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Prenatal Maternal Stress and Child Hair Cortisol Four Years Later:

Evidence from a Low-income Sample

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Abstract

Background. Maternal stress during pregnancy can influence the trajectory of fetal development, shaping offspring physiology and health in enduring ways. Some research implicates fetal programming of the hypothalamic-pituitary-adrenocortical (HPA) axis as a mediator of these effects. The present study is the first to examine child hair cortisol concentration (HCC) and maternal stress during pregnancy in a diverse, low-income sample.

Methods. The sample consisted of 77 healthy, low-income (*M* annual income: $13,321), mother-children pairs (*M* child age = 3.81 years, *SD* = .43). The children were 57% girls, 43% boys. Mothers were 65% Latina/Hispanic, 28% Non-Hispanic White, 7% Black/African American. Maternal prenatal stress was measured with the Perceived Stress Scale administered by interview in the second and third trimesters, and again approximately four years later when child hair samples for assaying HCC were collected.

Results. On average maternal perceived stress increased significantly across pregnancy, then returned to lower levels 4 years after birth. Regression analysis revealed that child HCC was not significantly predicted by maternal perceived stress at either single prenatal time point. Exploratory analysis revealed evidence of a relation between increases in maternal prenatal stress from second to third trimester and child HCC four years later ($r = .37$, $p = .04$).

Conclusions. These results suggest that measures of prenatal maternal stress at any one time point may not be predictive of offspring long-term HPA output in low-income child samples, but that increases in stress levels across pregnancy may provide important information undetected by individual time point measures.

Keywords: Prenatal stress, maternal stress, fetal programming, hair cortisol.

1. Introduction

Maternal stress during pregnancy can exert lasting influence on the developing fetus. Research suggests that exposure to prenatal maternal stress increases risk of various undesirable outcomes for offspring, including poor cognitive functioning, mental health disorders, and behavior problems (Van den Bergh et al., 2017). Fetal programming of the hypothalamic-pituitary-adrenocortical (HPA) axis has been suggested as a potential mechanism for these associations (Lupien, McEwen, Gunnar, & Heim, 2009). Fetal programming refers to the process whereby prenatal environmental characteristics alter the trajectory of fetal development, resulting in differential anatomy and physiology that persist into postnatal life (Glover, O'Conner, & O'Donnell, 2010). Past studies have found associations between prenatal maternal stress and offspring HPA activity (Pearson, Tarabulsy, & Bussiéres, 2015). However, it is not well understood which gestational period is most susceptible to "programming" of the HPA axis. The current prospective study utilized offspring hair cortisol concentration (HCC), a relatively novel method for measuring chronic HPA output over a three-month period, to investigate (1) if prenatal maternal stress could predict offspring chronic HPA output 4 years later, and (2) whether this association varied as a function of gestational period.

Dysregulation of the HPA axis is associated with several outcomes predicted by elevated prenatal maternal stress, such as poor physical health, impaired cognition, and behavior problems (Chrousos, 2009). Because of these associations, fetal programming of the HPA axis has been explored as a biological mechanism underlying the association between prenatal maternal stress and offspring outcomes. Elevated levels of prenatal maternal stress have been associated with dysregulated HPA activity, assessed via salivary cortisol, in infants (Davis, Glynn, Waffarn, & Sandman, 2011), young children (Yong Ping et al., 2015), and adolescents (O'Donnell et al., 2013). However, findings have been somewhat inconsistent with regard to the effect of gestational timing of maternal stress (Pearson et al., 2015).

Among the few studies that examined prenatal maternal stress during multiple gestational periods, one found that increased maternal anxiety at either 18- or 32-weeks gestation predicted reduced

adolescent cortisol awakening response (CAR; O'Donnell et al., 2013), suggesting no gestational timing influence. Another study found that a flattened daytime cortisol profile among adolescents was predicted by increased maternal anxiety in the first trimester, and not by maternal anxiety during the second and third trimesters (Van den Bergh et al., 2008). Davis and colleagues (2011), on the other hand, found that neonate cortisol response to a heel stick was positively associated with maternal prenatal cortisol levels in the second trimester, not in the third trimester. Prenatal maternal stress has also been shown to predict offspring cortisol response to a maternal/child separation paradigm at 2.5 years of age, with the effect being greatest when the stressor occurred in the third trimester (Yong Ping et al., 2015).

Inconsistent results may be due to variability in methodology (Pearson et al., 2015). For example, the varying use of diurnal slope, CAR, and reactivity methods when measuring HPA functioning makes comparing results difficult. Within the reactivity paradigms, the use of different tasks and methods for calculating reactivity also creates complexity. In addition, salivary cortisol levels vary according to daily fluctuations in several variables difficult to control, particularly with child and infant samples: time of awakening and nap schedules, ingested substances, as well as acute stressors that may occur the day of testing, all of which pose a challenge when trying to assess general HPA physiology (Clements, 2013; Larson, Gunnar, & Hertsgaard, 1991).

