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Long-Term Associations Between Prenatal Maternal Cortisol and Child Neuroendocrine-Immune Regulation

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Abstract

Background—Advancing understanding of the developmental origins of neuroendocrine-immune (NEI) functioning is key to elucidating the biological mechanisms involved in health and disease risk across the lifespan. This study examined whether prenatal maternal hypothalamic-pituitary-adrenal (HPA) activity moderates child NEI relations and explored the consistency of this moderating effect across gestation.

Methods—Pregnant women participated in five prenatal study visits from 24 to 38 weeks gestation. At each visit, women provided a saliva sample. In a 5-year follow-up study, children (n_{female} = 25, n_{male}=20) provided four saliva samples and participated in behavioral assessments and challenge tasks. Prenatal maternal saliva samples were assayed for cortisol. Child saliva samples were assayed for cortisol and cytokines (IL-1β, IL-6, IL-8, TNFα) as indices of HPA and inflammatory activity. Multilevel mixed-effects models examined the moderation of child NEI relations by prenatal maternal cortisol.

Results—Among males, average prenatal maternal cortisol did not moderate child NEI relations. Among females, average prenatal maternal cortisol moderated some child NEI relations with higher prenatal cortisol associated with more positive cortisol-cytokine relations at age five. When examined by gestational time point, there were more significant NEI moderation effects by maternal cortisol from later gestation (≥ 30 weeks) than earlier.

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Compliance with Ethical Standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest In the interest of full disclosure, DAG is founder and chief scientific and strategy advisor at Salimetrics LLC and Salivabio LLC and these relationships are managed by the policies of the committees on conflict of interest at the Johns Hopkins University School of Medicine and the University of California at Irvine.

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Conclusions—The findings suggest prenatal maternal HPA activity may moderate child NEI functioning. Additional research conducted with more heterogeneous and larger samples is needed to fully understand these relations. Furthering our knowledge of NEI development has important research and clinical implications, particularly for understanding and addressing conditions with inflammatory pathophysiologies, such as depression and cardiovascular disease.

Keywords

Prenatal maternal cortisol; Salivary cytokine; HPA axis; Neuroendocrine-immune; Sex differences

Introduction

Advancing understanding of the relations between endogenous maternal cortisol during pregnancy and child neuroendocrine and immune system functioning is key to illuminating the development of neuroendocrine-immune (NEI) system coordination. NEI system functioning is increasingly linked to disease pathophysiology, and elucidating the biological mechanisms underlying these associations is important for developing effective prevention and clinical intervention programs, as well as furthering our understanding of population health disparities [10, 31]. Maternal cortisol naturally increases with advancing gestation and is essential for fetal development and parturition [48]. The impact of prenatal maternal cortisol on fetal NEI coordination and the development of the immune system, however, has been largely unexamined in humans [35, 36, 59].

Characterized by rapid ontogenetic processes, the fetal period is a time of heightened environmental sensitivity. The development and calibration of the fetal neuroendocrine stress response and immunocompetence begin in utero [16, 59] making these systems, and coordination between them, particularly sensitive to environmental cues and experiences during the prenatal period. These environmental factors include exogenous exposures like pollutants and toxins, as well as endogenous factors like maternal hormones and immune processes. While multiple systems may be vulnerable, child stress physiology, and particularly hypothalamic-pituitary-adrenal (HPA) functioning, is one of the primary physiologic mechanisms investigated as a link between prenatal and early-life experiences and child health and behavioral outcomes [13, 19, 42, 58]. Although findings from animal studies demonstrate several potential mechanisms by which prenatal cortisol may influence fetal immune development and regulatory mechanisms, there is limited research on prenatal maternal cortisol and immune system development in children [2, 35, 36, 59].

Previous studies of prenatal maternal HPA activity and child outcomes have demonstrated the importance of accounting for both the timing of prenatal cortisol and fetal sex as key moderating factors in the relations between of prenatal maternal HPA activity, fetal development, and child physiology and behavior [2, 6, 8, 12, 13, 19, 21, 22, 26, 27, 46, 49, 55, 58]. In studies examining associations between prenatal maternal cortisol and child health and development, sex-specific effects are consistently found, and, in many studies, females are more sensitive to prenatal cortisol than males [2, 6, 19, 32, 55]. These findings are consistent with the “viability-vulnerability tradeoff’ theory [53] which posits that sex-dependent physiologic responses to in utero exposures, both endogenous and exogenous,

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result in increased fetal death among males and increased adaptation among females. These differential responses to in utero exposures result in a strong, homogenous cohort of surviving male infants, and a more variable cohort of female infants for whom adaptations made during gestation may increase vulnerably to poor outcomes later in life [53]. Support for sex-dependent effects of prenatal maternal cortisol also comes from animal models which have found sex-specific changes in neurobiology, gene expression, behavior, and cognitive function related to prenatal stress, such as repeated variable and restraint stress [3]. Importantly, while sex differences are commonly found, studies have also found that prenatal maternal HPA activity is associated with infant HPA physiology even in healthy, low-risk, and high socioeconomic samples [19]. This supports our understanding of maternal cortisol as a central mechanism influencing fetal development.

At approximately age 5, the final period of immunologic development ends and mature levels of immunocompetence and immune memory are achieved [59]. In a recent study, we explored relations between family adversity and child NEI function at the end of this window of vulnerability for immune system development. In a cross-sectional study of mothers and their 5-year-old children, we found evidence that maternal psychological distress may play a role in calibrating interactions between the child's neuroendocrine stress and immune system responses [52]. Specifically, children whose mothers were psychologically distressed demonstrated NEI relations suggesting a reduced sensitivity of inflammatory immune processes to cortisol's inhibitory effects [52]. Cortisol plays a critical role in moderating immune processes; it inhibits inflammatory cytokine activity thereby helping reduce excessive inflammation and the risk of tissue damage caused by prolonged, unregulated inflammation [28]. Inefficient regulation of inflammatory cytokines by cortisol may contribute to a wide range of diseases, such as asthma, depression, and cardiovascular disease [1, 10, 11]. This cross-sectional study, however, only allowed us to look at relations between maternal psychological distress and child NEI relations when the child was 5 years old.

To gain additional insight into the developmental origins of NEI function, in the current paper, we examined a subset of these 5-year-old children for whom repeated measures of maternal HPA activity during gestation are available. In the current study, we examined the moderating role of prenatal maternal HPA activity on child HPA axis and immune system coordination at 5 years old. Acknowledging the importance of both overall prenatal cortisol exposure and the gestational timing of exposure, we examined child NEI moderation by the average prenatal maternal cortisol level across pregnancy, as well as explored whether the moderation of child NEI relations by prenatal maternal HPA activity varies by the gestational timing of maternal HPA activity assessments. Based on previous research and the findings from our cross-sectional study, we hypothesized that prenatal maternal HPA activity would moderate child HPA-immune system relations 5 years later and that this moderation effect would be stronger among female children than male [6, 19, 32, 52, 55].

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Methods

Study Sample

The sample was drawn from a longitudinal study of pregnant women who participated in a five-visit prenatal study ($n = 124$) in 2006–2007 and a 5-year follow-up visit in 2011–2012. The study sample consisted of 45 mother/child pairs who had data from at least three of the five prenatal visits and complete salivary biomarker data from the 5-year visit. While women included in the 5-year follow-up study ($n = 45$) were slightly older than prenatal study participants who were not included in the follow-up study (mean difference = 1.76 years; $t(110) = -2.01$, $p < .05$), the two groups were similar on measures of race, education, pre-pregnancy body mass index (BMI), parity, and fetal sex. Women in the follow-up study also exhibited similar levels of salivary cortisol during pregnancy compared to women who were not included in the follow-up study.

