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Maternal cortisol during pregnancy is related to infant cardiac vagal control

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Abstract

Background: Prenatal exposure to maternal psychological distress and glucocorticoids result in neurobiological adaptations within the fetus that increase risk for developing exaggerated emotional, behavioral, and stress responses to novelty and challenges in childhood. The current study investigated the influence of maternal depressed mood and cortisol during pregnancy on infant cardiac vagal control (CVC) to standardized laboratory challenge tasks.

Methods: The sample comprised 194 women and their infants. Maternal reports of depressed mood and salivary cortisol were assessed at 14 and 32 weeks gestational age. Linear regression was used to examine associations between maternal measures during early and late pregnancy, and infant CVC indexed via respiratory sinus arrhythmia (RSA) at rest and in response to laboratory tasks designed to elicit frustration when infants were 6 months of age. It was hypothesized that maternal depressed mood and cortisol would be associated with lower basal RSA and smaller decreases in RSA from baseline to challenge.

Results: A significant decrease in infant RSA from baseline to frustration tasks indicated that laboratory tasks elicited a reliable decrease in RSA from baseline to frustration among infants which is characterized by reduction in vagal efferent activity on the heart in response to challenge. Higher maternal cortisol, but not depressed mood, was associated with lower basal RSA and greater decrease in RSA from baseline to frustration. Associations between maternal cortisol and infant basal RSA were observed for both early and late pregnancy whereas the associations between prenatal cortisol and decrease in RSA from baseline to frustration were observed for early, but not late, pregnancy.

Conclusions: Maternal cortisol during pregnancy was associated with infant CVC at 6-months of age. Such influences may have enduring impacts on the child and important implications for the development of physical and mental health outcomes.

Key Words: Cardiac Vagal Control; Fetal Programming; Depressed Mood; Cortisol; Respiratory Sinus Arrhythmia

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1.0 Introduction

There has been increasing awareness that many physical and psychological disorders commonly observed in adulthood such as hypertension, diabetes, and depression have origins in fetal development (Barker, 2002; Calkins and Devaskar, 2011; Glover, 2011). Vulnerability may be conferred through the fine-tuning of stress-regulatory systems that occurs following exposure to insults during a sensitive period in utero or early postnatal life (Van den Bergh, 2011). To date, human and animal evidence has convincingly reported that exposure to excess cortisol and maternal prenatal psychological distress (i.e., an amalgamation of comorbid subjective stress, anxiety, and depressed mood) alters the function of one of the co-acting stress-regulatory systems - the hypothalamic-adrenal-pituitary (HPA) (Braun et al., 2013; Field, 2011; Kajantie, 2006; Kapoor and Matthews, 2008; Seckl and Holmes, 2007). Relatively less attention has been paid to the influences of prenatal maternal cortisol and psychological distress on the second stress-regulatory system (i.e., the autonomic nervous system; ANS). The current study focuses on the associations between maternal prenatal cortisol and depressed mood on infant autonomic function

Empirical evidence suggests that exposure to prenatal maternal depressed mood results in neurological adaptations within the fetus that increase risk for low birth weight (Grote et al., 2010) and the development of exaggerated emotional (Davis et al., 2007; Hayes et al., 2013), behavioral (van der Wal et al., 2007), and HPA-axis responses (Fernandes et al., 2014; Oberlander et al., 2008) to stressors. While the mechanisms by which antenatal exposure to depressed mood confers future risk are not well understood, a central claim of the fetal programming hypothesis is that the maternal HPA-axis is a central component in the cascade from maternal depressed mood to fetal and infant development. Nevertheless, it is not clear whether depressed mood and glucocorticoids have combined (joint or interactive effects) or independent effects on infant development. Despite compelling theoretical reasons to believe that the effects of prenatal glucocorticoids may be greatest when they are accompanied by high levels of

psychological distress, the evidence to date suggests that they may be independent predictors of child outcomes (e.g., Davis and Sandman, 2010).

Glucocorticoids readily cross biological barriers such as the placenta (Seckl and Holmes, 2007) and exert a direct influence on fetal development. Although the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) regulates fetal exposure by converting cortisol to inactive cortisone, human studies have indicated that increased cortisol consequent to depressed mood (Diego et al., 2009; Field et al., 2004b) and subsequent decreased efficiency of the placenta at converting cortisol to cortisone (Oberlander et al., 2008) may lead to elevated in-utero cortisol exposure. Given that cortisol receptors are highly expressed in the developing brain (Sánchez et al., 2000) and are important for maturation of the developing CNS (Kapoor et al., 2008), prenatal exposure to glucocorticoids can impair feedback mechanisms of the fetal HPA-axis, resulting in altered basal function and responsiveness in the offspring (Kapoor et al., 2008; Maccari and Morley-Fletcher, 2007; Seckl and Holmes, 2007). For example, among premature infants, prenatal maternal treatment with synthetic glucocorticoids is reported to suppress HPA-axis responses to heel lance and physical examination stressors (Davis et al., 2006).

1.1 The Autonomic Nervous System

Compared to our understanding of the associations between maternal depressed mood and cortisol during pregnancy and fetal/infant HPA-axis development, relatively little is known about the influences of depressed mood and cortisol on the developing ANS (Porges and Furman, 2011). The relative dearth of literature in this area is surprising for several reasons. First, many adult diseases that have origins in fetal development are related to cardiovascular/ANS health (Barker, 2002; Calkins and Devaskar, 2011). Second, catecholamine's (e.g., epinephrine) are synthesized by the adrenal gland when the sympathetic branch of the ANS predominates; these hormones act to down regulate 11β-HSD2 gene expression in human placental cells (Sarkar et al., 2001) and may increase cortisol exposure in-utero. Elevation of catecholamines have been noted among women with antenatal depression compared to non-depressed controls (Field et al., 2004b; Lundy et al., 1999). Finally, cortisol is involved in the maturation of neurobiological areas involved in ANS regulation (Harris and Seckl, 2011).

