

## Pregnancy anxiety and prenatal cortisol trajectories



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### ABSTRACT

Pregnancy anxiety is a potent predictor of adverse birth and infant outcomes. The goal of the current study was to examine one potential mechanism whereby these effects may occur by testing associations between pregnancy anxiety and maternal salivary cortisol on 4 occasions during pregnancy in a sample of 448 women. Higher mean levels of pregnancy anxiety over the course of pregnancy predicted steeper increases in cortisol trajectories compared to lower pregnancy anxiety. Significant differences between cortisol trajectories emerged between 30 and 31 weeks of gestation. Results remained significant when adjusted for state anxiety and perceived stress. Neither changes in pregnancy anxiety over gestation, nor pregnancy anxiety specific to only a particular time in pregnancy predicted cortisol. These findings provide support for one way in which pregnancy anxiety may influence maternal physiology and contribute to a growing literature on the complex biological pathways linking pregnancy anxiety to birth and infant outcomes.

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### 1. Introduction

Pregnancy anxiety is a highly specific type of maternal psychological distress defined as anxiety concerning a mother's own health, the baby's health, and labor and delivery in the context of a specific pregnancy (Dunkel Schetter, 2011). It is a potent and often more consistent predictor of birth, infant and child outcomes than general forms of psychological distress (Buss, Davis, Muftuler, Head, & Sandman, 2009; Dunkel Schetter & Glynn, 2011; Kramer et al., 2009; Lobel et al., 2008; Roesch, Dunkel Schetter, Woo, & Hobel, 2004). For example, pregnancy anxiety predicts preterm birth and gestational length (Kramer et al., 2009; Mancuso, Dunkel Schetter, Rini, Roesch, & Hobel, 2004; Roesch et al., 2004), infant mental and psychomotor development (Buitelaar, Huisink, Mulder, de Medina, & Visser, 2003; Davis & Sandman, 2010; Huizink, De Medina, Mulder, Visser, & Buitelaar, 2002), child temperament (Blair, Glynn, Sandman, & Davis, 2011; Huizink et al., 2002; Sandman, Davis, Buss, & Glynn, 2012), and brain morphology in young children (Buss et al.,

2009). However, little is known about the biological mechanisms that explain these effects.

Alterations in the maternal hypothalamic–pituitary–adrenal (HPA) axis and placenta are hypothesized to be an explanatory mechanism (Seckl & Holmes, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005). Strong evidence from animal models demonstrates that maternal distress predicts increased fetal glucocorticoid exposure via synthesis and release of maternal glucocorticoids (Maccari et al., 2003; Weinstock, 2005), but similar evidence in humans linking pregnancy anxiety (or general maternal distress) to increased levels of maternal cortisol during pregnancy is lacking. Thus, it is unclear if and how pregnancy anxiety is associated with maternal cortisol during pregnancy. The purpose of this study was to determine whether pregnancy anxiety is associated with cortisol levels and trajectories over the course of pregnancy in an effort to better understand the broader processes linking pregnancy anxiety to birth, infant and child outcomes.

During pregnancy, maternal cortisol increases 2- to 4-fold (Glynn, Dunkel Schetter, Chicz-DeMet, Hobel, & Sandman, 2007; Mastorakos & Ilias, 2003; Meulenberg & Hofman, 1990) and passes through the placenta. However, the amount of maternal cortisol that crosses the placental barrier is limited because its passage is regulated by the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD2: Benediktsson, Calder, Edwards, & Seckl, 1997). Still,

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maternal cortisol accounts for approximately 30–40% of the variability in fetal concentrations of cortisol (Gitau, Cameron, Fisk, & Glover, 1998; Gitau, Fisk, Teixeira, Cameron, & Glover, 2001).

Maternal cortisol levels can affect birth and infant outcomes in multiple ways. For one, cortisol stimulates the synthesis and release of placental corticotrophin-releasing hormone (pCRH) (King, Smith, & Nicholson, 2001). In humans, elevated cortisol early in pregnancy predicts pCRH levels later in pregnancy (Sandman et al., 2006) and pCRH predicts preterm birth (McLean & Smith, 2001). Maternal cortisol also acts directly on the fetus and its developing nervous system (for reviews, see Davis & Sandman, 2006; Sandman et al., 2012). For example, results of prospective studies have documented that relatively high levels of prenatal maternal cortisol predict greater behavioral and physiological stress reactivity in fetuses, infants and children (Davis, Glynn, Waffarn, & Sandman, 2011; De Weerth, Van Hees, & Buitelaar, 2003), decreased cognitive ability in infants (Davis & Sandman, 2010; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), increased affective problems in young girls (Buss et al., 2012), and altered amygdala volumes in young girls (Buss et al., 2012).