During pregnancy, the mean age of the mothers was 32 years and 67% of women were nulliparous (Table 1). The majority of women were white and the majority of families were upper-middle class (Table 1). Most women reported a healthy pre-pregnancy weight (58% healthy/normal BMI), and 38% reported being overweight or obese prior to pregnancy. At birth, children were, on average, 39.31 weeks gestation (SD = 21.19, range 35.86–41.14 weeks; one child was born before 37 weeks), and 87% of children weighed between 2500 and 4000 g (mean birth weight = 3450.20 g (SD = 437.85, range = 2250–4285 g)). Mean Apgar scores at 1 and 5 min were 7.77 and 8.84, respectively (1 min SD and range 1.64, 2–9; 5 min SD and range 0.43, 7–9). At the 5-year visit, the mean age of children was 64 months, and the majority of children were healthy (based on maternal report; Table 1).

Procedures

Pregnant women participated in five prenatal study visits at 3-week intervals across pregnancy (from 24 to 38 weeks gestation; $n = 124$). To be eligible, participants had to be non-smoking women with healthy, singleton pregnancies. Eligibility criteria excluded women who reported pre-existing chronic health conditions (e.g., diabetes, chronic hypertension) and cases with fetal anomalies or malformations at the time of enrollment. Entry into the prenatal study was staggered, so that women entered between 24 and 26 weeks gestation and returned every 3 weeks until weeks 36–38 of gestation. During each prenatal study visit, women provided a saliva sample and completed sociodemographic questionnaires. Saliva samples were collected approximately 30 min after arriving to the research laboratory during which time the women received an ultrasound and answered questions about their health. See DiPietro et al. [14] for details on prenatal study protocol.

Maternal-fetal pairs were re-contacted when children were 5 years of age. To be eligible for the 5-year follow-up study, mothers and children had to be fluent in English, and children had to be 5 years old. Children were excluded if their mothers reported that they had a significant health condition or developmental disability that impaired cognitive, motor, or regulatory functioning such as cystic fibrosis, autism, or mental retardation. A total of 58 mother/child pairs from the prenatal study participated in a single 90-min laboratory visit at age 5; the remainder participated remotely ($n = 15$), withdrew from the study or were

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ineligible (n = 12), or were lost to follow up owing to moving out of the area or scheduling conflicts (n = 39). Forty-five of the 58 mother/child pairs who participated in the 5-year follow-up lab visit had complete salivary data for the child participants. The children with complete salivary data (n = 45) were similar to those with missingness due to saliva collection and/or processing issues (n = 13) on measures of age, sex, race/ethnicity, and BMI, and the mothers of these children were of a similar age and reported similar levels of depressive symptoms.

During the 5-year on-site visit, children completed neuro-psychological and behavioral assessments. Children also participated in three emotional stressor tasks designed to elicit negative emotions and challenge behavioral and emotional control: the Disappointing Gift Game [9], the Not Sharing Game [54, 57], and the Delay of Gratification Task [39, 57]. Four saliva samples were collected from children. The first saliva sample was collected approximately 25 min after the start of the study. Saliva samples 1 and 2 were collected before the stressor tasks and saliva samples 3 and 4 were collected after the stressor tasks. Mothers provided sociodemographic and child health information. Mothers also completed a battery of psychological assessments, including an assessment of depressive symptoms. See Riis et al. [51] for detailed information about the 5-year study visit.

Biospecimen Collection and Biomarker Determination

Saliva samples were collected from mothers at each prenatal visit. All prenatal visits were conducted in the afternoon, and participants were instructed to eat no later than 90 min prior to the visit arrival and restrict fluid intake during the visit. Saliva samples were collected using filter paper which was air dried and stored at room temperature [33, 41]. Cortisol assays were performed using a commercial, high-sensitivity EIA kit (Salimetrics, LLC). The assay range of sensitivity was 0.003–3.0 μg/dl (0.083–82.77 nmol/L). After accounting for the extraction dilution, the detection limit was 0.018 μg /dl (0.50 nmol/L). Inter-assay coefficients of variation (CVs) were less than 9.0% for high-and low-range laboratory controls, and intra-assay CVs were less than 4.5%. Concentrations below the assay lower limit of detection (LLD; < 1% of samples) and missing values (12% of samples) were replaced with the mean cortisol concentration across pregnancy. See Kivlighan et al. [33] for additional details regarding prenatal maternal cortisol procedures.

Four saliva samples were collected from children at the 5-year visit. Mothers were instructed to restrict their child's food and drink intake for at least 30 min prior to the scheduled study visit. Child saliva samples were collected using passive drool and stored at − 20 °C until assayed. Cortisol was assayed in duplicate using a commercially available enzyme immune-assay (Salimetrics, Carlsbad, CA). Cortisol assay sensitivity ranged from 0.007 to 3.0 μg/dL. The intra-assay CV was less than 5%, and the inter-assay CV was less than 10%. Salivary cytokines were measured following Riis and colleagues [50] using multiplex electrochemiluminescence immunoassays by Meso Scale Discovery (Gaithersburg, MD) and following the manufacturer's protocol. Cytokine concentrations (pg/mL) were determined with MSD Discovery Workbench software (v. 3.0.17) using curve fit models (4-PL with a weighting function option of 1/y2). All lower limits of detection for the cytokines were below 0.20 pg/mL and intra-assay CVs ranged from 2.1%to 6.6%.

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Primary Measures

Prenatal Maternal HPA Activity—Cortisol determinations from the five maternal saliva samples from the prenatal study were used to index HPA activity during pregnancy. The mean of the five cortisol determinations was used to represent average afternoon salivary cortisol levels across pregnancy. To examine time-specific effects of prenatal cortisol across gestation, salivary cortisol data were also organized by visit, with visit 1 data representing cortisol collected during study visits at gestational weeks 24–26, visit 2 data representing cortisol collected during study visits at gestational weeks 27–29, and so on, until visit 5 data, which represented cortisol collected during study visits at gestational weeks 36–38. Descriptive statistics for prenatal maternal salivary cortisol are presented in Table 2.

Child Neuroendocrine-Immune Activity—Concentrations of cortisol and four inflammatory cytokines (IL-1β, IL-6, IL-8, TNFα) from the four child saliva samples from the 5-year study visit were used to index child HPA and immune activity, respectively. These cytokines represent four of the most commonly examined inflammatory indices in saliva. While they have shared synergistic inflammatory properties, the exact origin and specific mechanisms and properties of each cytokine in the oral cavity has not yet been established [5, 7, 24, 40]. Therefore, each cytokine was considered separately in our analyses. Descriptive statistics for child salivary biomeasures of HPA and immune activity by saliva sample are shown in Table 3.

Key Covariates

Maternal Covariates—Maternal pre-pregnancy BMI, parity (nulliparous vs. multiparous), and age during pregnancy were measured via questionnaires during the prenatal visits and assessed as confounders of prenatal HPA activity [33]. The time of the prenatal study visit was recorded by evaluators and used to examine the impact of diurnal changes on salivary cortisol concentrations.

Based on our findings from the cross-sectional study [52] and consistent with other studies examining the relation between prenatal maternal cortisol and child development [19],we also controlled for concurrent maternal depressive symptoms at the 5-year study visit. Self-reported depressive symptoms were assessed with the Center for Epidemiologic Survey Depression Scale (CES-D-20) at the 5-year visit. The CES-D is a widely used validated scale assessing self-reported depressive symptoms [44].