The ANS comprises sympathetic and parasympathetic branches which innervate most internal body organs and generally act in an opposing fashion – in general, the sympathetic branch acts as an accelerator system and the parasympathetic branch acts as a decelerator system. Considerable interest has been paid to the role of the parasympathetic nervous system in the development of physical and psychological disorders (Rottenberg, 2007; Thayer et al., 2009; Thayer and Lane, 2007). Parasympathetic function can be measured noninvasively using cardiac vagal control (CVC) - the neural regulation of heart rate (HR) by parasympathetic influences emanating from the vagus nerve. The vagus represents an integrated bidirectional neural system between visceral organs and the brain. Vagal stimulation of the heart serves as a "brake" to maintain HR below the heart's autonomous rhythm. In this sense, basal CVC can be thought of as a physiological reserve capacity with higher levels reflecting more efficient vagal control and a greater capacity to adjust to environmental demands. Basal CVC has been hypothesized to indicate physiological flexibility that predisposes engagement with the physical and social environments (Beauchaine, 2001). In support of this, higher basal CVC is associated with effortful control of attention (Taylor et al., 2014), socio-emotional competence (Liew et al., 2011), mental development (Feldman et al., 2014), and cognitive performance (Staton et al., 2009), while lower basal CVC is associated with disorganized attachment (Holochwost et al., 2014) and depression among adults (Kemp et al., 2010; Rottenberg, 2007).

According to *Porges' polyvagal theory* (Porges, 2007), CVC withdrawal to challenge reflects the reduction of vagal efferent activity on the sino-atrial node as a means of mediating metabolic output (e.g. respiration) by way of increasing HR. This process reflects the function of the ANS and a larger set of integrated neurobehaviorual systems thought to facilitates attention, regulation of affect, and social engagement (Thayer et al., 2009). Thus, basal CVC represents potential energy reserves while CVC reactivity indicates the efficiency with which such reserves can be accessed. Research has generally supported the notion that effective CVC regulation is characterized by CVC withdrawal (Graziano and Derefinko, 2013). Further, relatively more CVC withdrawal to challenge among children is associated with fewer internalizing, externalizing and cognitive/academic problems (Graziano and Derefinko, 2013).

1.2 Depressed Mood During Pregnancy and Fetal/Infant Cardiac Vagal Control

Previous studies investigating the associations between depressed mood during pregnancy and fetal/infant CVC have focused on basal CVC. Fetuses of mothers who reported higher depressed mood during the third trimester had higher basal HRs (Allister et al., 2001) and greater HR reactivity when exposed to acute stress (Monk et al., 2004). Studies using more direct assessment of infant parasympathetic function have reported similar results - maternal depression (Field et al., 2004a; Field et al., 1995) and maternal negative emotionality (Field et al., 2003; Ponirakis et al., 1998) were inversely associated with infant basal CVC. Similarly, one notably large prospective investigation of 528 infants confirmed an inverse association between prenatal symptoms of depression or anxiety and infant basal CVC (Dierckx et al., 2009).

1.3 Cortisol During Pregnancy and Cardiac Vagal Control

Preliminary evidence suggests that the exogenous administration of synthetic glucocorticoids during pregnancy reduces CVC in human fetuses. Fetal CVC was reduced following the administration of betamethasone delivered during the third trimester to 8 pregnant mothers at risk of preterm labor (Schneider et al., 2010). Further, short-term variability in fetal HR increased transiently after the administration of betamethasone followed by a significant reduction that spanned 2-7 days (Lunshof et al., 2005; Senat et al., 1998). In addition, cortical thinning was observed in children between 6 and10 years of age who were exposed to synthetic glucocorticoids during fetal development (Davis et al., 2013). Cortical thinning was most notable in the anterior cingulate cortex, an area associated with the autonomic regulation of HR. In sum, prenatal exposure to synthetic glucocorticoids may reduce CVC, but it is unclear whether endogenous cortisol has a similar effect.

1.4 The Present Investigation

The present study tested the hypotheses that maternal depressed mood and diurnal cortisol during pregnancy are associated with infant CVC at 6-months of age. Given that higher basal CVC and greater reduction in CVC from baseline to challenge are associated with more optimal child outcomes and that elevated maternal depressed mood and cortisol during pregnancy are associated with less optimal child

outcomes, we hypothesized that higher levels of maternal depressed mood and cortisol during pregnancy would be associated with lower basal CVC and less reduction in CVC from baseline to frustration in infants. Further, given that depressed mood and cortisol could have either combined (interactive) or independent effects on infant CVC, we included interaction terms in our models to determine whether the effects of cortisol depend on level of prenatal depressed mood. To test these hypotheses, we prospectively collected measures of depressed mood and diurnal salivary cortisol from pregnant women in early and late gestation and assessed infant CVC during rest and frustration tasks at 6 months of age.

2.0 Methods

2.1 Participants

The sample is comprised of women and their infants enrolled in a prospective longitudinal study of nutrition during pregnancy (see www.apronstudy.ca for further details). Women were not enrolled if they reported: (a) using steroid medication, (b) smoking, (c) consuming alcohol or illicit drugs, (d) recent dental work or tendency for oral bleeding (leading to false elevation of salivary cortisol; (Kivlighan et al., 2004), (e) known pregnancy or fetal complications (e.g., preeclampsia, fetal genetic anomalies), or (f) illness during data collection (e.g., fever). Infants were excluded if born preterm or chart reviews noted congenital heart defects (e.g., arrhythmias). Participants provided informed consent prior to each procedure. The study protocol was approved by the University of Calgary Health Research Ethics Board.

2.2 Procedures

Pregnant women collected saliva samples (for cortisol assay) and reported on their depressed mood in both early pregnancy (T1) at 14.65 (*SD* = 3.54) weeks gestational age, and in late pregnancy (T2) at 32.47 (*SD* = 0.94) weeks gestational age. Participants attended an individualized training session at T1 during which they received instructions about the saliva collection device and the personal digital assistant (PDA) data collection device. The PDA was programmed to ring as a reminder for participants to collect saliva samples, respond to standardized questionnaires (not reported here), and to record exact timing of saliva samples.

