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Richoux, Sarah Elizabeth

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2019

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The Relationship of Maternal Perinatal Stress and Stress Hormones to Birth Outcomes

by
Sarah Richoux

DISSERTATION

Submitted in partial satisfaction of the requirements for degree of
DOCTOR OF PHILOSOPHY

in

Nursing

in the

GRADUATE DIVISION
of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

DocuSigned by:
Sandra Weiss Sandra Weiss
29B9816487EE497... Chair

DocuSigned by:
Abbey Alkon Abbey Alkon

DocuSigned by:
Franck, Linda Franck, Linda
5475D2A5ED0E4FC...

Committee Members

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by
Sarah Richoux

Dedication

To my teachers who influenced my decision to pursue a career in nursing and research: Jane McAullife and Dr. Jennifer Doering.

Acknowledgements

I feel fortunate for the time that I have had as a student at the University of California, San Francisco. I knew as an undergraduate in Wisconsin that I wanted to pursue a career as a nurse scientist. When I graduated, I was encouraged to dream big and pursue a PhD at UCSF. I appreciated that potentially what will allow me to do the most good with a career in research is to learn how to conduct research from one of the best schools of nursing in the world.

I am grateful to have such supportive mentors around me that believe in an ability that I sometimes struggle to see in myself. My diverse and substantive experiences while at UCSF have helped me feel ready to continue to dream big and build on this experience to become an independent investigator. Thank you to Dr. Kathryn Lee, for being my initial advisor and meeting with me after I graduated from nursing school. And a special thank you to Dr. Sandra Weiss, for being my advisor, mentor, and dissertation chair. I appreciate how Dr. Weiss helped me cultivate my research skills by providing me with so many learning experiences. She's also provided me with support and kindness on a personal level. I look forward to having her as a lifelong mentor. I appreciate Dr. Abbey Alkon and Dr. Linda Franck for serving on my dissertation committee. Dr. Laura Wagner and Dr. Linda Park helped me learn how to educate future nurses during my teaching residency. I have gained valuable insights from the brilliant researchers I have worked had the pleasure of working with: Nina Ahlers, Sabra Bell, Dr. Crystal Epstein, Dr. Caryl Gay, Dr. Sharon Kidd, Dr. Cherry Leung, Dr. Midori Nakajima, and Dr. Sandy Niemann. I am grateful my family was supportive of my dedication to my academic pursuits. Finally, thank you to the participants whose data I used for my dissertation, it has been a privilege to be let into the lives of so many families, and I appreciate their help with advancing science and research.

The Relationship of Maternal Perinatal Stress and Stress Hormones to Birth Outcomes

Sarah Richoux

Abstract

Background and Purpose

Adverse birth outcomes such as preterm birth and low birth weight threaten both children's survival and their long term health. Current evidence suggests a potential link between stress during pregnancy and adverse birth outcomes. Some research suggests that high maternal perceived stress may shorten the length of gestation, and increase the risk of an infant being small for gestational age or low birth weight. However, findings are not consistent across studies. In addition, it is not clear whether perceived stress or biological measures of stress (such as cortisol) may best predict stress-related birth outcomes and whether these relationships are moderated by sex of the fetus. Lastly, research is not clear on whether perceived stress and cortisol are associated with one another or may represent uniquely different components of the stress response. The overall purpose of this study was to clarify the relationship between perceived stress and cortisol values, their roles as predictors of infant birthweight or gestational age, and the moderating role of fetal sex on stress-related birth outcomes.

Methods

The sample included 58 women who were recruited in obstetric clinics during their third trimester of pregnancy. They ranged in age from 23 to 47 years. Women completed Cohen's Perceived Stress Scale and provided 8 saliva samples across 2 days between 26 and 40 weeks gestation. Salivary samples were used to determine average cortisol level, cortisol awakening response (CAR), diurnal slope, and total cortisol excreted throughout the day (AUC_G). Electronic medical records were used to derive a measure of perinatal risk and acquire data on infant gestational age, weight, and sex at birth. Correlation coefficients were used to determine

relationships between perceived stress and the 4 cortisol parameters. Logistic regression models were computed to determine the odds of infants being born preterm or low birth weight as a result of their mothers' self-reported stress and cortisol parameters. The moderating role of fetal sex was also examined in these models.