Self-reported medication use during pregnancy was assessed at each prenatal visit and considered as a covariate to adjust for pharmacological related changes in HPA function [23, 25]. The majority of women, however, reported no over-the-counter or prescription medication use during pregnancy (at least 60% at each visit). Small sample sizes and limited variability in medication use during pregnancy precluded its inclusion as a covariate.

Child Covariates—Child age in months was confirmed at the 5-year study visit by maternal report. Child medication use in the 2 days prior (yes/no) was assessed by maternal report and examined as a possible covariate given potential confounds with child neuroendocrine-immune function and covariation with salivary measures [23, 25]. The

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amount of time from child waking to the start of the 5-year study visit was calculated using maternal report of child wake time and evaluator report of study visit start time. Time since waking was assessed as a potential covariate to adjust for natural diurnal changes in child salivary cortisol and cytokine concentrations [30, 47].

Maternal reports of child dental/periodontal problems (yes/ no) and current illness (yes/no response to the question “Is your child currently feeling sick or ill (ex. runny nose, fever, cough, aching, etc.)?”) were examined as potential covariates to adjust salivary analyte concentrations for differences related to oral and systemic health [40, 51]. Child BMI (calculated using evaluator measurements and the Centers for Disease Control and Prevention’s BMI percentile ranks by age and sex) was also considered as covariate. However, there was too little variability in the sample to include these child characteristics as covariates (Table 1).

Statistical Methods

In preliminary analyses, we examined the distribution of salivary analyte data. All salivary biomeasure data were positively skewed. Child cortisol concentrations were log-transformed to improve the normality of the distribution and meet the multilevel mixed-effects modeling assumptions (model details provided below). We also examined whether child salivary analyte levels and child, maternal, and family characteristics (listed in Table 1) varied by child sex using *t*-tests, Wilcoxon-Mann-Whitney tests, and chi-square tests.

Preliminary analyses also examined the significance of potential maternal and child covariates using Pearson’s and Spearman’s correlations and *t*-tests and Wilcoxon rank-sum tests to examine relations between the covariates and maternal and/or child salivary analyte concentrations. *T*-tests and Wilcoxon rank-sum tests were also used to assess differences in analyte concentrations and covariates by child sex. Covariates that were associated with maternal and/or child salivary analyte concentrations at $p < .10$, and conceptually important covariates, were included in the final models.

Multilevel mixed-effects linear regression models were used to examine the moderating role of prenatal maternal cortisol on child NEI relations. Four models, each with child salivary cortisol as the outcome, were constructed. In each model, the average prenatal maternal cortisol level and a single child inflammatory marker (either IL-1β, IL-6, IL-8, or TNFα) were included as independent variables. All models included a random intercept and a random slope to account for the nesting of salivary analyte data within child (four saliva samples per child) and variation in cortisol trajectories across the study visit. Models were adjusted for the child’s age, time from child waking to the 5-year study appointment, the time of prenatal study appointment, mother’s age during pregnancy, pre-pregnancy BMI, parity, and depressive symptoms at the 5-year visit. Models for female children were also adjusted for recent medication use. The main independent variables were the child inflammatory marker (either IL-1β, IL-6, IL-8, or TNFα), the average prenatal maternal cortisol level, and the inflammatory marker × prenatal cortisol interaction. This interaction term represents the moderating effect of prenatal maternal HPA activity on child HPA-immune relations at age 5. All models were performed separately for male and female children.

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Maternal cortisol data grouped by prenatal visit were used to explore the role of the gestational timing of prenatal HPA activity on child NEI relations. The models described above were estimated using each visit's individual prenatal cortisol value, rather than the average cortisol level across pregnancy, as a main independent variable. For each set of analyses for the inflammatory markers, tests of statistical significance were two-sided with an alpha of 0.05, and Bonferroni-corrected alpha levels (.05/5 tests) were also examined to control for family-wise error rate in each cytokine's set of five statistical tests (one test for each of the five prenatal visit time points).

After model estimation, the impact of extreme analyte determinations on model fit and parameter estimates was assessed by examining standardized residuals and estimates of influence (i.e., Cook's D). We also conducted sensitivity analyses that excluded cases with salivary cytokine values greater than four standard deviations from the mean, and analyses that excluded dyads with two missing prenatal maternal cortisol values. The robustness of the interactions between prenatal maternal cortisol and child inflammatory marker levels was tested by using a dichotomized variable for average prenatal cortisol across pregnancy (high vs. low cortisol based on a median split), rather than the continuous variable, in the interaction term.

Results

Preliminary Analyses

Log-transformed child salivary cortisol data were normally distributed (skew ranged from −0.16 to 0.64 and kurtosis ranged from 2.49 to 3.47). There were no sex differences in child, maternal, and family characteristics (Table 1). However, at saliva sampling points 2, 3, and 4, there were significant sex differences in salivary cytokine concentrations with female children exhibiting higher concentrations of the inflammatory markers than male (Table 3). At saliva sample 4, males displayed higher salivary cortisol concentrations than females (Table 3).

All prenatal study visits were conducted between 1:17 P.M. and 4:37 P.M., and the time of the visit did not vary by child sex. On average, the 5-year study visit was conducted 5.91 h (SD = 2.52; range = 1–11.92 h) after the child woke up. Time since waking did not vary by child sex. Ten children were reported to have used medications in the 2 days prior, seven of which were female. The most commonly reported medications were antihistamines (n_{female} = 4; n_{male} = 2), followed by anti-inflammatories/steroids (n_{female} = 1; n_{male} =1), and expectorants (n_{female} = 1) and Tylenol (n_{female} = 1)

Does Prenatal Maternal Cortisol Moderate Child Neuroendocrine-Immune Relations?

Average prenatal maternal cortisol did not moderate NEI relations among male children (all inflammatory marker × prenatal cortisol interaction terms were non-significant; Table 4). In addition, no significant associations were found between average prenatal maternal cortisol and child cortisol among males (Table 4). In contrast, among females, average prenatal maternal cortisol moderated child NEI relations for some cytokines (Table 4, Fig. 1) with higher prenatal cortisol associated with more positive NEI relations (Table 4). Prenatal

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cortisol was also marginally inversely associated with child cortisol levels among females (Table 4).

Does the Effect of Prenatal Maternal Cortisol on Child Neuroendocrine-Immune Relations Vary by Gestational Age?

Models examining relations between prenatal maternal cortisol at each gestational time point and child NEI relations were only estimated among females as no significant results were found in models using average prenatal cortisol among males. Results from these models revealed that higher prenatal cortisol during the later gestational weeks (e.g., on or after 30 weeks) tended to be associated with lower cortisol levels among female children (Table 5). There were also more significant NEI moderation effects by maternal cortisol collected during later gestation (≥ 30 weeks) compared to earlier. While the pattern of NEI moderation effects across gestation varied by inflammatory marker (Table 5), the interactions between the inflammatory markers and the prenatal maternal cortisol concentrations were consistently positive for all cytokines and at all gestational time points (Table 5) indicating that higher prenatal cortisol was associated with more positive NEI relations.

