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Stress and hair cortisol concentrations from preconception to the third trimester

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Abstract

Stress is an important and modifiable determinant of health, and its association with hair cortisol concentrations (HCC) during pregnancy remains unclear. We selected a random sample of 97 participants from a cohort of pregnant participants attending prenatal clinics in Lima, Peru. Each provided a hair sample at enrollment (mean gestational age=13.1 weeks) and again at full-term delivery. Hair samples were segmented to reflect HCC in preconception and each trimester. At enrollment, measures of stress included: difficulty accessing basic goods, educational attainment, exposure to violence, fair or poor general health, perceived stress, and symptoms of depression, general anxiety, and post-traumatic stress disorder. Linear mixed models evaluated the association between each stress measure and absolute and relative changes in HCC. Pearson correlation coefficients (r) assessed correlations between HCC and continuous stress scores. Educational attainment of ≤12 years was associated with higher HCC in preconception and the 1st trimester, and general anxiety with lower preconception HCC. When modeling HCC patterns across the 4 hair segments, an educational attainment of ≤12 years was associated with higher HCC, high perceived stress with lower HCC, and general anxiety with steeper increases in HCC (group by time p-value= 0.02). Only preconception HCC and GAD scores correlated (r= −0.22, p=0.04). We observed few associations between stress and HCC. However, those that were seen were generally restricted to the preconception and 1st trimester. Further investigations into the association between stress and changes in HCC across pregnancy are warranted, and should include the preconception where possible.

2–3 SENTENCE LAY SUMMARY

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DISCLOSURES
None.

Few associations between stress and hair cortisol concentrations (HCC) were observed from preconception to the 3rd trimester. Observed differences in HCC based on educational attainment and anxiety were generally restricted to the preconception and 1st trimester when circulating cortisol concentrations are at their lowest compared to later in pregnancy. Further investigations into the association between stress and HCC across pregnancy are warranted, and should consider the evaluation of overall increases in HCC and the addition of the preconception period were possible.

Keywords

Anxiety; Cortisol; Depression; Distress; Hair; Pregnancy; SES; Stress

1. INTRODUCTION

Stress is an important and modifiable determinant of health. Given its potential impacts during pregnancy (Coussons-Read et al., 2012; Davis and Sandman, 2010, 2012; Diego et al., 2006; Glover, 2015; Glynn et al., 2013; Manuck et al., 2015; Wadhwa et al., 2011), the American College of Obstetricians and Gynecologists advocates for screenings of stress-related risk factors at least once per trimester followed by the appropriate referrals (2006). In addition, an understanding of mediating neuroendocrine stress-response mechanisms remains a priority (Shannon et al., 2007). One such mechanism is the hypothalamic pituitary adrenal (HPA) axis, and cortisol is a key biomarker of HPA axis activity. Cortisol concentrations in hair reflect long-term cortisol secretion during pregnancy and are positively correlated with salivary cortisol (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009; Stalder and Kirschbaum, 2012). Findings for the association between hair cortisol concentrations (HCC) and stress during pregnancy remain unclear. Furthermore, the association between stress and HCC in the preconception has not been reported.

On balance, no consistent relationship has been observed between HCC and measures of stress. For example, a recent correlation-based meta-analysis found no consistent relationship between HCC and self-reported perceived stress or depression but found a 17% reduction in HCC with anxiety disorders (Stalder et al., 2017). When restricted to studies of pregnant participants or those who have recently delivered, reported findings are also mixed. For example, some studies report positive associations between HCC, perceived stress scores,and depression (Caparros-Gonzalez et al., 2017; Hoffman et al., 2016; Kalra et al., 2007). In contrast, a similar amount of studies report no such associations (Braig et al., 2016; Wikenius et al., 2016)(Scharlau et al., 2017). To help provide clarity on the topic, we performed a comprehensive evaluation of the relationship between HCC patterns from preconception to the third trimester and multiple measures of stress during pregnancy, and used hair samples collected from a cohort of pregnant women in Lima, Peru (once at enrollment and again at full-term delivery). In doing so, we sought to determine whether changes in cortisol secretion (as reflected in maternal scalp hair samples) differed based on measures of stress assessed in early pregnancy.

2. METHODS AND MATERIALS

2.1 STUDY PARTICIPANTS AND PROCEDURES

Data for this study was drawn from participants of the Pregnancy Outcomes, Maternal, and Infant Study (PrOMIS), a prospective cohort study consisting of pregnant women attending prenatal clinics and previously described elsewhere (Barrios et al., 2015). Briefly, the PrOMIS cohort was designed to examine maternal social and behavioral risk factors on the development of preterm birth and other adverse pregnancy outcomes among Peruvian women. The cohort was comprised of women attending prenatal care clinics at the Instituto Nacional Materno Perinatal (INMP) in Lima, Peru, the primary reference establishment for maternal and perinatal care operated by the Ministry of Health of the Peruvian government. Written informed consent was obtained from all participants, and the institutional review board of the INMP and the Office of Human Research Administration at the Harvard T.H. Chan School of Public Health approved all study procedures. Recruitment began in February of 2012, and scalp hair samples were collected from participants during the period of October 2014 to November 2015. Participants who were 18 years of age or older, were able to speak and read in Spanish, and initiated prenatal care in early pregnancy were invited to participate (mean gestational age (GA)=13.1 weeks, standard deviation (SD)=3.9). Participants were then followed until delivery, and analyses were restricted to participants with full-term delivery to help ensure that segmented hair segments at delivery reflected the 2nd and 3rd trimesters. At enrollment in the 1st trimester, structured interviewer-administered questionnaires were used to collect information on stress, hair and sociodemographic characteristics, and medical and reproductive history. Among enrolled participants (N=2,068), 96% provided a first hair sample at enrollment (N=1,991), and 32% contributed a second hair sample after full-term delivery while women were still in the hospital (N=653) (mean GA 39.0 weeks, SD=1.0). Participants who contributed two hair samples were comparable to the full cohort (e.g. mean age: 27.5 vs. 27.9 and mean GA at enrollment: 12.0 weeks vs. 11.1 weeks, respectively). Since it was not financially feasible to conduct biochemical analyses using hair samples from all participants, we randomly selected 100 individuals from the pool of eligible individuals with two hair samples. Our final sample size of 97 participants excluded three participants whose cortisol was either undetectable or exceeded 100pg/mg (>4 SD above the mean).

