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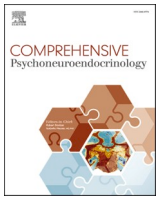

Within-person associations of cortisol, dehydroepiandrosterone, and testosterone hair hormone concentrations and psychological distress in pregnant and non-pregnant women

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A B S T R A C T

Prenatal hair cortisol concentration is inconsistently associated with maternal psychological distress. However, prior studies have not often measured hair cortisol and maternal psychological distress prospectively over time, examined within-person associations, nor concurrently considered the complex hormonal milieu in which cortisol operates during pregnancy. We addressed these limitations and tested associations against a similar non-pregnant comparison group. Participants included 68 women (34 pregnant and 34 non-pregnant; M_{age} = 29.14 and 83 % White) from the Midwestern United States. Pregnant women were assessed each trimester, at 12, 26, and 38 weeks and non-pregnant women were assessed three times on the same schedule. At each assessment, participants completed measures of psychological distress and provided hair samples. The first 3 cm (from the scalp) of hair was assayed using enzyme immune-assay kits to reflect cumulative levels within the given trimester/3-month time period of cortisol, dehydroepiandrosterone (DHEA) and testosterone. Within-person associations of hair cortisol and ratio of hair cortisol-to-DHEA and cortisol-to-testosterone with psychological distress were assessed using multilevel models. There were positive within-person associations of hair cortisol with cumulative psychological distress (γ = 0.01, s.e. = 0.003, p = .049), anxiety (γ = 0.09, *s.e.* = 0.04, p = .046), and pregnancy-related anxiety symptoms (γ = 0.10, *s.e.* = 0.05, p = .041) in the pregnant sample such that on occasions when hair cortisol was higher than average so were psychological distress symptoms. No within-person associations of hair cortisol were supported in non-pregnant women although there was a negative within-person association, such that on occasions of having lower testosterone level than typical, depression symptoms were higher. There were no within-person associations of psychological distress and cortisol-to-DHEA ratio or cortisol-to-testosterone ratio in either the pregnant or non-pregnant sample. At the between person-level for pregnant women, lower cortisol levels were associated with higher perceived stress (γ = -0.28, *s.e.* = 0.09, p = .003) and depression symptoms (γ = -0.11, *s.e.* = 0.06, p = .039), whereas higher cortisol levels were associated with higher psychological distress (γ = 0.03, *s.e.* = 0.01, p = .010), state anxiety (γ = 0.33, *s.e.* = 0.13, p = .010), and depression symptoms (γ = 0.23, *s.e.* = 0.09, p = .017) in non-pregnant women. Modeling hair cortisol at the within-person and between-person level revealed differential findings in pregnant and non-pregnant women.

Hair cortisol concentration, psychological distress, pregnancy, hormone coupling, within-person associations.

1. Within-person associations of cortisol, dehydroepiandrosterone, and testosterone hair hormone concentrations and psychological distress

Cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis in humans and non-human primates may play a mediating role between maternal psychological distress (experiencing symptoms of anxiety, depression, and stress broadly) and child outcomes (e.g. psychiatric conditions, cognitive abilities and temperament) [1,2]. The assessment of cortisol from hair is thought to be appropriate during pregnancy as it is insensitive to momentary fluctuations, requires fewer repeated assessments and is noninvasive compared to other biospecimens [3,4]. Subjective measures of psychological distress are most often captured using self-reports over the past month or a few weeks, and so basal, or longer-term cumulative measures of cortisol, may better map onto the timescale in which psychological distress is measured and the endocrine changes that unfold over the course of pregnancy [5]. Despite this, associations of maternal psychological distress and hair cortisol have been inconsistent [6].

We posit two key gaps in the literature that likely contribute to the inconsistency in the hair cortisol-psychological distress association during pregnancy. First, most studies have not assessed the correlation of cortisol as a biomarker of psychological distress at the correct level of analysis. That is, the underlying hypothesis (that women should have

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higher cortisol biomarkers when they are feeling distress) is actually posited at the within-person rather than the between-person level (i.e., women who have higher cortisol values are the women who also feel more distress). We note however, that the association at both levels may could be non-linear although non-linear effects in hair cortisol and psychological distress to our knowledge, have not been investigated. Second, there is a lack of attention to the hormonal milieu, which is known to change during pregnancy and be important for hormone-behavior associations [7]. We investigated levels of cortisol, dehydroepiandrosterone (DHEA), and testosterone, as well as ratios of cortisol to DHEA and cortisol to testosterone ratios based on their coupling and association with psychological distress during other developmental periods of hormonal changes [8–11].Therefore, the present study examines within-person associations of psychological distress and hair cortisol in a sample of pregnant women, in the context of DHEA and testosterone, and included a non-pregnant comparison sample to investigate whether any associations are pregnancy-specific or typical of women in adulthood.

1.1. Hair cortisol concentrations and psychological distress during pregnancy

A recent meta-analysis of 29 studies of the association between psychological distress (broadly defined as perceived stress, depression, anxiety) and hair cortisol during pregnancy and early post-partum reported an overall small, positive, but non-statistically significant effect of psychological distress [12]. Additionally, stronger, and statistically significant associations were found for associations examined during pregnancy (compared to post-partum) as were studies that examined psychological distress before hair cortisol concentration, but the timing or difference in frame of psychological stress and hair cortisol concentration did not moderate the results. In sub type analyses, there was no overall moderating effect of psychological distress type, but there were statistically significant and positive mean effects of perceived stress and depression but not for anxiety. However, none of these studies examined this association at the within person level and we are aware of only one other study that has examined the association between hair cortisol and any kind of psychological distress at the within person level. King and colleagues (2022) found a within person association of recent psychosocial adversity with hair cortisol concentration prenatally through 5–8 months postpartum but did not find the same for depression symptoms. The present study thus extends the current literature on psychological distress and hair cortisol concentrations across pregnancy by examining within-person associations of several types of psychological distress and hair cortisol during pregnancy, as well other key hormones during this period, prospectively measured during each trimester of pregnancy.

1.2. Hormonal milieu

The hormonal milieu, or endogenous hormonal environment, becomes increasingly complex and active during pregnancy to sustain pregnancy and support fetal development [7]. During pregnancy (similar to development just before pubertal onset) large increases in DHEA occur without corresponding increases in cortisol, despite their co-release during other periods of the lifespan [10]. Because of these interactions among hormones, failure to examine multiple hormones simultaneously may contribute to inconsistent findings of hair cortisol concentrations and psychological distress. Generally, the combinations of higher cortisol to DHEA ratio (i.e., levels of one hormone relative to another) and average levels of cortisol with low levels of DHEA have been associated with profiles of anxiety and depression symptoms in adolescents [13] and in pregnant women [14]. Ratios of high testosterone with low cortisol, on the other hand, have been linked to externalizing and aggressive behavior [15,16] and in females, testosterone-cortisol ratios may also be linked to depressive pathways to antisocial behavior [17]. Importantly, DHEA and testosterone have each been shown to change with or couple with cortisol at the within-person level at multiple time-scales (e.g., across timescales; [18]) across the day [11], and have been linked to indicators of psychological distress [19].

1.3. Present study

In the present study we sought to 1) examine within-person associations between hair cortisol concentrations and maternal psychological stress, 2) take into account a broader hormonal milieu during pregnancy (assessing DHEA and testosterone in addition to cortisol) both in terms of covariation and ratios, and 3) compare associations with a non-pregnant comparison group. Strengths of the current study include a prospective longitudinal design, clear mapping of the theoretical level of association (within-person) to the data analytic strategy, and accounting for the hormonal milieu by measuring and including multiple hormones together in the same models. The use of a comparison sample of non-pregnant women assessed with the same measures on the same timescale allows for greater understanding of whether associations are specific to or are obscured during pregnancy and during a life stage characterized by shifting hormonal milieu. We hypothesized that 1) positive within-person associations would be found between hair cortisol concentrations and psychological distress, that is, on occasions when individuals' hair cortisol is higher, so is psychological distress. We also hypothesized that 2) high cortisol-to-DHEA ratios would be associated with higher levels of psychological distress, also at the within-person level. We primarily examined a cumulative psychological distress variable, but in sensitivity analyses also examined components of psychological distress (i.e., stress, anxiety, depression) to test specificity of findings.