Model Diagnostics and Sensitivity Analyses

To interrogate the robustness of the findings, we examined the standardized residuals and the influence of extreme and missing data points in each of our models. There were no highly influential cases impacting the results of the models examining average prenatal maternal cortisol and child NEI relations (all Cook's D < 1). In models examining the moderation of child NEI relations by visit-specific prenatal maternal cortisol, there was one case with a Cook's D greater than or equal to one in four of the models (IL-1β models for visits 2 and 5, IL-8 model at visit 5, and TNFα model at visit 5). When this case was excluded, the moderation findings were maintained for prenatal cortisol and IL-1β at visit 2 and IL-8 at visit 5. However, excluding this case from the IL-1β and TNFα, visit 5 models weakened the moderation effects to marginally significant levels (IL-1β × prenatal maternal cortisol at visit 5: z= 1.94, $p = 0.05$; TNFα × prenatal maternal cortisol at visit 5: z= 1.73, $p = 0.08$).

All models had standardized residuals less than three. We tested the strength of the findings by excluding cases with residuals greater than two standard deviations from the mean (2–4 data points per model). Excluding these cases did not change the moderation of child NEI relations by average prenatal maternal cortisol. In visit-specific analyses, removing cases with residuals greater than two standard deviations from the mean weakened the moderating effect of prenatal maternal cortisol on child NEI relations to non-statistically significant levels for IL-8 at visit 2 and IL-1β at visit 5. Conversely, removing these cases strengthened the interactions between prenatal maternal cortisol and child IL-1β at visit 3 (z = 2.10, $p < .05$), IL-8 at visit 4 (z = 3.32, $p < 0.01$), and TNFα at visit 5 (z= 3.00,$p < .01$).

We also examined the sensitivity of the models to extreme data points by performing all analyses while excluding cases with cytokine concentrations greater than four standard deviations from the mean (1.7% of cytokine data). When excluding these cases, the moderation findings reported were largely unchanged or strengthened. For example, average prenatal maternal cortisol significantly moderated child cortisol-IL-1β relations (z = 2.68, p

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<0.01) when one case with extreme IL-1β concentrations was excluded from the analysis. Removing these cases also strengthened some moderation effects when relations were examined by visit (IL-1β × prenatal cortisol at visit 3: z= 3.06, $p < 0.01$; TNFα × prenatal cortisol at Visit 5: z = 2.83, $p < 0.01$); however, the moderation of child cortisol- IL-6 relations by prenatal maternal cortisol at visit 4 was weakened to a non-statistically significant level in these analyses.

We performed sensitivity analyses excluding dyads with two missing prenatal maternal cortisol values ($n = 5$), and the results from these models were similar to those presented above with no substantial changes in the moderation effects. Finally, we tested the strength of the interactions between prenatal cortisol and child inflammatory marker levels. We dichotomized average prenatal cortisol across pregnancy based on a median split and used this variable in the models in place of continuous average prenatal cortisol. Results from these models were similar to those reported above with no substantial changes in the moderation effects.

Discussion

Findings from this study provide insight into the sensitivity of child NEI functioning to prenatal maternal cortisol. In our small sample of young children, relations between child inflammatory cytokines and HPA activity in saliva varied by prenatal maternal cortisol activity among girls only. In addition, higher prenatal maternal cortisol was modestly associated with lower cortisol levels in 5-year-old girls. Systemically, cortisol plays an important regulatory role in preventing excessive and potentially dangerous inflammation by inhibiting inflammatory cytokine activity [1, 10, 11, 28]. Our findings suggest that higher prenatal maternal HPA activity may be associated with both lower cortisol levels and less efficient regulation of inflammatory mechanisms by cortisol during childhood among females. While our small sample size and limited measurement of prenatal maternal cortisol restrict the interpretation and generalizability of our results, our findings are consistent with the sex-dependent “viability-vulnerability tradeoff’ theory [53] and the notion that exposure to elevated levels of prenatal stress hormones can result in a desensitization of HPA and NEI regulatory mechanisms. Desensitization of these processes among females may represent an adaptation in response to cortisol exposure in utero that calibrates the developing HPA and NEI systems for later life. While the small sample of children examined in this study were largely healthy and low-risk, our findings provide insight into a potential mechanism by which prenatal maternal cortisol could potentiate reduced HPA activity and a “defensive phenotype” characterized by an over-active inflammatory response in children [37]. This study represents a first step in examining these relations, and additional research with larger and more diverse samples is needed to confirm these preliminary findings.

Advancing our understanding of the impact of in utero adaptations on child development and later-life health is paramount to promoting health and well-being across the lifespan. Prenatal cortisol is essential for fetal development and maturation, and elevated cortisol, particularly during late pregnancy, may be beneficial for neurodevelopment and cognitive performance [12, 58]. Therefore, fetal sensitivity and adaptations to prenatal cortisol are not necessarily risk factors for developmental and health problems. However, higher levels of

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prenatal cortisol have been associated with a wide range of child behavioral and health outcomes, including changes in fetal growth, lower birth weight, impaired cognitive and psychomotor development, affective problems, psychiatric symptoms, cardiometabolic disorders, brain morphology, negative temperament, and HPA function [4, 6, 8, 20, 26, 27, 29, 38, 42, 46, 48, 55]. Few studies have examined associations between prenatal cortisol and child immune functioning. However, prenatal adversity, such as maternal depression, psychosocial stress, and indices of prenatal stress physiology, has been linked with immune-related conditions among children later in life [17, 35, 36, 43, 48, 55, 59]. Using a multisystem approach and a small sample of low-risk, healthy pregnant women and their children, our study begins to advance understanding of potential mechanisms linking prenatal maternal cortisol with child health and development. Our eligibility criteria required all pregnant women be generally healthy, non-smoking, and carrying low-risk pregnancies, and the prenatal salivary cortisol determinations in our study are within the expected range for pregnant women. In addition, the vast majority of mothers in the study reported their child's health to be very good or excellent (91%) at the 5-year visit. Therefore, while not generalizable to the broader population, our findings provide insight into potential in utero adaptations that may link maternal physiology with child health and development. Elucidating these mechanisms in a low-risk, healthy sample may help us advance understanding of the mechanisms underlying poor child health outcomes in other higher-risk and disadvantaged populations. Our findings highlight the need for additional investigation into the long term, multisystem impacts of prenatal maternal cortisol exposure on child health.

Interestingly, the associations between average prenatal maternal cortisol and child NEI and HPA functioning were only found among girls. These findings extend our previously reported findings from a cross-sectional study of this sample that included an additional 90 children. Previously, we reported that concurrent maternal psychological distress moderated child NEI relations and that these effects were particularly strong among girls [52]. The current findings suggest that child NEI function may also be influenced by prenatal maternal cortisol levels, the effects of which persist throughout early childhood. Furthermore, they suggest that female NEI development may be more sensitive to external cues than male NEI development. Our findings are consistent with the sex- dependent "viability-vulnerability tradeoff" theory [53] and with findings from other studies of associations between prenatal cortisol and child stress physiology [2, 6, 13, 19, 32, 42, 46, 58]. Previous studies consistently report the relations between prenatal cortisol and child physiologic and behavioral outcomes vary by sex [2, 6, 19, 32, 55]. Our finding that higher salivary prenatal maternal cortisol is associated with less efficient regulation of salivary inflammatory processes among 5-year-old girls is consistent with sex differences in proinflammatory immune activity [34]. However, in interpreting the observed sex differences in NEI moderation, it is also important to note that female children exhibited higher salivary cytokine concentrations and greater variability at most sampling points than males. Sex differences in analyte distributions may be particularly salient factors in the statistical testing of our cytokine-prenatal maternal cortisol interaction terms. Additional research is needed to confirm the nature and distribution of salivary cytokine concentrations among young children, as well as salivary measures of NEI coordination and their potential moderation by

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environmental factors. Future research is also needed to understand the long-term impact of prenatal maternal HPA activity on child health and how these processes may contribute to disease risk and the perpetuation of population-level health disparities.