Results

On average, women reported a moderate amount of perceived stress. For the total sample, women's perceived stress was not related to any cortisol parameters. However, women who were ≤ 29 weeks of gestation in their pregnancy showed opposite and stronger associations between perceived stress and their mean cortisol level, cortisol awakening response, and AUC_G than did women who were ≥ 30 weeks of gestation when assessed. Perceived stress had no relationship to either low birth weight or preterm birth. One cortisol parameter did show a significant effect on birth outcome, but was moderated by fetal sex. Male infants whose mothers had higher salivary CAR during the third trimester of pregnancy had greater odds of being born low birth weight.

Conclusion

Findings indicate the need for further research on cortisol response at different pregnancy stages, as well as inclusion of self-report and different cortisol parameters when assessing stress. Results also suggest that a woman's elevated CAR during the 3rd trimester could serve as an indicator of risk for low birth weight male infants. Further research is needed to replicate these findings in a larger sample that has more heterogeneity and the power to examine moderating effects of fetal sex. If future findings support the role of CAR in predicting risk, salivary CAR sampling could be a valuable method to assist in mitigating women's risk for delivering an infant of low birth weight and related morbidities.

Table of Contents

Chapter 1.....	1
Introduction	
References.....	7
Chapter 2.....	10
The relationship among cortisol and perceived stress to birth outcomes: an integrative review	
References.....	33
Chapter 3.....	45
Cortisol and self-reported stress during the third trimester of pregnancy	
References.....	62
Chapter 4.....	76
Associations among women’s perceived stress and cortisol parameters during	
Pregnancy	
References.....	93
Chapter 5.....	106
Discussion	
References.....	117

List of Figures

Figure 2.1.....	41
Search Strategy	
Figure 3.1.....	75
Salivary Cortisol Values across the Day	

List of Tables

Table 2.1.....	42
Birth Outcome Data	
Table 2.2.....	43
Internal Validity	
Table 2.3	44
Control for Confounders	
Table 3.1.....	71
Demographic, Clinical and Stress Descriptors for the Sample	
Table 3.2.....	72
Salivary Cortisol Parameters	
Table 3.3.....	73
Pearson Correlations between Women’s Perceived Stress and their Salivary Cortisol Parameters	
Table 3.4	74
Pearson Correlations between Women’s Perceived Stress and their Salivary Cortisol Parameters by Stage of 3 rd Trimester	
Table 4.1.....	102
Demographic, Clinical, and Stress Descriptors for Women in the Sample	
Table 4.2	103
Clinical Descriptors for All Infants in the Sample and by Infant Sex	
Table 4.3	104
Logistic Regression for the Effects of Maternal Perceived Stress, Maternal Cortisol Awakening Response, and Fetal Sex on Delivery of a Low Birth Weight Infant	

Table 4.4 105

Pearson Correlations of Maternal Perceived Stress and Cortisol Parameters During Pregnancy
with Infant Gestational Age and Birth Weight

Introduction

The Study Problem

The prevalence of adverse birth outcomes in the U.S. represents a major public health concern. Nearly 9.85% of births are preterm, but the reasons for prematurity are not known more than 50% of the time (Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). In 2016, 8.17% of infants were born low birthweight, and 1.4% very low birthweight (Martin et al., 2018). Infants can also be small for gestational age (SGA), with their small size often a result of intrauterine growth restriction (Marconi et al., 2008).

Preterm birth and other adverse birth outcomes are leading contributors to neonatal morbidity and mortality (WHO, 2015). Cardiovascular disease, cognitive delays, autism and diabetes are potential issues infants may face later on in life when birth outcomes are suboptimal (Saigal & Doyle, 2008). Similarly, children born low birth weight often face behavioral challenges such as inability to focus and increased arousal (Debattista, Huffman, Alkon, Cooper, & Weiss, 2015; Weiss, 2005; Weiss, Wilson, & Morrison, 2004). In addition, infants born SGA have dysregulated autonomic reactivity in response to caregiving stress (Osterholm, Hostinar, & Gunnar, 2012; Schaffer et al., 2009). When SGA infants enter puberty, there is some evidence that males experience adrenal hypoactivity (Beck Jensen et al., 2011).