2. Methods

2.1. Participants and procedures

Participants included 68 women: 34 pregnant (average age = 29.14, SD = 5.06 years), and 34 non-pregnant (average age = 27.18, SD = 3.87 years). The sample was predominately (83 %) non-Hispanic White, and (75 %) above the poverty line as defined by the 2020 poverty guidelines (US-DHHS, 2020; see Table 1 for full demographics). To be eligible for enrollment in the study, pregnant women had to be less than 12 weeks pregnant (self-reported) and live within about a 1-h driving radius from Purdue University. Pregnant women were recruited from July 2017 through October 2018. Non-pregnant women were recruited from June 2018 through April 2019, from the same catchment areas and age ranges using the same strategies. Exclusion criteria for both samples included (1) unable to understand the elements of informed consent, (2) unable to understand English at an 8th grade level, and (3) being a minor (under the age of 18 years). Our target sample size was 30 participants in each group with a few extra anticipating sample attrition over time.

Pregnant women were followed longitudinally and assessed three times during pregnancy: 1) at 12 weeks (1st trimester, n = 34, M = 12.47, SD = 1.21), 2) at 26 weeks (during 2nd trimester, n = 33, M = 26.16, SD = 1.41), and 3) at 38 weeks (during the 3rd trimester, n = 31, M = 37.62, SD = 1.17). Non-pregnant women were assessed three times, at baseline (n = 34), 14 weeks later (n = 31, $M_{T1\text{-}T2} = 14.17$, $SD_{T1\text{-}T2} = 0.52$), and 12 weeks later (n = 29, $M_{T2\text{-}T3} = 12.23$, $SD_{T2\text{-}T3} = 0.54$) to mirror the assessment schedule of the pregnant group (aside from the six-month postpartum follow-up, not presented in this manuscript). The study was approved by the Purdue University IRB (#1704019124), and all participants provided informed consent. Please see Appendix 1 Part A: Recruitment, Attrition, and Missing Data for further details in Marceau and colleagues (2021).

Table 1
Sample demographic statistics.

	Pregnant Sample		Non-Pregnant Sample	
	Mean (SD)	Min-Max	Mean (SD)	Min-Max
Household Income	$65,000 ($48,000)	0-$230,000	$55,000 ($49,000)	0-$150,000
Age at first visit	29.14 (5.06)	19.55–39.74	27.18 (3.87)	18.13–41.78
Number of Children	1.14 (1.22)	0–4	0.72 (1.67)	0–3
Parity				
Number of Births	1.12 (1.23)	0–4	0.71 (1.19)	0–5
Previous Miscarriages	0.82 (1.64)[c]	0–9	0.31 (0.59)	0–2
Never Pregnant	–	–	0.59 (0.50)	
Financial Need	2.24 (2.03[d]	0–7	2.38 (2.67)	0–11
Financial Deprivation	2.00 (0.92)	1–4	2.21 (0.80)	1–4.67

Race[a]	N (%)	N (%)
White	25 (83.3)	24 (77.4)
Black or African American	3 (10.0)	1 (3.2)
Asian	1 (3.3)	3 (10.0)
Latinx or Hispanic	1 (3.3)	1 (3.2)
More than one/Other	0	2 (6.4)
Education		
Less than high school degree	1 (2.9)	0 (0.0)
High School degree/GED	11 (32.4)	13 (38.2)
2-year college degree	3 (8.8)	1 (2.9)
4-year college or university degree	11 (32.4)	15 (44.1)
Graduate Degree	8 (23.5)	5 (14.7)
Employment Status		
Unemployed/Student	12 (35.29)	20 (60.61)
Part Time	5 (14.71)	5 (15.15)
Full Time	17 (50.00)	8 (24.24)
Marital Status		
Single, never married	6 (17.6)	17 (50.0)
Married/Committed Living Together	28 (81.4)	14 (41.2)
Separated/Divorced	0 (0.0)	3 (8.8)
Contraception[b]		
Hormonal Contraception	–	19 (57.6)
None	–	13 (39.4)

[a] These are out of 31 because of missing data for race/ethnicity.
[b] Out of 33 because one participant was missing on contraception; all contraception was hormonally based, and most was continually taken, however, one non-pregnant participant took a plan B pill between the second and third visits. One mother non-pregnant participant was breastfeeding throughout the study.
[c] For the pregnant sample, 10 women experienced 1, 4 experienced 2–3, and one woman experienced 9 miscarriages (44 % experienced at least one). For the non-pregnant sample, only 25 % experienced at least one miscarriage (6 women experienced 1, two women experienced 2).
[d] Mean scores for financial need and financial deprivation are based principal component scores developed in a separate empirical paper using this sample, please see Rolan and colleagues [20] for more information on score creation.

2.2. Hair hormones

Hair samples were collected by a trained research assistant at the end of each trimester or corresponding time in the non-pregnant sample. Each of the three hair samples were segmented by 3 cm from the scalp end, each segment to reflect long-term hormone concentrations within approximately one trimester based on the common guideline of 1 cm of growth per month [21]. Thus, with three prospective assessments, we capture hair hormone concentrations across the entire pregnancy and corresponding nine-month period in non-pregnant women. The rule of thumb of one cm of hair growth per month is known to be an approximation of actual hair growth that varies among people and within person given reported fluctuations in growth rate due to race and ethnicity, seasonal changes and pregnancy status [22–25]. Thus, we measured hair growth for a small sub sample of pregnant and non-pregnant participants. Estimates were generally consistent with the 1 cm of growth per month although slightly faster (1.08–1.62 cm/month). Please see Marceau and colleagues for more hair collection detail, as well as for specific estimates and ranges of hair growth for the pregnant and non-pregnant sample by trimester and three month period and Appendix 1 Part B. Additional Assay Procedural Details for specifics on washing, drying, and extraction steps [26].

Our study design is aimed at tracking within-person variability and changes [27]. Thus, all three assessments for any given individual were assayed on the same plate in order to reduce intra-individual variability due to batch-related error. A plate was considered reliable if the standard curve had $R^2 > 0.996$. All samples were assayed using enzyme immune-assay kits (Salimetrics, PA) and tested in duplicate. Inter-assay variabilities were as follows: cortisol = 9.61 %, DHEA = 23.05 % (control highs = 9.26 %; control lows = 36.84 %), testosterone = 22.33 % (control highs = 10.24 %, control lows = 34.43 %). Further, one plate for DHEA and testosterone had a very low control low and if this control low is excluded the inter-assay variability for DHEA was 15.95 % and for testosterone was 14.47 %. Samples were re-assayed ($n = 4$ for testosterone) if duplicate test values that varied by more than 7 % (cortisol and DHEA) or 10 % (testosterone) error. Data cleaning included winsorizing outliers (to +3SD of the sample distribution) and removing batch-associated error from the hormone variables (see Ref. [27], Appendix 1 Part C for details).

2.3. Maternal psychological distress

Cumulative Maternal Psychological Distress was created by standardizing and summing the following measures of psychological stress and distress within each trimester: perceived stress, state anxiety, past three months anxiety and depression (each described below). Cronbach's alpha ranged from 0.93 to 0.77 across assessments for pregnant and non-pregnant women.

2.3.1. Perceived stress

The Perceived Stress Scale (PSS) [28] was collected at the end of each trimester or corresponding period (12, 26, and 38 weeks) for pregnant women and non-pregnant women. The scale is computed as the average of 10 items rated on a scale of 0 (never) to 4 (very often), such that higher scores reflect more perceived stress over the past month. Cronbach's alpha ranged from 0.84 to 0.94 across assessments for pregnant and non-pregnant women.

2.3.2. State anxiety

The State Trait Anxiety Inventory (STAI; [29]) was collected at the end of each trimester or corresponding period (12, 26, and 38 weeks) for pregnant women and non-pregnant women. Women rated "how they generally feel" by their extent of agreement with 20 positively (e.g., "I feel pleasant") and negatively (e.g., "I feel nervous and restless") worded items on a scale of almost never (0) to almost always (4). After reverse-coding positively worded items, scores were formed by averaging the items such that higher scores reflect greater anxiety. Cronbach's alpha ranged from 0.92 to 0.96 across assessments for pregnant and non-pregnant women.