Infants were brought to the laboratory at approximately 6-months of age (*M* = 24.39 weeks, *SD* = 2.62). In all cases infants were brought by their mothers and in some cases their fathers were also present. Figure 1 is a graphical depiction of the timeline and sequence of tasks included in the infant laboratory assessment. Upon arrival at the laboratory, infant behavioral state was assessed by the investigators using the Neonatal Behavior Assessment Scale (Brazelton and Nugent, 1995) which assesses infant arousal on a 7-point scale from 1 "quiet sleep" to 7 "crying." Infants underwent a 10-minute laboratory acclimation period and were then outfitted with an ambulatory heart rate monitor (described in more detail below). The heart rate monitor remained connected to the infant during the laboratory session and for 24-hour ambulatory monitoring (data from ambulatory monitoring not presented here).

Following the acclimation period, infants completed components of the Laboratory Temperament Assessment Battery (Lab-TAB prelocomotor version 3.1; (Goldsmith and Rothbart, 1996), which consisted of several phases completed in the following order:

Baseline. Infants were seated on their mothers' lap during a 3-minute baseline recording and mothers were asked to keep movement and interaction with their infant at a minimum.

Attention. Visual attention was assessed using a series of colorful images presented on a 30 X 38 cm computer screen. Data from this task are not included in the present analysis.

Frustration. A series of frustration tasks were presented. Previous research has shown that the selected tasks reliably elicit a decrease in CVC in infants (Bergman et al., 2007). The infant was seated in a highchair at the end of a table such that the infant could easily reach objects placed on the table. The infant's mother was seated to the child's left and the experimenter to the child's right. Frustration tasks were discontinued if the experimenter deemed that the infant was too distressed to continue (we used as a guiding rule 20 s of hard crying) or if the parent elected to stop.

The frustration series began with a toy retraction task. The child chose and then played with a novel toy for 15 s after which the mother gently moved the toy outside of the infant's reach and left it there for 30 s. The toy was then returned to the child for 15 s prior to administration of trails 2 and 3. At the end of the 3rd trial the infant was allowed to play with the toy during setup for the next task.

The second frustration task involved an attractive toy placed behind a clear plexiglas barrier. The infant was first allowed to engage with a stuffed rabbit. After 15 s of play, the experimenter placed a plexiglas barrier, approximately 9 cm in front of the infant, and asked the parent to remove the toy from the infant and place it directly behind the barrier. The toy remained behind the barrier for 30 s, after which the child was allowed to play with it for 15 s. This procedure was repeated 2 more times after which the infant was allowed to play with the toy during setup for the next task.

The final frustration task was a gentle arm restraint task. The parent was asked to stand behind the infant and gently grasp the infant's forearms and firmly hold them to the infant's side for 30 seconds. An attractive toy was placed directly in front of the infant. A 15 second recovery period was allowed prior to a 2nd trail during which the infant played with the toy or was comforted by the parent. At completion of the arm restraint task the parent and infant were invited to play with a box of toys for approximately 15 minutes and this marked the end of the laboratory assessment.

2.2 Measures

Prenatal and postnatal maternal depressed mood was assessed using the *Edinburgh Depression Scale (EPDS*; Cox et al., 1987). The EPDS is a reliable, valid, and sensitive 10-item self-report measure of depression symptoms in the perinatal period. High scores ($\geq$ 12) are strongly correlated with physician diagnosis of Major Depressive Disorder (Jomeen and Martin, 2007). While originally designed to measure symptoms of postnatal depression, the EPDS has been shown to be a reliable and valid measure of depression symptoms among pregnant women with adequate sensitivity (79%) and specificity (85%) (Jomeen and Martin, 2007).

Maternal cortisol during pregnancy was collected using whole saliva obtained from under the tongue with the Salimetrics Oral Swab (Salimetrics, State College, PA). Saliva collection was completed at home over 2 consecutive days (excluding weekends) at both T1 and T2, for a total of four days during pregnancy. Samples were obtained on the following schedule: upon waking, 30 min after waking, and at 1130h and 2100h. To facilitate adherence to study protocols, the PDA was programmed to allow a 20-minute response window following the signal, after which data were considered missing.

Participants were instructed to turn on the PDA upon awakening, record their waking time, and collect a saliva sample. The PDA was programmed to ring 30 minutes later at which time participants collected their second saliva sample of the day. This procedure was used to assess the cortisol awakening response (CAR). Each sampling event generated a unique code on the PDA corresponding to a prelabeled saliva tube and provided a precise time stamp for each saliva sample. The EPDS questionnaire was completed at home at the T1 and T2 assessments.

Pregnant women were asked to refrain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing their teeth within 30 minutes prior to saliva collection and to report adherence to these guidelines. Saliva samples were stored at -80°C until they were shipped frozen to Salimetrics, State College, PA. Samples were assayed for salivary cortisol using an enzyme immunoassay that has a lower limit of sensitivity of 0.007 μ*g*/dl, standard curve range from 0.012 to 3.0 μ*g*/dl, and average intra- and inter-assay coefficients of variation 3.5% and 5.1%, respectively. Method accuracy, determined by serial dilution was 100.8% and 91.7%. A random 10% of samples were assayed in duplicate to confirm reliability; the intra-assay coefficient of variation and correlation coefficient between the duplicate tests was 3.5%. Mean values from duplicate samples were used for analysis.

Infant CVC was indexed using ***Respiratory Sinus Arrhythmia (RSA)*** - variability in HR (i.e., interbeat or R-R intervals) that fluctuates in phase with spontaneous respiration [see (Grossman and Taylor, 2007)]. RSA was measured using continuous heart period recordings of R-R intervals sampled at a rate of 1000Hz. Infants wore a Firstbeat Bodyguard recording device (Firstbeat Technologies, Oy Jyvaskyla, Finland) connected to two leads by pre-gelled (Ag/AgCl) disposable electrocardiograph (ECG) electrodes attached beneath the right clavicle and to the left ribcage.

