,68]. Maternal perinatal psychological distress is reported to correlate with systemic inflammation, hormonal shifts, and gut dysbiosis [69–75]. Although such physiological processes were not assessed in the present study, elevated sialylated HMOs observed in association with higher self-reported symptoms may reflect pathways that have previously been linked to inflammatory responses, with sialic acid being proposed as a candidate biomarker [76,77]. It can be speculated that this increase in sialylated HMOs in distressed mothers may reflect a compensatory mechanism to protect the infant from inheriting risks from the maternal inflammatory state.

This study also reports negative associations between HMOs and maternal depressive symptoms, consistent with findings by Riedy et al. [24], who examined postnatal symptoms at a single time point. Our study builds on this by incorporating repeated assessments of maternal symptoms and multiple milk sampling time points, offering a more detailed view of how these associations may change over the course of lactation. Previously, it has been hypothesized that reduced HMO concentrations in mothers experiencing postpartum distress may result from decreased breastfeeding frequency, leading to incomplete duct emptying and increased mammary gland permeability [24,78,79]. Furthermore, prenatal proinflammatory states, potentially related to maternal distress, may impair mammary gland differentiation and permeability during pregnancy [78,80], whereas reduced local serotonin (5-HT) may further disrupt epithelial development and milk synthesis [81,82]; such mechanisms potentially support an explanation for the negative association of neutral fucosylated and nonfucosylated HMOs with symptom scores in this study.

Amino acids and protective nutrients

This study shows heterogeneous associations between maternal perinatal symptoms and amino acid concentrations: threonine was negatively associated with higher depressive symptoms, whereas glutamate, glutamine, and taurine were positively associated with symptoms of stress and depression. These findings align with 2 studies linking postnatal stress and depression to similar amino acids (alanine, leucine, phenylalanine, tyrosine); however, differences included maternal blood serum samples during pregnancy and milk samples taken $\leq$3 mo postpartum [27,56]. In contrast, 2 other studies reported no significant associations after multiple testing or found no correlations in milk sampled at 2.5 and 5 mo postpartum [8,83]. Variability in analytical approaches, as well as differences in study design such as focusing on either pre- or postnatal symptoms and relying on a single milk sample collected between 1 and 5 mo postpartum, may contribute to these contrasting results.

Our findings suggest that changes in milk in some free amino acids (FAAs) levels may relate to the type and source of amino acid. FAAs in milk, which account for 5%–10% of total amino acids, arise from 2 main sources: proteolytic breakdown of milk proteins synthesized de novo in the mammary gland, and from active transport of maternal plasma amino acids via mammary epithelial cell transporters [84–86]. Transporter activity is reported to be regulated across lactation and varies by amino acid type and size, indicating selective and dynamic control of FAA levels, potentially via intracellular signaling [86–88]. Maternal factors such as BMI may have modest effects on milk FAAs, whereas age, diet, and geography show minimal influence [86,89]. Furthermore, the lack of correlation between maternal plasma and milk FAA concentrations supports the concept of this selective regulation within mammary tissue [86,90].

Divergent patterns in glutamine, glutamate, and threonine may reflect their roles in inflammation and transporter regulation activity. Perinatal depressive, anxiety, and stress symptoms can be linked to low-grade inflammation and heightened energy needs, which may drive increased glutamate and glutamine via metabolic adaptation to energy demands and immunological responses [30,74,86,91–95]. Previously seen in animal studies, inflammatory-induced release of glucocorticoids can upregulate system L transporters (e.g., LAT1/SLC7A5), potentially enhancing glutamine secretion into human milk [27,85]. In contrast, threonine is likely to be transported via system A (SNAT2), which is less influenced by hormones [96]. It may also be catabolized to glycine for glutathione synthesis during oxidative stress [96], which is a process linked to psychological distress [97], potentially explaining lower concentrations. Longitudinal analysis shows that higher postnatal depressive symptoms were negatively associated with glutamate and taurine, which may reflect hormonal modulation of amino acid transporters by prolactin and oxytocin at this postnatal stage [81,85]. Oxytocin's documented inhibition of stress responses and cortisol release may reduce glutaminase-driven conversion of glutamine to glutamate [98]. Overall, our results suggest that variations in amino acid levels may reflect maternal physiological responses to distress.