2.3.3. Anxiety and depression

We assessed past three month's anxiety and depression symptoms using a subset of 10 items from the Beck Anxiety Inventory. Cronbach's alpha ranged from 0.91 to 0.96 across assessments for pregnant and non-pregnant women and 13 items from the Beck Depression Inventory (Beck & Steer, 1984). Cronbach's alpha ranged from 0.92 to 0.96 across assessments for pregnant and non-pregnant women [30]. For depression, participants chose the statement that describes their general feelings the best in the past three months for various feelings characteristic of depression (e.g., guilty, hopeless, sad; "I didn't feel particularly guilty", "I felt guilty a good part of the time", "I felt quite guilty most of

the time", "I felt guilty all of the time"). For anxiety, participants chose one of four statements that best characterized how they felt in the past three months (e.g., "Not at all", "Slightly", "Moderately", "Severely") for various feelings related to anxiety (e.g., nervousness, fear of losing control, heart pounding or racing).

2.3.4. Pregnancy-related anxiety

The short form of the Pregnancy Related Anxiety Questionnaire (PRAQ; [31]) was administered to the pregnant sample only. Pregnant women answered ten items related to common anxieties experienced while pregnant (e.g., feeling anxious about the delivery/pain, the health of the child, and their appearance), and were prompted to "select each answer that applies most accurately to your current situation". The scale is formed by averaging the 10 items that were rated on a scale of 0 (absolutely not relevant) to 4 (very relevant), such that higher scores reflect women reporting greater levels of pregnancy-related anxiety. Cronbach's alpha ranged from 0.80 to 0.85 across assessments.

2.3.5. Covariates

We included age (date of first assessment – date of birth, rounded to the hundredth decimal), socioeconomic adversity, negative life events, and BMI (self-reported $703 \times \text{weight}_{\text{lbs}}/\text{height}_{\text{inches}}$) at the first assessment as covariates for both pregnant and non-pregnant women. Additionally, we adjusted for any psychiatric medication use in the pregnant and non-pregnant sample. Finally, in separate sensitivity models for the pregnant and non-pregnant sample we included pregnancy complications and fetal sex (pregnant-only), and hormonal contraceptive use (non-pregnant only) as additional covariates.

Negative Life Events. The Life Events Checklist was collected at the end of the first trimester (38 weeks) in the pregnant sample specifically for events that happened in the past year and in the non-pregnant sample for the same time period. Women checked yes or no to a series of 55 events to report whether they had occurred. If yes was checked, women were then asked to report the extent to which they were positively and negatively impacted (on a four-point Likert scale for each emotion). The negative life events score is the sum of negative impacts of endorsed items.

Pregnancy complications were coded in terms of potential risk to the fetus on a scale of 1 = not harmful or relevant to 6 = very great harm to or deviation in offspring development according to the McNeil-Sjöström obstetric complications [32,33]. Questions about pregnancy complications as defined by that scale (e.g., maternal age, maternal infections) were each coded with the appropriate risk-level weight, and if the level of risk surpassed a "3" (potentially but not clearly harmful or relevant) the risk scores were summed. This yielded a weighted risk score indicating more pregnancy complications on which zero indicated the absence of all measured complications.

Socioeconomic Adversity was a composite score derived from Principal Component Analysis (PCA) based on family income, education, and employment status, as well as financial need and financial deprivation at the first assessment. One factor was extracted (Eigenvalue = 2.31), which explained 46 % of the variance. Factor loadings were: 0.85 for financial deprivation, −0.77 for income, 0.74 for financial need, −0.67 for education, and −0.09 for occupation. Higher scores indicate relatively higher levels of socioeconomic adversity. See Rolan and colleagues [20] for more information on score creation.

2.4. Analytic strategy

2.4.1. Data preparation

In order to test our first hypothesis of positive within-person associations of hair cortisol concentrations with psychological distress (herein referred to as coupling) in the context of a broader hormonal milieu, we within person centered hair hormone variables. Specifically, we calculated and retained the average of each person's scores across the three assessments (termed *person average* and indexing between-person differences overall). Then, we subtracted each person's *person average* scores from their raw scores at each assessment, yielding a vector of three within-person centered scores (*within-person* variability). To test our second hypothesis that higher cortisol relative to DHEA would be positively coupled with psychological distress in the context of the broader hormone milieu, we created hormone ratio variables by taking the log of the division of hair cortisol concentration by hair DHEA and testosterone concentration, respectively (i.e., creating two separate logged ratio scores) [34]. The value of the quotient therefore represents the logarithm of the ratio between cortisol and each hormone, with higher scores representing higher cortisol relative to DHEA (or testosterone) and lower scores represent lower cortisol relative to DHEA (or testosterone). These scores were subsequently within person centered (i.e., across trimesters), and the *person average* values were specified as a level 2 covariate, as described above.

2.4.2. Sample descriptive statistics

Hormone levels and changes in this sample have been previously published [27]. Raw, windsorized hormone concentrations are presented in Table 2. Here, we also describe the hormonal milieu in terms of between person correlations within trimester and within-person correlations across the study. We also describe the between-person correlations within trimester and within-person correlations across the study amongst the various measures of psychological distress. For completeness, we examined correlations of psychological distress measures and cortisol levels separately within each trimester, as these data may be useful for the line of work examining potential sensitive periods of associations of cortisol and psychological distress among pregnant women (e.g., anticipating future meta-analyses). Due to the large number of correlations (available in supplemental materials), we consider the overall pattern of findings and effects sizes in order to contextualize the results of the main hypothesis tests, rather than interpreting specific correlation coefficients.

2.4.3. Main hypotheses tests

For the main tests of study hypotheses, pregnant and non-pregnant women were analyzed separately in two-level multilevel models where assessments (level 1) were nested within individuals (level 2). Models were fit within a structural equation modeling framework using M*plus* (version 8.1) in order to accommodate missing data on both outcomes and predictors via Full Information Maximum Likelihood. We included within-person and person-average indexes of hormone levels (aim 1) and hormone ratios (aim 2) as predictors of the psychological distress composite. Effects of the within-person cortisol variables (i.e., within-person correlations) were of interest; *person-average* variables were included as covariates. Within- and between-person indices of DHEA and testosterone levels were included as level 1 and 2 covariates to account for a broader hormonal milieu. We additionally included assessment (0, 1, 2) as a within-person (level 1) covariate to account for any linear systematic change in the outcome variable over time and age, BMI and socioeconomic adversity at baseline, as well as negative life events in the past year reported at the first visit as between-person (level 2) covariates. We also added sample specific covariates (e.g., total pregnancy complications, and fetal sex in pregnant women; hormonal contraception in non-pregnant women). Finally, because of the number of parameters estimated by the structural equation parameterization of the multilevel models relative to the sample size, we fixed the estimates of the means for within-person centered variables to zero (whose means are by definition zero), which sufficiently reduced the number of parameters estimated in order for the models to be identified.

2.4.4. Sensitivity analyses

The first set of sensitivity analyses we conducted were identical to those described above, except that we included the four subscales of our psychological distress variable each as a unique outcome (perceived stress, state anxiety, anxiety and depression symptoms). Additionally,

Table 2
Hormone concentrations.

	Pregnant Sample					Non-Pregnant Sample				
Cortisol (pg/mg)	N	Mean	(Std. Dev.)	Min	Max	N	Mean	(Std. Dev.)	Min	Max
Trimester 1	33	9.31	(9.35)	0.60	39.30	34	8.78	(5.17)	3.90	34.05
Trimester 2	32	8.74	(11.70)	1.05	68.11	31	10.66	(9.81)	4.05	47.55
Trimester 3	29	12.80	(15.07)	2.55	68.11	28	10.24	(6.30)	4.80	37.05
DHEA (pg/mg)										
Trimester 1	30	14.10	(9.56)	2.65	32.71	33	22.75	(8.62)	7.13	39.16
Trimester 2	26	10.86	(6.45)	1.47	23.87	31	24.85	(13.90)	7.51	66.85
Trimester 3	24	11.53	(4.74)	3.51	21.71	28	26.70	(14.61)	11.36	66.85
Testosterone (pg/mg)										
Trimester 1	29	1.88	(1.06)	0.67	4.80	34	1.79	(0.66)	0.82	3.28
Trimester 2	25	1.96	(1.00)	0.21	4.80	31	1.78	(0.58)	1.04	3.25
Trimester 3	24	2.08	(0.89)	0.81	4.80	27	1.82	(0.58)	1.10	3.53

we examined pregnancy-specific anxiety as an outcome for the pregnant sample only.