In the literature, the association between pregnancy anxiety and maternal cortisol is inconsistent at best. For example, a study of 603 women found that worries about pregnancy-related complications were associated with high evening maternal cortisol late in pregnancy (Obel et al., 2005), but with lower morning cortisol early in pregnancy. Several other studies reported no associations between maternal cortisol and pregnancy anxiety (Bolten et al., 2011; Davis & Sandman, 2010; Goedhart et al., 2010; Harville, Savitz, Dole, Herring, & Thorp, 2009; Kivlighan, Dipietro, Costigan, & Laudenslager, 2008; Pluess, Bolten, Pirke, & Hellhammer, 2011; Sikkema et al., 2001). However, the majority of these studies assessed maternal cortisol at only 1 or 2 time points during pregnancy, conducted only cross-sectional analyses, or did not test changes in levels or trajectories of cortisol, thus limiting their ability to detect potential effects.

The specific aim of the present study was to test predicted links between pregnancy anxiety and both maternal cortisol levels at specific time points in pregnancy and cortisol trajectories across pregnancy using multilevel modeling techniques. We hypothesized that higher levels of pregnancy anxiety would be associated with higher levels of cortisol and steeper increases in cortisol trajectories over pregnancy. We further hypothesized that pregnancy anxiety would be an independent predictor of cortisol after adjusting for other commonly studied measures of psychological distress (i.e., state anxiety and perceived stress).

## 2. Method

### 2.1. Study design and procedure

Study participants were women from a prospective longitudinal study assessing prenatal psychosocial and behavioral factors in pregnancy, the Multi-Site Behavior in Pregnancy Study (MSBIPS). Women were recruited in the Los Angeles area by research nurses at prenatal clinics in two large urban medical centers. Data for this study were collected on 4 occasions over the course of pregnancy separated by six week intervals (T1: 18–20 weeks,  $M=19.29$ ,  $SD=.80$ , T2: 24–26 weeks,  $M=24.98$ ,  $SD=.83$ , T3: 30–32 weeks,  $M=30.98$ ,  $SD=.76$ , and T4: 36–38 weeks,  $M=36.75$ ,  $SD=.70$ ). At each assessment, women completed semi-structured interviews, questionnaires and provided one saliva sample for cortisol assessment. All study protocols and procedures were approved by each institution's Institutional Review Boards.

### 2.2. Participants

Inclusion criteria for the sample were 18 years of age, English ability, and singleton intrauterine pregnancy. Exclusion criteria were tobacco, alcohol, or drug use during pregnancy, and medical conditions involving dysregulated neuroendocrine,

**Table 1**  
Sample socio-demographics and medical history.

Variables	N (%)
Age at Time 1	$M=30$ ( $SD=5.20$ , range = 18–43)
Ethnicity	
Non-Hispanic White	221 (49.3%)
Latina	99 (22.1%)
African-American	61 (13.6%)
Asian	40 (8.9%)
Multi-ethnic/other	27 (6.9%)
Income	
<\$10,000	20 (4.5%)
\$10,001–30,000	67 (15.0%)
\$30,001–50,000	87 (19.4%)
\$50,001–70,000	91 (20.3%)
\$70,001–90,000	60 (13.4%)
>\$90,000	123 (27.5%)
Education	
High School or less	70 (15.6%)
Some college	175 (39.1%)
Bachelor's degree	123 (27.5%)
Graduate degree	80 (17.9%)
Married or cohabitating	396 (88.4%)
Parity	
Nulliparous	242 (54.0%)
Multiparous	206 (46.0%)
Current obstetric risk factors	$M=1.15$ ( $SD=1.40$ , range = 0–7)
Historical obstetric risk factors	$M=0.75$ ( $SD=.99$ , range = 0–6)
Medical history risk factors	$M=1.20$ ( $SD=1.14$ , range = 0–5)

Note:  $N=448$ .

cardiovascular, hepatic or renal functioning.<sup>1</sup> Sixty-three percent of the 1189 women screened met eligibility criteria and 67% of these women consented to participate in the study ( $N=498$ ). The primary reasons for declining to participate in the study included work or school conflict, scheduling difficulties, child care issues and lack of interest. Participants were included in the current analysis if they had cortisol and pregnancy anxiety data for at least one time-point during the course of the study ( $N=494$ ).<sup>2</sup> Forty six women were excluded from the current sample due to missing demographic data or missing medical risk factor variables. Therefore, 448 women comprised the final sample.<sup>3</sup>

Table 1 shows demographic and medical risk data for this sample. Participants were 30 years of age on average. The sample was composed of 49% non-Hispanic white, 22% Latina, 14% African American, and 9% Asian and was fairly socio-economically diverse. The majority of the women were married or cohabitating (88%). Slightly over half of the women were carrying their first child (54%).