2.2 HAIR COLLECTION AND LABORATORY ANALYSIS

Trained research staff collected hair samples from the posterior vertex region as close to the scalp as possible at enrollment and again at full-term delivery. Prior to lab analysis, individual’s hair samples were randomly ordered and segmented for a total of four 3cm hair segments per individual. Given that the average hair growth in humans is approximately one centimeter per month (Astore et al., 1979; Barman et al., 1965; Barth, 1986; Loussouarn et al., 2005; Pecoraro et al., 1967), the four hair segments reflected the following time periods: preconception (3–6cm from the scalp at enrollment), 1st trimester (3cm from the scalp at enrollment), 2nd trimester (3–6cm from the scalp at delivery), and 3rd trimester (3cm from the scalp at delivery) (Figure 1). Segments further from the scalp were excluded due to concerns of measurement error from cortisol degradation and washout (Hamel et al., 2011; Li et al., 2012; Russell et al., 2012). To remove variability due to batch from within-subject contrasts, hair segments from the same woman were extracted and assayed in the same batch. The immunoassay used was the Cortisol Saliva Luminescence Immunoassay, IBL International ®, for which the lower limit of detection was 0.1 pg/mg. Six blinded quality control (QC) samples were randomly dispersed per batch to assess variability (Tworoger and Hankinson, 2006). Briefly, Segmented hair samples were washed in in 2.5 milliliters (ml) of isopropanol twice for three minutes each time, and then dried for 12 hours in an extractor hood. After drying, 7.5mg of whole non-pulverized hair was weighed out per segment and then minced into small pieces. Hair samples were then incubated for 18 hours in 1.8ml high-grade methanol at room temperature, after which 1.6ml of clear supernatant was transferred to a vial and the methanol evaporated off at 55°C using a steady stream of nitrogen for thirty minutes. The residue from each segment was then resuspended in 225 microliters (μl) of distilled water, and 20μl of internal standard (cortisol-d_4) was added. Calculated inter-assay and intra-assay coefficients of variation were 19.4% and 11.9%, respectively, and within the acceptable range (Tworoger and Hankinson, 2006). Since cortisol concentrations in hair may be influenced by ultraviolet light (UV) exposure (Hansen et al., 2001; Li et al., 2012), UV categories of low, intermediate, and high were established using dates of enrollment and meteorological data.

2.3 STRESS MEASURES

Stress measures were identified using criteria from Miller et al. 2007 and Stalder et al. 2017 (Miller et al., 2007; Stalder et al., 2017) which required exposure to conditions that would reasonably be appraised as threatening and exceeding coping resources, or psychological in nature. Based on these criteria, at enrollment we assessed economic stressors, exposure to violence, perceived health and stress, and psychological distress. Economic stressors included: difficulty accessing basic goods (yes/no), unemployment (yes/no), and educational attainment (≤12 vs. >12 years). We assessed a participant’s exposure to violence by asking about their history of physical or sexual abuse prior to age 18 (yes/no) and their history of intimate partner violence across their lifetime (yes/no). We assessed a participant’s perceived health by asking their current general health status (fair/poor vs. good/excellent). Measures of psychological distress were assessed at enrollment using previously translated scales validated for use in Spanish-speaking populations, and included: perceived stress in the past month using the 14-item Perceived Stress Scale (PSS) (Cohen et al., 1983), general anxiety symptoms in the past two weeks using the Generalized Anxiety Disorder-7 scale and a cutoff score of 7 (Zhong et al., 2015), depressive symptoms in the past week using the Patient Health Questionnaire-9 scale and a cutoff score of 10 (Zhong et al., 2014), and post-traumatic stress disorder (PTSD) symptoms in the past month using the Civilian version of the Posttraumatic Stress Disorders Checklist (PCL-C) and a cutoff score of 26 (Gelaye et al., 2017).

2.4 STATISTICAL ANALYSIS

The Shapiro-Wilk test statistic was used to evaluate skewness of HCC (pg/mg). Based on findings of right-skewness, values were transformed on the natural logarithm scale to approximate normality (logHCC), and mean logHCC and standard deviations (SD) are reported. Pair-wise differences in mean logHCC across hair segments were evaluated using paired t-tests. Pearson correlation coefficients (r) were used to assess correlation of logHCC across hair segments and with continuous scores from psychological distress scales (perceived stress, general anxiety, and depression). Differences in mean logHCC across categorical measures of maternal stress were evaluated using independent t-tests and linear mixed models. For categorical analyses of perceived stress, the median score of 29.0 was used as the cutoff for distinguishing high vs. low perceived stress. For depression, general anxiety, and PTSD the aforementioned cutoffs were used to create categories. Likelihood ratio tests (LRT) were used to determine best fitting models for both the logHCC means and within-subject covariance. Based on LRT results, the final model contained a linear time trend across the 4 hair segments (0,1,2,3) and an unstructured covariance matrix. Each categorical index of stress was included as a main group effect with time. In separate models each stress measure (or group) was evaluated for its interaction with time (group x time) which characterizes the extent to which the slope of HCC across the time points (preconception to the 3rd trimester) differ according to categories of that stress measure. Multivariable models adjusted for factors that could influence cortisol concentrations in hair, such as: early-pregnancy body mass index, alcohol consumption, hair structure, chemical hair treatment and UV exposure (Stalder et al., 2017). In sensitivity analyses we further adjusted for gestational age (Wikenius et al., 2016). All statistical analyses used SAS® version 9.4 software (SAS Institute, Inc., Cary, North Carolina) and p-values are two-sided at the alpha 0.05 level.