Current evidence suggests a link between stress during pregnancy and adverse birth outcomes (Lilliecreutz, Larén, Sydsjö, & Josefsson, 2016; McEwen, 2007; Wadhwa, Entringer, Buss, & Lu, 2011). There is evidence that maternal perceived stress may impact length of gestation, risk of preterm birth, and birthweight (Gavin, Nurius, & Logan-Greene, 2012; Kane, Dunkel Schetter et al., 2014; Lau, 2013; Sable & Wilkinson, 2000). However, the mechanisms for how stress impacts fetal growth and birth timing remain poorly understood.

Cortisol and Birth Outcomes

Biomarkers of stress that indicate the functioning of the Hypothalamic-Pituitary-Adrenal (HPA) axis include hormones such as cortisol, ACTH, and corticosterone, as well as inflammatory markers such as cytokines. However, it is not known what may be the best biomarker measure of stress in predicting risk for adverse birth outcomes. Although there are many stress biomarkers of interest, a common one that is studied is cortisol because of its central role in functioning of the HPA axis and its ease of measurement (Corwin et al., 2013; Kirschbaum & Hellhammer, 1994). This dissertation will focus on cortisol as a key stress biomarker and examine its relationship to self-reported stress.

Seven studies have found that women whose cortisol profiles are different from expected cortisol norms (i.e., high overall cortisol values, elevated cortisol awakening response (CAR), dampened afternoon trajectory) have greater risk for delivery of infants who are lower birth weight and fewer weeks gestation (Baibazarova et al., 2013; Bolten et al., 2011; Buss et al., 2009; D'Anna-Hernandez et al., 2012; Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011; Ghaemmaghami, Dainese, La Marca, Zimmermann, & Ehlert, 2014; Hoffman, Mazzoni, Wagner, Laudenslager, & Ross, 2016). One study found no relationship between cortisol and birth outcome (Goedhart et al., 2010).

Perceived Stress and Birth Outcomes

In four studies, findings indicate that women who report greater stress during pregnancy are more likely to deliver a low birthweight infant and have an infant born early (Gavin et al., 2012; Hoffman et al., 2016; Lau, 2013; Sable & Wilkinson, 2000). Two studies found no relationship between perceived stress and birth outcome (Ghaemmaghami et al., 2013; Goedhart et al., 2010).

Perceived Stress versus Cortisol as Predictors of Birth Outcome

Although four studies above show a positive relationship between self-reported stress and adverse birth outcomes, different findings emerge when perceived stress and cortisol are examined together as predictors. Research shows that cortisol trajectories or cortisol means are more predictive of low birthweight and shorter gestation than responses on self-administered, perceived stress questionnaires (Baibazarova et al., 2013; Bolten et al., 2011; Cheng & Pickler, 2010; Entringer et al., 2011; Hoffman et al., 2016; Kivlighan et al., 2008) In these studies, perceived stress has not been as strong a predictor of birth outcomes. In addition, when studies use both a self-evaluation of stress and cortisol, an individual's self-appraisal of stress is *not* always predictive of cortisol values (Baibazarova et al., 2013; Bolten et al., 2011; Ghaemmaghami et al., 2014; Harville, Savitz, Dole, Herring, & Thorp, 2009; Hoffman et al., 2016; Kivlighan et al., 2008; Kramer et al., 2013; Salacz, Csukly, Haller, & Valent, 2012; Shaikh et al., 2011; Valladares, Pena, Ellsberg, Persson, & Hogberg, 2009).

