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Pregnancy-specific anxiety and gestational length: The mediating role of diurnal cortisol indices

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Abstract

Background: Preterm birth or shorter gestation is a common adverse pregnancy outcome. Pregnancy-specific anxiety is robustly associated with risk for shorter gestation. Hypothalamic-pituitary-adrenal (HPA) dysregulation, indicated by diurnal cortisol index variability [slope, area-under-the-curve (AUC) or cortisol awakening response (CAR)], could mediate associations between pregnancy-specific anxiety and shorter gestation. The purpose of this study was to explore whether diurnal cortisol index variability mediates associations between pregnancy-specific anxiety and gestational length.

Methods: A sample of 149 women from the Healthy Babies Before Birth study reported pregnancy-specific anxiety in early pregnancy. Saliva samples were taken at three times during pregnancy, for two days each, at wake, 30 min post wake, noon, and evening. Diurnal cortisol indices were calculated using standard approaches. Pregnancy cortisol index variability was calculated across pregnancy timepoints. Gestational length was derived from medical charts. Covariates were sociodemographics, parity and obstetric risk. Mediation models were tested using SPSS PROCESS.

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Author contributions

Ross and Mander are co-first authors. This manuscript is based on Mander's Honours thesis, which was supervised by Ross. Ross conceptualized the project, completed statistical analyses and oversaw manuscript writing. Mander cleaned the cortisol data, worked with Ross to calculate the cortisol indices, conducted the literature review, and wrote the manuscript. Rinne contributed to the Introduction material on cortisol during pregnancy and extensively reviewed the manuscript. Okun completed the cortisol assays and provided comments on the manuscript. Hobel, Coussons-Read and Dunkel Schetter are HB3 PIs and provided comments on the manuscript.

Declaration of interest

The authors have no financial or personal conflicts of interest to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106114.

Results: There was a significant indirect effect of pregnancy-specific anxiety on gestational length via CAR variability, *b(SE)*= −0.102(0.057), *.95CI* [− 0.227,− 0.008]. Higher pregnancy-specific anxiety was associated with lower CAR variability, *b(SE)*= −0.019(0.008), *p* = .022, and lower CAR variability was associated with shorter gestation, *b(SE)*= 5.29(2.64), *p* = .047. Neither AUC or slope variability mediated associations between pregnancy-specific anxiety and gestational length.

Conclusion: Lower CAR variability during pregnancy mediated the association between higher pregnancy-specific anxiety and shorter gestational length. Pregnancy-specific anxiety could dysregulate HPA axis activity, as indicated by lower CAR variability, demonstrating the importance of the HPA axis system in regulating pregnancy outcomes.

Keywords

Diurnal cortisol indices; Pregnancy; Gestational length; Preterm birth; Pregnancy-specific anxiety; Cortisol variability

1. Introduction

Approximately 1 in 10 infants in the US are born preterm and 70% of infant deaths are due to preterm birth (Centers for Disease Control and Prevention, 2022). Preterm birth or shorter gestation is associated with adverse outcomes, such as complications at birth (Barker, 2007; O'Connor et al., 2002), increased infant mortality (Hedegaard et al., 1993), developmental disorders (Glynn et al., 2008), and adult health risks. Pregnancy-specific anxiety is a consistent predictor of preterm birth and shorter gestation (Dole et al., 2003; Dunkel Schetter, 2011; Kane et al., 2014; Kramer et al., 2009; Roesch et al., 2004). One potential pathway linking pregnancy-specific anxiety and shorter gestation is the hypothalamic-pituitary-adrenal (HPA) axis (Dunkel Schetter, 2011), as captured by diurnal cortisol indices. This pathway, however, is not well explored in the literature.