3. RESULTS

Sociodemographic and hair characteristics of study participants are described in Table 1, and stress was common. For example, 41.2% of participants had difficulty paying for basic goods, 39.2% a low educational attainment (≤12 years), 53.6% were unemployed during pregnancy, 68.0% reported fair or poor general health during the current pregnancy, 29.9% had a history of physical or sexual abuse as a child, and 16.5% had a history of intimate partner violence. Further, the mean perceived stress score was 29.0 (SD=4.9), 23.7% presented with depression (PHQ-9 score ≥10), 34.0% presented with generalized anxiety disorder (GAD-7 score ≥7), and 19.6% presented with PTSD (PCL-C score ≥26). LogHCC values on the same hair sample were highly correlated (r= 0.83 on hair sample 1, and r=0.75 on hair sample 2), while LogHCC values across hair samples were not (r’s ranged from 0.08 to 0.23, p-values >0.11) (Table 2). Mean logHCC values significantly increased from preconception (1.28, standard deviation (SD)=1.0) to the 1st trimester (1.64, SD=0.96) and with each subsequent trimester (2nd trimester=1.75, SD=0.89; 3rd trimester=2.22, SD=0.88) (Figure 2). With the exception of the 1st and 2nd trimester difference, all pairwise differences in logHCC were statistically significant (Table 3).

On average, mean logHCC values and overall patterns from preconception to the 3rd trimester did not differ according to categories of stress, and differences that were seen were generally restricted to segments reflecting the preconception and the 1st trimester (Figure 3 and Table 3). For example, an educational attainment of ≤12 years was associated with higher mean logHCC values in both preconception (1.58 vs. 1.09, p-value=0.02) and the 1st trimester (2.01 vs. 1.39, p-value=0.002), while general anxiety (GAD-7 score ≥7) was associated with lower mean logHCC in preconception (0.88 vs. 1.49, p-value=0.004) (Table 3). In adjusted models, increases in mean logHCC by categories of time were evident across all categories of stress (p values <0.0001), and although slopes were similar, mean logHCC values were generally higher with low education (Beta=0.32, p=0.01) and low with high perceived stress (Beta= −0.28, p-value=0.02) (Table 4). The overall rate of increase in logHCC from preconception to the 3rd trimester was steeper among individuals with general anxiety at enrollment (group x time p-value=0.02) (Table 4). With the exception of preconception HCC and general anxiety scores (r= −0.22, p-value=0.04), correlations of HCC with continuous scores of psychological distress were not observed (Table 5). General anxiety scores and depression scores were highly correlated (r=0.75, p-value <0.0001). Models adjusted for gestational age did not appreciably differ (data not shown).

4. DISCUSSION

In this sample of participants with a high prevalence of stress in early pregnancy, we observed expected increases in HCC with pregnancy progression and few associations between measures of stress and absolute and relative changes in HCC. With the exception of general anxiety, overall patterns of HCC from preconception to the 3rd trimester were similar across the selected measures of stress. Additionally, observed differences in HCC by education and general anxiety were generally restricted to the preconception and 1st trimester when cortisol concentrations are lower relative to later in pregnancy, and closer to when measures of stress were assessed. Unexpectedly, participants with high perceived stress had lower HCC from preconception to the 3rd trimester.

On average, our HCC values were lower than other studies that collected hair samples at comparable times in pregnancy, although observed increases were generally similar. For example, one study of 103 participants who provided hair samples at delivery observed mean HCC values of 13, 20, and 40 pg/mg in 1st, 2nd, and 3rd trimester hair segments, respectively (add Kirschbaum 2009). Another study of 21 participants who provided hair samples at each trimester observed mean HCC values of 25, 33, and 40 pg/mg in 1st, 2nd, and 3rd trimester hair segments, respectively (add D’Anna Hernandez 2012). For comparison, the geometric mean HCC values observed in our study of 97 participants were approximately: 5, 6, and 9 pg/mg in 1st, 2nd, and 3rd trimester hair segments, respectively. In contrast, our values were higher than a study of 180 participants who observed geometric mean HCC values of 3–4 pg/mg across the trimesters (Schrier et al. 2016). Despite the fact that our study observed lower absolute values of HCC, the approximately 2-fold increase in HCC from the 1st trimester to the 3rd trimester in our study is within range of the 1 to 3-fold increases observed in the aforementioned studies. We found no studies reporting on preconception HCC levels.