Statement of the Problem

Experiencing high levels of stress in the year leading up to pregnancy and stress during pregnancy have been linked to delivering an infant who weighs less, is born early and at a risk of being born small for gestational age (SGA) (Dole et al., 2003; Heaman et al., 2013; Khashan et al., 2014). However, findings are not consistent across studies (Bolten et al., 2011; Kivlighan, DiPietro, Costigan, & Laudenslager, 2008; Larsen et al., 2013; Pryor et al., 2003; Wakeel, Wisk, Gee, Chao, & Witt, 2013). One reason for conflicting findings across studies may be differences in how stress is operationalized and measured. Little is known about whether specific methods of assessing stress during pregnancy may be better predictors of birth outcomes than others. The incongruence across findings could be related to differences in timing of when data collection

occurred during pregnancy, procedures for collecting and calculating cortisol, and variations in controlling for potential confounders of perceived stress, cortisol expression, or birth outcomes. In addition, it is not clear whether perceived stress and biological measures of stress (such as cortisol) are associated with one another, or instead may measure entirely different components of stress.

Purpose of Study

The aims of this dissertation were threefold:

- 1) To determine if there is a relationship between women's self-reported stress and four salivary cortisol values: a) overall mean cortisol level, b) Cortisol Awakening Response (CAR), c) Cortisol Diurnal Trajectory, and d) Cortisol Area Under the Curve (AUC_G).
- 2) To determine whether women's self-reported stress or their cortisol parameters during the third trimester of pregnancy best predict an infant being born preterm or low birth weight;
- 3) To examine the moderating role of fetal sex in the relationship of perceived stress and cortisol to preterm birth or low birth weight.

Operational Definitions

Perceived Stress for this study was defined as a feeling of overload that occurs when an individual's coping abilities are exhausted and was measured by Cohen's PSS-10 (Cohen, Kamarck, & Mermelstein, 1983).

Overall Mean Cortisol Level was the average cortisol value across 2 days of sampling; samples were collected 4 times daily from wake time through 4pm in the evening.

Cortisol Awakening Response (CAR) was the difference between cortisol levels from wake time to 45 minutes following wake time (Alder et al., 2011).

Diurnal trajectory or decline (slope) was the linear degree of change in cortisol levels across the day from morning to evening, excluding the morning awakening response.

Area under the curve across the day (AUC_G) measures the total cortisol output across samples collected during the day, considering the difference between individual cortisol samples and the time between each sampling period. The formula developed by Pruessner, et al. (2003) was used to calculate this index.

Preterm birth was defined as birth prior to 37 weeks' gestation.

Low birth weight (LBW) was defined as 2,500 grams or less at birth (Martin, Hamilton, Osterman, Driscoll, & Mathews, 2017).

Significance of the Study

This dissertation research adds to the current body of knowledge in the field by attempting to clarify relationships among perceived stress, cortisol parameters, and birth outcomes. The study included women who are at high risk of an adverse birth outcome. Other studies have typically excluded women who may have a complicated, high risk pregnancy. Findings may help identify the best measures for assessment and prediction of stress-related effects on adverse birth outcomes. More targeted stress screening for women during pregnancy may help identify women at particular risk so they can receive interventions to mitigate adverse birth outcomes. This dissertation research used data from a NIH-funded cohort study (Weiss, RO1 HD081188).

Organization of the Dissertation

The dissertation includes 4 chapters following this introductory chapter. The first chapter is a review of the literature guided by the STROBE criteria for cohort studies. The review examined evidence to date regarding a) the relationship between reported perceptions of stress and cortisol values during pregnancy, and b) the relationship of perceived stress and cortisol

parameters to adverse birth outcomes. The third chapter describes the rationale, methods and results related to Aim 1 of the dissertation. A discussion of implications of the findings is also provided. The fourth chapter describes the rationale, methods and results related to Aims 2 and 3 of the dissertation, as well as a discussion of their potential meaning and implications. The final chapter represents a synthesis and overall integration of the dissertation findings.

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**Relationships among Cortisol, Perceived Stress, and Birth Outcomes:
An Integrative Review**

Abstract

Objective: Individual studies have shown that both a woman's self-reported stress during pregnancy and her cortisol values may impact length of gestation and birthweight. However, the literature has not been integrated to determine the degree to which studies converge regarding the effects of maternal perceived stress and cortisol on birth outcomes or whether these potential predictors of outcome are associated with one another.

Methods: Using STROBE guidelines, studies were reviewed to determine the current state of knowledge in this field. The terms prenatal stress, psychological stress, cortisol, pregnancy and birth were searched on PubMed and Embase for publications between 2010 and 2019.