Studies have shown HCC to be stable across repeated measurements and relatively robust against a range of possible confounding influences; HCC therefore may represent a reliable method for indexing long-term cortisol output, and general HPA physiology (Stalder & Kirschbaum, 2012). The growth rate of scalp hair varies by individual, based on sex, age, and race, though an average of 1 cm per month is generally accepted in adult and pediatric samples (Barth, 1987; Wennig, 2000). Assuming the average hair growth rate, the 3 cm of hair closest to the scalp, for example, may therefore be used to approximate general cortisol output over the previous 3 months (Stalder & Kirschbaum, 2012). HCC has been shown to have modest correlation with salivary cortisol (Vanaelst et al., 2012). However, in children, HCC was found to be more strongly predicted by distal self-reported stress, when compared to salivary or serum methods, suggesting HCC may be a better measure of long-term chronic stress (Vanaelst et al., 2012). In

addition, HCC levels have been found to correlate with demographic variables associated with chronic stress, such as neighborhood socioeconomic status (SES; Vaghri et al., 2013), and parent education level (Vliegenthart et al., 2016). There is very limited research examining the associations between prenatal maternal stress and offspring HCC. Prenatal maternal stress throughout pregnancy has been previously associated with offspring HCC levels in rhesus monkeys (Kapoor, Lubach, Ziegler, & Coe, 2016), and in human neonates related to prenatal maternal stress in the third trimester (Romero-Gonzalez, Caparros-Gonzalez, Gonzalez-Perez, Delgado-Puertas, & Peralta-Ramirez, 2018). We are not aware of any past research examining prenatal maternal stress and HCC in childhood. Further investigations into the potential relation between prenatal stress and offspring long-term HPA output may help clarify previous mixed results obtained primarily through short-term salivary methods. In addition, testing offspring HCC in childhood may contribute to our understanding of potential long term fetal programming effects and chronic HPA output.

The current study aimed to test the relation between prenatal maternal stress, measured at two gestational time points (mean gestational age at time 1 = 20.3 weeks; at time 2 = 32.6 weeks), and offspring HPA output, using offspring HCC measures at age 4, to (1) investigate changes in maternal reported stress in pregnancy and four years after birth, (2) add to knowledge about prenatal maternal stress and offspring HPA physiology, and (3) investigate whether gestational timing of maternal stress was associated with different child outcomes. Substantial evidence has documented changes in maternal perceived stress throughout pregnancy, though findings have been mixed with regard to direction of change (Costa, Larouche, Dritsa, & Brender, 1999; Goletzke et al., 2017; Silveira, Pekow, Dole, Markenson, & Chasan-Taber, 2013). We therefore hypothesized that maternal stress, on average, would change throughout pregnancy and after delivery, although this was a non-directional hypothesis. Given past research linking higher levels of maternal stress during pregnancy to heightened HPA output in offspring, we hypothesized that increased prenatal maternal stress would predict increased child HCC levels. Analyses examining effects of gestational timing were considered exploratory. The current study utilized data from a larger longitudinal study investigating cognitive outcomes at age 4; hair cortisol

sampling at age 4 coincided with the collection of cognitive data at this time point (cognitive data were not yet available for the current analysis). We utilized the Perceived Stress Scale (PSS; Cohen & Williamson, 1988) to index maternal perceived stress, because it was specifically designed to be generalizable among community samples from various sub-populations and of varying socioeconomic status (Cohen, Kamarck, & Mermelstein, 1983). It has also been used in maternal samples in prior research in both English and Spanish. In addition, the use of this scale allowed us to measure maternal stress both during and after pregnancy, whereas other prenatal stress instruments are designed for either pregnancy or postpartum. We also assessed child salivary circadian cortisol as a validation method for HCC values.

2. Methods

2.1. Participants

Participants in the current study were a subset of individuals from a larger study titled the "Preconception and Prenatal Stress: Pathways to Child Biology and Behavior Study", that followed families from 3 sites in the Community Child Health Network (CCHN) 5-site study, funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The goal of the study was to investigate preconception and prenatal influences on health disparities in pregnancy, fetal-programming, parent health, and child health and development (for study conceptual framework, design, and methods, see Ramey et al., 2015). Initial recruitment of participants and subsequent retention are described elsewhere (Guardino et al., 2016). One-hundred and twenty-seven mother-child dyads participated in all three waves of data collection. Of these dyads, 29 children did not provide a hair sample at Time 3 (14 children had a hair style that prevented collection, 10 children had hair that was too short, three parents refused, and two children refused). Of the 98 children that provided a hair sample, 21 hair samples were declared too small for reliable assaying by the lab that performed the assays. This resulted in a final sample of 77 mother-child dyads with the primary dependent variable data available for analysis. Compared to children with available hair cortisol data, those without available data were more

likely to have an African American mother, and to have a mother with a lower education level. No other differences between participants with and without hair cortisol data were observed.