A recent correlation-based meta-analysis showed no consistent association of HCC with perceived stress or depression, and 17% lower HCC with anxiety disorders (Stalder et al., 2017). When restricted to studies that evaluated the association between stress and HCC during pregnancy or the postpartum, similar inconsistencies are observed (Braig et al., 2016; Caparros-Gonzalez et al., 2017; Hoffman et al., 2016; Kalra et al., 2007; Scharlau et al., 2017; Wikenius et al., 2016)(Supplemental Table). For example, in a study of 44 participants recruited during prenatal visits in Spain, Caparros-Gonzalez and colleagues report higher HCC using 3cm hair segments reflecting each trimester when comparing participants with and without depression in the postpartum period (Caparros-Gonzalez et al., 2017). In contrast, a larger study (N=181) using a similar recruitment strategy in Norway and a similar screening instrument found no association between HCC from 1cm hair segments reflecting the 2nd trimester and depression scores assessed in the 2nd trimester (Pearson correlation coefficient=0.1, p-value not reported; multivariable beta coefficient not reported, p-value=0.38)(Wikenius et al., 2016). A U.S. study recruited 90 participants at prenatal clinics and found significant correlations between HCC from 3cm hair segments and scores of perceived stress, depression, and anxiety in all trimesters (Hoffman et al., 2016). They found no association between HCC and education or socioeconomic status. In the largest study to date, 768 participants were recruited during hospital stays after delivery in Germany and 3cm hair segments reflecting the 3rd trimester were collected(Braig et al., 2016). No association was observed between HCC and scores of anxiety, depression, and chronic stress (Spearman correlation coefficients ranged from 0.0–0.1, p-values >0.06, beta coefficients not reported). The chronic stress scale in that study included questions regarding employment and social burdens. Lastly, in a study using a sample of 25 callers requesting medication safety information in Canada, modest correlations between HCC from 1–1.5cm hair segments reflecting the late 1st to early 2nd trimester and perceived stress scale scores were observed (Spearman correlation coefficient=0.47, p<0.05) (Kalra et al., 2007). Recently, although no association was observed between HCC from 1cm hair segments reflecting the 2nd and 3rd trimester and depression scores, a negative correlation with cortisone was observed (Scharlau et al., 2017). We found no studies reporting on stress and HCC in the preconception. On balance, our study's findings of few associations between stress and HCC during pregnancy are consistent with the literature. Potential reasons for discrepancies across studies include differences in the trimesters reflected by cortisol and stress, country, and screening instruments.

Lower HCC levels among individuals with anxiety have been previously reported (Steudte et al., 2011) (Stalder et al., 2017)and are consistent with hypotheses of wear-and-tear due to chronic stress (McEwen, 1998; Pruessner et al., 1999), specifically cortisol hyposecretion subsequent to a period of hypersecretion (Trickett et al., 2010). In our study, lower HCC values among participants with general anxiety were restricted to the preconception and 1st trimester, while 2nd and 3rd trimester HCC levels did not differ. Therefore, it is likely that lower HCC in the preconception and 1st trimester explain our observation of a steeper slope of increase in HCC values from preconception to the 3rd trimester. We know of no comparable study reporting on such changes in HCC across pregnancy by measures of stress. However, similar findings of steeper cortisol increases across pregnancy among those with anxiety have been previously reported using salivary cortisol (Kane et al., 2014). Overall, the results from our study in conjunction with prior research suggest that measures of stress may not play a strong role in determining absolute levels of HCC in during pregnancy, and that future researchers should instead examine their association with their changes during pregnancy (including the preconception) and their health outcomes. Similar recommendations were reported in a study of non-pregnant women and salivary cortisol (Vedhara et al., 2003). Further, the association between cortisol changes during pregnancy and depression status is an important gap in the literature (Orta et al., 2018).

Our findings of differences in HCC restricted to preconception and 1st trimester hair segments may be explained by several reasons. First, higher placental-derived corticotropin releasing hormone during the 2nd and 3rd trimesters of pregnancy lead to substantial increased maternal circulating cortisol concentrations upwards of twice that of pre-pregnancy levels (Goland et al., 1986). This, in conjunction with research suggesting that physiologic responses to psychosocial stressors attenuate with advancing gestation (Entringer et al., 2010), suggests that the impact of stress may no longer be physiologically detected using hair cortisol in late pregnancy. This implies a potential "ceiling effect", a commonly observed phenomenon in which discrimination is only detectable at low to moderate levels (Austin and Brunner, 2003). Further, in our study, instruments used to assess distress asked participants to reflect on symptoms in the weeks prior to enrollment. Despite the trait stability of distress symptoms such as anxiety during pregnancy (Schubert et al., 2017), the preconception and 1st trimester period may have been the most biologically relevant time window for such comparisons. Furthermore, since stress levels were assessed only once at enrollment, we do not know if symptoms persisted throughout pregnancy.

In an effort to evaluate whether measures of stress were associated with absolute HCC and its patterns, our study assessed multiple aspects of the stress experience that encompassed socioeconomic status, history of violence, and psychological distress using validated instruments. This comprehensive evaluation addresses some of the issues of previous studies that focused on only some potential stressors, or one, and helps lay the foundation for the role of multiple stressors on cortisol secretion patterns across pregnancy. Our study also used multiple hair collections using standardized collection techniques and trained personnel. Our inclusion of hair segments reflecting the preconception period is novel and helps fill an important gap in the literature. An additional strength to our study was our statistical approach, which evaluated differences in absolute HCC and HCC patterns while accounting for covariance across hair segments and potential covariates.