In the non-pregnant state, the HPA axis is a critical regulatory and stress response pathway (Adam and Kumari, 2009). When activated, the hypothalamus secretes corticotropin-releasing hormone (CRH) into the hypophyseal portal system, which in turn acts on the pituitary to stimulate release of adrenocorticotropic hormone (ACTH) into the general circulation. ACTH acts on the adrenal glands to stimulate the release of cortisol, which can be measured in saliva. HPA axis activity is often captured using diurnal cortisol indices. Cortisol awakening response (CAR) measures the increase in cortisol concentration that occurs 30–45 min after awakening. Heightened and blunted CARs are associated with physical health risks, dysregulation of the immune system (Chida and Steptoe, 2009; Clow et al., 2010) and higher depressive symptomology (Bhagwagar et al., 2005; Pruessner et al., 2003). The diurnal slope captures linear changes in cortisol levels across the day from morning to evening, and is typically negative or declines across the day (Adam et al., 2006; Adam and Gunnar, 2001; Ross et al., 2014). The Area Under the Curve (AUC) captures the total daily cortisol output across the day or cumulative cortisol exposure. High AUC values indicate greater exposure to cortisol, increased "wear and tear," and with possible adverse downstream repercussions on physiological systems and increased risk for disease (McEwen, 2000; Ross et al., 2014). In the non-pregnant state, higher anxiety is associated

with diurnal cortisol indices, including heightened and blunted CARs (Pruessner et al., 2003; Walker et al., 2011), higher total AUC (Mantella et al., 2008), and flatter diurnal cortisol slopes (Doane et al., 2013).

During pregnancy, HPA axis activity changes (Pascual and Langaker, 2022), largely due to the development of the placenta, which serves as a temporary endocrine organ (Howland et al., 2017; Sandman, 2018). The mother-fetus-placenta axis comprises a new mechanism that regulates and directs the progression of pregnancy, fetal development, and childbirth. Notably, the placenta expresses the gene for and produces corticotropin-releasing hormone (CRH) that in turn contributes to substantial changes to HPA axis regulation. Placental CRH (pCRH) production shifts regulation of the maternal HPA axis from a negative feedback loop to a tissue-specific positive feedback loop. Specifically, adrenal release of cortisol stimulates release of pCRH, resulting in simultaneous increases in CRH, ACTH, and cortisol over the course of pregnancy, that reaches peak levels near the time of labor and delivery, then returns to pre-pregnancy levels within five to seven days of delivery (Duthie and Reynolds, 2013; Glynn et al., 2018; Howland et al., 2017; Pascual and Langaker, 2022). These increases in cortisol, ACTH, and CRH are mirrored by changes in diurnal indices of cortisol, including a dampening of the cortisol awakening response and flattening of the cortisol slope from early to late pregnancy (Duthie and Reynolds, 2013; Entringer et al., 2010), though the diurnal rhythm of cortisol is still preserved (Kivlighan et al., 2008). Increases in cortisol levels and changes to diurnal indices in pregnancy facilitate important processes during this period, including fetal development and childbirth (for reviews, see Glynn et al., 2018; Howland et al., 2017; Sandman, 2018); dysregulation in these processes are associated with adverse birth outcomes, including shorter length of gestation (Duthie and Reynolds, 2013). For example, higher pregnancy cortisol, as assessed using a single measure (Entringer et al., 2011; Erickson et al., 2001; Mazor et al., 1994), higher CAR levels (Buss et al., 2009; Entringer et al., 2011), and flatter diurnal cortisol slopes in late pregnancy (Gilles et al., 2018) are associated with shorter gestation (for an exception, see Ruiz et al., 2001).