Results: After screening abstracts for inclusion criteria and STROBE standards, 11 articles were included in the final review. Substantial heterogeneity existed in the demographic characteristics, geographical locations and level of clinical risk across samples of the 11 studies. Only a few studies found a relationship between self-reported stress and cortisol.

Conclusions: Although some studies found a relationship of perceived stress to birth outcomes, most research found that cortisol was more strongly correlated with birth outcomes than self-reports of stress. Comparison across studies was challenging because cortisol was acquired from varied sources (e.g. saliva, hair, blood) that used different metric values of cortisol in different ways. The timing of sampling during pregnancy also was inconsistent. This integrative review suggests that knowledge regarding the effects of stress on birth outcomes would be advanced if studies examine both self-reports of stress and cortisol parameters as

uniquely different stress indicators, and measure varied sources of cortisol (e.g. saliva and hair) at several time points during pregnancy.

Introduction

The prevalence of adverse birth outcomes (preterm birth, low birth weight, and small for gestational age) in the U.S. represents a major public health concern. Nearly 10% of births are preterm, but the reasons for prematurity are not known more than 50% of the time (Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). In 2016, 8.2% of infants were born low birthweight, and 1.4% very low birthweight (Martin et al., 2018). Infants can also be small for gestational age (SGA), often a result of intrauterine growth restriction (Marconi et al., 2008).

Adverse birth outcomes are leading contributors to neonatal morbidity and mortality (WHO, 2015). Cardiovascular disease, cognitive delays, autism and diabetes are potential issues infants may face later in life when birth outcomes are suboptimal (Saigal & Doyle, 2008). Similarly, children born with low birth weight often face behavioral challenges such as inability to focus and increased arousal (Debattista, Huffman, Alkon, Cooper, & Weiss, 2015; Weiss, 2005; Weiss, Wilson, & Morrison, 2004).

Current evidence suggests a link between stress during pregnancy and adverse birth outcomes (Lilliecreutz, Larén, Sydsjö, & Josefsson, 2016; McEwen, 2007; Wadhwa, Entringer, Buss, & Lu, 2011). Research indicates that maternal perceived stress may impact length of gestation, risk of preterm birth, and birthweight (Gavin, Nurius, & Logan-Greene, 2012; Kane, Dunkel Schetter, Glynn, Hobel, & Sandman, 2014; Lau, 2013; Sable & Wilkinson, 2000). Experiencing high levels of stress in the year leading up to pregnancy and stress during pregnancy also have been linked to delivering SGA infants (Dole et al., 2003; Heaman et al., 2013; Khashan et al., 2014). However, findings are not consistent across studies (Kivlighan,

DiPietro, Costigan, & Laudenslager, 2008; Larsen et al., 2013; Pryor et al., 2003; Wakeel, Wisk, Gee, Chao, & Witt, 2013). One reason for conflicting findings across studies may be differences in how stress is operationalized and measured. Some studies assess perceived stress through a variety of self-report methods, while others assess various biomarkers of stress. Little is known about whether specific methods of assessing stress during pregnancy may be better predictors of birth outcomes than others. In addition, it is not clear whether perceived stress and cortisol are associated with one another, or instead may measure entirely different components of stress.

Six previous reviews of the literature were identified that examined how stress during pregnancy affects birth outcomes (Cherak et al., 2018; Duthie & Reynolds, 2013; Entringer, Buss, & Wadhwa, 2015; Graignic-Philippe et al., 2014; Shapiro et al., 2013; Zijlmans, 2015). Three reviews were systematic and were limited to an examination of the effects of cortisol secretion on birth outcomes (Cherak et al., 2018; Graignic-Philippe et al., 2014; Zijlmans, 2015), while three others were structured as non-systematic reviews (Duthie & Reynolds, 2013; Entringer, Buss, & Wadhwa, 2015; Shapiro et al., 2013). Overall, the conclusion of these reviews affirmed the lack of consistent findings across studies and that much is not understood about the effects of either exposure to cortisol or perceived stress on birth outcomes. The reviews also noted the lack of clarity regarding how alterations in cortisol may be affected or affect perceptions of stress at different times during gestation.