### 2.3. Measures

#### 2.3.1. Maternal cortisol

Saliva samples were scheduled for collection at least 1 h after the participant had eaten. The samples were collected using a cotton gauze pad placed into a syringe and then clarified by depressing the plunger. They were then stored at  $-20^{\circ}\text{C}$  until assayed. Mean collection time for the samples was 2:20 pm ( $SD=1.92$  h). Thawed samples were centrifuged at 3000 rpm for 15 min before assay. All samples were assayed in duplicate using a competitive solid-phase radioimmunoassay (Coat-A-Count; Diagnostic Product Corp.). The test has a minimum detectable level of 0.02  $\mu\text{g}/\text{dl}$  and the intra- and inter-assay coefficients of the variance were 5.5% and 7.6%, respectively. Cortisol data were log-transformed [ $\ln(\text{cortisol} \times 27.6)$ ] and values outside  $\pm 4$  standard deviations from the mean were removed from the current analyses (2 data points were removed from T1, 1 from T2, 2 from T3 and 1 from T4).

<sup>1</sup> Despite screening procedure efforts, it was revealed from information abstracted from medical records and self-report questionnaires that a few ( $n=28$ ) women smoked up to the first assessment (Time 1). By the 2nd assessment (Time 2), no women reported any smoking. Because they were already enrolled in the study and occasional smokers, these women were retained in the study. A dummy coded variable was created, 1 (smoking) and 0 (no smoking) and entered into all analyses. The results remained unchanged and smoking was not a significant predictor of initial cortisol levels or cortisol trajectories. This variable was, therefore, not reported in the analyses.

<sup>2</sup> Three participants were removed due to lack of cortisol data. One participant was removed due to lack of pregnancy anxiety data.

<sup>3</sup> Women not included in the final sample were not significantly different from women who were included in the final sample on any study variables (all  $ps > .10$ ).

**Table 2**  
Descriptive statistics for primary variables at each assessment.

Gestational age	18–20 weeks (Time 1)	24–26 weeks (Time 2)	30–32 weeks (Time 3)	36–38 weeks (Time 4)
Pregnancy anxiety				
Mean (SD)	1.91 (.53)	1.79 (.48)	1.78 (.48)	1.73 (.44)
(N)	(445)	(398)	(401)	(344)
State anxiety				
Mean (SD)	2.04 (.60)	1.96 (.61)	1.99 (.65)	2.06 (.63)
(N)	(442)	(400)	(407)	(333)
Perceived stress				
Mean (SD)	2.26 (.64)	[Not assessed]	2.27 (.70)	2.27 (.71)
(N)	(444)		(401)	(344)
Salivary cortisol <sup>a</sup>				
Mean (SD)	0.22 (.18)	0.30 (.23)	0.34 (.24)	0.40 (.22)
(N)	(438)	(414)	(401)	(341)

<sup>a</sup> Raw values, µg/dl.

2.3.2. *Pregnancy anxiety*

Pregnancy anxiety was measured with a published 10-item scale at each study visit ( $\alpha_{T1} = .80, \alpha_{T2} = .80, \alpha_{T3} = .82, \alpha_{T4} = .78$ ) assessing the extent to which women worried about their health during pregnancy, their baby's health, and their upcoming labor and delivery (Guardino & Dunkel Schetter, 2014; Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999). Sample items included: "I am fearful regarding the health of my baby" and "I am afraid that I will be harmed during delivery." Participants responded on a 4-point scale from 1 (never or not at all) to 4 (almost all of the time or very much). This instrument has been used in prior pregnancy studies in English and Spanish speaking women with evidence for its reliability and validity (Glynn, Dunkel Schetter, Hobel, & Sandman, 2008; Rini et al., 1999). Pregnancy anxiety scores were quite stable over the course of pregnancy in this study ( $r_s = .65-.80$ )

2.3.3. *Perceived stress*

Perceived stress was measured with a modified 12-item version of the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983) at each visit except for Time 2 (24–26 weeks) ( $\alpha_{T1} = .91, \alpha_{T3} = .93, \alpha_{T4} = .93$ ). This scale assessed how often participants felt stressed, upset, overwhelmed by daily hassles, or unable to cope with changes or challenges in the last 7 days. Sample items included: "How often have you felt that you were on top of things?" (reverse coded) and "How often have you found that you could not cope with all the things you had to do?" Participants responded on a 5-point scale from 1 (never) to 5 (almost always). Perceived stress scores were moderately to strongly correlated across visits ( $r_s = .51-.63$ )

2.3.4. *State anxiety*

State anxiety was measured with a 10-item brief version of the state subscale of the State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) assessing symptoms of anxiety over the past few days at each visit ( $\alpha_{T1} = .89, \alpha_{T2} = .90, \alpha_{T3} = .91, \alpha_{T4} = .91$ ). Sample items included "I am worried" and "I feel at ease" (reverse coded). Participants responded on a 4-point scale from 1 (not at all) to 4 (very much). State anxiety scores were strongly correlated across visits ( $r_s = .60-.63$ ).