Participants in the current study included 77 mother-child dyads (57% girls, 43% boys; 65.8% Latina/Hispanic, 27.6% White/non-Hispanic, 6.6% Black/African American mothers), who participated in two waves of prenatal assessment (mean gestational age at time 1 = 20.3 weeks; at time 2 = 32.6 weeks), and provided both maternal stress data and child hair samples approximately 4 years after the child's birth (mean child age = 3.8 years, *SD* = .43 years). Children were born on average at a gestational age of 38 weeks (SD = 1.9 weeks), and weighed on average 3176 grams (*SD* = 629.7 grams) at birth. At Time 1, mothers were on average 28.5 years old (*SD* = 5.2 years), and reported a yearly per capita income of $13,321 (*SD* = $14,270). Maternal education varied widely: 31% had less than a high school education, 38% graduated high school or obtained a GED, 12% had some college attendance, and 19% earned a 4-year college degree or higher.

2.2. Measures

2.2.1. Prenatal Maternal Stress. Prenatal maternal stress was measured using the Perceived Stress Scale (PSS), a scale developed to assess perceived stressfulness of participants' lives over the previous month (Cohen & Williamson, 1988). The PSS was specifically designed for use among community samples with at least a junior high school education, and contains language that is general in nature, so as to avoid content specific to any subpopulation (Cohen, Kamarck, & Mermelstein, 1983). Participants were presented with 10 questions that assess the general unpredictability and uncontrollability of their lives (e.g., *in the past month, how often have you been upset because of something that happened unexpectedly?*), and answer on a 5-point Likert type scale ranging from *not at all* (0) to *very often* (4), such that higher scores indicate higher levels of perceived stress. Mothers completed the PSS at four time points: during the second trimester (Cronbach's α = .87), during the third trimester (α = .86), approximately 13 weeks after birth (Cronbach's α = .84), and during a follow up visit four years after birth (α = .80). Participants in the current study were among those in the CCHN study who became pregnant again; prenatal maternal stress data varied due to when mothers recognized they

were pregnant and reported this to the local site (second trimester PSS, $n = 37$; third trimester PSS, $n = 57$; 4 years after birth PSS, $n = 77$).

2.2.2. Hair Cortisol Concentration. Hair cortisol concentration (HCC) was obtained from children during a home visit four years after birth (Mean age = 3.81 years). Hair samples were cut from the posterior vertex by trained researchers, with the 3 cm of hair closest to the scalp being retained for later assay. Samples were stored in aluminum foil and kept in a dark, cool location until shipped to the University of Massachusetts for analysis. Hair samples weighed on average 5.26 mg ($SD = 4.35$ mg). Hair samples were assayed at the University of Massachusetts. Complete washing and assaying protocol, and validation of procedures, are reported in detail elsewhere (Meyer, Novak, Hamel, & Rosenberg, 2014). Briefly, samples were weighed, washed twice in isopropanol, in order to rid samples of external contaminants, and dried for a minimum of two days before assaying. Samples were then ground to a fine powder using a stainless steel ball mill, and immersed in methanol overnight for extraction, after which methanol was evaporated using a vacuum evaporator. Dried extractions were then reconstituted in assay and assayed in duplicate using Arbor Assays (Ann Arbor, MI) cortisol enzyme immunoassay kit. Assay readouts were then converted to pg cortisol per mg hair. Intra- and inter-assay coefficients of variation for this sample were 8.9% and 5.6% respectively[1].

2.2.3. Covariates. We obtained the following additional health and demographic data for bivariate correlational analysis: child age, sex, body mass index (BMI), maternal education level, and maternal race/ethnicity. Child age and BMI correspond to when the hair sample was obtained. Child's height and weight were measured without shoes using a stadiometer and scale, allowing the calculation of BMI. Maternal education level was coded into a 4-level ordinal variable: 0 = *less than high school,* 1 = *high school diploma or GED,* 2 = *some college but no 4-year degree,* 3 = *4-year degree or higher*.

2.2.4. Salivary Cortisol. Child salivary cortisol data were collected at age 4 using take-home methods. Complete description of salivary cortisol methods are reported elsewhere (Guardino et al., 2016). For the current study child salivary cortisol was used primarily as a validation method for HCC values. Briefly, research staff provided saliva sampling kits along with verbal and written instructions on

procedures for collecting saliva at three times over the course of three consecutive days (upon waking, 30

minutes after waking, and bedtime; nine samples total per participant). Cortisol values were log-

transformed to normalize skew and values above 3 *SD* were Winsorized to the highest value within 3 *SD*

from the mean. Area under the curve (AUC) without awakening response was calculated using the first

and third daily sample. AUC values across the three days were averaged together. The intra- and

interassay coefficients of variance for salivary cortisol were 5.5% and 7.6%, respectively.