For this integrative review, perceived stress is conceptualized as a process involving feelings of overload and an inability to feel control over unexpected occurrences (Cohen, Kamarck, & Mermelstein, 1983), without any specification or labeling of particular stressors that may cause the stress. The review will focus on cortisol as a key biomarker of stress. Although there are many stress biomarkers of interest, a common one that is studied is cortisol because of its central

role in functioning of the Hypothalamic-Pituitary-Adrenal axis and its ease of measurement (Corwin et al., 2013; Kirschbaum & Hellhammer, 1994). The purpose of this paper is to provide a synthesis and analysis of published research to date to determine the degree to which studies converge regarding the effects of perceived stress and cortisol on birth outcomes, and whether self-reported stress and cortisol measures are associated with one another. Recommendations for future research will also be made.

Methods

An integrative review of the literature was performed in December, 2017 using two electronic databases. Studies were included if the following were examined: anthropometric data, subjective stress, cortisol sampling during the pregnancy period, and involvement of human participants. The evaluation of the quality of publications included was informed by the STROBE checklist. The STROBE checklist is a guide that outlines desirable reporting standards in cohort, case-control and cross-sectional studies (von Elm et al., 2014).

The search was limited to nine years, to capture studies most relevant to the US President signing the Prematurity Research Expansion and Education for Mothers who deliver Infants Early (PREEMIE) Act from 2006 and its Reauthorization (P.L. 113-55) in 2013 (Williamson et al., 2008). This act called on the research community to work with health departments and other key stakeholders to identify potential antecedents to preterm birth (PTB) (Health, 2017). The search was limited to humans, since we cannot measure psychological stress in animals, animal HPA activity does not resemble that of humans over time, and psychological stress is a core concept for this literature review. Using PubMed and Embase, the following terms were searched: stress, psychological, hydrocortisone, cortisol, pregnancy, and infant.

Results

The process used in the search is shown in **Figure 1.1**. The search yielded 94 publications from PubMed and 25 publications from Embase. Eleven articles were duplicates; therefore 108 abstracts were screened for inclusion. Eighty-one publications were eliminated because they did not include anthropometric data. Four publications had no subjective stress measure. Four publications captured cortisol outside of the pregnancy period. Two publications used animals in the research. Seven articles were reviews, and one was not written in English. Two additional publications were identified through a hand search and included in the review (Cheng & Pickler, 2010; Hoffman, Mazzoni, Wagner, Laudenslager, & Ross, 2016). The final group of 11 studies were each approved by the appropriate ethics committees for human research at their institutions. The design and main findings from each study are described in the overview that follows.

Overview of the Studies: Design and Main Findings

The purpose of Baibazarova et al.'s (2013) longitudinal study of 158 women (aged 32.7 ± 5.1) in Utrecht, Netherlands was to explore relationships between maternal stress during pregnancy and birth outcomes and early infant temperament. The study included 37.2% multiparas expecting to deliver a singleton fetus. Exclusion criteria were maternal endocrine disorders and congenital fetal anomalies. Women completed the PSS-14 to assess stress. Cortisol was collected from amniotic fluid during an amniocentesis and plasma cortisol was collected immediately following an amniocentesis around 16 weeks of gestation. No relationship was found between reports of stress and plasma or amniotic cortisol. Perceived stress was not correlated with gestational age or infant birth weight. A positive correlation between maternal

plasma and amniotic cortisol was found, and elevated amniotic cortisol was related to lower birth weight (Baibazarova et al., 2013).

Bolten et al.'s (2011) prospective study of 94 women (aged 31.4 ± 5.3) in Germany investigated the impact of maternal distress during pregnancy on birth outcomes. The study included 62.8% multiparas expecting to deliver a singleton fetus. Inclusion criteria controlled for maternal endocrine disorders, including diabetes, and fetal anomalies. Women completed the 14-item Perceived Stress Scale (PSS-14) to assess stress and across two consecutive working days collected four passive drool samples within an hour of wake time (8 samples across two days). These were collected to assess the area under the curve (AUC_G) from the cortisol awakening response (CAR) during the second and third trimesters. Higher reports of stress were not related to the women's AUC_G . No relationships were found between self-reports of stress and birth outcomes. The AUC_G derived from the morning cortisol samples explained 19.8% of the variance in birth weights of the infants. The higher the fetus' exposure to cortisol, the less the baby weighed at birth (Bolten et al., 2011).