The second set of sensitivity analyses tested pregnant and non-pregnant sample differences by conducting the analyses together in the same model. Changes to the base models described above included dropping the sample specific covariates, including pregnant status as a level 2 indicator, and including a cross-level interaction to test differences between the groups in the within-person cortisol, cortisol-to-DHEA ratio, and cortisol-to-testosterone coupling parameters with pregnancy status. Given the large number of models and parameters tested, we used a Bonferroni adjusted p value for each model for the number of parameters tested. Adjusted p values rounded to 0.003 and are noted in the results tables below.

3. Results

3.1. Description of psychological distress and hormonal milieu

Between-person correlations of hormones each trimester. Correlations among all measures of hormone concentrations within and across trimesters are provided in Supplemental Table S2. Correlations among hormones within trimester were sparse, with testosterone and DHEA correlated for both samples at T1 (r = 0.60-0.62) and for the non-pregnant sample in T2 (r = 0.32), and cortisol and DHEA correlated in the pregnant sample at T2 (r = 0.45). Generally, there was moderate stability (r values typically in the 0.40–0.60 range) within hormone concentrations across consecutive trimesters. However, there was no correlation between T1 and T2 cortisol and a very high correlation between testosterone at T2 and T3 (r = 0.86) in the pregnant sample. In the non-pregnant sample, there was no correlation of DHEA across time, or of testosterone at T1 and T3 in the non-pregnant sample. Hormone ratios were generally not associated except for T1 and T3 cortisol-to-testosterone ratio in the pregnant and non-pregnant sample (r = −0.83, 0.58, respectively) and T1 and T3 cortisol-to-DHEA ratio in the pregnant sample (r = 0.71).

Between-person correlations of psychological distress each trimester. Correlations among all measures of psychological distress, within and across trimesters, are provided in Supplemental Table S3. Generally, in both samples, all measures of psychological distress were highly correlated (r = 0.35–0.91) and became somewhat more highly correlated in later trimesters than earlier trimesters. Pregnancy-related anxiety was less correlated than the other measures in the pregnancy sample, particularly in the first trimester (r = 0.20–0.36 at T1; r = 0.40–0.61 at T3). Measures were also fairly stable over time, with most r values between 0.60 and 0.80.

Between-person correlations of hormones and psychological distress each trimester. There were no between-person correlations of psychological distress measures and hormone levels within any trimester for the pregnant sample except for between pregnancy related anxiety and cortisol-to-DHEA ratio at T1 (r = −0.46) and T2 (r = −0.45; Supplemental Table S4). A higher cortisol-to-DHEA ratio was correlated with past three-month anxiety at T1 only (r = 0.37). In the non-pregnant sample, correlations were also sparse (Supplemental Table S5): with respect to the hypothesized correlations, only perceived stress was associated with cortisol at T1 (r = 0.34) and only anxiety symptoms were associated with cortisol-to-DHEA ratio at T1 (r = −0.47).

Within- and between-person correlations of hormones across time. There were no within-person correlations among any hormones or hormone ratios in the pregnant sample (Supplemental Material S6). However, there were between-person correlations of overall (*person average*) cortisol and DHEA and DHEA and testosterone. There were also positive within-person and overall between-person correlations of DHEA and testosterone for non-pregnant women (but neither were correlated with cortisol) (Supplemental Material S7).

Within- and between-person correlations of psychological distress across time. For psychological distress at the within-person level for the pregnant sample, there were correlations of state anxiety with perceived stress and depression symptoms, perceived stress and depression symptoms, depression and anxiety symptoms, as well as anxiety symptoms and pregnancy related anxiety (Supplemental Material S6). For pregnant women, all psychological distress measures were correlated at the overall (*person average*) between-person level except for a) perceived stress and pregnancy related anxiety, and b) depression symptoms and pregnancy related anxiety. All measures of psychological distress (excluding pregnancy-related anxiety) were positively correlated within-person in the non-pregnant sample (Supplemental Material S7). All 'overall' (*person average*) measures were also correlated at the between-person level except for a) state anxiety and anxiety symptoms, b) depression and anxiety, and c) perceived stress and anxiety.

3.2. Aim 1: within-person associations of hair cortisol and psychological distress

In the pregnant group, there was positive coupling of hair cortisol with psychological distress, γ = .01, *s.e.* = 0.003, p = .049, anxiety, γ = 0.09, *s.e.* = 0.04, p = .046 and pregnancy related anxiety, γ = 0.10, *s.e.* = 0.05, p = .041 (see Table 3). In contrast, in the non-pregnant group there was no coupling of hair cortisol with any form of psychological distress (see Table 4). There was also no coupling for DHEA or testosterone with any form of psychological distress in either the pregnant or non-pregnant women, except for testosterone and symptoms of depression in non-pregnant women, γ = -3.24, *s.e.* = 1.15, p = .005.

In sensitivity analyses, there was a nominal interaction between pregnant status and within-person cortisol predicting state anxiety, γ = 0.26, *s.e.* = 0.12, p = .039 (Supplemental Table S1), however, in the main analysis the within-person associations of cortisol and state anxiety did not differ from zero for either the pregnant or non-pregnant sample. Despite evidence of coupling of hair cortisol with cumulative psychological distress and anxiety symptoms in pregnant women, we did not find evidence that these effects were different from the non-pregnant

Table 3
Test of aim 1 in pregnant sample.

	Psych Distress			State Anxiety			Perceived stress			Depression			Anxiety			Pregnancy Anxiety		
	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.
Fixed Effects:																		
Intercept	−0.08		0.16	36.54	***	2.35	15.06	***	1.46	17.52	***	1.05	13.93	***	1.37	21.84	***	2.12
Linear Slope	−0.04		0.05	0.03		0.55	0.41		0.41	−0.58	†	0.35	−0.87	†	0.47	−0.48		0.61
WP Cortisol	0.01	*	0.003	0.02		0.03	0.02		0.03	0.02		0.03	0.09	*	0.04	0.10	*	0.05
WP DHEA	−0.01		0.01	−0.09		0.10	−0.13		0.13	0.08		0.08	0.02		0.11	−0.13		0.14
WP Testosterone	0.01		0.07	−0.26		1.13	0.42		0.79	−0.67		0.55	0.42		0.60	0.91		0.71
BP Cortisol	−0.02		0.01	−0.35	†	0.18	−0.28	**	0.09	−0.11	*	0.06	0.01		0.07	−0.15		0.14
BP DHEA	0.03		0.03	0.24		0.45	0.09		0.20	0.20		0.23	0.03		0.19	0.47	†	0.24
BP Testosterone	−0.13		0.17	−2.36		2.77	0.38		1.28	−1.35		1.14	−0.37		1.06	−2.46		1.55
Age	−0.01		0.03	0.15		0.43	−0.07		0.19	−0.15		0.14	−0.19		0.17	0.06		0.34
BMI	0.01		0.01	−0.03		0.15	0.00		0.10	−0.02		0.06	−0.05		0.08	0.26	†	0.13
NLE	0.02	**	0.01	0.38	***	0.10	0.18	**	0.06	0.16	***	0.03	0.06	*	0.03	0.06		0.12
SES Adversity	−0.15		0.14	−3.45	†	1.89	−2.35	*	1.14	−0.31		0.57	−0.39		0.62	−1.47		1.72
Fetal Sex	0.07		0.27	3.68		4.56	−0.46		1.95	2.06		2.14	0.56		2.07	−0.07		2.24
Pregnancy Comp	0.06		0.08	−0.05		1.24	0.75		0.72	0.36		0.44	−0.24		0.42	0.45		0.82
Random Effects:																		
Intercept	0.27	**	0.09	75.71	*	29.3	14.63	***	3.87	12.97	†	7.48	6.69		4.26	23.08	***	6.11
Residual	0.12	***	0.02	15.38	***	3.32	12.98	***	0.09	5.76	***	1.50	13.65	**	4.35	17.20	**	5.10
Model Fit																		
-2LL	−1506.89			−1755.04			−1725.79			−1695.08			−1717.39			−1740.58		
AIC	3079.78			3576.08			3517.58			3542.79			3500.78			3547.15		
BIC	3166.17			3662.71			3604.20			3542.79			3587.41			3633.78		

Note: †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$; WP= Within-person; BP=Between-person; Psych Distress = Psychological distress; BMI=Body mass index; NLE=-Negative life events; SES Adversity = Socioeconomic adversity; Pregnancy comp = Pregnancy complications; Bonferroni adjusted p value: 0.003; *** or ** bolded survives adjusted p value; These models are not adjusted for medication use because of a warning produced by Mplus suggesting the standard errors for the adjusted model were untrustworthy due to their being more parameters in the model than the sample size, however, the adjusted model with the warning produced the same pattern of findings with near identical effect estimates.