Energy and metabolism

Lactose and succinate emerged as energy metabolites of interest, with increased symptoms of perinatal maternal depression and anxiety showing negative associations with lactose concentrations and positive associations with succinate. Few studies have examined lactose specifically; most group it with other carbohydrates and report no association with postpartum symptoms [25,34]. However, aligning to this study, Ziomkiewicz et al. [99], who studied milk composition at 5 mo postpartum and in a sample of 146 healthy mothers, found psychosocial stress and cortisol to be negatively associated with human milk lactose.

Lactose synthesis in the mammary gland depends on maternal plasma glucose, which is metabolized to UDP-galactose via key enzymes regulated by hormones including prolactin, glucocorticoids, insulin, thyroid hormones, progesterone, and estrogens [100]. Lower lactose levels have been linked to elevated cortisol, which may divert glucose toward energy demands during stress, limiting its availability for lactose synthesis [99]. This mechanism, together with the inhibitory effects of proinflammatory cytokines such as TNF-α on glucose transporters like GLUT1, may explain the stress- and depression-related changes in lactose levels [56,91,92,101]. On the other hand, succinate has been elevated in human milk in subjects with inflammatory bowel disease, potentially due to gut dysbiosis, and has therefore been suggested as a

proinflammatory stimulus to regulate local stress and immune responses [102,103]. This provides a potential explanation for the increase in succinate in mothers' milk who have higher levels of perinatal psychological distress symptoms.

Lipids/fatty acids

Caprylate (caprylic acid), a medium-chain saturated fatty acid present in dairy and certain plant oils, showed significant positive associations in this study. Previous research on maternal stress and human milk fatty acid composition has produced inconsistent findings [22,26,29,104]. Despite the studies using various targeted milk metabolite analyses, differences in analytical platforms, sample preparation, milk sampling time points, and timing and type of maternal questionnaires can still contribute to variability in reported results. Studies have identified that human milk fatty acid composition is influenced by maternal diet, BMI, and genetics [105]. Caprylate, as a medium-chain fatty acid, increases with low maternal fat intake due to upregulated de novo lipogenesis in the mammary gland [22,106]. Therefore, lower caprylate levels associated with postnatal symptoms may reflect dietary shifts, such as reduced carbohydrate or increased fat intake, which may suppress de novo lipogenesis. Other explanations for the divergent patterns prenatally and postnatally may be due to cortisol, which plays a key role in lipolysis and energy metabolism but also acts as a hormonal mediator of the physiological response to stress and inflammatory states [22,26]. In animal studies, caprylate is also reported to have anti-inflammatory effects through suppression of proinflammatory cytokines (e.g., TNF-α) and upregulation of anti-inflammatory markers [107]. A recent study further links lower caprylate levels in infants to disease states such as atopic dermatitis [108]. This may suggest that elevated caprylate levels in human milk may also reflect a maternal compensatory response aimed at supporting fetal immune development and mitigating inflammatory risk.

Implications for infant health

It is generally considered that human milk provides universal benefits to infants regardless of maternal health status. Our study adds to this understanding by showing that even in the context of maternal distress symptoms, a range of human milk metabolites, including HMOs, amino acids, lipids, and energy metabolites considered beneficial, were positively associated with increased maternal symptoms. One can speculate that human milk may serve as a buffering mechanism between maternal distress and infant immune and developmental processes. Perinatal mood disorders have been associated with altered maternal and infant gut microbiome, often characterized by reduced beneficial microbes and a proinflammatory state [73,109–111]. Increased concentrations of HMOs in this study, such as 3′SL, 6′SL, and LNnT (sialyated) and 2'FL (neutral), promote beneficial bacteria, including *Bifidobacterium longum* and *Phocaeicola vulgatus*, support gut barrier function, and aid intestinal maturation, contributing to newborn health [112]. Similarly, increases of FAAs such as glutamine enhance beneficial bifidobacteria and reduce potentially pathogenic species in Pseudomonadota [86,95]. Together, these components may help prevent or correct early-life dysbiosis, suggesting a compensatory role of altered human milk composition [32,95,113–115].