Exploratory models that examined whether the moderating role of prenatal maternal cortisol on child NEI relations was consistent across pregnancy suggested time-specific relations between prenatal cortisol and child HPA and, to a lesser extent, NEI functioning among females. Although limited by our sample size, our results suggest that HPA function among females may be particularly sensitive to maternal HPA activity later in gestation. The relations between prenatal cortisol and child NEI relations were less specific to gestational timing and varied by inflammatory marker. Future studies examining these relations in larger samples are needed to understand the time-specific relations found in our study. However, our findings are consistent with prior reports of differential effects of prenatal cortisol by timing and fetal sex [2, 6, 8, 12, 13, 19, 26, 32, 55, 58]. Several investigations have found later pregnancy may be a critical period for prenatal HPA activity [8,22, 45]. The second half of pregnancy is important for fetal glucocorticoid receptor development, making later pregnancy important for the development and calibration of the HPA response [48]. In addition, fetal sensitivity to maternal cortisol may be heightened during late gestation due to decreases in the placental enzyme (11beta-hydroxysteroid dehydrogenase type 2) that inactivates maternal cortisol [45].

Limitations and Strengths

Our sample size restricted our statistical power and the range of models that were available to answer our research questions. Our findings were generally modest or trending, and some were not robust to sensitivity analyses that excluded influential data points. The exploratory models examining the role of gestational timing of prenatal maternal cortisol on child NEI relations were especially sensitive to the exclusion of influential and extreme data points because individual prenatal cortisol determinations had more variation and skew than the average prenatal cortisol composite variable. Also, it is important to note that while we highlight the statistically significant moderation effects, the biological and clinical significance of these effects, some of which are very small in magnitude, is not known. With very limited power to detect main, and especially interaction, effects, our findings should be interpreted as a first step in the examination of prenatal influences on child NEI function. Further studies are needed to confirm and extend our findings and advance our understanding of the clinical significance of these relations. Future researchers should also examine the relations observed in our study using a larger, more diverse sample as our sample was very homogenous with little variation in race/ethnicity and socioeconomic status.

Additional research is also needed to better develop our understanding of salivary cytokines, their relations with salivary cortisol, and the meaning of salivary immune and NEI indices for health and disease risk over time. The extent to which salivary cytokine and NEI measures reflect systemic immune and NEI activity is not known. Given this lack of understanding of salivary cytokines, we chose to model each cortisol-cytokine relation separately, rather than combine the cytokines into a single inflammatory index. Despite

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positive correlations among the cytokines, we found that the nature of NEI relations, and their moderation by prenatal maternal cortisol, varied by inflammatory marker. Additional studies are needed to understand the role of each salivary cytokine, alone and in coordination with other salivary immune markers, in indexing oral and systemic inflammation. It is important to note that oral inflammation and oral health problems have been linked with several health conditions, including cardiovascular disease, stroke, and diabetes [56]. Therefore, the finding that prenatal maternal cortisol may potentiate dysregulation in salivary NEI function that could result in increased oral inflammation is an important finding for the study of oral and systemic health conditions.

Our study's protocol and saliva sampling schema, and the associated potential implications for salivary analyte measurements and interpretation, also warrant discussion. Child salivary data were collected across a series of three emotional challenge tasks. While the order of these tasks was the same for all participants, the time between saliva samples was determined by the child's pace in completing the study tasks and consequently varied by child. Therefore, changes in child salivary analyte concentrations across the study visit reflect cumulative HPA and inflammatory activity, rather than task-specific responses. We used a random-effects modeling approach to statistically account for variability in child cortisol trajectories across the study visit. We also assessed the impact of sample collection timing on our findings by controlling for the elapsed time between child saliva samples 1 and 2,2 and 3, and 3 and 4 in our models (data not shown). The results from these models were similar to those reported above. Additional research is needed, however, to confirm the NEI patterns observed in this study and assess whether these activation patterns are evident in resting, as well as stress-related activity.

Finally, our study is limited by the availability of a single cortisol determination at each prenatal visit. All prenatal saliva samples in the current investigation were collected in the afternoon when diurnal cortisol levels are naturally more stable. However, findings from other studies examining prenatal maternal cortisol suggest that more robust associations between prenatal maternal HPA activity and child outcomes are found when examining fluctuations in maternal cortisol across the day (e.g., stress-related change, cortisol awakening responses, diurnal slopes) rather than average or baseline cortisol levels [19, 22, 48]. Also, participant wake time was not recorded during the prenatal study visits. While we adjusted our analyses for the time of the study visit, without wake time data, we cannot directly assess the impact of individual diurnal patterns on salivary cortisol measurements. We suggest that, despite these limitations in the interpretation of our prenatal salivary cortisol measurement, the observed relations support the notion that maternal HPA function in pregnancy may be related to child NEI function later in life and these changes may be measurable with child salivary biomeasures. Our findings do not, however, identify specific aspects of HPA activity that may be more or less important for fetal development, such as cortisol's morning rise or diurnal slope, and these aspects of activation may or may not show similar relations with child NEI function. Future research should further examine the relations observed in this study using more meaningful measures of prenatal maternal HPA function such as diurnal or stress-related change in cortisol. Larger studies of these relations could also use more complex statistical modeling strategies, such as latent state-trait

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modeling, to parse variation in salivary cortisol related to stable, person-specific, and fluctuating, state-dependent factors (e.g., [18]).

It is also important to note that HPA activity is only one potential mechanism by which maternal health, physiology, and experience may impact fetal development. Future studies employing multisystem measurement of potential mechanisms, such as maternal catecholamine and immune function and oxidative stress, will provide a more comprehensive understanding of the links between maternal health and experience, fetal development, and child health [45]. Additional insight into the impact of prenatal maternal cortisol on the developing fetus may also come from studies of children exposed to antenatal exogenous glucocorticoids [38]. These studies have found significant associations between high levels of prenatal glucocorticoids and child neurologic development; however, the long-term impact of exogenous glucocorticoids on child and adult health and functioning is not clear [38]. The generalizability of these findings to associations between naturally occurring, endogenous prenatal cortisol and child health is also not known.

Despite these limitations, there are notable strengths to the study. The findings are bolstered by a longitudinal design and tight laboratory controls during the prenatal and child visits. It is important to note that our sample excluded children with significant health conditions and developmental disabilities, and the majority of children were the result of full-term pregnancy and were a healthy weight and reported healthy by their mothers at age 5. Our relatively homogeneous sample of healthy, low-risk, mother/child dyads allowed us to examine healthy developmental processes. Finally, our multisystem approach to examining differences in adaptation and physiology contributes to the novelty of our findings and provides a more nuanced understanding of the relations between prenatal maternal HPA activity and child development.

Conclusion

This study begins to advance our understanding of the developmental origins of child NEI functioning and potential mechanisms linking prenatal maternal cortisol and later-life health and disease among children. Our study contributes to the literature by examining prenatal maternal HPA activity using repeated biologic assessments during pregnancy and assessing cross-system physiologic relations in children using salivary biomeasures. Although preliminary due to our small sample size, the findings suggest that prenatal maternal HPA activity may moderate NEI functioning in children during early childhood by altering HPA activity and the sensitivity of inflammatory immune processes to the inhibitory effects of cortisol. This desensitization may represent one mechanism by which in utero adoptions alter NEI functioning with potential impacts on child health and disease risk [10]. The prenatal and early childhood periods offer the opportunity to examine the origins of HPA and immune system interactions. Despite well-documented and mounting evidence of the role of NEI dysregulation in health conditions that emerge early in life, such as asthma and obesity, as well as later-life conditions like depression and cardiovascular disease [1, 10, 11], little is known about the origins of individual differences in HPA-immune system coordination. Our findings present opportunities for future researchers to continue these

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investigations using multisystem, potentially minimally invasive biomeasures of NEI function in multi-generational studies of maternal and child health.