Table 4
Test of aim 1 in non-pregnant sample.

	Psych Distress			State Anxiety			Perceived stress			Depression			Anxiety		
	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.
Fixed Effects															
Intercept	−0.05		0.14	39.69	***	1.59	14.73	***	1.69	16.98	***	0.96	13.66	***	1.27
Linear Slope	0.07		0.06	0.55		0.72	0.82	†	0.49	0.11		0.42	−0.004		0.58
WP Cortisol	−0.02		0.02	−0.23	†	0.13	−0.13		0.14	−0.002		0.07	−0.09		0.10
WP DHEA	0.003		0.01	0.04		0.08	−0.02		0.05	0.09		0.07	0.004		0.07
WP Testosterone	−0.20		0.14	−1.58		1.62	−0.81		1.25	−3.24	**	1.15	0.05		1.76
BP Cortisol	0.03	*	0.02	0.33	*	0.13	0.19		0.20	0.23	*	0.09	0.16	†	0.09
BP DHEA	0.01		0.01	−0.01		0.12	0.22	†	0.12	0.11		0.12	0.07		0.08
BP Testosterone	0.98	***	0.19	11.96	***	2.31	8.55	***	1.81	5.36	***	1.51	4.37	*	1.83
Age	0.04	*	0.02	0.21		0.19	0.17		0.16	0.39	*	0.15	0.33	*	0.12
BMI	−0.01		0.02	0.08		0.21	0.09		0.18	−0.13		0.14	−0.17	*	0.08
NLE	0.02	***	0.01	0.35	***	0.06	0.20	***	0.05	0.17	***	0.04	0.09	*	0.04
SES Adversity	−0.58	***	0.12	−5.87	***	1.41	−4.35	***	1.13	−4.44	***	1.10	−2.42	*	0.92
Contraception	0.29		0.20	3.89		2.93	3.44	†	1.90	2.79	*	1.39	−0.04		1.81
Psychiatric Medication	−1.16		0.20	−11.76	***	2.62	−11.62	***	1.64	−6.66	***	1.77	−5.14	*	1.91
Intercept	0.11	*	0.04	15.18		8.22	9.58	*	6.66	7.49	*	3.28	2.70		2.34
Residual	0.20	***	0.05	25.82	***	5.52	15.38	***	3.21	10.06	***	2.87	15.27	***	3.88
Model Fit															
-2LL	−1486.00			−1718.47			−1693.27			−1673.94			−1676.86		
AIC	3038.01			3502.94			3452.54			3413.88			3419.72		
BIC	3124.63			3589.57			3539.16			3500.51			3506.34		

Note: †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$; Est = Estimate; S.E. = Standard Error; WP= Within-person; BP=Between-person; Psych Distress = Psychological distress; BMI=Body mass index; NLE=Negative life events; SES Adversity = Socioeconomic adversity; Bonferroni adjusted p value: 0.003; *** or ** bolded survives adjusted p value.

women, $\gamma = 0.02$, *s.e.* $= 0.01$, $p = .113$, $\gamma = 0.15$, *s.e.* $= 0.10$, $p = .123$, respectively. Thus, our hypothesis of within-person associations for aim 1 was partially supported in pregnant women but not non-pregnant women.

3.3. Aim 2: within-person associations of hair cortisol-to-DHEA ratio and psychological distress

There was no cortisol-to-DHEA ratio coupling with psychological distress in the pregnant and non-pregnant sample (see Tables 5 and 6). Thus, our hypothesis that higher cortisol-to-DHEA ratio levels was not supported, and exploration of cortisol-to-testosterone ratios yielded no findings.

3.3.1. Between-person hormone associations and psychological distress

In the pregnant sample, lower between-person levels of cortisol were associated with greater levels of perceived stress and higher depression symptoms, $\gamma = -0.28$, *s.e.* $= 0.09$, $p = .003$, $\gamma = -0.11$, *s.e.* $= 0.06$, $p = .039$, respectively (See Table 3). In other words, individuals who had lower cortisol levels generally were also likely to report higher perceived

Table 5
Test of aim 2 in pregnant sample.

	Psych Distress		State Anxiety			Perceived stress			Depression			Anxiety			Pregnancy Anxiety		
	Est	S.E.	Est	S.E.		Est	S.E.		Est	S.E.		Est	S.E.		Est	S.E.	
Fixed Effects																	
Intercept	−0.22	0.34	33.62	5.25	***	14.89	1.31	***	15.62	2.65	***	12.92	2.65	***	21.02	4.70	***
Linear Slope	−0.02	0.04	0.18	0.52		0.47	0.41		−0.47	0.30		−0.53	0.50		−0.20	0.60	
WP cortisol/DHEA ratio	0.02	0.19	0.45	1.94		1.76	1.77		−1.65	0.94	†	−1.32	1.94		1.94	1.59	
WP cortisol/testosterone ratio	0.08	0.20	0.77	1.93		−0.70	1.83		2.00	1.05	†	2.10	2.13		0.54	2.47	
BP cortisol/DHEA ratio	−0.03	0.20	−1.23	2.90		−1.00	1.70		−1.32	1.50		0.07	1.26		1.32	2.05	
BP cortisol/testosterone ratio	0.06	0.14	0.86	2.16		−0.86	1.07		0.68	1.07	*	1.03	0.82		1.32	2.05	
Age	−0.03	0.03	−0.21	0.42		−0.18	0.19		−0.31	0.15		−0.15	0.14		−0.19	0.29	
BMI	0.004	0.01	−0.05	0.16		0.01	0.10		−0.02	0.06	***	−0.08	0.08		0.23	0.14	
NLE	0.02	0.01	0.34	0.07	***	0.19	0.05	***	0.14	0.03		0.07	0.03	*	0.07	0.10	
SES Adversity	−0.06	0.11	−1.42	1.60		−1.96	1.09	†	0.26	0.49		−0.34	0.55		−0.60	1.25	
Fetal Sex	−0.04	0.26	2.92	4.14		−0.30	2.10		1.70	1.73		−0.27	1.82		−2.89	2.50	
Pregnancy Comp	0.09	0.08	0.40	1.27		1.01	0.74		0.49	0.46		−0.26	0.38		0.90	0.88	
Psychiatric Medication	0.28	0.26	6.53	4.71		3.38	2.94		1.97	1.37	†	−1.97	1.10	†	0.13	3.16	
Random Effects:																	
Intercept	0.28	0.10	79.24	28.71	**	15.71	4.74	***	13.47	8.09	†	5.67	4.17		24.78	5.59	***
Residual	0.12	0.02	15.18	3.24	***	13.80	2.46	***	5.66	1.45	***	13.93	4.42	**	17.58	5.12	**
Model Fit																	
-2LL	−827.08		−1074.51			−1045.67			−1014.29			−1036.08			−1061.87		
AIC	1708.15		2203.01			2145.34			2082.58			2126.16			2177.74		
BIC	1779.02		2273.89			2216.22			2153.46			2197.03			2248.62		

Note: †$p < .10$, *$p < .05$, **$p < .01$,***$p < .001$; Est = Estimate; S.E. = Standard Error; WP= Within-person; BP=Between-person; Psych Distress = Psychological distress; BMI=Body mass index; NLE=Negative life events; SES Adversity = Socioeconomic adversity; Pregnancy comp = Pregnancy complications; Bonferroni adjusted p value: 0.003; *** or ** bolded survives adjusted p value.

stress and depression symptoms. In contrast, higher between-person levels of cortisol were associated with higher cumulative psychological distress, $\gamma = 0.03$, *s.e.* $= 0.01$, $p = .010$, higher state anxiety, $\gamma = 0.33$, *s.e.* $= 0.13$, $p = .010$, higher depression symptoms, $\gamma = 0.23$, *s.e.* $= 0.09$, $p = .017$, respectively in non-pregnant women (See Table 4). Additionally, higher between-person levels of testosterone in non-pregnant women were associated with higher cumulative psychological distress, $\gamma = 0.98$, *s.e.* $= 0.19$, $p = .000$, state anxiety, $\gamma = 11.96$, *s.e.* $= 2.3$, $p = .000$, perceived stress, $\gamma = 8.55$, *s.e.* $= 0.1.81$, $p = .000$, depression symptoms, $\gamma = 5.36$, *s.e.* $= 1.51$, $p = .000$, and anxiety symptoms, $\gamma = 4.37$, *s.e.* $= 1.83$, $p = .017$ respectively.