There is evidence that pregnancy-specific anxiety is associated with prenatal cortisol levels (Kane et al., 2014; Obel et al., 2005); these studies examined a single cortisol measure per day, and it is not clear how or whether pregnancy-specific anxiety is associated with diurnal cortisol indices (e.g., CAR, AUC and slope). There were also no studies found that directly examined whether diurnal cortisol indices mediate associations between pregnancy-specific anxiety and gestational length. Studies of other pregnancy outcomes and sources of distress, however, have been examined. For example, a study of 405 pregnant women tested associations between distress, an aggregate of perceived stress, specific prenatal worries, negative life events, depression symptoms, trait anxiety, and neuroticism, and diurnal cortisol, and diurnal cortisol and gestational length in two separate models. The study reported an association between higher late-gestation distress and flatter diurnal cortisol slopes in one model, and an association between flatter diurnal cortisol slopes and shorter gestational length in the other (Gilles et al., 2018). However, whether associations between distress and gestational length were mediated by diurnal cortisol slopes was not directly tested. Another study of 98 pregnant women tested whether associations between second trimester psychological distress, an aggregate of depressive symptoms, general anxiety and daily hassles and birth weight was mediated by maternal urinary cortisol using

structural equation modelling (Diego et al., 2006). An indirect effect was detected, such that higher distress was associated with higher urinary cortisol levels, and higher maternal urinary cortisol levels were in turn associated with lower birth weight (Diego et al., 2006). Collectively, these studies suggest that diurnal cortisol could mediate associations between pregnancy-specific anxiety and shorter gestation.

In non-pregnant samples, diurnal cortisol indices are not stable over time (Kuhlman et al., 2019; Ross et al., 2014; Segerstrom et al., 2017) and diurnal cortisol index variability could be a more direct indicator of HPA axis adaptability, plasticity or regulation (Sannes et al., 2017; Segerstrom et al., 2014). For example, mental states, such as neuroticism, are associated with diurnal cortisol variability (Segerstrom et al., 2017), and differences in diurnal cortisol variability are associated with health outcomes, such as low-grade inflammation (Herriot et al., 2017; Segerstrom et al., 2014) and poor mental health (Havermans et al., 2010; Sannes et al., 2017). Diurnal cortisol index variability is particularly important to consider in the context of pregnancy given the dynamic adaptations that occur in the HPA axis as pregnancy progresses.

Evidence from non-pregnant samples suggests that mental states could affect diurnal cortisol index variability, and that diurnal cortisol index variability could be associated with health outcomes. However, no studies to date have examined diurnal cortisol index variability within the context of pregnancy. The purpose of this study is to explore whether diurnal cortisol index variability could mediate associations between pregnancy-specific anxiety and gestational length. A sample of pregnant women from Denver, CO, Los Angeles, CA, was used to test this question. Pregnancy-specific anxiety was assessed in the early second trimester; saliva samples for cortisol assay were collected at three time points between the second and third trimesters, and labor and delivery (gestational length) occurred after the third assessment.

2. Methods

2.1. Participants

The study data was collected as part of the Healthy Babies Before Birth study, and focused on a a sample of 149 women. These women were recruited from the Denver Health and Hospital Authority (DHHA) in Denver, Colorado, and the Cedars Sinai Medical Center (CSMC) in Los Angeles, California. Recruitment and data collection took place between 2013 and 2018. The women included in the study had intrauterine, singleton pregnancies and were 18 years of age or older; recruited at less than 16 weeks of gestation and gave birth to live-born infants. The exclusion criteria included current substance use, a current substance abuse diagnosis, being pregnant with multiple gestation, and having a chronic infection, e.g., HIV positive. Pregnant women were identified by trained study team members at prenatal care visits, and if the women were eligible, they were invited to take part in the study. The majority of the recruiting took place through direct patient contact in the prenatal clinics of major medical centres, with some by placing study pamphlets in prenatal care facilities. Participants were recruited in Los Angeles at a major medical facility where associated prenatal clinics provided care to women from a range of socioeconomic backgrounds. Participants were recruited in Denver at one prenatal clinic associated with

a major medical facility that mostly served low-income women. On average, at both sites, of the women who were approached and qualified for the study, 23% agreed to participate. Reasons to decline included time commitment, transportation, and lack of interest. All participants provided informed consent, and protocols and procedures were reviewed and approved by IRBs at the University of California – Los Angeles, University of Colorado – Colorado Springs, Denver University and Cedars-Sinai Medical Centre in Los Angeles.