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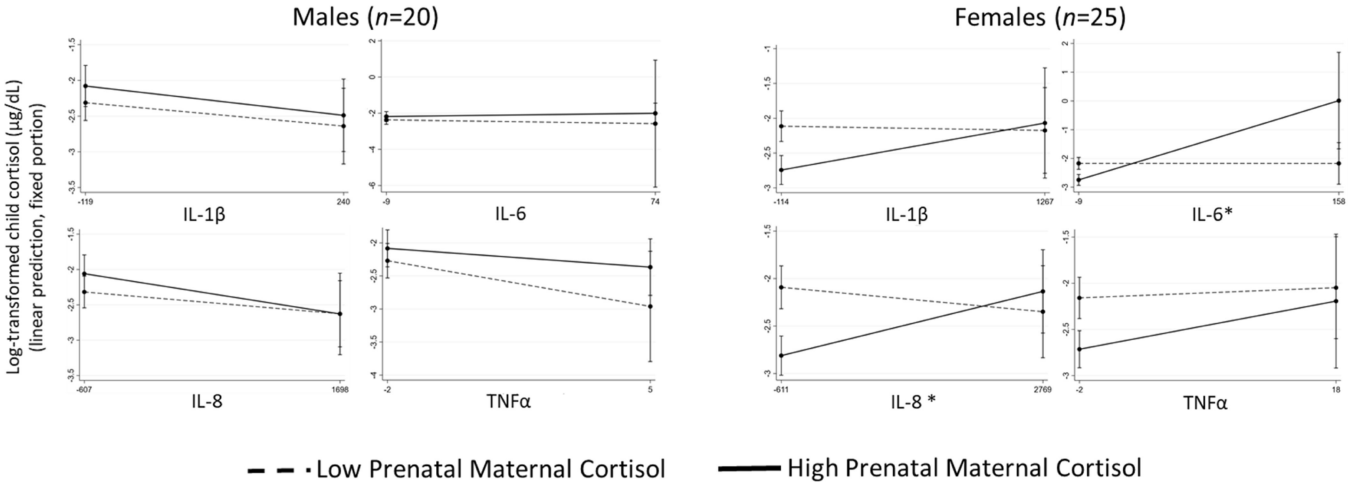

Fig. 1.

Among males, mean maternal cortisol across pregnancy did not moderate child NEI relations at 5 years old. Among females, higher prenatal maternal cortisol was associated with more positive relations between child cortisol and child IL-6 and IL-8 at 5 years old. Note: Results are from eight multilevel mixed-effects linear regression models for child salivary cortisol (dependent variable). Each model includes a random intercept and a random slope to account the nesting of salivary analyte data within child (four saliva samples per child) and variation in cortisol trajectories. All models are adjusted for maternal pre-pregnancy body mass index, age, and parity, child age, maternal depressive symptoms at the 5-year visit, time of the prenatal visit, and time since waking for children. Female models are also adjusted for recent medication use. Child cortisol values are log-transformed, and child cytokine values are mean centered. Maternal prenatal cortisol is categorized using a median split of the full sample. *Prenatal maternal cortisol significantly moderates the relation between child cortisol and the inflammatory marker (p <0.05)

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Table 1

Demographic and health characteristics of mother and child participants by child sex ($N = 45$ dyads)

	Male ($n = 20$)		Female ($n = 25$)	
	Frequency	**Percent**	**Frequency**	**Percent**
Maternal characteristics				
Age during pregnancy, mean years (SD; range)	32.00 (3.97; 23–40)		32.48 (5.36; 21–43)	
Pre-pregnancy body mass index	24.46 (5.00; 18.51–36.31)		24.28 (4.50; 16.64–37.10)	
Education, mean years (SD; range)	17.30 (2.18; 12–20)		17.20 (1.98; 13–20)	
Nulliparous	12	60%	18	72%
White	17	85%	20	80%
Black/African American	2	10%	3	12%
Asian/Pacific Islander	1	5%	2	8%
Depressive symptoms at 5-year visit, mean CES-D score (SD; range)	7.95 (7.19, 1–24)		7.52 (7.02, 0–29)	
Child characteristics at age 5				
Age, mean months (SD; range)	63.59 (3.13; 59.84–72.76)		64.67 (3.60; 59.61–72.89)	
Current illness	4	20%	4	16%
Currently overweight or obese	1	5%	2	8%
Current periodontal or dental issues	1	5%	1	4%
Annual family income at child age 5				
$35,000–49,999	1	5%	1	4%
$50,000–74,999	2	10%	1	4%
$75,000–99,999	3	15%	0	0%
$100,000–149,999	7	35%	11	44%
≥ $150,000	7	35%	12	48%

Current illness indicates that the mother responded “yes” to the question “Is your child currently feeling sick or ill (ex. runny nose, fever, cough, aching, etc.)?” CES-D scale range = 0–60, scores ≥ 16 indicate risk of clinical depression. There were no statistically significant differences in any of the above characteristics by child sex

SD standard deviation, *CES-D* Center for Epidemiologic Survey Depression Scale

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Table 2

Descriptive statistics for prenatal maternal salivary cortisol (μg/dL) assessed five times across pregnancy by child sex

Prenatal Visit	Male			Female		
	Mean	SD	Range	Mean	SD	Range
Visit 1 (weeks 24–26)	0.23	0.09	(0.02, 0.35)	0.20	0.08	(0.04, 0.34)
Visit 2 (weeks 27–29)	0.24	0.11	(0.11, 0.60)	0.21	0.06	(0.11, 0.33)
Visit 3 (weeks 30–32)	0.24	0.15	(0.07, 0.62)	0.23	0.09	(0.09, 0.38)
Visit 4 (weeks 33–35)	0.21	0.13	(0.02, 0.52)	0.24	0.12	(0.08, 0.68)
Visit 5 (weeks 36–38)	0.27	0.16	(0.09, 0.74)	0.28	0.09	(0.08, 0.44)

Sample size for visits 1–5 for women carrying male fetuses = 19, 19, 14, 18, 16, respectively; sample size for visits 1–5 for women carrying female fetuses = 25, 21, 21, 22, 19, respectively. These data are part of a larger study; see DiPietro et al. [15] for detailed information about maternal cortisol changes across pregnancy and sex-specific cortisol trajectories [15]

weeks weeks gestation, *SD* standard deviation

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Table 3.