2.3. Analysis

Bivariate correlations and paired samples t-tests were analyzed using IBM SPSS Statistics version

25; regression analyses were performed using R 3.6.1 with the following package: *brms* 2.10.0.

Preliminary analysis of HCC data revealed a positive skew, so a log transformation was performed to

normalize HCC values, though for clarity HCC descriptive data are presented in their raw metric in Table

1. Within the HCC dataset 3 outliers (>3 SD above the mean) were identified. In order to preserve sample

size, outliers were Winsorized to within 3 SD of the mean, a method previously used with HCC data

(Rietschel et al., 2017; Vaghri et al., 2013). Bivariate correlations were computed among the main study

variables and the following demographic variables: child age, sex, BMI, maternal race/ethnicity, and

maternal education. To test if prenatal maternal stress changed throughout pregnancy and after birth, we

conducted two paired samples t-tests, examining changes from the second trimester to the third trimester,

and from the third trimester to four years after giving birth.

To test our primary aims, whether maternal prenatal stress predicted child HCC, and whether a

specific gestational period was a stronger predictor, we employed a simple slopes comparison approach.

In this approach, perceived stress scores (PSS) at Times 1-3 (second trimester, third trimester, and 4 years

after birth, respectively) each predict (log) hair cortisol concentration (HCC) measured four years after

birth in separate regression equations. More specifically, all three linear models are simultaneously

estimated in a single model, each with one intercept, one coefficient, and one residual variance. This

allows us to compute the difference in the coefficients of interest: $\beta_\Delta = \beta_{11} - \beta_{12}$, where β_{1t} represents the

PSS coefficient at time t. This is identical to computing the moderating effect of time point on the

coefficient of interest, but easily allows any contrast of interest and permits varying residual variances in a simple manner. PSS scores were standardized prior to analysis.

Additional exploratory analyses were conducted to test whether change in perceived stress levels during pregnancy predicted child HCC levels, and whether maternal perceived stress measured at approximately 13 weeks after birth, predicted child HCC levels. For the change in perceived stress analysis, we computed change scores by subtracting second trimester PSS from third trimester PSS, such that higher scores represent greater increases in perceived stress from second to third trimester. PSS change scores were standardized. We then regressed child log HCC onto prenatal PSS change scores.

2.3.1 Missingness. According to a Little's MCAR test (Chi-square = 18.74, df = 17, p = .34) we retained the null hypothesis that data were missing completely at random (MCAR). However, aspects of the study design suggest that data may not be MCAR. For example, some participants may have been less likely to report the subsequent pregnancy to the local site during early pregnancy, thus preventing the collection of PSS during the second trimester. In the interest of transparency, we took two approaches to analyzing the data. The first approach was to simply use pairwise deletion. The second approach was to use auxiliary variables to model the missing values. That is, perceived stress values were simultaneously modeled with demographic variables (child sex, child BMI, maternal ethnicity, and maternal education level). During the model estimation, the missing PSS values are imputed, and the estimates are integrated across uncertainty in the missing values. Results and inferences for the primary model were the same with and without missing data estimation, we therefore report findings from the simpler pairwise deletion approach. In the change score model, missing PSS values at Time 1 or Time 2 were each imputed. The imputed and observed values were then combined, and difference scores were computed.

3. Results

3.1. Demographic Characteristics and Correlations

Table 1 presents study sample descriptive statistics. Table 2 presents complete bivariate correlational results. PSS scores during pregnancy (second trimester PSS Mean = 14.05, SD = 6.27; third trimester PSS Mean = 17.49, SD = 6.09) were within the normative range, with third trimester levels at

the higher end of normative values (Cohen & Williamson, 1988). As expected, PSS scores over the three waves correlated highly. PSS in second trimester correlated with third trimester PSS ($r = .53$, $p = .002$) and with PSS 4 years after birth ($r = .44$, $p = .006$). Third trimester PSS also correlated with PSS 4 years after birth ($r = .41$, $p = .002$). Child HCC values correlated with child salivary cortisol AUC values ($r = .40$, $p = .004$), consistent with previous research (Stalder & Kirschbaum, 2012). Child HCC levels also correlated with being female ($r = .26$, $p = .02$) and having a Black/African American mother ($r = .31$, $p = .006$), such that girls and children of Black/African American mothers had higher levels of HCC at age 4 than boys or children of other race/ethnicity mothers. Child salivary cortisol AUC values correlated with third trimester maternal PSS ($r = .36$, $p = .03$), but were not significantly correlated with either second trimester maternal PSS or concurrent maternal PSS.