2.4 Data Reduction

Infant RSA was quantified using Nevrokard advanced Heart Rate Variability (HRV) analysis software. Heart period recordings were screened for artifacts according to recommendations (Berntson et al., 1990). An algorithm was set to detect ectopic beats, defined as interbeat intervals below 200 ms, above 2000 ms, and those that differed from the previous and subsequent 50 interbeat intervals by a value

greater than 20%. Interpolation was used to correct ectopic and missing beats. No participant in the final sample required more than 5% data interpolation. After scanning for artifacts, HRV was quantified using Fast Fourier Transformations (FFT). R-R intervals during each laboratory challenge were re-sampled and partitioned into 45-second segments (3 segments occurring during each task). Each segment was linearly detrended and subject to a Hamming window. Power spectral density functions were then assembled and estimates of power were adjusted to account for the attenuation produced by the Hamming window. Infant RSA was quantified as the average power spectral density of R-R fluctuations occurring in the respiratory band (.24 – 1.04 Hz) recommended for use with infants (Bar-Haim et al., 2000). RSA for each task was calculated as the mean RSA value occurring across the three 45 second segments composing each task. The variable $RSA_{Frustration}$ was created as the average value (ms^2/Hz) for $RSA_{Toy\ Retraction}$, $RSA_{Toy\ Barrier}$, and $RSA_{Restraint}$. $\Delta RSA_{Frustration}$ was calculated by subtracting $RSA_{Frustration}$ from $RSA_{Baseline.}$ Using this formula, higher values reflect greater decrease in RSA from baseline to frustration. $RSA_{Baseline}$ and $\Delta RSA_{Frustration}$ were log transformed to adjust for skew and kurtosis.

Maternal cortisol during pregnancy was used to calculate two summary measures of HPA axis function: the cortisol awakening response (CAR) as a measure of the morning increase in cortisol and the area under the curve from ground (AUCg) as a measure of total cortisol secretion (Fekedulegn et al., 2007). Cortisol AUCg has been conceptualized as a measure of total hormone output (Pruessner et al., 2003) and the CAR reflects the increase in cortisol typically observed following waking and is hypothesized to occur in preparation for the demands of the upcoming day (Fries et al., 2009). Samples used to calculate the CAR were excluded if they were taken more than 15 minutes after waking (for the waking sample) and more than 50 minutes after waking for the 2nd sample (Okun et al., 2010). The AUCg calculation included the waking, 1130h and 2100h samples. Cortisol AUCg values were log transformed to adjust for skew.

2.5 Statistical Procedures.

Data Screening. Data from 203 mother-infant dyads were screened for potential outliers. Univariate outliers were identified as values that exceeded a z-score of 3.29 and adjusted according to

recommendations (Tabachnick and Fidell, 2012). No more than three values (1.5%) were adjusted for any variable. Missing data was handled using multiple imputation (Little and Rubin, 2002) with 10 imputations. Multivariate outliers were assessed using Mahalanobis distances. Nine participants (4.4%) had values that exceeded $\chi^2(8)_{critical}$ of 26.12 and were removed from analyses resulting in a final sample size of 194.

Prenatal predictors of $RSA_{Baseline}$ and $\Delta RSA_{Frustration}$. Associations between maternal depressed mood and cortisol and infant RSA were examined using hierarchical linear models. Infant RSA was entered as the criterion variable, covariates (gestational age, infant sex, infant birth weight, infant behavioral state, maternal age, maternal parity, socioeconomic status, and maternal postnatal depressed mood) were entered in STEP1, prenatal cortisol and depressed mood were entered in STEP 2, and the interaction terms between cortisol and depressed mood were entered in STEP 3. Models for early and late pregnancy predictors were run separately to allow for different effects of early and late exposures on infant RSA. Gestational age at T2 was not entered as a covariate in the model for late pregnancy because of the homogeneous age at measurement. The final models reported below did not include infant sex, infant birth weight, socioeconomic status, and maternal parity because these variables were not associated with the outcomes and did not influence the associations between the predictors and the outcomes.

3.0 Results

The initial sample of 301 pregnant mothers and their infants was reduced to a final sample of 203 because of preterm birth (n = 18), problems with data acquisition (n = 36), unusable data (i.e., required > 10% data interpolation; n = 20), incomplete recordings (n = 11) and infant crying or fussing during baseline recording (n = 13).

3.1 Adherence to ambulatory saliva collection

Adherence to ambulatory saliva collection was excellent. Women returned 97.5 percent (1,591 out of a possible 1,632) of salivary samples during early pregnancy and 90 percent (1,469 out of a possible 1,632) during late pregnancy.

3.2 Change in Infant RSA from Baseline to Frustration

In order to determine whether the frustration tasks elicited a vagal response, we conducted a paired sample *t*-test which indicated that infant RSA decreased from baseline to frustration, M_{Diff} = -54.66, t (193) = 2.14, $p < .05$, Cohen's d = 0.16, see Table 1 for mean values.

3.3 Association between Maternal Depressed Mood and Cortisol

Table 2 presents bivariate correlations among study variables. Maternal depressed mood was not associated with AUCg or CAR during early or late pregnancy, all r's < .12, all p's > .05. Given that mood and cortisol were independent, we included both within the regression models.

3.4 Early Pregnancy Predictors of $LogRSA_{Baseline}$ and $Log\Delta RSA_{Frustration}$

Table 3 presents the results of regression models assessing the associations between early-pregnancy maternal characteristics and infant RSA. The CAR was not associated with $RSA_{baseline}$ (β = -0.013, p = .13). However, a relatively larger maternal CAR was associated with greater infant decrease in RSA from baseline to frustration, $\Delta RSA_{Frustration}$ (β = 0.012, p = .015), accounting for 3.2% of variance.

Maternal AUCg during early pregnancy was associated with lower infant $RSA_{Baseline}$ (β = -0.57, p = .016) and greater decrease in RSA from baseline to frustration, $\Delta RSA_{Frustration}$ (β = 0.32, p = .028). Semipartial correlations indicated that maternal AUCg during early pregnancy accounted for 2.9% and 2.6% of unique variance in infant $RSA_{baseline}$ and $\Delta RSA_{Frustration}$, respectively. Figures 2a and 2b illustrates the association between maternal cortisol during early pregnancy and the predicted change in infant RSA between baseline and recovery using non-transformed RSA values to aid with interpretability.

Maternal depressed mood during early pregnancy was not associated with infant $RSA_{Baseline}$ or $\Delta RSA_{Frustration}$. Further, there were no early pregnancy depressed mood by cortisol interactions on infant $RSA_{Baseline}$ or $\Delta RSA_{Frustration}$.