Notably, in this study, higher maternal depressive or stress symptom scores were associated with an increased sialylated-to-fucosylated HMO ratio. Given the role of sialylated HMOs in supporting infant neural growth and cognitive functioning [63–65], this shift could reflect another potential mechanism to assist infant cognitive function in potentially stressful or inflammatory environments. However, this remains speculative and requires further investigation. Moreover, HMOs that were positively associated with maternal distress in this study, 3′SL, 3′FL, and 6′SL, modulate immune cell functions, regulate immune responses, and exhibit direct antiviral activity against pathogens including rotavirus, respiratory syncytial virus (RSV), and influenza A [33,112,116–119]. DSLNT, one of the most abundant sialylated HMOs, shows anti-inflammatory effects relevant to necrotizing enterocolitis [32,59]. Similarly, increases in FAAs such as glutamine along with medium-chain fatty acids such as caprylate reduce proinflammatory and increase anti-inflammatory cytokines [1,35], whereas succinate acts as an inflammatory signal via macrophage and dendritic cell activation [102,103]. Therefore, increases in certain sialylated HMOs, FAAs, lipids, and energy metabolites in human milk that we observed to be positively associated with higher depressive, stress, or anxiety symptom scores may regulate infant immune activity.

On the other hand, the reduction in concentrations of other key metabolites should also be considered when evaluating its potential impact on infant health. Lactose, here negatively associated with higher maternal depressive, stress, or anxiety scores, for example, has been shown to enhance gut immunity by upregulating antimicrobial peptides, supporting intestinal homeostasis, and serving as an energy source for beneficial bacteria such as Bifidobacteria and Lactobacillus [120,121]. Lactose is also positively linked to human milk volume and increased infant growth [122]. The amino acid threonine, also negatively associated with maternal distress in this study, has been shown to support gut health by promoting a protective mucus layer in the gut, microbial production of beneficial metabolites such as short-chain fatty acids, and by protecting from potential pathogens [123].

HMO composition in human milk is highly variable and influenced by multiple factors, including maternal stress [57]. In this study, maternal symptoms were associated with specific shifts in HMO composition, particularly the sialylated-to-fucosylated ratio, as well as changes in other metabolites. Some of these, such as nonfucosylated HMOs like LNnT and fucosylated derivatives such as LNFP1 and LNDFH II, are considered to have probiotic properties, prevent pathogen adhesion, and support immune function [112,124]. Changes in these metabolites may therefore influence infant microbiome development and subsequent health trajectories. Our findings demonstrate the dynamic nature of human milk composition in relation to maternal distress, suggesting several directions for future research. Longitudinal studies examining whether differences in milk metabolite exposure influence infant outcomes such as growth, immune function, neurodevelopment, and the gut microbiome could clarify their potential impact on infant health. Studies exploring interventions targeting maternal diet, physical activity, or psychosocial support may reveal ways to optimize milk composition under maternal stress. Future research, including mothers with clinically significant stress,

anxiety, or depressive symptoms, may help determine whether associations with milk metabolites are more pronounced at higher symptom severity. In addition, qualitative research exploring maternal knowledge and beliefs about milk composition and its determinants could help contextualize biological findings and support the translation of research evidence into educational gaps. Finally, population-level and mechanistic studies could explore social, environmental, and biological determinants of metabolite variation, informing maternal–child nutrition and public health strategies.