Maternal education level was inversely associated with maternal perceived stress during the third trimester ($r = -.42$, $p = .001$) and with perceived stress 4 years after birth ($r = -.36$, $p = .001$). Maternal education and perceived stress during the second trimester were only marginally correlated ($r = -.32$, $p = .06$), but revealed the same trend – i.e., mothers who were more educated reported lower levels of perceived stress.

3.2. Changes in Maternal Perceived Stress

We conducted two paired samples t-tests to investigate change in maternal perceived stress from second trimester to third trimester, and from third trimester to four years after giving birth. Results revealed that maternal perceived stress increased significantly from the second trimester ($M = 13.92$, $SD = 5.99$) to the third trimester ($M = 16.23$, $SD = 5.45$), $t(30) = 2.30$, $p = .03$, and then decreased significantly from the third trimester to 4 years after giving birth ($M = 13.74$, $SD = 5.09$), $t(56) = 4.61$, $p < .001$.

3.3. Prenatal Maternal Perceived Stress and Offspring Hair Cortisol Concentration

Estimates, standard errors, and 95% confidence intervals are provided in Table 3. We additionally include a Bayes factor comparing two models: One in which the slope is exactly zero, and the second in which the predicted slope is uncertain with a Normal (0, .5) distribution. A BF_{01} is a marginal likelihood ratio of the null hypothesis and the alternative hypothesis. If $BF_{01} > 1$, then the null hypothesis is

supported *relative* to the alternative hypothesis. Conversely, if $BF_{01} < 1$, then the alternative hypothesis is supported relative to the null hypothesis. For example, $BF_{01} = 6$ implies the data are six times more likely under the *null* hypothesis than under the alternative. For an introduction to Bayes factors, see Andraszewicz et al. (2015). The BF_{01} gives the reader insight into support of the null hypothesis relative to the alternative; if the BF_{01} is small (e.g., $1/10 < BF_{01} < 10$), then there may not be sufficient evidence in support of the null nor the alternative.

Results revealed that child HCC was not significantly predicted by maternal PSS score during either trimester of pregnancy. In addition, child HCC was not significantly associated with concurrent maternal PSS. Scatter plots of maternal PSS scores and child HCC are presented in Figure 1. The Bayes factors for these three estimates only slightly favored the null hypothesis (all BFs < 5), suggesting that we cannot be confident in supporting either the null or the alternative hypothesis. In other words, the data were not sufficiently evidentiary in favor of either hypothesis. The estimated difference in coefficients between second and third trimester PSS scores was: $\beta_\Delta = .22$, *SE* $= .18$, 95% CI [-.14, .56], $BF_{01} = 1.76$. Therefore, the predictive coefficients during the second and third trimester were not significantly different from one another. However, the BF again implies that neither the null nor the alternative hypotheses can be reasonably supported over the other.

3.3.1 Exploratory Analysis

At the bivariate level child HCC levels were significantly correlated with change in maternal PSS from second to third trimester ($r = .37$, $p = .04$), such that children of mothers who reported greater increases in perceived stress from second to third trimester, exhibited higher HCC levels in childhood. A scatter plot of maternal PSS change scores and child HCC is presented in Figure 1d. Controlling for child age, sex, BMI, maternal race/ethnicity, maternal education, and concurrent maternal PSS, increases in prenatal maternal PSS still predicted higher child HCC, $\beta = .53$, *SE* $= .23$, $p = .04$. However, using missing data estimation, this relation was no longer significant, $\beta = .23$, *SE* $= .23$, 95% CI [-.23, .63], $BF_{01} = 1.37$, with a Bayes factor that only slightly favored the null hypothesis. Results from the linear

regression predicting child HCC from post-natal maternal PSS revealed a non-significant relation between maternal PSS and child HCC levels, β = -.16, *SE* = .13, 95% CI [-.42, .09], BF_{01} = 1.90.

4. Discussion

This prospective study's aim was to test the relation between maternal perceived stress during pregnancy and a marker of long-term cumulative HPA output in offspring as measured by child hair cortisol concentrations (HCC) at age four. The assessment of maternal perceived stress at two time points in pregnancy allowed us to also examine potential differential effects of gestational timing by independently testing the associations between second and third trimester maternal perceived stress with child HCC. Results revealed that offspring HCC in childhood was not significantly predicted by maternal perceived stress at either second or third trimester of pregnancy. This is in contrast to previous findings using salivary cortisol (Davis et al., 2011; O'Donnell et al., 2013; Yong Ping et al., 2015). This discrepancy may be explained best by the use of hair cortisol concentration. The majority of significant findings linking maternal prenatal stress to offspring HPA activity have utilized salivary methods (Pearson et al., 2015). This may be a reflection of the novelty of hair cortisol methods, i.e.– less evidence is available using HCC methods due to the fewer number of studies that have utilized this methodology. It also may reflect a true difference in these HPA output indices. For example, prenatal stress effects on HPA axis development may lead to differential cortisol output that is more easily captured by dynamic, short-term measurements (e.g., CAR, acute stress induced reactivity). Due to the long-term nature of HCC levels, high variability may be expected, as the HCC value is a representation of the average cortisol output over a 3-month period (Gray et a., 2018), and we might expect high variability in what occurs during that period. A recent systematic review found that maternal perceived stress during pregnancy was inconsistently associated with maternal hair cortisol during pregnancy (Mustonen et al., 2018), providing additional evidence that HPA activity indexed through hair sampling methodology may be only weakly related to prenatal maternal perceived stress.