3.5 Late Pregnancy Predictors of $LogRSA_{Baseline}$ and $Log\Delta RSA_{Frustration}$.

Table 4 presents the results of regression models assessing the associations between late-pregnancy maternal characteristics and infant RSA. In contrast to observations in early pregnancy, a larger maternal CAR during late pregnancy was not associated with $\Delta RSA_{Frustration}$ (β = 0.005, p = .99), but

it was associated with lower infant $RSA_{Baseline}$ (β = -0.02, p = .006) accounting for 3.8% of unique variance.

There was a marginally significant association between maternal AUCg during late pregnancy and infant $RSA_{Baseline}$ (β = -0.51, p = .062), such that higher AUCg was associated with lower infant $RSA_{Baseline}$. There was no association between maternal AUCg during late pregnancy and infant $\Delta RSA_{Frustration}$ (β = 0.07, p = .72).

Maternal depressed mood during late pregnancy was not associated with infant $RSA_{Baseline}$ or $\Delta RSA_{Frustration}$. Further, there were no late pregnancy maternal depressed mood by cortisol interactions on infant $RSA_{Baseline}$ or $\Delta RSA_{Frustration}$.

4.0 Discussion

We prospectively investigated the impact of maternal depressed mood and cortisol during pregnancy on infant basal CVC and CVC reactivity to frustration at 6-months postpartum. Maternal cortisol, but not maternal depressed mood, was associated with infant parasympathetic cardiac regulation at rest and in response to frustration, albeit the proportion of variance accounted for was small and varied by stage of pregnancy and metric of cortisol. In early pregnancy it was cortisol AUCg that predicted infant basal CVC whereas in late pregnancy the CAR was associated with basal CVC. Associations between cortisol measures and infant CVC reactivity to frustration were observed only for early pregnancy cortisol AUCg and CAR. Taken in the context of other studies showing that maternal cortisol during pregnancy is involved in programming of the fetal/infant HPA axis, these results suggest that maternal cortisol has broad effects on the development of infant stress physiology that extend to ANS function.

The results support the hypotheses that maternal cortisol was associated with infant CVC. The findings for basal CVC were consistent across cortisol measures and timing – higher maternal cortisol was associated with lower infant basal CVC. Both elevations of total cortisol (AUCg) in early-pregnancy and a larger CAR in late pregnancy were associated with lower basal CVC among infants. Lower basal CVC is indicative of less well developed cardiac vagal control and suggestive of less efficient vagal

control and reduced physiological capacity to adjust to environmental demands (Beauchaine, 2001). Greater exposure to maternal cortisol during gestation may reduce infant ability to develop the physiological reserve capacity that is needed to regulate responses to physical and social environments; and this reduced capacity may help to explain the association between lower basal CVC and reduced socio-emotional competence (Liew et al., 2011) and effortful control of attention (Taylor et al., 2014) in children with elevated cortisol exposure. These results are also consistent with previous reports of an inverse association between exposure to synthetic glucocorticoids in utero and fetal basal CVC (Lunshof et al., 2005; Schneider et al., 2010; Senat et al., 1998). Our findings suggest that the effects of exogenous glucocorticoids on fetal CVC are similar to the effects of endogenous maternal cortisol on infant CVC.

The results for changes in CVC from baseline to frustration were limited to early pregnancy. Both AUCg and CAR in early pregnancy were associated with *greater* decreases in infant CVC from baseline to challenge. The direction of this effect was the reverse of what we expected based on the majority of previous literature. Decreases in CVC between baseline and task are thought to facilitate attention, regulation of affect, and social engagement (Thayer et al., 2009) and children with greater decreases in CVC subsequent to challenge have fewer internalizing, externalizing and cognitive/academic problems compared to children with smaller decreases in CVC (Graziano and Derefinko, 2013). Given that greater internalizing, externalizing and cognitive/academic problems are associated with elevated maternal cortisol we reasoned that higher cortisol exposure would be associated with *less* reduction in CVC from baseline to frustration. The fact that greater maternal cortisol was associated with *greater* decreases in CVC from baseline to frustration seems to suggest that greater cortisol exposure has a salutary effect on CVC regulation during challenge. However, recent theoretical and empirical works suggest that studying basal CVC and CVC reactivity in isolation may obscure the relationship between parasympathetic regulation and adaptive functioning and that some combination of these indicators may be needed to clarify the role of vagal regulation in the development of cognitive and socio-emotional competencies in children (Del Giudice et al., 2011; Hinnant and El-Sheikh, 2013; Yaroslavsky et al., 2014). In fact, Yaroslavsky and colleagues (Yaroslavsky et al., 2014) report that the combination of low

basal CVC and relatively large decreases in CVC from baseline to challenge is an 'atypical' pattern of CVC regulation that is associated with heightened risk for psychopathology. Considering combinations of CVC indicators to predict developmental outcomes is a relatively new approach that requires replication. Nevertheless, the current results are consistent with this approach and highlight the potential importance of considering patterns of parasympathetic regulation across multiple indicators.

The pathophysiological mechanisms through which maternal cortisol might influence cardiac vagal control of the fetus are not yet well understood. One possibility is through hormone-mediated reduction in myelination of the vagus nerve and neural areas associated with stress reactivity (Harris and Seckl, 2011). According to the *model of neurovisceral integration* (Thayer and Lane, 2000), the vagus nerve serves as a structural and functional link between the heart and a highly integrated constellation of neural structures known as the central autonomic network (CAN; (Benarroch, 1993). Glucocorticoids readily cross the placenta (Seckl and Holmes, 2007), affect the expression of more than one thousand genes in fetal brain cells (Salaria et al., 2006), and play a role in the maturation of the CAN (see (Braun et al., 2013; Harris and Seckl, 2011). Exposure to glucocorticoids in-utero has been reported to reduce cerebral myelination in animals (Antonow-Schlorke et al., 2009) and cortical thickness in human adolescents (Davis et al., 2013), likely due to the catabolic effects of steroids. Interestingly, fetal development is also the time during which myelination of vagal fibers begins (Porges and Furman, 2011), and this process may be sensitive to the effects of glucocorticoids.