2.3.5. *Medical risk variables*

Three medical or obstetric risk factors were created from information abstracted from medical charts by summing the total number of risks for (1) general health (e.g.,

diabetes, pulmonary disease), (2) baseline and historical medical factors that pose risk in pregnancy (e.g., pre-pregnancy weight under 100 lbs, pelvic inflammatory disease, previous myomectomy, previous abortion) and, (3) risk factors in the index pregnancy (e.g., anemia, threatened abortion, bacterial vaginosis, asthma, vaginal bleeding, high blood pressure). These medical risk factors were based on known risks of preterm birth and other adverse pregnancy outcomes. The number of risk factors ranged from 0 to 7 with means close to 1 in each category (see Table 1).

2.3.6. *Sociodemographics*

Demographic variables including age, self-identified ethnicity, education, income and parity were measured at the Time 1 visit (see Table 1).

2.4. *Statistical analyses*

Multilevel modeling (MLM), using HLM 7 software (Raudenbush et al., 2011), was used to explore the effects of pregnancy anxiety on initial levels of maternal cortisol at 19 weeks and trajectories of maternal cortisol. MLM treats the data in a hierarchical manner with observations nested within persons and it accounts for both *between-person* variation (between-person differences) and *within-person* variation (within-person changes).

*Time-invariant* predictors, those measured only once, were centered so that 0 was a meaningful value (e.g., the average age at the initial assessment, no obstetric or medical risk factors). The primary *time-varying* predictors, those measured multiple times during pregnancy (pregnancy anxiety, state anxiety and perceived stress) were parameterized as two variables to represent between-person differences and within-person variation (Hoffman & Stawski, 2009). These variables were parameterized in two different ways. In one set of analyses, the between-person variables reflected the means of pregnancy anxiety, perceived stress and state anxiety for each woman averaged across all four assessments. These variables were centered so that 0 was the sample mean. The within-person variables had occasion-specific values at each time point that reflected deviance from the woman's mean on that occasion. For example, if a woman's mean level of pregnancy anxiety was 2 and her level of pregnancy anxiety at Time 4 was 3, then her within-person value of pregnancy anxiety at Time 4 would be 1. In another analysis, between-person differences in pregnancy anxiety reflected differences in initial or baseline levels of pregnancy anxiety at 19 weeks

**Table 3**  
Bivariate correlations among independent variables.

Independent variable	1	2	3	4	5	6	7	8	9	10
1. Time 1 age	1.00									
2. Ethnicity (white 0, non-white 1)	-.20***	1.00								
3. Income	.41***	-.32***	1.00							
4. Education level	.42***	-.33***	.50***	1.00						
5. Parity (nulliparous 0)	.14**	.21***	-.06	-.15**	1.00					
6. General medical risk	.15**	-.03	-.03	.01	.05	1.00				
7. Historical obstetric risk	.21***	.10*	.01	.04	.22***	.23***	1.00			
8. Current obstetric risk	-.02	.16**	-.13**	-.12**	.10*	.25***	.25***	1.00		
9. Pregnancy anxiety (PA) <sup>a</sup>	-.07	.16**	-.19***	-.05	-.20***	.18***	-.02	.09 <sup>†</sup>	1.00	
10. State anxiety <sup>a</sup>	-.06	.12*	-.24***	-.13**	-.01	.15**	.12*	.10*	.57***	1.00
11. Perceived stress <sup>a</sup>	-.03	.20***	-.24***	-.18***	.07	.15**	.11*	.11*	.45***	.78***

Note: N = 448, \*\*\*  $p < .001$ , \*\*  $p < .01$ , \*  $p < .05$ , <sup>†</sup>  $p < .10$ , <sup>a</sup> Mean across all assessments (T1–T4).

of gestation. Within-person variation reflected deviations or changes over time from the baseline assessment (Berg et al., 2009). Weeks of gestation, the measure of time, was centered at 19 weeks. Cortisol collection time was a covariate, so it was grand-mean centered. Missing data was accommodated using maximum likelihood estimation. Two-hundred and seventy-two women had complete data, 114 were missing complete data from one assessment, 37 from two assessments and 25 had complete data for only one assessment.