The only other previous study to test prenatal stress programming effects on offspring HPA activity using hair cortisol methods tested offspring HCC in early infancy (Romero-Gonzalez et al.,

2018). Although this suggests that there is a relation between prenatal stress exposure and offspring HCC levels, it is possible that the effect is small and potentially subject to 'washout' throughout the subsequent years after birth, as individuals are exposed to other environmental factors. Alternatively, our different results may be due to the demographic characteristics of our sample. The study by Romero-Gonzalez and colleagues (2018) had a relatively highly educated sample. In comparison, the participants in our study had on average low-education and low-income levels. It is possible that among low-SES populations perceived stress may not adequately capture total variations in chronic stress exposure. This may have prevented us from identifying links between prenatal stress and offspring HCC levels. Future studies may benefit from addressing a larger number of maternal stress factors and postnatal environmental factors in children.

In our bivariate correlational analysis, we did find a significant relation between third trimester maternal perceived stress and child salivary cortisol levels at age 4. This provides some evidence that the third trimester may be a sensitive period of fetal development, consistent with prior literature (Yong Ping et al., 2015), and that salivary methods may better tap into fetal programming effects. Of note, the association of saliva cortisol and hair cortisol in a small exploratory analysis here found moderate association validating the hair measures. However, testing salivary cortisol was not an aim of this study and should therefore be viewed as exploratory. Future research testing both hair and salivary methods in larger samples could further test differences in these indices.

In addition, we did not find a significant relation between concurrent maternal perceived stress and child HCC levels. This is consistent with past findings showing no relation between child HCC and parent self-reported stress (Liu, Snidman, Leonard, Meyer, & Tronick, 2016; Olstad et al., 2016; Ursache, Merz, Melvin, Meyer, & Noble, 2017) and potentially points to the difficulty in capturing meaningful variations in chronic stress exposure through self-report measures that relate to chronic HPA output (Gray et al., 2018). We did, however, find that maternal perceived stress scores were consistently associated with socioeconomic status, such that less educated mothers reported higher perceived stress at all three time points as would be expected.

In bivariate analyses, we found some preliminary significant associations between child HCC and demographic variables. Children of African American mothers exhibited higher HCC levels, which is consistent with past findings showing higher HCC levels in Black adults compared to other ethnic groups (Abell et al., 2016). Though this racial disparity in HPA output is not likely a result of differential perceived stress in the current sample, other chronic stressors previously found to relate to HCC levels, such as discrimination (O'Brien, Meyer, Tronick, & Moore, 2017), may better explain this association. In addition, we found higher HCC levels among girls. This is in contrast to a recent meta-analysis in adults documenting higher HCC levels in male adults (Stalder et al., 2017). However, among children more heterogeneous results have been found, with a majority of studies finding no significant gender differences in HCC levels (Gray et al., 2018). Given that testing for demographic differences in child HCC levels was not a primary aim of this study, these results need to be replicated before drawing firm conclusions. In addition, considering the small proportion of children of African American mothers in the current study, future research could benefit from replicating these findings in samples that include a larger proportion of African American children.

The third perceived stress assessment four years after birth provided a unique opportunity to look at stability or change in maternal stress over a long time frame, and showed remarkable significant effects and moderate effect sizes. Specifically, we found that on average maternal perceived stress increased from second trimester to third trimester, and then decreased from third trimester to approximately four years after birth. Significant changes in perceived stress throughout pregnancy have been previously documented (Da Costa, Larouche, Dritsa, & Brender, 1999; Silveira, Pekow, Dole, Markenson, & Chasan-Taber, 2013), and have been shown to be related to psychosocial factors, with mothers who report less mastery exhibiting increasing stress throughout pregnancy (Gurung, Dunkel Schetter, Collins, Rini, & Hobel, 2005), and whether the pregnancy is the first pregnancy, with primipara mothers reporting decreasing stress from the second to third trimester (Goletzke et al., 2017). The present sample was primarily composed of low-SES women, all of whom had previously given birth, which may explain why we saw increases in stress from second to third trimester.