Despite our strengths, our study has some limitations. First, multiple assessments of stress at each trimester of pregnancy would have provided opportunities to examine HCC patterns in relation to changes in stress during pregnancy. Second, our study may not have been sufficiently powered to detect small group differences and group by time interactions. Further, we did not correct for multiple tests. Third, poor correlations across hair samples in our study may have influenced why observed differences on hair sample one did not extend to hair sample two. Fourth, in some cases psychological distress scales assessed time windows shorter than the 3cm three-month windows reflected by hair cortisol in the 3cm hair segments reflecting the past 3-months. However, observed differences based on general anxiety, a generally stable trait (Schubert et al., 2017), may not have been as impacted as some of the other psychological distress scales. Furthermore, although our CV's were on the higher end of the acceptable range, high batch to batch variation may have impacted our findings. However, our study design ensured that segments from all women were assayed in the same batch, and that random ordering ensured that individuals with high and low stress were randomly distributed across batches. Therefore, potential measurement error is likely non-differential with respect to stress. Furthermore, our findings may be impacted by residual confounding from factors associated with both stress and cortisol secretion during pregnancy. Lastly, although HCC in segments up to 6cm from the scalp are thought to be least impacted by cortisol “washout” over time, HCC in preconception and second trimester hair segments (3–6cm from the scalp) are likely somewhat attenuated, compared to if they were collected as proximal segments (3cm from the scalp). Therefore, confirmation of such findings in larger studies designed to assess stress measured at multiple time points in pregnancy using proximal hair segments (3cm from the scalp) in all trimesters and the preconception using our statistical approach is warranted.

5. CONCLUSION

To our knowledge, we are the first to evaluate the association between measures of stress with both absolute HCC levels and their changes from preconception to the 3rd trimester. With the exception of educational attainment, perceived stress, and general anxiety, few indicators of stress were observed to be associated with hair cortisol and its patterns. Further, observed differences were restricted to the preconception and first trimester when cortisol levels are low relative to later in pregnancy. Future studies seeking to assess the impacts of stress on biomarker secretion patterns in hair during pregnancy should consider the addition of the preconception time window where possible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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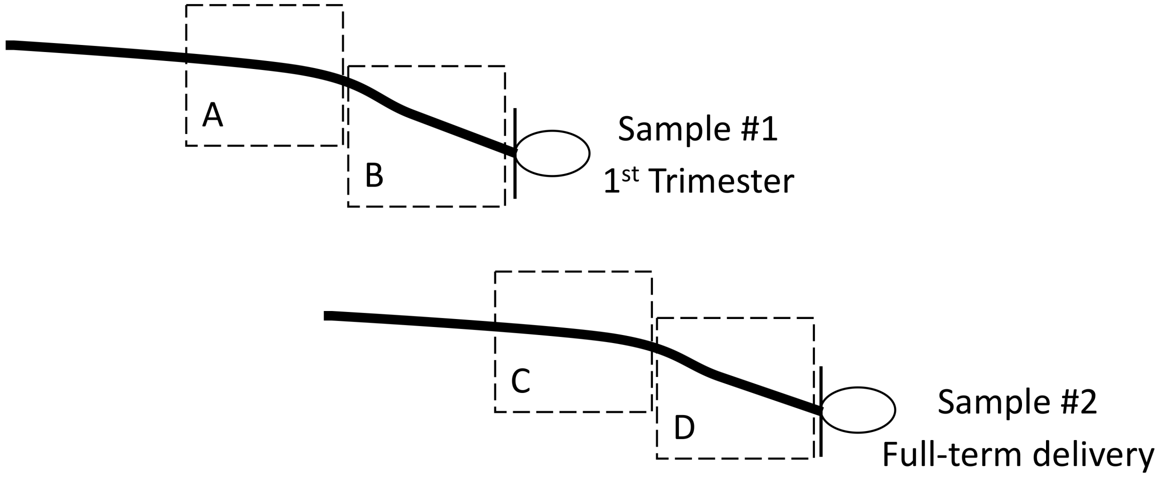

Figure 1: Diagram showing the four hair segments used in analyses

Dashed boxes indicate segments on each hair sample used in analyses: A) 3–6cm from the scalp on hair sample 1 reflecting the preconception period, B) 3cm from the scalp on hair sample 1 reflecting the 1st trimester, C) 3–6cm from the scalp on hair sample 2 reflecting the 2nd trimester, and D) 3cm from the scalp on hair sample 2 reflecting the 3rd trimester.