We estimated a series of nested models using maximum likelihood estimation for fixed effects and restricted maximum likelihood for random effects. First, we estimated an unconditional means model to determine the amount of variation in cortisol due to between-person differences and within-person changes or variation over the course of pregnancy. Then we estimated an unconditional growth model with the addition of weeks of gestation to estimate initial levels of cortisol and change in cortisol over gestation. Cortisol sample collection time was included in all models. To ensure that any associations between pregnancy anxiety and cortisol were not due to actual medical risk or demographic factors, we first assessed if any of these variables were independently associated with cortisol. Only significant potential third variables (covariates) were included in the primary analyses. We then ran models with only the between-person and within-person pregnancy anxiety predictors followed by a model that included state anxiety, perceived stress and any significant demographic or medical risk covariates.

### 3. Results

Descriptive statistics for the primary variables illustrate the predicted rise in maternal cortisol over gestation (see Table 2). Bivariate correlations among the independent variables indicated that perceived stress and state anxiety were highly inter correlated, whereas pregnancy anxiety was moderately correlated with each of these variables (see Table 3).

Partial correlations adjusted for cortisol collection time indicated that pregnancy anxiety at Time 1, Time 2 and Time 3 were significantly correlated with maternal cortisol levels at Time 3 (see Table 4). Based on these results, we regressed cortisol at Time 3 over all three respective pregnancy anxiety measurements adjusting for sample collection time in order to test whether pregnancy anxiety at a particular time period in pregnancy independently predicted cortisol levels at Time 3. Results indicated that no single time point was independently associated with cortisol at Time 3 (all  $ps > .10$ ).

#### 3.1. Maternal cortisol trajectories

Consistent with the general increase in cortisol over gestation, the unconditional means model indicated that 78% of the total variation in cortisol was attributable to within-person variation and 22% of the total variation in cortisol was attributable to differences between women ( $ICC = .22$ ). The unconditional growth model indicated that on average there was a significant increase in cortisol over the course of pregnancy ( $\gamma = .034$ ,  $SE = .002$ ,  $p < .001$ ). The correlation between initial status and rate of change ( $r = -.93$ ) showed that higher cortisol levels at the initial assessment were associated with a decreased rate of change. The addition of the quadratic rate of change term was not significant, nor did its addition significantly improve model fit.

Demographic variables (age, level of education, self-reported income and self-identified ethnicity/race), parity and the medical risk indices were added into the model individually. Race/ethnicity was coded white (0) or non-white (1) in order to retain the largest sample and because race/ethnicity was not the main focus of these analyses. Neither demographic variables, medical risk indices nor parity were associated with either initial cortisol levels or cortisol trajectories ( $ps > .10$ ). Therefore, we did not adjust for any of these variables in the primary analyses.<sup>4</sup>

<sup>4</sup> Although medical risk was not a significant predictor of cortisol levels or trajectories in our preliminary analyses, we ran a model that included the medical risk variables. Mean levels of pregnancy anxiety remained a significant predictor cortisol trajectories ( $\gamma = .014$ ,  $SE = .004$ ,  $p = .002$ ).

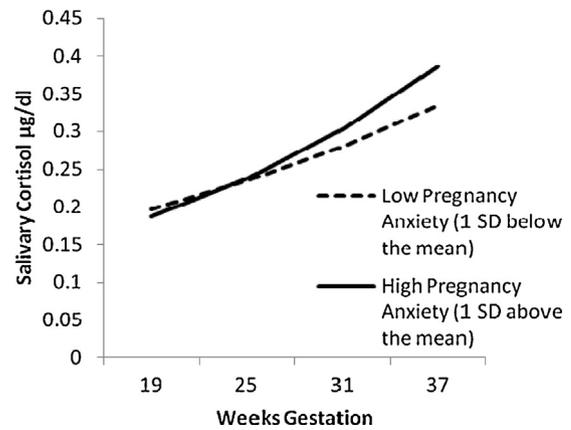


Fig. 1. Cortisol trajectories over gestation by between-person pregnancy anxiety.