The effects of maternal cortisol on infant CVC appear to be sensitive to timing of exposure. In general, more associations between maternal cortisol and infant CVC were observed during early pregnancy than during late pregnancy. This was especially true for vagal reactivity which was associated with maternal cortisol only in early pregnancy. This result, along with typical developmental timelines, suggests that early exposure to cortisol may exert relatively greater influence on infant CVC during basic development of neural and cardiac structures than on myelination of the vagus nerve. Primary formation and development of the cardiovascular and nervous systems occur within early pregnancy (Moore et al., 2008) whereas myelination of the vagal efferents occur primarily from 32 weeks gestational age until 6-

months post-partum (Porges and Furman, 2011). Thus, it seems likely that the effects of maternal cortisol in early pregnancy on infant CVC derive, at least in part, from the effects of cortisol on the formation and development of neural and cardiac structure. This interpretation is consistent with the general developmental principle that biological systems are most vulnerable to external influence during periods of rapid cell division (Davis et al., 2013; Moore et al., 2008). It is well known that biological systems are particularly vulnerable to environmental exposures during periods of rapid cell division (Kajantie, 2006) and both the timing and duration of fetal exposures have important implications for understanding developmental outcomes (Davis and Sandman, 2010; Moore et al., 2008).

The hypotheses that maternal depressed mood during pregnancy is associated with lower infant basal CVC and less decrease in CVC from baseline to frustration challenge was not supported. These results are contrary to previous studies that reported significant effects of depressed mood and anxiety during pregnancy on infant basal CVC (Field et al., 1995; Ponirakis et al., 1998) and HRs (Monk et al., 2004). There are several key differences between the current and previous reports that may account for these differences in results. First, depressed mood was used as a measure of psychological distress in this investigation whereas previous studies have measured a cluster of maternal mood variables (e.g., anxiety, depressed mood, stress, anger) and it is not yet clear which prenatal mood variables have the greatest impact on infant CVC. Our data suggest that aspects of psychological distress other than depression may be driving the association with infant CVC. Second, the majority of mothers in our sample reported high SES and low levels of depressed mood whereas previous studies have assessed pregnant mothers who reported greater adversity and psychiatric symptoms (e.g., low SES, clinically relevant levels of anxiety or depression, teenage pregnancy). That is, the associations between psychological distress and infant CVC reported in clinical populations may not generalize to community samples of healthy women. Finally, prior studies have compared groups of pregnant mothers with and without clinically significant psychiatric symptoms (Field et al., 1995; Monk et al., 2004), reporting differences in the level or developmental trajectory of infant basal CVC. The association between maternal depressed mood and infant autonomic function may be confined to clinical elevation in depression.

4.1 Limitations

There are several limitations to the present investigation. First, respiration could influence CVC (Grossman and Taylor, 2007) but infant respiration was not assessed and CVC was not adjusted for respiratory change. Nevertheless, a meta-analysis of 44 studies reported no association between infant CVC reactivity and respiration (Graziano and Derefinko, 2013). Second, while suggestive of prenatal programming, the observed results are observational and it is not possible to determine the cause of our observations. It is known, for example, that early parent-infant interactions influence infant CVC (Feldman and Eidelman, 2003) and although we accounted for maternal postnatal depressed mood this variable may not capture important aspects of the postnatal relational environment that are relevant to change in CVC from baseline to challenge. Future studies should consider including measures of postnatal maternal cortisol to better account for maternal experiences of stress in the postnatal period. Third, participants in this study were primarily White, middle to upper class, married women and caution should be taken when generalizing these results to low SES and ethnically diverse populations. Finally, although a range of scores were observed on the measure of depressed mood, the sample mean indicated that the majority of women were not significantly depressed. The distribution of depressed scores was positively skewed and kurtotic. Non-normal distributions tend to result in an underestimation of variance and may obscure a linear relationship between maternal depressed mood and infant CVC. Nevertheless, statistical procedures conducted on large samples ($n > 200$) are usually robust against skewness and kurtosis (Tabachnick and Fidell, 2012) .

4.2 Conclusions

This prospective longitudinal investigation found that maternal cortisol during pregnancy was associated with reduced basal CVC and greater decreases in CVC from baseline to frustration among infants at 6-months of age, albeit the associations were small and depended on the metric used to summarize cortisol. The results suggest that prenatal endogenous maternal cortisol alters the function of stress regulatory systems in the offspring, independent of postnatal characteristics and in a manner similar to that of exposure to exogenous glucocorticoids. Such influence may have enduring impacts on the

children's adaptation to their environments and important implications for the development of chronic physical health (e.g., cardiovascular disease and hypertension) and mental health disorders (Barker, 2002; Glover, 2011).

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Highlights

- Assessed maternal cortisol during pregnancy in relation to infant vagal control
- Higher cortisol was associated with lower basal cardiac vagal control
- The effect was independent of maternal prenatal/postnatal depressed mood
- The results suggest that prenatal cortisol has widespread effects on infant development

Table 1

Sample and Descriptive Characteristics

Variable	***Percent***	
Nulliparous	37.9	
Married or common law	99.5	
Annual household income		
More than $100,000/year	56.8	
$70,001 - $100,000	23.9	
$40,001 - $70,000	12.9	
Less than $40,000/year	5.8	
Variable	***M(SD)***	***Missing data (%)***
$RSA_{Baseline}$ (ms^2/Hz)	461.93 (425.53)	0
$RSA_{Frustration}$ (ms^2/Hz)	396.92 (320.24)	1.4
CAR T1 (μ*g*/dl)	1.79 (3.39)	4.4
CAR T2 (μ*g*/dl)	3.02 (3.64)	9.8
CortAUCg T1	176.93 (51.65)	2.5
CortAUCg T2	257.76 (63.44)	6.9
Maternal depressed mood T1	5.25 (3.94)	13.3
Maternal depressed mood T2	5.08 (3.63)	8.9
Maternal age	31.80 (4.06)	0
Gestational age at birth (weeks)	39.17 (1.71)	0
Gestational age at T1 (weeks)	14.65 (3.52)	0
Gestational age at T2 (weeks)	32.47 (0.94)	0
Infant age at stress testing (weeks)	24.43 (2.65)	0
Maternal postnatal depressed mood	3.60 (3.50)	10.8
Infant birth weight (g)	3356.91 (564.92)	0