The aim of Cheng & Pickler's (2010) repeated measures study of 46 women (aged 26.9 ± 6.4) was to determine the relationships between salivary cortisol and measures of distress and happiness during the pregnancy and postpartum periods. The study included women who were expecting a singleton fetus. Women collected passive drool twice in one day at or after 36 weeks of gestation to assess CAR, and completed the PSS-10. CAR was inversely related to stress, but the relationship was not significant. Prenatal stress was not correlated with birth outcomes. Cortisol was not related to infant birthweight.

D'Anna-Hernandez et al.'s (2012) prospective study of 55 women (aged 28 ± 6) explored how acculturation was associated with cortisol and birth outcomes. The study occurred in

Colorado and included 87.7% multiparas, with individuals being excluded who had a current or past history of using illicit substances. Women provided three saliva samples across three days during each trimester of pregnancy and postpartum. Greater acculturation was associated with a blunted diurnal slope as well as with lower infant birth weight. A secondary finding was that women with flattened diurnal cortisol slopes were more likely to give birth to a low birth weight infant (D'Anna-Hernandez et al., 2012).

Entringer et al.'s (2011) cross-sectional study of 25 women (no maternal age or parity status reported) in California examined the relationships between ecological momentary assessments of affect and salivary cortisol in a naturalistic setting during pregnancy and how these two measures related to birth outcomes. Negative affect was a proxy for perceived stress. Exclusion criteria included illicit drug use, endocrine disorders, and multiple gestation. Women collected four saliva samples across four days (two days on the weekend and two days during the week) at 23.4 (± 9.1 , range 10-35) weeks of gestation. Elevated cortisol across the day was associated with more negative affect. Negative affect was not associated with birth outcomes. Flatter diurnal slopes for cortisol were associated with shortened length of gestation (Entringer et al., 2011).

Goedhart et al.'s prospective study of 2,810 women ($M = 31.4$ years) in the Netherlands aimed to determine the association between maternal cortisol and infant birth weight. Women who carried their pregnancy to term and were free from endocrine disorders were included. Serum cortisol was collected and women completed a battery of psychosocial questionnaires at 22 weeks of gestation (range 13-29). Questionnaires included measures of depressive symptoms with the Center for Epidemiologic Studies Depression Scale, anxiety symptoms with the State-Trait Anxiety Inventory, pregnancy related anxiety with the Pregnancy Related Anxiety Questionnaire, and parenting stress with the Frequency of Parenting Daily Hassles. 42% of

women were multiparous. No association between maternal psychosocial stress and cortisol was found. No report was provided on the relationship between perceived stress and birth outcomes. A non-significant association was found between cortisol and both birthweight and length of gestation, but this relationship did not exist once other covariates were entered into the model (e.g. infant gender, ethnicity, maternal age, parity, BMI, and smoking) (Goedhart et al., 2010).

Hoffman et al.'s (2016) prospective cohort study of 92 women in Colorado (aged 28.2 ± 6.1 in term group, $n=79$, and 32.4 ± 5.8 in preterm group, $n=11$) examined the relationships among scores on the PSS, hair cortisol, and birth outcomes. The study included 63% nulliparas with uncomplicated pregnancies who were expecting singletons. Women were included if they were at least 18 years of age, not using illicit drugs or abusing alcohol, and had no corticosteroid use. Hair cortisol was evaluated in this study across all three trimesters of pregnancy. Perceived stress was related to elevated hair cortisol but only during the second trimester. Greater perceived stress was associated with gestational age of delivery, but only when assessed at 16 weeks of pregnancy. Higher mean cortisol concentrations in hair were associated with preterm and early deliveries (Hoffman et al., 2016).