#### 3.2. Primary analyses on pregnancy anxiety and maternal cortisol

First, we tested a model that included both mean levels of pregnancy anxiety averaged across all four assessments and within-person deviations from mean levels at each assessment simultaneously. We examined the degree to which mean pregnancy anxiety levels and being higher or (lower) on pregnancy anxiety relative to mean levels at each assessment predicted cortisol. Mean levels of pregnancy anxiety (between-person differences) were not associated with initial cortisol levels at 19 weeks ( $\gamma = -.060$ ,  $SE = .063$ ,  $p = .34$ ). However, as indicated by a significant between-person pregnancy anxiety by weeks of gestation interaction ( $\gamma = .013$ ,  $SE = .004$ ,  $p = .002$ ), higher mean levels of pregnancy anxiety were associated with steeper increases in cortisol trajectories over the course of pregnancy. To further explore this interaction and to determine when in gestation differences in cortisol levels emerged, we centered each week of gestation at 0 and tested the intercept differences in pregnancy anxiety. Significant differences in cortisol levels (intercepts) emerged beginning between 30 and 31 weeks of gestation ( $\gamma = .077$ ,  $SE = .040$ ,  $p = .052$  and  $\gamma = .090$ ,  $SE = .040$ ,  $p = .024$ , respectively) and persisted until the end of pregnancy (see Fig. 1). In contrast to predictions, within-person deviations from each woman's individual mean level of pregnancy anxiety did not predict cortisol ( $\gamma = .048$ ,  $SE = .050$ ,  $p = .33$ ). Thus, being higher (or lower) than a woman's individual mean pregnancy anxiety level on each measurement occasion was not associated with her cortisol levels.

In a second analysis, we tested a model that included both baseline levels of pregnancy anxiety at 19 weeks of gestation and within-person changes from baseline levels at each subsequent assessment (Berg et al., 2009).<sup>5</sup> We examined the degree to which baseline pregnancy anxiety levels and increases or decreases from baseline levels predicted cortisol. Results of this analysis were quite similar to the results of the previous analysis. Baseline levels of pregnancy anxiety (between-person differences) were not associated with initial cortisol levels at 19 weeks ( $\gamma = -.016$ ,  $SE = .052$ ,  $p = .76$ ). However, as indicated by a significant between-person pregnancy anxiety by weeks of gestation interaction ( $\gamma = .008$ ,  $SE = .003$ ,  $p = .016$ ), higher baseline levels of pregnancy anxiety were associated with steeper increases in cortisol trajectories. Changes (increases or decreases) in pregnancy anxiety from baseline were not associated with cortisol ( $\gamma = .070$ ,  $SE = .046$ ,  $p = .13$ ).

Finally, to determine if the effect of mean pregnancy anxiety on cortisol was being driven by pregnancy anxiety at any particular

<sup>5</sup> The sample for this analysis was 445 women because 3 cases were missing pregnancy anxiety at Time 1.

**Table 4**

Partial correlations between pregnancy anxiety and cortisol adjusting for cortisol sample collection time.

	Cortisol 18–20 weeks	Cortisol 24–26 weeks	Cortisol 30–32 weeks	Cortisol 36–38 weeks
Pregnancy anxiety T1 (N)	.00 (436)	–.03 (411)	.11* (397)	.05 (339)
Pregnancy anxiety T2 (N)		–.04 (389)	.16** (372)	.06 (313)
Pregnancy anxiety T3 (N)			.17** (388)	.08 (330)
Pregnancy anxiety T4 (N)				.06 (338)

Notes: \*\* $p < .01$ , \* $p < .05$ , different sample sizes reflect different numbers of cases of both pregnancy anxiety and cortisol at each measurement occasion.**Table 5**

Pregnancy anxiety predicting cortisol trajectories adjusting for state anxiety and perceived stress.

Parameters	Estimate	SE	p-Value
Intercept (initial status at 19 weeks)	1.647	.03	<.001
Between-person (BP) pregnancy anxiety	–.021	.08	.79
Between-person (BP) state anxiety	.032	.09	.73
Between-person (BP) perceived stress	–.047	.07	.52
Linear slope (gestational age)	.036	.00	<.001
BP pregnancy anxiety	.011	.00	.032
BP state anxiety	–.002	.01	.73
BP perceived stress	.003	.01	.55
Time collected	–.097	.01	<.001
Within-person pregnancy anxiety	.096	.06	.094
Within-person state anxiety	–.053	.04	.21
Within-person perceived stress	.038	.04	.35
Variances/covariances	Estimate	SE	
Intercept variance	.148	.02	
Covariance	–.005	.00	
Slope variance	.000	.00	

Notes:  $N = 447$  in this model because of one case with missing state anxiety. Between-person variables represent means across all 4 assessments. Within-person variables represent the deviation from each woman's individual mean at each assessment.

measurement occasion, we tested three additional models to determine if pregnancy anxiety at each remaining assessment (Time 2, Time 3, Time 4) was associated with cortisol trajectories. The results of these analyses revealed that pregnancy anxiety measured at Time 2 ( $\gamma = .012$ ,  $SE = .003$ ,  $p = .002$ ,  $n = 398$ ), Time 3 ( $\gamma = .010$ ,  $SE = .003$ ,  $p = .009$ ,  $n = 401$ ), and Time 4 ( $\gamma = .012$ ,  $SE = .004$ ,  $p = .006$ ,  $n = 344$ ) each predicted cortisol trajectories.<sup>6</sup>