There were three pregnancy assessments (8–16 weeks, 20–26 weeks, and 30–36 weeks). At each assessment, interviews were conducted (Spanish or English) and biological samples were collected, including saliva for cortisol assay. For the purpose of these analyses, measures and timepoints were selected to ensure that variables were temporally ordered. As such, only pregnancy-specific anxiety from the first assessment was included; saliva samples from the first, second and third assessment were included; and labor and delivery (gestational length) data were thereafter for all cases. A total of 235 pregnant women consented to participate in the study and completed baseline measures (pregnancy-specific anxiety). Of those, 172 participants provided enough saliva samples to calculate diurnal cortisol index variability across the three pregnancy assessments (see below) and had gestational length data available. A total of 149 had complete data on all key variables and covariate variables.

2.2. Pregnancy-specific anxiety

Pregnancy specific anxiety was obtained from the first pregnancy assessment (8–16 weeks gestation). It was measured using a 10 -item scale that assesses the frequency with which participants worried or felt concerned about their health, their baby's health, labour and delivery, and caring for a baby (Rini et al., 1999). This scale is responded to on a 4-point scale from 1 (never or not at all) to 4 (almost all the time or very much). The score is calculated by taking the mean across all the items. This measure has been used in prior studies of pregnancy and is a reliable and valid measure (Rini et al., 1999), *Cronbach's α* = 0.80.

2.3. Gestational length

Gestational length (weeks) was derived from medical charts at birth, which occurred after the third pregnancy assessment. Gestational length was analyzed as a continuous variable.

2.4. Salivary samples

Saliva samples were obtained immediately following each assessment during pregnancy. Salivary samples were collected at home, for two consecutive days, at waking, 30 min post-awakening, noon, and evening (maximum 8 samples per assessment; maximum 24 prenatal samples total). Participants were provided with verbal and written instructions that asked participants to collect saliva samples by chewing on a cotton roll included in the Salivette tube (Sarstedt, Rommelsdorf, Germany) for 60 s until the roll was saturated. Participants were also instructed to collect saliva samples before brushing teeth, eating or drinking (except water and medications), and before exercise. Participants recorded time of awakening and the time of each saliva collection in a provided log and were asked to keep saliva samples refrigerated until the kit was mailed back to the lab.

Saliva samples were spun at 3000 rpm for 10 min then aliquoted and stored at − 70°F (−57 °C) until they could be processed in batches using ELISA kits purchased from Salimetrics (State College, PA). The assay is an expanded range high sensitivity competitive immunoassay where cortisol in standards and samples competed with cortisol conjugated to horseradish peroxidase (HRP) for antibody binding sites on the microtiter plate. After incubation and washing, bound HRP-streptavidin cortisol conjugate was measured by its enzymatic reaction with tetramethylbenzidine (TMB), as detected with the plate reader at 450 nm. The amount of HRP-streptavidin cortisol conjugate detected is inversely proportional to the amount of cortisol in the sample (Chard, 1990). The assay range is 0.012 – 3.000 ug/dL, with an assay sensitivity of < 0.007 ug/dL. All samples were analyzed in duplicate. The intra-assay coefficient of variability was within acceptable limits (8.83%; "Calculating Inter- and Intra-Assay Coefficients of Variability – Salimetrics," n.d.) and the inter-assay coefficient of variability was 1.49%.

2.5. Cortisol indices and variability

Cortisol data was cleaned and diurnal cortisol indices were calculated at each of the three pregnancy timepoints as per standard practices (Adam and Kumari, 2009). Briefly, CAR was calculated by subtracting the 30 min post-wake values from the wake values and then averaging them across the two days of assessment. Diurnal slope was calculated through linear regression analysis of cortisol levels across the day from morning to evening. AUC was calculated via a trapezoidal function technique to calculate the total area under the cortisol curve throughout the day, minus 30-min post-wake samples (Pruessner et al., 2003). Diurnal cortisol index values for each of the three pregnancy assessments was calculated by averaging across the two days of sampling.