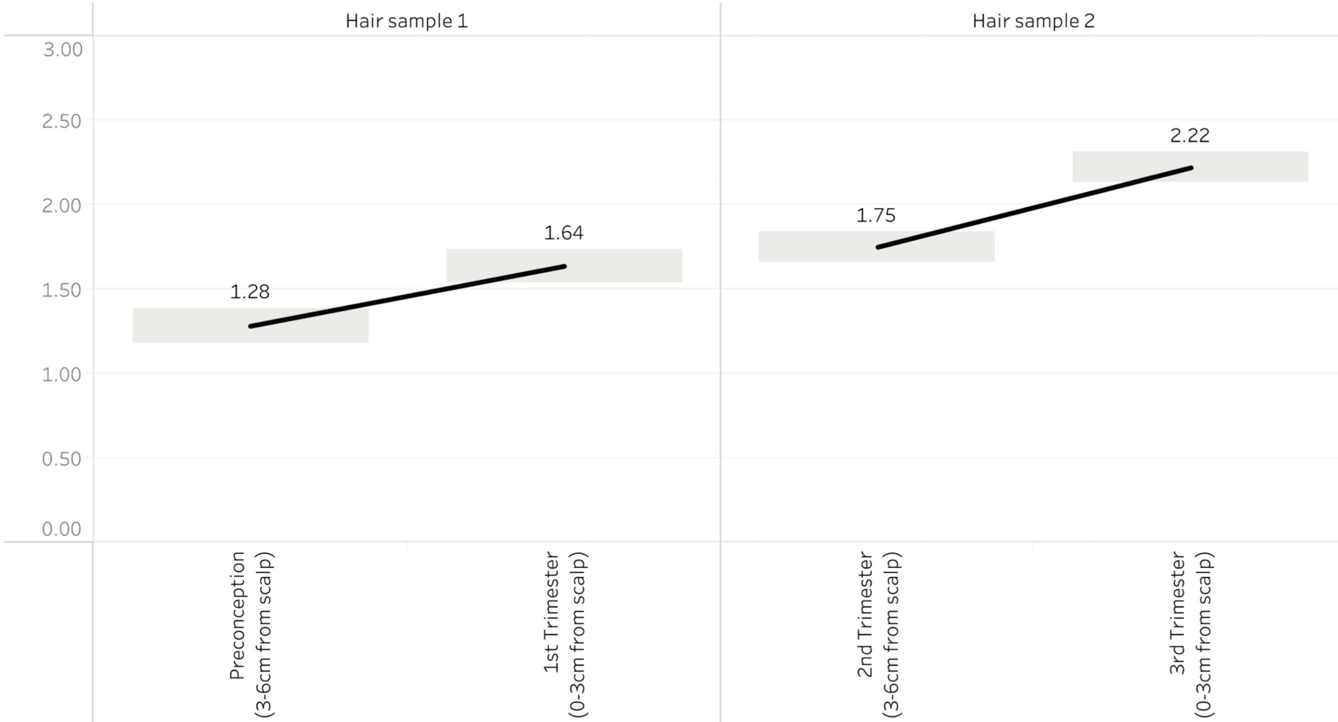

Figure 2: Mean log-transformed hair cortisol concentrations at the four hair segments (preconception, 1st trimester, 2nd trimester, 3rd trimester), by hair sample (1 or 2) (N=97 participants).

Gray bars indicate 95% error bars. The difference in mean logHCC comparing preconception to the first trimester was 1.28 (standard deviation (SD)=1.00) vs. 1.64 (SD=0.96), p-value <0.0001. The difference in mean logHCC comparing the first and second trimesters was 1.64 (SD=0.96) vs. 1.75 (SD=0.89), p-value >0.05. The difference in mean logHCC comparing the second and third trimesters was 1.75 (SD=0.89) vs. 2.22 (SD=0.88), p-value <0.0001.

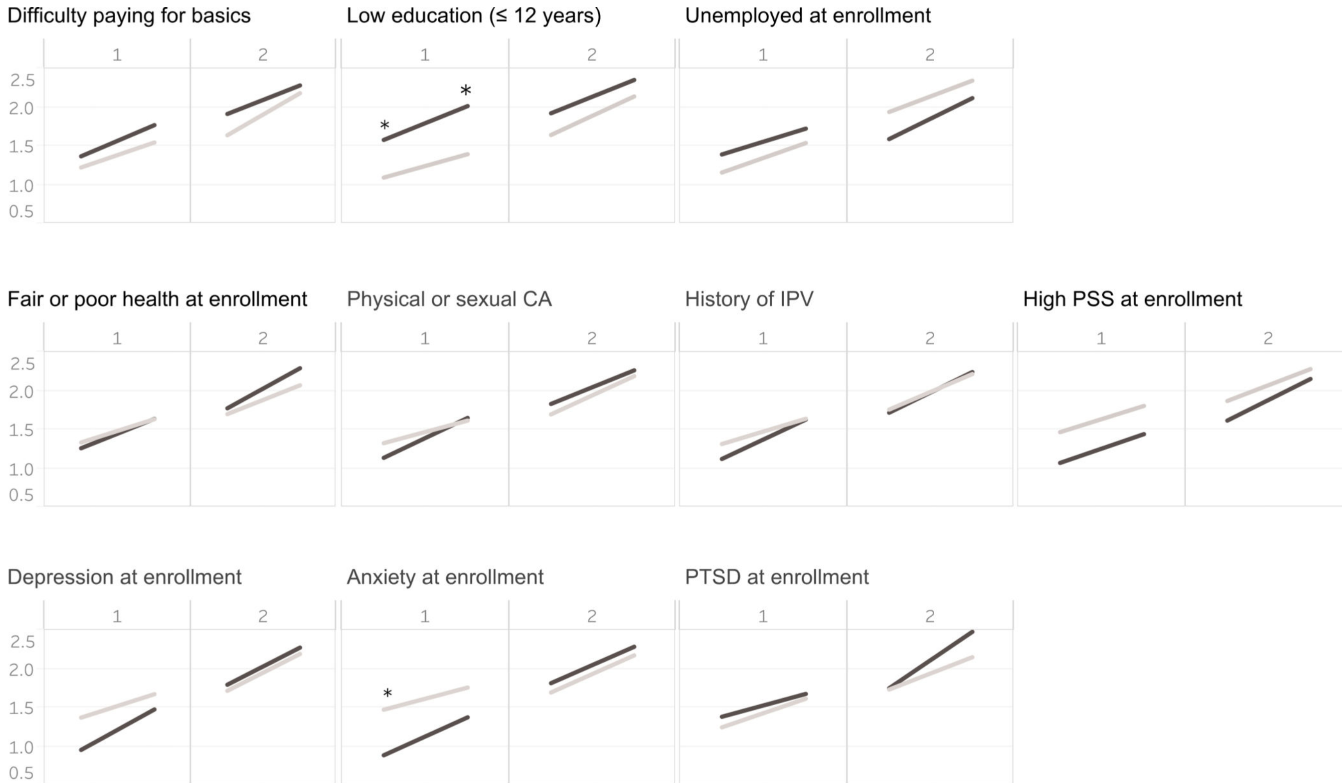

Figure 3: Mean log-transformed hair cortisol concentrations at the four hair segments (preconception, 1st trimester, 2nd trimester, 3rd trimester), by hair sample (1 or 2) and measure of stress assessed (gray=no, black=yes).