### 3.3. Pregnancy anxiety as an independent predictor of maternal cortisol

To test whether mean levels of pregnancy anxiety remained a significant predictor of cortisol trajectories after adjusting for other measures of general distress, state anxiety and perceived stress were each added to the model individually. The results revealed that mean levels of pregnancy anxiety remained a significant predictor of cortisol trajectories after adjusting for both state anxiety ( $\gamma = .013$ ,  $SE = .005$ ,  $p = .009$ ) and perceived stress ( $\gamma = .01$ ,  $SE = .005$ ,  $p = .027$ ). Neither mean levels, nor deviations from mean levels of state anxiety or perceived stress were significant predictors of cortisol trajectories in these models. When state anxiety and perceived

stress were entered into the model together, pregnancy anxiety again remained a significant predictor of cortisol trajectories (see Table 5).

## 4. Discussion

In this study, pregnancy anxiety and maternal salivary cortisol levels were assessed in a sample of 448 women on four occasions during pregnancy. Repeated assessments of both pregnancy anxiety and cortisol permitted the use of multilevel modeling to test the extent to which within-person changes and between-person differences in pregnancy anxiety predicted prenatal cortisol trajectories. Results revealed that women with higher mean levels of pregnancy anxiety over the course of the study exhibited steeper increases in cortisol compared to women with lower mean levels. Furthermore, women who reported higher mean pregnancy anxiety had significantly elevated cortisol levels beginning between 30 and 31 weeks of gestation, and these elevations persisted for the rest of pregnancy. Of note, pregnancy anxiety also remained an independent predictor of cortisol after adjusting for state anxiety and perceived stress.

This study is the first to demonstrate that mean levels of pregnancy anxiety from mid to late pregnancy predict maternal cortisol trajectories across pregnancy and pinpoint when in gestation (30–31 weeks) these trajectories diverge resulting in cortisol level differences. Although we do not know why these trajectories diverge later rather than earlier in pregnancy, the present findings support the premise that pregnancy anxiety plays a role in modulating HPA activity in pregnant women. The results are consistent with previous studies demonstrating positive associations between pregnancy anxiety and prenatal HPA and placental activity (Mancuso et al., 2004; Obel et al., 2005).

By simultaneously taking into account both between- and within-person variation in pregnancy anxiety, the present findings clarify which type of variation is predictive of maternal cortisol. In doing so, the possibility of between- and within-person effects being confounded is reduced. Mean levels of pregnancy anxiety and baseline levels of pregnancy anxiety (between-person differences) predicted cortisol, whereas changes in pregnancy anxiety relative to mean levels or baseline levels (within-person changes) did not predict cortisol. Furthermore, regression analyses and additional multilevel modeling analyses suggested that mean pregnancy anxiety was a consistent predictor of cortisol trajectories. The regression analyses demonstrated that pregnancy anxiety measured at three occasions during pregnancy (T1: 18–20 weeks, T2: 24–26 weeks, and T3: 30–32 weeks) all predicted maternal cortisol, but not independently of each other. The multilevel analyses demonstrated that pregnancy anxiety at each time point predicted cortisol trajectories. Thus, the effect of mean pregnancy anxiety was not being driven by pregnancy anxiety at any particular measurement occasion.

<sup>6</sup> We note that each of these models includes a slightly different sample size due to differences in the number of cases with pregnancy anxiety scores at each assessment. Results did not significantly change when using Empirical Bayes estimates of the intercepts (pregnancy anxiety levels) at each assessment utilizing the entire sample of 448 women.

Although a few prior studies have shown that prenatal distress measures averaged across pregnancy are predictive of gestational length (Lobel, Dunkel Schetter, & Scrimshaw, 1992; Roesch et al., 2004), the majority of studies show that pregnancy anxiety at specific times in gestation or changes over time in pregnancy anxiety are predictive of birth and infant outcomes (e.g., Buitelaar et al., 2003; Buss et al., 2009; Mancuso et al., 2004). There are several potential explanations for why changes in pregnancy anxiety were not predictive of cortisol in the current study. First, some measures of pregnancy anxiety may be more sensitive to changes in pregnancy anxiety. For example, Mancuso et al. (2004) found a timing effect of pregnancy anxiety predicting HPA activity (pCRH) using a measure that assessed pregnancy anxiety with an adjective checklist (e.g., worried, jittery) and a specified timeframe of the past week. Second, although pregnancy anxiety measured in this study was relatively stable from mid to late gestation, pregnancy anxiety decreased over gestation on average. Thus, outcomes measured in other studies demonstrating timing effects such as infant development may be more sensitive than cortisol to small changes in pregnancy anxiety assessed with this measure. Additionally, the present study only assessed changes in pregnancy anxiety from mid to late gestation and potentially predictive changes from early in gestation were not included. Finally, because significant changes in cortisol occurred early in the third trimester, it is possible that there is an accumulation effect of pregnancy anxiety on cortisol levels.