Of the women who consented to participate in the study at baseline, 222 provided baseline saliva samples, and 172 provided saliva samples at each of the three pregnancy assessments. The majority (99%) provided all requested saliva samples (eight samples) at each assessment; two participants returned only seven saliva samples, one for the first assessment and the other for the second assessment. Both were missing a wake sample from one of the two days of sample collection; given that times of sample collection and cortisol concentrations were consistent for the other available time points and samples at the same assessment, the available wake value and time for these two participants were used to impute the missing value.

For each diurnal cortisol index, diurnal cortisol index variability was calculated by taking the standard deviation across the three pregnancy assessments. Given that variability can be limited by floor or ceiling effects, average diurnal cortisol index values were also calculated across the three assessments and included in models as covariates.

2.6. Covariates

Participants age, race and ethnicity (Hispanic or not Hispanic), per capita household income (socioeconomic status), marital status (married or single), gestational age (calculated through subtraction of conception date, determined through ultrasounds, from the blood collection assessment date), parity (nulliparous or parous), pre-pregnancy body mass index

(BMI) (kg/m^2), obstetric risk (yes or no), fetal sex (male, female), any substance use (use of any alcohol, tobacco or other recreational substances during pregnancy or not) and recruitment site (Los Angeles, Denver) were included as covariates. Pre-pregnancy BMI was calculated by taking self-reported pre-pregnancy weight and dividing by height measured at baseline using a balance-beam scale. Obstetric risk was determined using information from prenatal and labor and delivery records; participants were coded as having an obstetric risk if records indicated current or past presence of well-established relevant risk factors, i.e., severe infection, hypertension, diabetes, any vascular risk infection, oligohydramnios, and polyhydramnios (Glynn et al., 2008; Hobel, 1982).

2.7. Analytic strategy

All analyses were conducted using IBM SPSS (IBM, 2018). Descriptive statistics and bivariate correlations were generated. Power analyses for indirect effects are based on previously published mediation model simulations for power (1-β) equal to 0.80 (Fritz and MacKinnon, 2007). For mediation models that use bias-corrected bootstrapping, a sample size of 71 is needed to detect an indirect effect comprised of medium effect size paths (0.39) and a sample size of 148 is needed to detect an indirect effect comprised of small-to-medium effect size paths (0.26). The sample size for this study is 149, and is powered to detect small-to-medium indirect effect sizes.

Mediation models were tested using the PROCESS Macro v.4.2 (Hayes, 2018) in SPSS (IBM Corp, 2018). Three separate models were tested for each diurnal cortisol index (CAR, slope, and AUC). Pregnancy-specific anxiety was entered as the predictor (X), and gestational length as the outcome (Y). The diurnal cortisol index variability was entered as the mediator (Z).[1] Mediation was tested using Model 4 (Fig. 1). Each model adjusted for all covariates. PROCESS produces (1) linear regression models testing associations between pregnancy-specific anxiety and diurnal cortisol variables (path ‘a’), (2) linear regression models testing associations between pregnancy-specific anxiety, diurnal cortisol variables, and gestational length (paths ‘b’ and ‘c’), and (3) bootstrapped estimates of the indirect effect (‘ab’ paths) for each combination of predictor, mediator, and outcome. Indirect effects were calculated by bootstrapping estimates 5000 times. Significant indirect effects were probed by examining the direct paths.

3. Results

3.1. Sample characteristics and correlations

Descriptive statistics are presented in Table 1. Bivariate correlations are presented in Suppl. Table 2. Higher pregnancy-specific anxiety was significantly associated with lower CAR variability, $r = -0.160$, $p = .033$. Higher CAR variability was significantly associated with longer gestational length, $r = 0.156$, $p = .041$.

[1]For analytic purposes, average diurnal cortisol indices were also tested as mediators. None of the average diurnal cortisol index values significantly mediated associations between pregnancy-specific anxiety and gestational length (results not shown).