Descriptive statistics for child salivary biomeasures assessed four times across a 90-min laboratory visit at age 5 years

Saliva sample	Analyte	Male (*n* = 20)			Female (*n* = 25)		
		Mean	SD	Range	Mean	SD	Range
1	Cortisol	0.11	0.06	(0.05, 0.27)	0.12	0.09	(0.05, 0.42)
	IL-1β	74.33	84.09	(13.54, 360.87)	191.48	294.16	(14.34, 1388.09)
	IL-6	6.23	17.08	(0.00, 77.65)	13.58	34.56	(0.29, 166.28)
	IL-8	572.86	548.91	(106.74, 2379.88)	1001.36	918.39	(124.25, 3450.76)
	TNFα	0.97	0.97	(0.01, 3.28)	3.33	5.50	(0.14, 18.81)
2	Cortisol	0.11	0.05	(0.03, 0.19)	0.10	0.06	(0.05, 0.33)
	IL-1β *	77.24	86.81	(16.65, 359.32)	195.66	291.08	(11.38, 1274.61)
	IL-6	5.93	14.72	(0.10, 67.45)	10.70	23.55	(0.42, 117.01)
	IL-8 *	472.08	326.76	(98.62, 1500.20)	871.27	800.81	(106.78, 3190.14)
	TNFα *	0.77	0.59	(0.10, 2.43)	1.77	2.54	(0.14, 12.99)
3	Cortisol	0.10	0.03	(0.04, 0.16)	0.09	0.04	(0.04, 0.19)
	IL-1β *	75.36	69.41	(9.58, 281.33)	161.37	178.43	(11.39, 745.39)
	IL-6 *	7.03	15.57	(0.34, 52.34)	9.01	13.34	(0.34, 52.50)
	IL-8 *	519.47	402.52	(134.04, 1883.74)	881.30	730.91	(103.75, 3210.41)
	TNFα	1.44	1.58	(0.02, 5.81)	1.77	2.08	(0.10, 10.49)
4	Cortisol *	0.14	0.08	(0.06, 0.38)	0.10	0.05	(0.04, 0.23)
	IL-1β *	44.25	38.31	(3.39, 157.51)	109.08	107.25	(7.59, 380.24)
	IL-6 *	6.05	18.19	(0.32, 82.76)	10.36	13.15	(0.57, 50.23)
	IL-8 *	273.41	182.61	(75.35, 833.65)	687.75	747.82	(72.10, 3386.79)
	TNFα *	0.65	0.63	(0.00, 2.69)	1.65	1.76	(0.06, 6.38)

Children participated in three emotional challenge tasks during the study visit; saliva samples 1 and 2 were collected before the challenge tasks, and saliva samples 3 and 4 were collected after the challenge tasks. All units in pg/mL with the exception of cortisol in μg/dL

IL-1β interleukin 1-beta, *IL-6* interleukin 6, *IL-8* interleukin 8, *TNFα* tumor necrosis factor-alpha, *SD* standard deviation

* Statistically significant difference in concentration levels by sex, $p < 0.05$

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Table 4

Adjusted relations between child cortisol, child inflammatory markers, and average prenatal maternal cortisol among 5-year-old male (n = 20) and female children (n = 25)

Main independent variables:	Males					Females				
	Coefficient	**SE**	***Z***	**95% CI**	***p***	**Coefficient**	**SE**	***Z***	**95% CI**	***p***
(a)										
Prenatal maternal cortisol	0.70	1.07	0.66	− 1.39, 2.80	0.51	− 2.43	1.34	− 1.81	− 5.06, 0.21	0.07
Child IL-1β	− 0.00	0.00	− 1.68	− 0.00, 0.00	0.09	0.00	0.00	1.59	− 0.00, 0.00	0.11
Child IL-1β × prenatal maternal cortisol	− 0.00	0.01	− 0.17	− 0.01, 0.01	0.87	0.00	0.00	1.32	− 0.00, 0.01	0.19
(b)										
Prenatal maternal cortisol	1.48	1.23	1.20	− 0.94, 3.90	0.23	− 1.43	1.33	− 1.07	− 4.04, 1.18	0.28
Child IL-6	− 0.00	0.01	− 0.18	− 0.01, 0.01	0.86	*0.01*	*0.00*	*3.41*	*0.01, 0.02*	*<0.01*
IL-6 × prenatal maternal cortisol	0.07	0.11	0.64	− 0.14, 0.28	0.52	*0.12*	*0.04*	*3.05*	*0.04, 0.20*	*<0.01*
(c)										
Prenatal maternal cortisol	0.70	0.97	0.73	− 1.20, 2.61	0.48	− 2.23	1.31	− 1.70	− 4.80, 0.33	0.09
Child IL-8	*− 0.00*	*0.00*	*− 2.07*	*− 0.00,* − 0.00	*0.04*	*0.00*	*0.00*	1.65	− 0.00, 0.00	0.10
Child IL-8 × prenatal maternal cortisol	− 0.00	0.00	− 0.70	− 0.00, 0.00	0.49	*0.00*	*0.00*	*2.49*	*0.00, 0.00*	*0.01*
(d)										
Prenatal maternal cortisol	1.50	1.10	1.36	− 0.65, 3.65	0.18	− 2.34	1.33	− 1.76	− 4.95, 0.27	0.08
Child TNFα	− 0.06	0.03	− 1.87	− 0.12, 0.00	0.06	0.02	0.01	1.53	− 0.01, 0.05	0.13
Child TNFα × prenatal maternal cortisol	0.43	0.50	0.86	− 0.54, 1.40	0.39	0.15	0.22	0.69	− 0.28, 0.58	0.49

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Results from eight multilevel mixed-effects linear regression models for child salivary cortisol (dependent variable; log transformed) are shown. Each model includes a random intercept and a random slope to account for the nesting of salivary analyte data within child (four saliva samples per child) and variation in cortisol trajectories. All models are adjusted for maternal pre-pregnancy body mass index, age, and parity, child age, maternal depressive symptoms at the 5-year visit, time of the prenatal visit, and time since waking for children. Female models are also adjusted for recent medication use. Analytes and continuous covariates are mean centered. Statistically significant results at $p < 0.05$ are in italics

IL-1β interleukin 1-beta, *IL-6* interleukin 6, *IL-8* interleukin 8, *TNFα* tumor necrosis factor-alpha, *SE* standard error, *CI* confidence interval

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Table 5

Adjusted relations between child cortisol, child inflammatory markers, and prenatal maternal cortisol at five time points across pregnancy among 5-year old female children

Main independent variables:		Coefficient	SE	Z	95% CI	*p* value
(a)						
Prenatal cortisol at visit 1 (weeks 24–26)	Prenatal maternal cortisol	− 1.50	1.33	− 1.12	− 4.11, 1.11	0.26
	Child IL-1β	0.00	0.00	1.39	− 0.00, 0.00	0.17
	IL-1β × prenatal maternal cortisol	0.00	0.00	0.89	− 0.00, 0.01	0.38
Prenatal cortisol at visit 2 (weeks 27–29)	Prenatal maternal cortisol	− 3.35	2.71	− 1.24	− 8.66, 1.96	0.22
	Child IL-1β	0.00	0.00	0.62	− 0.00, 0.00	0.54
	IL-1β × prenatal maternal cortisol	**0.02**	**0.01**	**2.89**	**0.01, 0.04**	< **0.01**
Prenatal cortisol at visit 3 (weeks 30–32)	Prenatal maternal cortisol	*− 2.45*	*1.14*	*− 2.16*	*− 4.67, − 0.22*	*0.03*
	Child IL-1β	0.00	0.00	1.24	− 0.00, 0.00	0.22
	IL-1β × prenatal maternal cortisol	0.01	0.00	1.88	− 0.00, 0.02	0.06
Prenatal cortisol at visit 4 (weeks 33–35)	Prenatal maternal cortisol	*− 1.44*	*0.72*	*− 2.00*	*− 2.84, − 0.03*	*0.05*
	Child IL-1β	0.00	0.00	0.84	− 0.00, 0.00	0.40
	IL-1β × prenatal maternal cortisol	0.00	0.00	1.23	− 0.00, 0.01	0.22
Prenatal cortisol at visit 5 (weeks 36–38)	Prenatal maternal cortisol	**− 4.17**	**0.89**	**− 4.68**	**− 5.92, − 2.42**	< **0.01**
	Child IL-1β	0.00	0.00	1.53	− 0.00, 0.00	0.13
	IL-1β × prenatal maternal cortisol	*0.01*	*0.00*	*1.97*	*0.00, 0.02*	*0.05*
(b)						
Prenatal cortisol at visit 1 (weeks 24–26)	Prenatal maternal cortisol	− 1.06	1.32	− 0.80	− 3.65, 1.53	0.42
	Child IL-6	**0.01**	**0.00**	**2.64**	**0.00, 0.02**	< **0.01**
	IL-16 × prenatal maternal cortisol	0.06	0.03	1.95	− 0.00, 0.13	0.05
Prenatal cortisol at visit 2 (weeks 27–29)	Prenatal maternal cortisol	− 2.72	2.67	− 1.02	− 7.96, 2.52	0.31
	Child IL-6	0.00	0.01	0.68	− 0.01, 0.02	0.50
	IL-6 × prenatal maternal cortisol	*0.27*	*0.11*	*2.41*	*0.05, 0.50*	*0.02*
Prenatal cortisol at visit 3 (weeks 30–32)	Prenatal maternal cortisol	− 1.03	1.02	− 1.02	− 3.03, 0.96	0.31
	Child IL-6	**0.01**	**0.00**	**4.36**	**0.01, 0.02**	< **0.01**
	IL-6 × prenatal maternal cortisol	**0.24**	**0.05**	**4.82**	**0.14, 0.34**	< **0.01**
Prenatal cortisol at visit 4 (weeks 33–35)	Prenatal maternal cortisol	− 1.13	0.71	− 1.60	− 2.52, 0.25	0.11