Note. N = 194 (100 male); CAR = Cortisol Awakening Response; CortAUCg = Cortisol Area Under the Curve from Ground; RSA = Respiratory Sinus Arrhythmia; T1 = Early Pregnancy; T2 = Late Pregnancy

Table 2

Bivariate Correlations between Infant RSA, Maternal Cortisol, and Covariates

Variable	1	2	3	4	5	6	7	8	9	10	11
1. $LogRSA_{Baseline}$											
2. $Log\Delta RSA_{Frustration}$	.31**										
3. CAR T1 (µg/dl)	-.08	.11									
4. CAR T2 (µg/dl)	-.17*	-.06	.38**								
5. LogCortAUCg T1	-.16*	.10	-.14	.01							
6. LogCortAUCg T2	-.10	.00	-.04	-.10	.36**						
7. Depressed mood T1	.06	.12	.03	-.04	.04	-.02					
8. Depressed mood T2	-.03	-.03	.00	-.05	-.04	-.10	.57**				
9. Postnatal depressed mood	-.13	.03	-.03	-.03	.09	-.06	.34**	.39**			
10. Infant behavioral state	-.03	.02	-.11	.01	.12	.07	.05	.10	.10		
11. GAT1	.02	.10	-.09	-.04	.22**	.00	.11	.08	.03	.05	
12. Maternal age	.06	.00	.08	.11	.02	.12	-.17*	.21**	-.05	-.06	.05

Note. * $p < .05$; ** $p < .01$. CAR = cortisol awakening response; CortAUCg = cortisol area under the curve from ground; GA = gestational age; T1 = early pregnancy; T2 = late pregnancy.

Table 3

Regression Models for Early Pregnancy Predictors of Infant $RSA_{Baseline}$ and $\Delta RSA_{Frustration}$ (ms^2/Hz)

	Effect	Estimate (SE)	*t*-value (df)
$LogRSA_{Baseline}$	Intercept	3.47(0.61)	
	Gestational age at T1	0.003(0.008)	0.71(186)
	Infant behavioral state	-0.009(0.06)	0.15(186)
	Maternal age	0.007(0.007)	1.04(186)
	Postnatal depressed mood	-0.018(0.24)	1.77(186)
	CAR T1 (µ*g*/dl)	-0.013(0.008)	1.60(186)
	LogCortAUCg T1	-0.53(0.24)	2.24*(186)
	Depressed mood T1	0.01(0.008)	1.52(186)
	CAR T1(µ*g*/dl) by depressed mood T1	0.003(0.003)	1.22(184)
	LogCortAUCg T1 by depressed mood T1	-0.004(0.07)	0.06(184)
$Log\Delta RSA_{Frustration}$	Intercept	1.52(0.40)	
	LogRSABaseline	0.22(0.044)	4.98**(185)
	Gestational age at T1	0.005(0.005)	0.99(185)
	Infant behavioral state	0.008(0.037)	0.21(185)
	Maternal Age	-0.001(0.004)	0.24(185)
	Postnatal depressed mood	0.0053(0.006)	0.43(185)
	CAR T1 (µ*g*/dl)	0.012(0.005)	2.44*(185)
	LogCortAUCg T1	0.32(0.14)	2.20*(185)
	Depressed mood T1	0.004(0.005)	0.77(185)
	CAR T1(µ*g*/dl) by depressed mood T1	-0.001(0.001)	0.72(184)
	LogCortAUCg T1 by depressed mood T1	-0.003(0.039)	0.07(184)

Note. † $p < .10$; * $p < .05$; ** $p < .01$. CAR = cortisol awakening response; CortAUCg = cortisol area under the curve from ground; T1 = early pregnancy.

Table 4

Regression Models for Late Pregnancy Predictors of Infant $RSA_{Baseline}$ and $\Delta RSA_{Frustration}$ (ms^2/Hz)

	Effect	Estimate (SE)	*t*-value (df)
$LogRSA_{Baseline}$	Intercept	3.56(0.71)	
	Infant behavioral state	0.00(0.06)	0.00(187)
	Maternal age	0.009(0.007)	1.26(187)
	Postnatal depressed mood	-0.016(0.10)	1.66(187)
	CAR T2 (µ*g*/dl)	-0.02(0.008)	2.73**(187)
	LogCortAUCg T2	-0.52(0.28)	1.86†(187)
	Depressed mood T2	0.003(0.01)	0.28(187)
	CAR T2(µ*g*/dl) by depressed mood T2	-.001(0.00)	0.30(185)
	LogCortAUCg T2 by depressed mood T2	0.04(0.07)	0.62(185)
$Log\Delta RSA_{Frustration}$	Intercept	2.21(0.54)	
	LogRSABaseline	0.20(0.045)	4.47**(186)
	Infant behavioral state	0.009(0.037)	0.24(186)
	Maternal age	-0.001(0.004)	0.33(186)
	Postnatal depressed mood	0.006(0.006)	1.01(186)
	CAR T2 (µ*g*/dl)	0.000(0.005)	0.00(186)
	LogCortAUCg T2	0.07(0.20)	0.35(186)
	Depressed mood T2	-0.004(0.005)	0.76(186)
	CAR T2(µ*g*/dl) by depressed mood T2	-.001(0.001)	0.61(184)
	LogCortAUCg T2 by depressed mood T2	-0.007(0.04)	0.17(184)

Note. † $p < .10$; ** $p < .01$. CAR = cortisol awakening response; CortAUCg = cortisol area under the curve from ground; T2 = late pregnancy.

Figure 1

Timeline and sequence of infant laboratory assessment at 6 months.