Strengths and limitations

Strengths of this study include its longitudinal cohort design with a large sample of 424 mothers, with repeated self-report questionnaires in both prenatal and postnatal time periods and multiple time points for milk samples. This allowed the application of both cross-sectional and longitudinal models that accounted for relevant confounding factors, as well as the use of robust testing, attrition analysis, and adjusted *P* values. In addition, although we could not study colostrum, we had the ability to study human milk composition later during lactation, albeit with a more limited sample size. In our protocol, we did not implement any breast or nipple cleaning, which may cause contamination of skin (products) in the sample; however, potential contaminants were not included in the metabolomics assays. The human milk sample describes just a snapshot of human milk composition, which changes throughout the feeding, day, and lactation, and hence, the sample does not fully reflect the nutrition received by the infant.

In addition, postnatal depressive symptoms were assessed at 4 time points, whereas anxiety only at 3. Later time points included fewer milk samples, particularly at 24 mo, which may have reduced statistical power for some analyses in model 1 (multiple linear regression). Effect sizes, as visualized in our forest plots (Supplemental Files 3 and 4), show greater variability at this time point compared with earlier ages, suggesting that results should be interpreted with caution. In contrast, our LMMs are suited for unbalanced data and can account for variability across time points, supporting the robustness of the main longitudinal findings. Moreover, we could not address all factors that are potentially related to milk composition or mediating/moderating the link between maternal distress and milk composition, such as maternal diet. Overall symptom levels in this sample were predominantly mild to moderate, and findings may therefore not generalize to populations with severe or clinically diagnosed mental health conditions. In addition, no direct physiological markers of stress were assessed. References to biological mechanisms throughout the Discussion section are intended to provide potential biological context based on prior literature, recognizing that even mild-to-moderate psychological symptoms may co-occur with physiological responses, although these were not directly measured in the present study. As such, the biological pathways underlying the observed associations, such as inflammation, hormonal modulation, or oxidative stress, and their relevance for infant development remain to be investigated in future research.

In conclusion, our findings indicate that both prenatal and postnatal maternal distress symptoms were associated with differential metabolomic signatures in human milk metabolites, although the patterns were variable across metabolite classes and milk sampling time points. There was a minimal change in the links between distress and milk composition across early (0–6 mo) and later (6–24 mo) stages of lactation. Importantly, this study demonstrates that even subclinical or low-to-moderate maternal anxiety and depressive symptoms, which are common in the perinatal period, are reflected in measurable alterations in milk metabolites. This highlights the potential sensitivity of human milk to maternal psychological state. These alterations further represent the dynamic nature of human milk, which may provide a protective medium buffering infants from maternal distress while simultaneously reflecting downregulation of other key metabolites. The findings emphasize the importance of addressing maternal psychological and inflammatory states during the perinatal period. Although perinatal stress, anxiety, and depression symptoms are often unavoidable, public health efforts can support maternal mental health by targeting modifiable factors such as diet, physical activity, and psychosocial support, which may help mitigate their effects on both maternal well-being and infant health outcomes [125].

Author contributions

The authors' responsibilities were as follows – NR, A-KA, SMO, HM, PL-W, LP,LFP LK, HK, H-MK: designed research study; H-MK, HK, LK, A-KA: collected the data; NR, A-KA, UKS: analyzed data; NR, A-KA, SMO: authored the paper; NR, A-KA, SMO, AMD, SL, LP, ST: interpreted data; A-KA, LP: supervised the data analyses; NR, A-KA, LP: visualized the data; NR: wrote the initial manuscript; A-KA, SMO, NR: majorly revised the manuscript; and all authors: read and approved the final manuscript.

Conflict of interest

H-MK is currently employed by International Foods and Flavors Inc.

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Data availability

Individual-level data cannot be openly shared due to national legislation on personal data protection and the consent given by the study participants. The research data can be shared as part of a research collaboration with relevant research agreements in place. Please contact Linnea Karlsson (linnea.karlsson@utu.fi). R analytic code and scripts are available at Doi https://doi.org/10.5281/zenodo.17242045.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to revise R scripts. After using this tool, the

authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2026.101531.

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