Post hoc exploratory analyses revealed evidence that increases in maternal perceived stress over pregnancy predicted child chronic HPA output. This is consistent with previous research that found that adverse birth outcomes (i.e., preterm birth) were predicted by changes in perceived stress during pregnancy, and not by stress levels at any individual gestational period (Glynn, Dunkel Schetter, Hobel, & Sandman, 2008). In this previous study, mothers on average reported decreases in perceived stress (PSS) over pregnancy. However, mothers who reported increases in stress from second to third trimester were more likely to have a preterm birth compared to mothers who reported decreases in stress (Glynn et al., 2008). Similar to these results, increases in perceived stress from second to third trimester within our sample predicted higher HCC output for offspring. These results could not be explained by gestational age or preterm birth, as post hoc analysis found no relation between these variables and either PSS change or child HCC levels. In addition, we found no relation between PSS change and child or maternal demographic variables (e.g., maternal education level), making it unlikely that this relation could be explained by any of the measured environmental characteristic variables. We also found that increases in prenatal maternal PSS predicted higher offspring BMI, further supporting the potential long term health consequences of increasing perceived stress throughout pregnancy. Although we did not find significant relations between child HCC and BMI in the current sample, higher HCC has been linked to higher BMI in a recent meta-analysis (Stalder et al., 2017). Future research is needed to fully understand the potential relations between increases in prenatal perceived stress, offspring BMI, and offspring hair cortisol.

On average, perceived stress tends to decline from second to third trimester (Glynn et al., 2008; Goletzke et al., 2017; Silveira et al., 2013). It is therefore possible that increases in perceived stress represent a marked deviation from normative psychological trends during pregnancy. This may help explain why increases in stress throughout pregnancy predict adverse health outcomes for offspring (Glynn et al., 2008). However, it is important to keep in mind the exploratory nature of these results. In addition, the relation between increases in maternal prenatal PSS and child HCC was rendered non-significant when missing data imputation was implemented; we therefore recommend against strong

interpretation of these results. Future research could benefit from further investigating the predictive value of changes in maternal stress during pregnancy.

Important limitations warrant mention. First, the sample size was somewhat small, which made it difficult to test more complex models involving moderators such as offspring sex. Past research suggests that fetal programming effects may be moderated by offspring sex (Braithwaite et al., 2017; Cuffe, O'Sullivan, Simmons, Anderon, & Moritz, 2012). In addition, our Bayesian analysis allowed us to test for the likelihood that there is a null effect. The Bayes factors obtained suggested that our null results may be due to sample size, as the data were not sufficiently evidentiary in favor of either the null or the alternative hypothesis. Our perceived stress variables during pregnancy also exhibited substantial missingness because the parent study was of preconception periods and pregnancies were not always identified sufficiently early to obtain second or third trimester assessments though the mothers were then included in the longer term follow-up of their children. Nonetheless, missing prenatal perceived stress data may have limited our ability to test our primary study aims. Future studies could benefit from testing these relations using larger samples. In addition, investigation of HCC missingness revealed that children of African American mothers, and less educated mothers, were more likely to decline to provide a hair sample, which may have biased results. Future researchers must that take into account specific challenges associated with hair sampling in African American and lower-education communities.

Future research could also benefit from collecting maternal salivary cortisol levels during pregnancy in addition to measuring maternal perceived stress to directly test whether changes in circulating maternal cortisol during and over the course of pregnancy mediate associations between PSS and child HCC. Given very limited maternal salivary cortisol data available, due to participant reenrollment methods described above in addition to participant failure to complete take home salivary samples (second trimester, $N = 18$; third trimester, $N = 27$), we were unable to adequately test mediation via maternal cortisol. Lastly, the correlational design of this study precludes definitive causal inferences, thus future studies should consider employing an experimental design that could test the clinical

application benefits of randomly assigning women at risk for high stress levels to an evidence-based stress reduction intervention during pregnancy.

Despite these limitations, there are several strengths to this prospective study. We tested long-term outcomes of prenatal maternal stress in a predominantly low SES, minority sample. Research on prenatal stress among low SES and minority populations may be of particular importance as low SES and minority communities may face disproportionately higher levels of chronic stress (O'Campo et al., 2016; Ramey et al., 2015), particularly during pregnancy (Dunkel-Schetter, 2011). By measuring prenatal maternal stress at two time points, we were able to test the effect of gestational timing on the relation between prenatal maternal stress and offspring HPA output, and investigate how changes in prenatal stress relate to offspring stress biology. In addition, this is only the second study to test the relation between maternal perceived stress during pregnancy and offspring HPA physiology using hair cortisol concentration (HCC) levels, expanding upon previous research to look at HCC in childhood. HCC methodology is gaining increasing attention, as it is relatively non-invasive, easy to administer among child populations, and robust to influence by momentary or daily variation in a number of covariates (Abell et al., 2016). However, more research is needed to determine the factors that predict HCC levels in childhood.