It is difficult to directly compare these results to previous studies examining pregnancy anxiety and cortisol because of the use of different measures of pregnancy anxiety, different assessments of cortisol (cortisol awakening response (CAR), single assessments, early morning and evening cortisol) and different cortisol collection methods (saliva, plasma). In this study, cortisol trajectories diverged between 30 and 31 weeks of gestation suggesting why some cross-sectional studies measuring cortisol and pregnancy anxiety earlier in pregnancy did not find an association (e.g., Harville et al., 2009; Sikkema et al., 2001). However, one study did examine cross-sectional associations later in pregnancy with the same pregnancy anxiety measure as in the present study and still found no association (Davis & Sandman, 2010), but this study had a relatively smaller sample of women.

Our findings underscore the necessity of longitudinal designs to better understand how different types of prenatal distress are associated with maternal physiology and birth and infant outcomes. The use of slightly different conceptualizations and measures of pregnancy anxiety may result in inconsistent results with respect to effects on maternal physiology and birth and infant outcomes. Pregnancy anxiety is now conceptualized as a risk factor that is related to yet distinct from general distress, state and trait anxiety. Ongoing research is examining the properties and correlates of pregnancy anxiety (Dunkel Schetter & Glynn, 2011; Guardino & Dunkel Schetter, 2014). Elucidating when and how pregnancy anxiety affects these outcomes is critical for the development of theory and ultimately intervention.

In addition to implications for birth outcomes such as PTB (Dunkel Schetter, 2011), these results are relevant to understanding fetal and infant development because fetal exposure to excessive maternal cortisol has been shown to affect fetal HPA axis development and resultant infant stress regulation (Sandman et al., 2012). Even small changes in maternal cortisol may have a meaningful effect on fetal concentrations of cortisol. Fetal cortisol concentrations are much lower than maternal cortisol concentrations, so the addition of any maternal cortisol crossing the placental barrier can greatly alter fetal cortisol concentrations. For this reason, maternal cortisol has been thought to have a large impact (Gitau et al., 1998). Furthermore, it appears that maternal cortisol may influence fetal cortisol concentrations to an even greater extent when a woman

is highly anxious during pregnancy through the down-regulation of the enzyme 11 $\beta$ -HSD2 (Glover, Bergman, Sarkar, & O'Connor, 2009; O'Donnell et al., 2012). It is possible that women who are high in pregnancy anxiety not only produce more cortisol, but these increased levels may also affect the fetus to a greater extent because more cortisol is allowed to pass through the placental barrier.

A strength of this study was the careful characterization of medical and obstetric risks. An alternative explanation for our findings is that women with greater medical and obstetric risk factors may have higher pregnancy anxiety creating a spurious association between pregnancy anxiety and maternal cortisol. However, pregnancy anxiety was not associated with obstetric risk factors in prior pregnancies and was only marginally associated with obstetric risk factors in the current pregnancy. Moreover, adjusting for medical and obstetric risk factors did not alter the reported results. Therefore, it is unlikely that medical and obstetric risk factors explain the association of pregnancy anxiety and cortisol in this sample. However, continuing to explore these associations is important for future research.

Although the assessment of maternal cortisol at multiple time points during pregnancy is a strength of the study design, the collection of a single assessment at each time point is a potential limitation. To help mitigate this limitation, we carefully recorded the time of day each sample was collected and statistical adjusted for the sample collection time in all analyses. Nevertheless, future research should examine changes in diurnal cortisol over pregnancy and consider how diurnal cortisol slopes might change as a function pregnancy anxiety. For example, in one study, flatter diurnal cortisol slopes were associated with higher trait anxiety late in pregnancy (Kivlighan et al., 2008), but changes in diurnal slopes over pregnancy were not assessed.

## 5. Conclusion

Pregnancy anxiety is a relatively new concept and a seemingly powerful prenatal risk factor. Although strong evidence from animal models identifies cortisol as a key physiological mechanism explaining the effects of prenatal distress on birth and infant outcomes, there is not sufficient evidence in human studies. These results provide initial evidence for the association between pregnancy anxiety and maternal cortisol in humans. They demonstrated that higher mean levels of pregnancy anxiety from mid to late pregnancy were associated with steeper increases in maternal cortisol compared to lower levels and identified differences in cortisol levels beginning early in the third trimester. This association is a critical first link in understanding the physiological mechanisms through which pregnancy anxiety may exert its effects on birth and infant outcomes. Finally, these findings further highlight the need to understand how pregnancy anxiety affects maternal physiology – not just whether or not it does.

## Conflict of interest

No conflicts of interest declared.

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