3.2. Diurnal cortisol index variability

Separate mediation models were used to test whether pregnancy-specific anxiety was associated with gestational length via diurnal cortisol index variability (CAR, AUC and slope), independent of average diurnal cortisol index and covariates (Suppl Table 1). For the model that examined CAR variability as a mediator, pregnancy-specific anxiety was not independently or directly associated with gestational length, $b = 0.055$, $SE = 0.260$, $p = .832$. However, a significant indirect effect of pregnancy-specific anxiety on gestational length by CAR variability was detected, $b = -0.102$, $SE = 0.057$, $.95CI[-0.227, -0.008]$ (Fig. 2). Specifically, high pregnancy-specific anxiety was associated with low CAR variability, $b = -0.019$, $SE = 0.008$, $p = .022$. And low CAR variability, in turn, was associated with shorter gestational length, $b = 5.29$, $SE = 2.64$, $p = .047$.

Similar models were run testing cortisol slope variability and AUC variability as mediators (Suppl. Table 2). However, neither cortisol slope variability, $b = -0.012$, $SE = 0.039$, $.95CI[-0.096,0.065]$, nor AUC variability, $b = -0.011$, $SE = 0.035$, $.95CI[-0.094,0.052]$, significantly mediated associations between pregnancy-specific anxiety and gestational length, independent of respective average diurnal cortisol index values and covariates.

4. Discussion

The current study examined whether prenatal diurnal cortisol index variability mediated associations between pregnancy-specific anxiety and gestational length, a robustly reported pattern in the literature. An indirect effect of CAR variability was detected, such that higher pregnancy-specific anxiety was associated with lower CAR variability, which in turn was associated with shorter gestational length. Neither slope nor AUC variability were directly or indirectly associated with gestational length. This study is the first to assess diurnal cortisol indices as a mediator between pregnancy-specific anxiety and gestational length, and to assess diurnal cortisol index variability during pregnancy.

The association between pregnancy-specific anxiety and gestational length was only significantly mediated by CAR variability. This may be consistent with previous research. One study of 101 pregnant women assessed changes in CAR during the second and third trimesters of pregnancy and associations with gestational length (Buss et al., 2009). Awakening cortisol levels increased between the two timepoints, resulting in flatter or attenuated CAR over time. Moreover, relative to women with longer gestation, women with shorter gestation had less attenuation of CAR or more consistent CAR patterns between the second and third trimesters (Buss et al., 2009). This pattern is consistent with less variability in CAR over this period. Our findings extend this work by suggesting that pregnancy-specific anxiety could be associated with less variability in CAR during pregnancy, which in turn is associated with shorter gestation. Higher pregnancy-specific anxiety could restrict or limit HPA axis adaptability, or ability to respond to changes in regulation or physiological conditions, as indexed by lower CAR variability. This could be problematic in the context of a dynamic physiological period like pregnancy, where HPA axis adaptations, such as increasing basal cortisol levels and flatter CARs, are a normative physiological adaptation (Buss et al., 2009). Interruption in normative HPA axis adaptations, in turn, could indicate or confer increased risk for shorter gestation.