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Main independent variables:		Coefficient	SE	Z	95% CI	*p* value
	Child IL-6	*0.01*	*0.00*	*2.33*	*0.00, 0.02*	*0.02*
	IL-6 × prenatal maternal cortisol	*0.07*	*0.03*	*2.46*	*0.01, 0.13*	*0.01*
Prenatal cortisol at visit 5 (weeks 36–38)	Prenatal maternal cortisol	**− 2.65**	**0.75**	**− 3.52**	**− 4.13, − 1.18**	< **0.01**
	Child IL-6	**0.01**	**0.00**	**5.09**	**0.01, 0.02**	< **0.01**
	IL-6 × prenatal maternal cortisol	**0.24**	**0.04**	**5.57**	**0.16, 0.33**	< **0.01**
(c)						
Prenatal cortisol at visit 1 (weeks 24–26)	Prenatal maternal cortisol	− 1.71	1.31	− 1.31	− 0.00, 0.85	0.19
	Child IL-8	0.00	0.00	1.88	− 0.00, 0.00	0.06
	IL-8 × prenatal maternal cortisol	0.00	0.00	1.39	− 0.00, 0.00	0.16
Prenatal cortisol at visit 2 (weeks 27–29)	Prenatal maternal cortisol	− 4.24	2.67	− 1.59	− 9.48, 1.00	0.11
	Child IL-8	*0.00*	*0.00*	*2.06*	*0.00, 0.00*	*0.04*
	IL-8 × prenatal maternal cortisol	*0.00*	*0.00*	*2.33*	*0.00, 0.00*	*0.02*
Prenatal cortisol at visit 3 (weeks 30–32)	Prenatal maternal cortisol	*− 2.39*	*1.00*	*− 2.40*	*− 4.34, − 0.44*	*0.02*
	Child IL-8	0.00	0.00	0.77	− 0.00, 0.00	0.44
	IL-8 × prenatal maternal cortisol	**0.00**	**0.00**	**3.64**	**0.00, 0.00**	< **0.01**
Prenatal cortisol at visit 4 (weeks 33–35)	Prenatal maternal cortisol	− 1.30	0.72	− 1.81	− 2.70, 0.10	0.07
	Child IL-8	0.00	0.00	0.76	− 0.00, 0.00	0.45
	IL-8 × prenatal maternal cortisol	*0.00*	*0.00*	*2.09*	*0.00, 0.00*	*0.04*
Prenatal cortisol at visit 5 (weeks 36–38)	Prenatal maternal cortisol	**− 4.31**	**0.76**	**− 5.71**	**− 5.80, − 2.83**	< **0.01**
	Child IL-8	0.00	0.00	1.16	− 0.00, 0.00	0.25
	IL-8 × prenatal maternal cortisol	**0.00**	**0.00**	**3.72**	**0.00, 0.00**	< **0.01**
(d)						
Prenatal cortisol at visit 1 (weeks 24–26)	Prenatal maternal cortisol	− 1.33	1.34	− 0.99	− 3.96, 1.30	0.32
	Child TNFα	0.02	0.01	1.39	− 0.01, 0.05	0.17
	TNFα × prenatal maternal cortisol	− 0.01	0.15	− 0.08	− 0.31, 0.28	0.93
Prenatal cortisol at visit 2 (weeks 27–29)	Prenatal maternal cortisol	− 3.27	2.80	− 1.16	− 8.76, 2.23	0.24
	Child TNFα	0.02	0.02	1.22	− 0.01, 0.05	0.22
	TNFα × prenatal maternal cortisol	0.29	0.36	0.80	− 0.42, 0.99	0.43
Prenatal cortisol at visit 3 (weeks 30–32)	Prenatal maternal cortisol	*− 2.30*	*1.04*	*− 2.21*	*− 4.33, − 0.26*	*0.03*
	Child TNFα	0.02	0.02	1.45	− 0.01, 0.06	0.15
	TNFα × prenatal maternal cortisol	*0.58*	*0.30*	*1.97*	*0.00, 1.16*	*0.05*

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Main independent variables:		Coefficient	SE	Z	95% CI	*p* value
Prenatal cortisol at visit 4 (weeks 33–35)	Prenatal maternal cortisol	− 1.36	0.72	− 1.91	− 2.77, 0.04	0.06
	Child TNFα	0.02	0.02	1.22	− 0.01, 0.05	0.22
	TNFα × prenatal maternal cortisol	0.17	0.16	1.02	− 0.15, 0.49	0.31
Prenatal cortisol at visit 5 (weeks 36–38)	Prenatal maternal cortisol	**− 3.88**	**0.84**	**− 4.61**	**− 5.53, − 2.23**	< **0.01**
	Child TNFα	*0.03*	*0.01*	*2.24*	*0.00, 0.05*	*0.03*
	TNFα × prenatal maternal cortisol	*0.65*	*0.30*	*2.16*	*0.06, 1.23*	*0.03*

Results from 20 multilevel mixed-effects linear regression models for child salivary cortisol (dependent variable; log transformed) are shown. Each model includes a random intercept and a random slope to account the nesting of salivary analyte data within child (four saliva samples per child) and variation in cortisol trajectories. All models are adjusted for maternal pre-pregnancy body mass index, age, and parity, child age and recent medication use, maternal depressive symptoms at the 5-year visit, time of the prenatal visit, and time since waking for children. Analytes and continuous covariates are mean centered. Participants with missing prenatal cortisol concentrations were excluded from visit-specific analyses. Sample sizes by visit: visit 1 $n = 25$; visit 2 $n = 21$; visit 3 $n = 22$; visit 4 $n = 22$; visit 5 $n = 19$. Statistically significant results at the $\alpha = 0.05$ level are in italics; results that are statistically significant at a Bonferroni-corrected alpha level ($\alpha = 0.01$) are in bold

IL-1β interleukin 1-beta, *IL-6* interleukin 6, *IL-8* interleukin 8, *TNFα* tumor necrosis factor-alpha, *SE* standard error, *CI* confidence interval