Abbreviations: CA= child abuse, IPV = intimate partner violence, PSS= perceived stress score, PTSD= Post-traumatic Stress Disorder

*An asterisk indicates that the difference in mean logHCC values comparing the two groups at that segment was statistically significant at the alpha 0.05 level

For depression at enrollment (N=96)

Table 1:

Distribution of participant characteristics at enrollment (N=97 participants)

Characteristics	Mean (SD) or %
Sociodemographics	
Mean age in years (SD)	26.5 (5.8)
Mestizo ethnicity	85.6%
Married or living with a partner	79.4%
Nulliparous	44.3%
Mean BMI at enrollment (kg/m^2) (SD)	25.4 (3.6)
Smoked prior to index pregnancy	14.4%
Consumed alcohol prior to index pregnancy	22.7%
Ever diagnosed with asthma	10.3%
Hair characteristics	
Color	
Black	55.7%
Brown	44.3%
Hair structure	
Straight	68.0%
Curly	32.0%
Hair washing frequency (per week)	
1–2	5.2%
3–5	78.4%
6–7	16.5%
Shampoo or conditioner use when washing	
Shampoo only	34.0%
Shampoo and conditioner	66.0%
Chemical hair treatment (tint, dye, or perm)	39.2
Ultraviolet light exposure at enrollment	
Low UV	20.6%
Intermediate Low and High UV	62.9%
High UV	16.5%
Stress measures	
Difficulty paying for basics	41.2%
Low education (≤12 years)	39.2%
Unemployed during pregnancy	53.6%
Self-reported fair or poor health during index pregnancy	68.0%
History of physical or sexual child abuse	29.9%
History of intimate partner violence	16.5%
Mean perceived stress scale (PSS)	29.0 (4.9)
Mean depression score [a,b]	6.5 (4.1)
PHQ-9 Score ≥10	23.7%
Mean generalized anxiety disorder score [c]	5.0 (3.4)

Characteristics	Mean (SD) or %
GAD-7 Score ≥7	34.0%
Post-traumatic stress-disorder symptoms [d]	19.6%

[a] 9-item Patient Health Questionnaire

[b] One missing value

[c] 7-item Generalized Anxiety Disorder

[d] Civilian version of the Posttraumatic Stress Disorders Checklist score ≥26

Table 2:

Pearson correlation coefficients (r), and mean differences (∂) and standard deviations (SD) of log-transformed hair cortisol concentrations from preconception to the 3rd trimester (N=97 participants).

			Hair sample #1		Hair sample #2	
			Preconception (3–6cm)	**1st Trimester(3cm)**	**2nd Trimester(3–6cm)**	**3rd Trimester(3cm)**
Hair sample #1	Preconception (3–6cm)	r	1.00	0.83 **	0.23 *	0.15
		∂ (SD)	REF	0.35 (0.57) ***	0.47 (1.17) **	0.94 (1.23) ***
	1st Trimester (3cm)	r		1.00	0.19	0.08
		∂ (SD)		REF	0.11 (1.18)	0.58 (1.25) ***
Hair sample #2	2nd Trimester (3–6cm)	r			1.00	0.75 ***
		∂ (SD)			REF	0.47 (0.63) ***
	3rd Trimester (3cm)	r				1.00
		∂ (SD)				-

*** =p-values <0.0001

** =p-values <0.01

* =p-values <0.05

Table 3:

Mean log-transformed hair cortisol concentrations at the four hair segments (preconception, 1st trimester, 2nd trimester, 3rd trimester) by each stress measure (N=97 participants).

Participant characteristics	N (%)	Mean logHCC (Standard Deviation)			
		Preconception (3–6cm)	1st Trimester (0–3cm)	2nd Trimester (3–6cm)	3rd Trimester (0–3cm)
All participants	97 (100%)	1.28 (1.00)	1.64 (0.96)	1.75 (0.89)	2.22 (0.88)
Difficulty paying for basics					
No	57 (85.8%)	1.22 (1.06)	1.54 (0.99)	1.64 (0.89)	2.18 (0.73)
Yes	40 (41.2%)	1.37 (0.90)	1.77 (0.91)	1.91 (0.89)	2.28 (1.07)
P-value		0.48	0.26	0.14	0.59
Education ≤ 12 years					
No	59 (60.8%)	1.09 (1.07)	1.39 (1.02)	1.64 (0.95)	2.14 (0.93)
Yes	38 (39.2%)	1.58 (0.80)	2.01 (0.72)	1.92 (0.78)	2.35 (0.79)
P-value		**0.02**	**0.002**	0.13	0.25
Unemployed during pregnancy					
No	45 (46.4%)	1.16 (1.09)	1.54 (1.07)	1.94 (0.80)	2.34 (0.71)
Yes	52 (53.6%)	1.39 (0.90)	1.72 (0.85)	1.59 (0.95)	2.11 (1.00)
P-value		0.25	0.34	0.054	0.21
Fair or poor health					
No	31 (32.0%)	1.33 (1.07)	1.63 (1.14)	1.70 (0.77)	2.07 (0.69)
Yes	66 (68.0%)	1.26 (0.97)	1.64 (0.87)	1.77 (0.95)	2.29 (0.96)
P-value		0.73	0.98	0.70	0.25
History of physical or sexual child abuse					
No	68 (70.1%)	1.32 (0.96)	1.61 (0.97)	1.69 (0.83)	2.19 (0.80)
Yes	29 (29.9%)	1.18 (1.09)	1.69 (0.95)	1.88 (1.03)	2.29 (1.06)
P-value		0.52	0.74	0.36	0.59
History of IPV [a]					
No	81 (83.5%)	1.31 (1.03)	1.64 (0.98)	1.76 (0.92)	2.22 (0.90)
Yes	16 (16.5%)	1.12 (0.79)	1.63 (0.89)	1.72 (0.75)	2.24 (0.82)
P-value		0.48	0.97	0.87	0.92
High perceived stress					
No	52 (53.6%)	1.47 (0.86)	1.80 (0.84)	1.87 (0.86)	2.28 (0.90)
Yes	45 (46.4%)	1.07 (1.10)	1.44 (1.06)	1.61 (0.92)	2.15 (0.87)
P-value		0.0501	0.06	0.17	0.48
Depression [b]					
No	73 (75.2%)	1.37 (0.99)	1.67 (1.00)	1.72 (0.93)	2.19 (0.91)
Yes	23 (23.7%)	0.95 (0.94)	1.48 (0.79)	1.79 (0.73)	2.27 (0.80)
P-value		0.08	0.36	0.22	0.70
Generalized Anxiety Disorder [c]					
No	64 (66.0%)	1.49 (0.90)	1.77 (0.87)	1.72 (0.90)	2.19 (0.86)