Although these findings are consistent with previous research, it is not clear why variability in CAR specifically – and not AUC or slope – mediates associations between pregnancy-specific anxiety and gestational length. It is possible that these findings are consistent with previous assertions that the CAR reflects different neurobiological processes than diurnal cortisol indices that capture values from across the day, such as AUC and slope, and which is reflected in unique patterns of association with psychological and health outcomes (Fries et al., 2009; Kumari et al., 2011; Segerstrom et al., 2014). However, other studies did report findings consistent with associations between greater pregnancy distress and lack of variability in cortisol levels in pregnancy (AUC). A study of 250 pregnant women collected blood samples for cortisol assay at four time points during pregnancy (Peterson et al., 2020). General growth mixture modelling was used to identify cortisol trajectory groups: Typical increases in cortisol levels, accelerated increases in cortisol levels, and relatively flat cortisol levels due to higher early-pregnancy cortisol. Women with higher distress scores (composite of pregnancy anxiety, state anxiety, perceived stress and depressive symptoms) were more likely to be in the flatter cortisol levels group (Peterson et al., 2020). Moreover, although associations between cortisol trajectory group and pregnancy outcomes were not reported, separate analyses explored associations with infant growth. Infants of women in the flatter cortisol levels group demonstrated faster increases in BMI percental between birth and six months, and higher BMI percental at three and six months, compared to infants of women in the other trajectory groups (Hahn-Holbrook et al., 2023). Flatter plasma cortisol levels during pregnancy could be consistent with less variability in total cortisol load over the day, or AUC. There are several possible reasons for why a pattern consistent with less variability in AUC was detected elsewhere but not here, including differences in approach with respect to pregnancy distress, cortisol assessment, and outcomes considered. Regardless, future research should compare and contrast the role of different cortisol index approaches (e.g., single values, slope, AUC, CAR) and different analytic approaches (e.g., variability, trajectory groups, within-person changes) when exploring how cortisol variables could mediate associations between pregnancy distress and outcomes.

The current study has several limitations. Foremost, although the current study was designed as per best practice in cortisol sampling at the time (in 2013), standards for cortisol collection have changed since then. First, current CAR guidelines recommend assessing CAR using between four and five samples in the morning (Stalder et al., 2016). Here, only two saliva samples were collected to calculate CAR. With respect to variability, minimum recommended days of sampling is five to ten days for diurnal slope and three days for AUC (Segerstrom et al., 2014). Variability was calculated from three assessments, which meets minimum requirements for AUC but not slope. Despite these limitations, the current study included more saliva samples and days of assessment than some previous studies, some of which included a single saliva assessment or one or two days of assessment. The novelty of our analyses and findings suggest that our methods are defensible despite these limitations; nonetheless, future research should replicate these analyses in data that adheres to the since-published guidelines. Also, current guidelines recommend use of objective measures of sample timing, e.g., MEMS caps (Stalder et al., 2016). Self-reported sample timing was used here, which is consistent with recommendations at the time of study design (Clements, 2013). It is possible that there were inaccuracies in reported timing of saliva

sample collection. This is a limitation, however, that is common in saliva sampling methods generally, and there is evidence that use of objective timing measures, such as MEMS caps, cannot completely correct for reported timing inaccuracies in at-home saliva collection, particularly with respect to waking samples (Dockray et al., 2008; Rotenberg and McGrath, 2014).

Another potential limitation is that the sample consisted of mostly term pregnancies, with few women delivering before 37 weeks gestation (preterm). Future research should determine whether associations with shorter gestational length here generalize in larger and high risk studes of preterm delivery. Finally, it is possible that additional variables, such as fetal sex, race/ethnicity, socioeconomic status or BMI, could moderate associations between pregnancy-specific anxiety, diurnal cortisol index variability and gestational length. Although each of these variables was included as a covariate in analyses, it was not possible to test moderated-mediation here. It would be valuable to examine mediated-moderation in larger samples or in future data collections.

In summary, prenatal variability in CAR, but not slope or AUC, mediated associations between higher pregnancy-specific anxiety and shorter gestational length. These findings suggest that examining indicators that capture the dynamic changes in cortisol and HPA axis activity during pregnancy could provide additional insight on associations between pregnancy-specific anxiety and gestational length.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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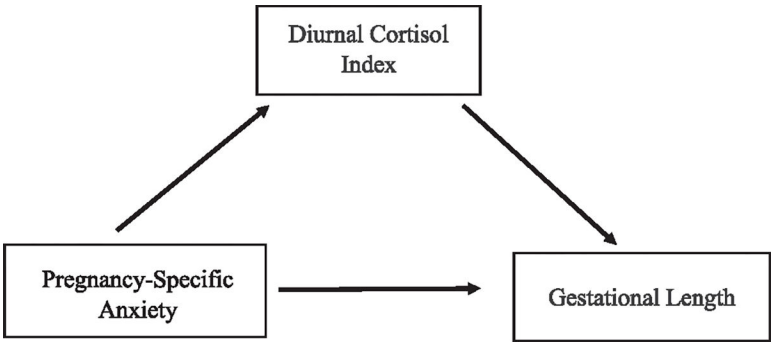

Fig. 1.
Mediation Model.