4. Discussion

We examined within- and between-person associations of hair cortisol concentrations and maternal psychological distress, in the context of the broader hormonal milieu during pregnancy. This included controlling for covariation with other hair hormones, cortisol-DHEA and cortisol-testosterone ratios, as well as comparing associations with a non-pregnant comparison group. Overall, hair cortisol concentrations were positively coupled with some indicators of psychological distress over time in pregnant women only and were negatively correlated with other indicators of psychological distress at the between-person level. Evidence of correlations of hormone ratios and psychological distress were unsupported in pregnant and non-pregnant women alike. The coupling correlations in the pregnant sample also suggest the importance of modeling the associations at both the within- and between-person level of analysis. Careful interpretation of hormone-behavior associations in accordance with the level at which they are observed may ultimately clarify the mixed support for theorized hormone-behavior associations that have been largely tested via between-person associations.

4.1. Within-person hair hormone-behavior associations

Accumulating evidence suggests that modeling hormone-behavior associations at the within-person level more accurately represents the correspondence between pregnant women's lived experience of psychological distress and underlying physiology [35]. In pregnant women, effects were found for three of out six types of psychological distress tested here (cumulative psychological distress, anxiety, and pregnancy specific anxiety symptoms). Similarly, the only other study of within-person associations of hair cortisol during pregnancy found within-person associations of recent experiences of adversity but not depression symptoms which is consistent with our findings. It may be that during pregnancy, cortisol is more closely linked to anxiety symptoms framed in specific contexts (i.e., the last three months, pregnancy-specific, but not trait). Our findings tentatively clarify that within-person correlations of perceived stress and depression may be less time-specific and rather occur on a stable between-person level across pregnancy [12,36] It is also important to note that although we found evidence of coupling in pregnant women for hair cortisol with cumulative psychological distress and anxiety symptoms, we did not find evidence that these effects were different than the non-pregnant comparison group, indicating weak pregnancy specific effects (although this analysis does suffer a lack of statistical power to detect the interactions in the full model). It is also unclear why we were not able to detect within-person associations of hair cortisol and psychological distress in non-pregnant women and if the pregnancy context is what lead to the differential findings in our sample groups. Nonetheless, hair cortisol levels were most robustly associated with psychological distress among pregnant women and at the within-person level. However, these interpretations must be held in the context of our small, relatively low risk and generalizable sample, and considering that none of the within-person associations between hair hormones and psychological distress survived the adjusted p values corrected for multiple testing. We

Table 6
Test of aim 2 in non-pregnant sample.

	Psych Distress			State Anxiety			Perceived stress			Depression			Anxiety		
	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.	Est		S.E.
Fixed Effects															
Intercept	0.51		0.58	49.32	***	1.79	21.01	***	4.97	17.71	***	3.75	21.01	***	4.97
Linear Slope	0.04		0.06	0.02		0.72	0.53		0.57	0.27		0.39	0.53		0.57
WP cortisol/DHEA ratio	−0.02		0.09	−0.03		2.37	−0.11		0.67	−0.09		0.79	−0.11		0.67
WP cortisol/testosterone ratio	−0.05		0.26	−1.65		2.37	−0.70		1.55	0.53		2.13	−0.70		1.55
BP cortisol/DHEA ratio	0.10		0.24	2.32		2.71	1.04		1.83	0.61		2.38	1.04		1.83
BP cortisol/testosterone ratio	−0.22		0.37	−4.32		4.39	−2.80		3.21	0.28		2.25	−2.80		3.21
Age	0.001		0.02	−0.26		0.21	−0.17		0.14	0.13		0.13	−0.17		0.14
BMI	−0.01		0.02	0.13		0.29	0.10		0.23	−0.17		0.19	0.10		0.23
NLE	0.02	*	0.01	0.30	**	0.09	0.16	*	0.07	0.14	*	0.06	0.16	*	0.07
SES Adversity	−0.42	*	0.15	−3.95	†	2.13	−3.04	*	1.29	−3.43	**	1.18	−3.04	*	1.29
Contraception	−0.11		0.22	−0.68		2.99	0.11		2.00	−0.07		1.66	0.11		1.99
Psychiatric Medication	−0.62	*	0.29	−5.50		3.99	−6.94	*	2.71	−3.30	*	1.58	−6.94	*	2.71
Random Effects:															
Intercept	0.28	**	0.09	38.09	***	15.12	20.81	**	6.66	14.74	*	6.29	20.81	**	6.66
Residual	0.22	***	0.05	26.85	***	5.74	16.01	***	3.55	11.44	**	3.61	16.01	***	3.55
Model Fit															
-2LL	−840.09			−1071.37			−1045.00			−1026.70			−1045.00		
AIC	1730.18			2192.73			2140.01			2103.40			2140.01		
BIC	1716.84			2258.36			2205.63			2169.02			2205.63		

Note: †$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$; Est = Estimate; S.E. = Standard Error; WP= Within-person; BP=Between-person; Psych Distress = Psychological distress; BMI=Body mass index; NLE=Negative life events; SES Adversity = Socioeconomic adversity; Bonferroni adjusted *p* value: 0.003; *** or ** bolded survives adjusted p value.

conducted a power analysis based on King and colleagues that suggested we were 10–18 % powered to detect our coupling effects in pregnant women. Despite this, we do demonstrate internal replication of the coupling effects with three separate measures of similar yet distinct measures of psychological distress with effect sizes similar to that presented in the King and colleagues' paper which lend confidence to their robustness and utility as preliminary data.

4.2. Hormone ratios

This is the first study to examine associations of within- and between-person measures of cortisol-to-DHEA and cortisol-to-testosterone ratios with measures of psychological distress in pregnant and non-pregnant women over time. Examination of cortisol-to-DHEA ratio and cortisol-to-testosterone ratio at the within-person and between-person levels generally yielded no findings. Our results are thus not consistent with past literature and theories of hormone-behavior associations reporting higher psychological distress with greater cortisol-to-DHEA ratios [9,10]. Future investigations of the hormonal milieu in much larger samples might continue to focus on hormone ratios or interactive models rather than simply controlling for levels of other hormones to investigate the implications of the shifting hormonal milieu during pregnancy further.

4.3. Between-person hair hormone-behavior associations

Counter to the prevailing literature, we found that when within- and between-person variance in hair cortisol levels were partitioned, pregnant women with lower hair cortisol levels experienced more perceived stress and depression symptoms, on average. Prior studies have previously reported a negative association between perceived stress and depression symptoms with hair cortisol [37,38], however the recent meta-analysis on this topic failed to detect an overall moderating effect of distress type but perceived stress and depression had positive mean effect estimates [12]. Thus, the significance of this finding in the broader literature is precedented but it is unclear how much significance should be placed on it. In contrast, higher levels of cortisol were associated with higher cumulative psychological distress, higher state anxiety, and depression symptoms in non-pregnant women. Finally, higher testosterone levels were associated with greater psychological distress across all measures studied. These were the strongest findings of our investigation with all effects surviving the adjusted *p* value except for anxiety symptoms. These findings are relatively novel for testosterone measured from hair samples but is consistent with existing literature reporting elevated testosterone being associated with higher depression symptoms in premenopausal women in studies using serum levels although low testosterone has also been associated with higher depression symptoms in women [39].