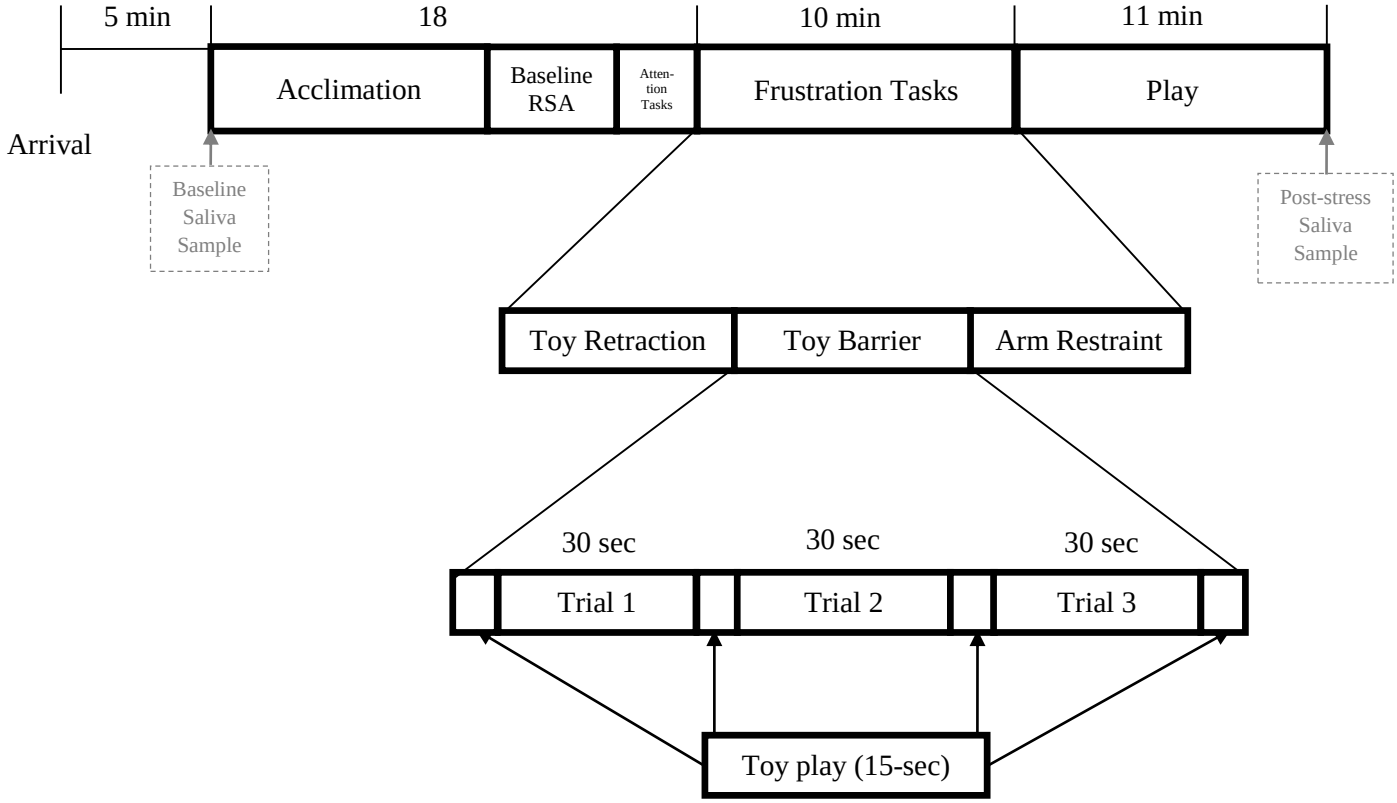
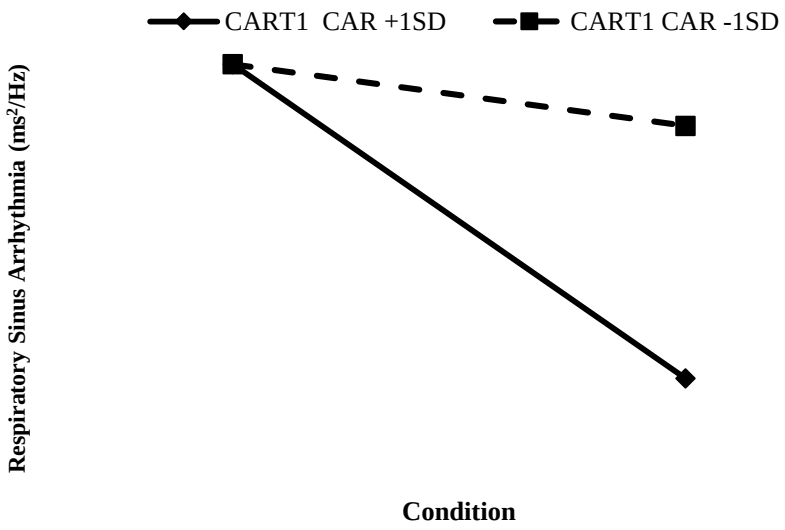


Figure 2a

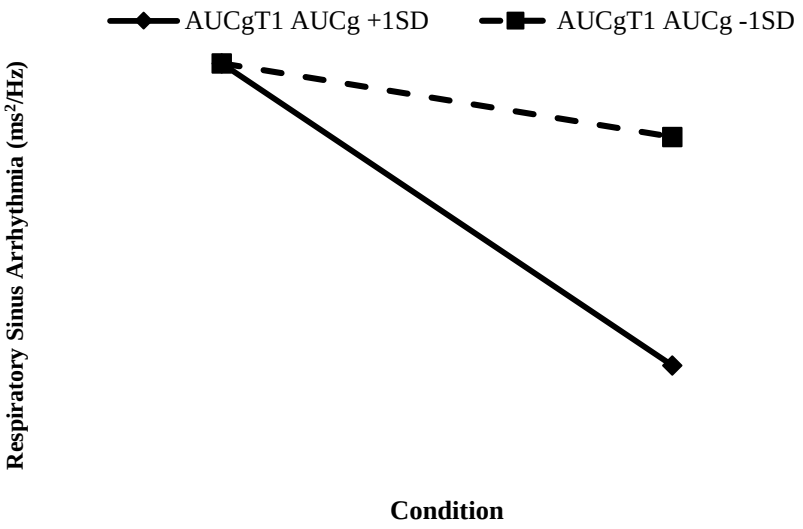
Predicted change in RSA from Baseline to Frustration as a function of maternal CAR in early pregnancy.



Note: CAR = cortisol awakening response. The dashed and solid lines represents ΔRSA for infants of mothers with a CAR one standard deviation below and above the sample mean, respectively.

Figure 2b

Predicted change in RSA from Baseline to Frustration as a function of maternal AUCg in early pregnancy.



Note: AUCg = area under the curve from ground. The dashed and solid lines represents ΔRSA for infants of mothers with a CAR one standard deviation below and above the sample mean, respectively.