4.1. Conclusion.

We found minimal evidence for the hypothesis that maternal prenatal perceived stress is associated with child HCC levels four years later, and some preliminary evidence that increases in maternal prenatal stress from the second to third trimester may predict increased offspring HCC 4 years after birth. Dysregulation of the HPA axis is associated with poor mental and physical health outcomes (Chrousos, 2009); thus, understanding the etiology of differential HPA functioning is critical. A better understanding of how dynamic changes in stress during pregnancy relate to child outcomes has important implication for both research methodology and intervention.

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Footnotes

[1]The inter-assay CV was determined using a quality control with a mid-level cortisol concentration, whereas the intra-assay CV was calculated by averaging the CVs of the duplicate wells of all the study samples analyzed for the paper. Since the study samples included a number of values with low cortisol concentrations that yield more variability in the assay, the result was a greater average intra-assay CV.

Table 1. *Sample Demographics and Variable Characteristics.*

Variable	M	SD	Range
Child HCC	29.10	85.27	0.68–556.70
Child (ln)HCC	2.07	1.28	-0.38–5.65
PSS Time 1	14.05	6.27	1–29
PSS Time 2	17.49	6.09	5–33
PSS Time 3	13.89	5.54	2–29
PSS Change	2.30	5.58	-13–16
Child Salivary AUC	65.87	11.52	46.84–93.10
Child Age	3.81	0.43	3.46–5.48
Child BMI	16.62	1.81	13.73–24.80
Mother Age	26.48	5.21	18.19–39.32
Annual Income	13,321.16	14,270.12	60–56,252

	Category	N	%
Child Sex			
	Female	44	57.1
	Male	33	42.9
Maternal Education			
	Less than high school	23	31.1
	High school	28	37.8
	Some college	9	12.2
	4-year degree or higher	14	18.9
Maternal Ethnicity			
	Latina	50	65.8
	Caucasian	21	27.6
	African American	5	6.6

Note. HCC = hair cortisol concentration. (ln)HCC = log transformed hair cortisol concentration, Winsorized to within 3 *SD* from the Mean. PSS = perceived stress scale. AUC = area under the curve. Time 1 = second trimester, Time 2 = third trimester, Time 3 = childhood, PSS Change = PSS Time 2 – PSS Time 1. Household income is yearly income adjusted for family size.

Table 2. *Bivariate Correlations Between Major Study Variables and Covariates.*

	2	3	4	5	6	7	8	9	10	11	12
1. Child (ln)HCC	-.11	.16	-.02	.37*	.40**	-.11	.26*	-.03	.03	-.12	.31**
2. PSS Time 1	–	.53**	.44**	-.56**	.16	.09	.19	-.09	-.32	.16	-.06
3. PSS Time 2		–	.41**	.41*	.36*	.19	.10	.16	-.42**	.23	-.09
4. PSS Time 3			–	-.18	-.07	.10	.10	.16	-.36**	.13	.05
5. PSS Change				–	.23	.04	.08	.51**	-.27	.08	-.01
6. Child Salivary AUC					–	-.06	.20	.28*	-.14	-.02	.00
7. Child Age						–	-.17	-.18	.01	-.07	.07
8. Child Sex							–	-.05	-.14	-.03	.12
9. Child BMI								–	-.32**	.19	.05
10. Maternal Education									–	-.51**	-.05
11. Maternal Ethnicity (Latina)										–	-.36**
12. Maternal Ethnicity (African American)											–

Note. (ln)HCC = natural log transformed hair cortisol concentration. PSS = perceived stress scale. AUC = area under the curve. Time 1 = second trimester, Time 2 = third trimester, Time 3 = 4 years after birth. PSS Change = PSS Time 2 – PSS Time 1.
* $p < .05$, ** $p < .01$.

Table 3. *Simple Slopes Estimates for Maternal PSS on Child HCC*

Predictor	Intercept	Slope	*SE*	95% CI	Residual *SD*	BF$_{01}$
PSS Time 1	0.00	-.09	.14	[-.36, .19]	.90	2.92
PSS Time 2	0.00	.14	.11	[-.09, .36]	.95	2.14
PSS Time 3	-0.01	-.02	.12	[-.25, .21]	1.00	4.51

Note. PSS = perceived stress scale. HCC = hair cortisol concentration. BF$_{01}$ = Bayes factor. Time 1 = second trimester, Time 2 = third trimester, Time = 4 years after birth.

Figure Captions

Figure 1. Scatterplots of child log hair cortisol concentration (lnHCC) and (a) second trimester maternal perceived stress (PSS; $N =$ 37, $r =$ -.11, $p =$ .54), (b) third trimester maternal PSS ($N =$ 57, $r =$ .16, $p =$ .24), (c) concurrent maternal PSS ($N =$ 77, $r =$ -.02, $p =$ .85), and (d) changes in maternal PSS from second to third trimester ($N =$ 31, $r =$ .37, $p =$ .04). Shaded area represents 95% confidence interval.