4.4. Psychological distress

Some researchers have called attention to the mismatch between the timescale in which cortisol is measured (most often approximately an entire trimester) and psychological distress is measured (most often in the past 2 weeks or past month) as a possible reason for null or mixed associations in the literature [5]. When we assessed anxiety and depression symptoms, we asked participants to reflect on the past trimester or past three months and our measure of perceived stress asked participants to reflect on the past month. Additional measures such as the STAI and pregnancy related anxiety are not time specific. Thus, in general, we used measures that aligned with the timescale of hair hormone concentration to provide a better test of the associations between psychological distress measures and hair hormones concentrations. Interestingly, we did not find that the measures in which we specifically asked participants to reflect on the last three months/trimester were more strongly associated with hormone measures than the more general (or one-month time scale) measures. Although it is not possible to know if the specific phenotype of psychological distress is confounded with the time frame in the investigation of these associations, this provides some ancillary evidence that the timing for which participants rate their psychological distress is unlikely to solely explain the overall lack or mix of associations in the literature.

In general, it is important to bear in mind that findings were sparse. It is possible that despite initial excitement, hair hormone models of stress may not be the single most ideal stress model for understanding psychological distress during pregnancy. King and colleagues (2022) argued that changes in hair cortisol concentration are unlikely to change on the timescale of trimesters and so examined hair cortisol concentration from 1 cm segments of hair (approximately one month's exposure) but as previously mentioned, they also did not find a within-person

association of hair cortisol with depression symptoms. It is also possible that efforts to uncover timing effects are hampered by measurement error in the timing of cortisol exposure. That is, the timing of hair samples is known to not be particularly accurate, given evidence that hair does not grow at the same rate across individuals or across time within individuals, which may differ even more during pregnancy [27]. Improving the collection procedures for hair to be segmented based on actual growth rate to capture specific periods of time may help attenuate the issue of mis-matched timing of assessments. Together, measuring hair cortisol concentration at a more finely grained timescale and matching psychological distress measures to this timescale may yield more consistent findings. As a future direction for this area of research, non-hormonal biomarkers may offer a complementary perspective in studying pregnant populations as changes in the hormonal milieu may obscure associations of hair hormones and experienced psychological distress.

This study has limitations that warrant attention and discussion. The sample is small (with observations from 68 individuals), comes from a limited recruitment area, is lacking in racial and ethnic diversity, and has relatively low socioeconomic adversity. Thus, the generalizability of the findings from this sample are narrow. This work is best characterized as a pilot sample, and the novel information gleaned should be interpreted with caution and used primarily to generate novel findings to be tested in larger and more representative samples, as well as in samples with higher levels of socioeconomic adversity or other types of adversity where these effects may be larger and more applicable to populations who on average experience higher amounts of psychological distress during pregnancy. Finally, our linear multilevel modeling of the association between psychological distress and hair cortisol does not allow for the possibility that associations may be non-linear in reality [40]. Based on our descriptive exploration of the data we concluded that a linear model would fit the data best and that a non-linear model would stretch the capacity of our already small sample. However, future studies, particularly with much larger sample sizes and greater variability should investigate the potential non-linear association between psychological distress and hair cortisol.

4.5. Conclusions

In summary, this study contributed a novel investigation of within-person associations of hair cortisol and hormone ratios while taking into account the broader hormonal milieu in a prospectively followed sample of pregnant and non-pregnant women. In general, coupling of hair cortisol and psychological distress were supported in pregnant women for cumulative psychological distress, anxiety symptoms and pregnancy specific anxiety symptoms, whereas negative correlations of hair cortisol with perceived stress and depression symptoms occurred at the between person level. In contrast, we found no evidence of within-person associations in non-pregnant women, although we did find between-person associations of higher cortisol with greater psychological distress measures consistent with the existing literature. Additionally, investigating hormone ratios yielded no findings, accounting for hormonal milieu seemed to do little to stabilize mixed findings in the literature, and the timeframe of questions on psychological distress is unlikely to fully account for mixed findings. Critically, we add to a small but growing body of literature that shows that hair hormone-behavior associations can occur at both the within- and between-person level in pregnant women, although whether these associations exist in non-pregnant populations needs further investigation. Future studies should examine these associations in larger, higher risk samples, continue to consider hormones other than cortisol, and take care to match the timescale of hair hormone exposure to the measure of psychological distress.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2023.100214.

Beck & Steer, 1984, US-DHHS, 2020.

References

[1] K. Bergman, P. Sarkar, T.G. O'Connor, N. Modi, V. Glover, Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy, J. Am. Acad. Child Adolesc. Psychiatry 46 (11) (2007) 1454–1463, https://doi.org/10.1097/chi.0b013e31814a62f6.

[2] C. Buss, E.P. Davis, B. Shahbaba, J.C. Pruessner, K. Head, C.A. Sandman, Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems, Proc. Natl. Acad. Sci. USA 109 (20) (2012) E1312, https://doi.org/10.1073/pnas.1201295109.

[3] S.J. Short, T. Stalder, K. Marceau, S. Entringer, N.K. Moog, E.A. Shirtcliff, P. D. Wadhwa, C. Buss, Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures, Psychoneuroendocrinology 71 (2016) 12–18, https://doi.org/10.1016/j.psyneuen.2016.05.007.

[4] T. Stalder, C. Kirschbaum, Analysis of cortisol in hair–state of the art and future directions, Brain Behav. Immun. 26 (7) (2012) 1019–1029, https://doi.org/10.1016/j.bbi.2012.02.002.

[5] P. Mustonen, L. Karlsson, E.-L. Kataja, N.M. Scheinin, S. Kortesluoma, B. Coimbra, A.J. Rodrigues, N. Sousa, H. Karlsson, Maternal prenatal hair cortisol is associated with prenatal depressive symptom trajectories, Psychoneuroendocrinology 109 (2019), 104383, https://doi.org/10.1016/j.psyneuen.2019.104383.

[6] P. Mustonen, L. Karlsson, N.M. Scheinin, S. Kortesluoma, B. Coimbra, A. J. Rodrigues, H. Karlsson, Hair cortisol concentration (HCC) as a measure for prenatal psychological distress - a systematic review, Psychoneuroendocrinology 92 (2018) 21–28, https://doi.org/10.1016/j.psyneuen.2018.03.019.

[7] T. Napso, H.E.J. Yong, J. Lopez-Tello, A.N. Sferruzzi-Perri, The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation, Front. Physiol. 9 (1091) (2018), https://doi.org/10.3389/fphys.2018.01091 [Review].

[8] E. Barel, R. Abu-Shkara, R. Colodner, R. Masalha, L. Mahagna, O.C. Zemel, A. Cohen, Gonadal hormones modulate the HPA-axis and the SNS in response to psychosocial stress, J. Neurosci. Res. 96 (8) (2018) 1388–1397, https://doi.org/10.1002/jnr.24259.

[9] F.R. Chen, A. Raine, D.A. Granger, Tactics for modeling multiple salivary analyte data in relation to behavior problems: additive, ratio, and interaction effects, Psychoneuroendocrinology 51 (2015) 188–200.

[10] H.S. Kamin, D.A. Kertes, Cortisol and DHEA in development and psychopathology, Horm. Behav. 89 (2017) 69–85, https://doi.org/10.1016/j.yhbeh.2016.11.018.

[11] K. Marceau, P.L. Ruttle, E.A. Shirtcliff, P.D. Hastings, B. Klimes-Dougan, C. Zahn-Waxler, Within-person coupling of changes in cortisol, testosterone, and DHEA across the day in adolescents, Dev. Psychobiol. 57 (6) (2015) 654–669, https://doi.org/10.1002/dev.21173.

[12] J.E. Khoury, L. Giles, H. Kaur, D. Johnson, A. Gonzalez, L. Atkinson, Associations between psychological distress and hair cortisol during pregnancy and the early postpartum: a meta-analysis, Psychoneuroendocrinology 147 (2023), 105969, https://doi.org/10.1016/j.psyneuen.2022.105969.