Participant characteristics	N (%)	Mean logHCC (Standard Deviation)			
		Preconception (3–6cm)	1st Trimester (0–3cm)	2nd Trimester (3–6cm)	3rd Trimester (0–3cm)
Yes	33 (34.0%)	0.88 (1.07)	1.37 (1.08)	1.81 (0.88)	2.28 (0.93)
P-value		**0.004**	0.052	0.62	0.62
PTSD [d]					
No	78 (80.4%)	1.24 (1.01)	1.61 (0.99)	1.73 (0.85)	2.15 (0.87)
Yes	19 (19.6%)	1.44 (0.94)	1.73 (0.81)	1.83 (1.07)	2.51 (0.88)
P-value		0.44	0.63	0.68	0.11

Values in bold are statistically significant at the alpha 0.05 level.

[a] IPV= intimate partner violence

[b] 9-item Patient Health Questionnaire score ≥10

[c] 7-item Generalized Anxiety Disorder Scale score ≥7

[d] Post-traumatic stress disorder (score ≥26)

Table 4:

Linear model results for the effect of stress (group), time (preconception, 1st trimester, 2nd trimester, 3rd trimester), and group by time on mean logHCC patterns from preconception to the 3rd trimester.

Dependent variable LogHCC	**Unadjusted Model 1**		**Adjusted Model 2** [a]	
	Beta (SE)	**P**	**Beta (SE)**	**P**
Time (all participants)	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Difficulty Paying for Basics				
Group	0.19 (0.14)	0.18	0.07 (0.13)	0.57
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time [b]	-	-	-	0.68
Education ≤ 12 years				
Group	0.40 (0.13)	**0.003**	0.32 (0.13)	**0.01**
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.63
Unemployment				
Group	−0.04 (0.14)	0.77	-0.10 (0.13)	0.45
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.34
Self-reported Fair or Poor Health				
Group	0.06 (0.15)	0.70	−0.01 (0.14)	0.96
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.16
History of Physical or Sexual CA				
Group	0.06 (0.15)	0.72	0.04 (0.14)	0.78
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.31
History of IPV				
Group	−0.05 (0.18)	0.77	−0.09 (0.17)	0.62
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.31
Perceived stress score > median				
Group	−0.29 (0.13)	**0.04**	−0.28 (0.12)	**0.02**
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.23
Depression (1 missing)				
Group	−0.11 (0.16)	0.48	−0.14 (0.15)	0.34
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.09
Generalized Anxiety Disorder				
Group	−0.20 (0.14)	0.16	−0.17 (0.13)	0.22
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**

Dependent variable LogHCC	**Unadjusted Model 1**		**Adjusted Model 2 [a]**	
	Beta (SE)	**P**	**Beta (SE)**	**P**
Group x Time	-	-	-	**0.02**
Post-traumatic stress disorder				
Group	0.19 (0.17)	0.26	0.20 (0.16)	0.22
Time	0.36 (0.03)	**<0.0001**	0.36 (0.03)	**<0.0001**
Group x Time	-	-	-	0.39

Abbreviations: SE= standard error, CA= child abuse, and IPV= intimate partner violence

[a]= Adjusted for BMI (continuous), asthma, alcohol consumption, hair structure, chemical hair treatment, and UV exposure in early pregnancy.

[b] = All group by time interactions were evaluated in models separately from the main effects of group and time

Table 5:

Pearson correlation coefficients (r) of log-transformed hair cortisol concentrations (logHCC) from preconception to the 3rd trimester with continuous scores of psychological distress at enrollment (N=97 participants).

	Hair sample #1				Hair sample #2		
	Preconception (3–6cm)	1st Trimester (0–3cm)	2nd Trimester (3–6cm)	3rd Trimester (0–3cm)	Perceived stress	Depression	Generalized anxiety
Perceived stress [a]	−0.11P [b]=0.27	−0.12P=0.25	−0.06P=0.54	0.01P=0.92	1.00	0.19P=0.06	0.14P=0.17
Depression [c]	−0.17P=0.10	−0.07P=0.48	0.03P=0.78	0.05P=0.66	0.19P=0.06	1.00	**0.75P<0.0001**
Generalized Anxiety [d]	**−0.22P=0.04**	−0.13P=0.20	0.08P=0.42	0.09P=0.38	0.14P=0.17	0.75P<0.0001	1.00

Values in bold are statistically significant at the alpha 0.05 level

[a] 14-item Perceived Stress Scale

[b] P= p-value

[c] 9-item Patient Health Questionnaire

[d] 7-item Generalized Anxiety Disorder scale