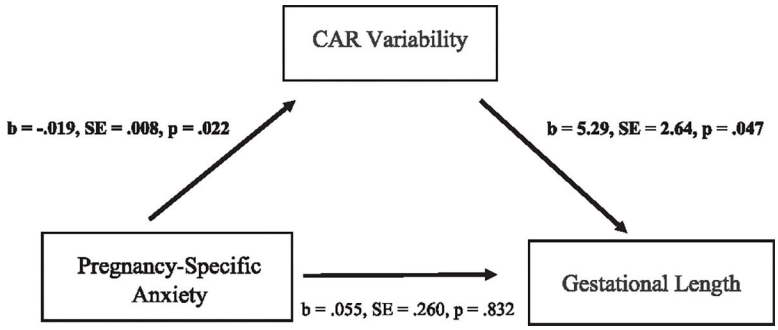

Fig. 2.

Significant indirect effect (b = −0.102, SE =0.057,.95CI(−0.227, −0.008)) of elevated pregnancy-specific anxiety on shorter gestational length, mediated by lower CAR variability.

Table 1

Descriptive Statistics (N = 149).

Variable	Mn + /− SD or % (N)			*p*
	Total Sample	Los Angeles, CA 51% (76)	Denver, CO 49% (73)	
Age (years)	30.4 + /− 5.96	33.3 + /− 4.70	27.4 + /− 5.65	< 0.001
Married or cohabiting	68% (101)	87% (66)	49% (36)	< 0.001
Race/ethnicity (Hispanic)	37% (55)	27% (21)	47% (34)	0.002
Per capita household income ($1000)	24.4 + /− 23.7	30.0 + /− 22.6	18.4 + /− 23.5	< 0.001
Education (years)	15.5 + /− 3.38	17.1 + /− 2.73	13.8 + /− 3.18	< 0.001
Pre-pregnancy BMI (kg/m2)	26.3 + /− 6.84	24.6 + /− 6.03	28.1 + /− 7.17	< 0.001
Gestational age at study entry (weeks)	13.9 + /− 1.82	14.8 + / − 0.985	12.7 + /− 1.96	< 0.001
Nulliparity	55% (82)	61% (46)	48% (35)	0.043
Obstetric Risk (any)	44% (65)	46% (35)	41% (30)	0.440
Fetal Sex (male)	51% (76)	58% (44)	43% (31)	0.025
Tobacco, alcohol or substance use (any)	25% (37)	36% (27)	15% (11)	< 0.001
CAR mean	0.009 + / − 0.063	0.010 + / − 0.054	0.008 + / − 0.071	0.799
CAR SD	0.046 + / − 0.048	0.051 + / − 0.056	0.038 + / − 0.031	0.079
AUC mean	1.39 + / − 0.719	1.51 + / − 0.777	1.25 + / − 0.627	0.009
AUC SD	0.589 + / − 0.576	0.670 + / − 0.635	0.464 + / − 0.448	0.021
Slope mean	−0.005 + / − 0.007	−0.004 + / − 0.007	−0.005 + / − 0.007	0.207
Slope SD	0.006 + / − 0.004	0.006 + / − 0.004	0.005 + / − 0.005	0.390
Pregnancy-specific anxiety	1.76 + / − 0.493	1.80 + / − 0.489	1.72 + / − 0.500	0.234
Gestational length (weeks)	39.3 + /− 2.02	39.4 + /− 1.68	39.3 + /− 2.38	0.868