[13] I.M. Goodyer, R.J. Park, C.M. Netherton, J. Herbert, Possible role of cortisol and dehydroepiandrosterone in human development and psychopathology, Br. J. Psychiatry 179 (3) (2001) 243–249, https://doi.org/10.1192/bjp.179.3.243.

[14] P. Leff-Gelman, M. Flores-Ramos, A.E.Á. Carrasco, M.L. Martínez, M.F. S. Takashima, F.M.C. Coronel, B.F. Labonne, J.A.Z. Dosal, P.B. Chávez-Peón, S. G. Morales, I. Camacho-Arroyo, Cortisol and DHEA-S levels in pregnant women with severe anxiety, BMC Psychiatr. 20 (1) (2020) 393, https://doi.org/10.1186/s12888-020-02788-6.

[15] A.D. Grotzinger, F.D. Mann, M.W. Patterson, J.L. Tackett, E.M. Tucker-Drob, K. P. Harden, Hair and salivary testosterone, hair cortisol, and externalizing behaviors in adolescents, Psychol. Sci. 29 (5) (2018) 688–699, https://doi.org/10.1177/0956797617742981.

[16] D. Terburg, B. Morgan, J. van Honk, The testosterone-cortisol ratio: a hormonal marker for proneness to social aggression, Int. J. Law Psychiatr. 32 (4) (2009) 216–223, https://doi.org/10.1016/j.ijlp.2009.04.008.

[17] E.M. Cooke, E.J. Connolly, D.L. Boisvert, T.A. Armstrong, R.H. Lewis, N. Kavish, M. Woeckener, J. Wells, J. Harper, Examining how testosterone and cortisol influence the relationship between strain, negative emotions, and antisocial behavior: a gendered analysis, Crime Delinquen. 66 (10) (2020) 1470–1501, https://doi.org/10.1177/0011128720903047.

[18] A.R. Dismukes, E.A. Shirtcliff, J.L. Hanson, S.D. Pollak, Context influences the interplay of endocrine axes across the day, Dev. Psychobiol. 57 (6) (2015) 731–741.

[19] K. Marceau, E.A. Shirtcliff, P.D. Hastings, B. Klimes-Dougan, C. Zahn-Waxler, L. D. Dorn, E.J. Susman, Within-adolescent coupled changes in cortisol with DHEA and testosterone in response to three stressors during adolescence, Psychoneuroendocrinology 41 (2014) 33–45, https://doi.org/10.1016/j.psyneuen.2013.12.002.
[20] E.P. Rolan, O. Robertson, N. Nonkovic, K. Marceau, Reliability of prospective and retrospective maternal reports of prenatal experiences, BMC Pregnancy Childbirth 22 (1) (2022) 968, https://doi.org/10.1186/s12884-022-05286-7.
[21] M.A. LeBeau, M.A. Montgomery, J.D. Brewer, The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair, Forensic Sci. Int. 210 (1–3) (2011) 110–116.
[22] M.J.E. Greff, J.M. Levine, A.M. Abuzgaia, A.A. Elzagallaai, M.J. Rieder, S.H.M. van Uum, Hair cortisol analysis: an update on methodological considerations and clinical applications, Clin. Biochem. 63 (2019) 1–9, https://doi.org/10.1016/j.clinbiochem.2018.09.010.
[23] S.N. Moody, L. van Dammen, W. Wang, K.A. Greder, J.M. Neiderhiser, P.A. Afulani, A. Willette, E.A. Shirtcliff, Impact of hair type, hair sample weight, external hair exposures, and race on cumulative hair cortisol, Psychoneuroendocrinology 142 (2022), 105805, https://doi.org/10.1016/j.psyneuen.2022.105805.
[24] J. Nissimov, U. Elchalal, Scalp hair diameter increases during pregnancy, Clin. Exp. Dermatol. 28 (5) (2003) 525–530, https://doi.org/10.1046/j.1365-2230.2003.01331.x.
[25] V.A. Randal, F.J.G. Ebling, Seasonal changes in human hair growth, Br. J. Dermatol. 124 (2) (1991) 146–151, https://doi.org/10.1111/j.1365-2133.1991.tb00423.x.
[26] W. Wang, S.N. Moody, J. Kiesner, A. Tonon Appiani, O.C. Robertson, E.A. Shirtcliff, Assay validation of hair androgens across the menstrual cycle, Psychoneuroendocrinology 101 (2019) 175–181, https://doi.org/10.1016/j.psyneuen.2018.10.029.
[27] K. Marceau, E. Rolan, O.C. Robertson, W. Wang, E.A. Shirtcliff, Within-person changes of cortisol, dehydroepiandrosterone, testosterone, estradiol, and progesterone in hair across pregnancy, with comparison to a non-pregnant reference group, Comprehensive Psychoneuroendocrinology 5 (2021), 100024, https://doi.org/10.1016/j.cpnec.2020.100024.
[28] S. Cohen, T. Kamarck, R. Mermelstein, Perceived stress scale, Measuring stress: A guide for health and social scientists 10 (1994) 1–2.
[29] C.D. Spielberger, State-Trait anxiety inventory, The Corsini encyclopedia of psychology (2010) pp 1-1.
[30] R.A. Steer, A.T. Beck, Beck Anxiety Inventory, 1997.
[31] A.C. Huizink, M.J. Delforterie, N.M. Scheinin, M. Tolvanen, L. Karlsson, H. Karlsson, Adaption of pregnancy anxiety questionnaire-revised for all pregnant women regardless of parity: praq-R2, Arch Womens Ment Health 19 (1) (2016) 125–132, https://doi.org/10.1007/s00737-015-0531-2.
[32] K. Marceau, M. De Araujo-Greecher, E.S. Miller, S.H. Massey, L.C. Mayes, J. M. Ganiban, D. Reiss, D.S. Shaw, L.D. Leve, J.M. Neiderhiser, The perinatal risk index: early risks experienced by domestic adoptees in the United States, PLoS One 11 (3) (2016), e0150486, https://doi.org/10.1371/journal.pone.0150486.
[33] T.F. McNeil, E. Cantor-Graae, K. Sjöström, Obstetric complications as antecedents of schizophrenia: empirical effects of using different obstetric complication scales, J. Psychiatr. Res. 28 (6) (1994) 519–530, https://doi.org/10.1016/0022-3956(94)90042-6.
[34] S. Sollberger, U. Ehlert, How to use and interpret hormone ratios, Psychoneuroendocrinology 63 (2016) 385–397, https://doi.org/10.1016/j.psyneuen.2015.09.031.
[35] L.S. King, K.L. Humphreys, D.A. Cole, I.H. Gotlib, Hair cortisol concentration across the peripartum period: documenting changes and associations with depressive symptoms and recent adversity, Comprehensive Psychoneuroendocrinology 9 (2022), 100102, https://doi.org/10.1016/j.cpnec.2021.100102.
[36] M.C. Hoffman, S.E. Mazzoni, B.D. Wagner, M.L. Laudenslager, R.G. Ross, Measures of maternal stress and mood in relation to preterm birth, Obstet. Gynecol. 127 (3) (2016) 545–552, https://doi.org/10.1097/AOG.0000000000001287.
[37] L. Jahangard, T. Mikoteit, S. Bahiraei, M. Zamanibonab, M. Haghighi, D. Sadeghi Bahmani, S. Brand, Prenatal and postnatal hair steroid levels predict post-partum depression 12 Weeks after delivery, J. Clin. Med. 8 (9) (2019), https://doi.org/10.3390/jcm8091290.
[38] S. Kalra, A. Einarson, T. Karaskov, S. Van Uum, G. Koren, The relationship between stress and hair cortisol in healthy pregnant women, Clin. Invest. Med. 30 (2) (2007) E103–E107, https://doi.org/10.25011/cim.v30i2.986.
[39] D.T. Maharjan, A.A.S. Syed, G.N. Lin, W. Ying, Testosterone in female depression: a meta-analysis and mendelian randomization study, Biomolecules 11 (3) (2021), https://doi.org/10.3390/biom11030409.
[40] S.J. Lupien, B.S. McEwen, M.R. Gunnar, C. Heim, Effects of stress throughout the lifespan on the brain, behaviour and cognition, Nat. Rev. Neurosci. 10 (6) (2009) 434–445.