Kramer et al.'s case-control, prospective cohort study included 648 women. The study aimed to measure plasma cortisol, cortisol-binding globulin, ACTH, oestradiol and progesterone in women during their second trimester, and determine how these hormones were associated with stressors and distress. Study participants were at least 18 years old from Montreal, Canada with a singleton pregnancy. Women who had cervical insufficiency or diabetes were not included. At 24-26 weeks gestation, women provided plasma cortisol and completed several stress-distress measures (16 measures: 9 related to stress, 7 related to distress). A short form of Cohen's Perceived Stress Scale was completed by the women in the study. No associations between

cortisol and the stress measures were found. Data on how perceived stress related to birth outcomes was not provided. No relationship was identified between preterm birth and cortisol.

The purpose of Stewart et al.'s RCT (2015) was to examine the effects of prenatal nutrient supplementation on salivary cortisol, and explore how cortisol and perceived stress impact birth outcomes. 1,372 women were included from a rural area of Malawi. They were between 15-49 years of age and 19.6% were primiparous. Women with pregnancy complications were excluded. Saliva samples were collected at a single time point during the day at 28 and 36 weeks' gestation. Women completed the PSS-10 at 28 weeks of gestation. No relationship was found between perceived stress and cortisol across pregnancy. Elevated perceived stress at 36 weeks' gestation was significantly associated with shorter newborn length. Length at birth is an anthropometric indicator of growth during gestation. Elevated cortisol was associated with earlier delivery and smaller infants.

Su et al.'s case-control study included 142 women who were between 20-34 years old from Xi'an, China examined umbilical cord plasma ACTH, cortisol, norepinephrine, and epinephrine levels, and determined if there was an association between these hormones with maternal perceived stress, birth outcomes, or neurodevelopment of infants. Women who were primiparous were included, but women with pregnancy complications and certain medical complications were excluded. Immediately following birth, the umbilical cord blood was collected. Women completed a life events checklist to examine psychological stress (the Life Events Scale for Pregnant Women; LESPW) during their stay in the hospital after their infant's birth. Different stressors had different weights assigned, and participants selected if they had any exposure to the stressor in the past year. Cases in this study were women with elevated stress scores from the LESPW. Cortisol in the cord blood was lower among the women in the elevated stress group (Su

et al., 2015). The women in the elevated stress group had infants who weighed significantly less than infants of women in the control group.

Valladares's et al.'s cross-sectional study of 147 women (aged 14-40) aimed to examine cortisol patterns during pregnancy in women and explored how perceived stress and violence relate to cortisol and how these exposures affect the length of the pregnancy and growth of the fetus. Women from Nicaragua who were 40% primiparous were included. Between 18 and 39 weeks gestation, women provided 2 saliva samples: one between 7am and 8am and a second sample between 2pm and 3pm. They also completed a perceived stress scale (no data on iteration or name of scale used). Greater perceived stress was associated with elevated afternoon cortisol values. The relationship between perceived stress and birth outcomes was not reported. Women who had elevated levels of cortisol had infants who weighed significantly less than the infants who were exposed to less cortisol at the time of the study sampling.

Synthesis of Study Findings

Cortisol and Birth Outcomes. Eight studies found that women whose cortisol profiles are different from expected cortisol norms (i.e., high overall cortisol values, elevated cortisol awakening response (CAR), dampened afternoon trajectory) had greater risk for delivery of infants who were lower birth weight and of fewer weeks gestation (Baibazarova et al., 2013; Bolten et al., 2011; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Hoffman et al., 2016; Stewart et al., 2015; Su et al., 2015; Valladares et al., 2009). Two studies found no relationship between cortisol and birth outcomes (Goedhart et al., 2010; Kramer et al., 2013). Birth outcome data and infant weight from each study are outlined in **Table 2.1**.

Perceived Stress and Birth Outcomes. All of the studies that were described earlier on cortisol and birth outcomes collected data on women's perception of stress as well. However,

some of those studies did not analyze the relationship between stress and birth outcomes (Baibazarova et al., 2013; Bolten et al., 2013; Valladares et al., 2009). Of those that did analyze a relationship, two studies did not find an association between perceived stress and birth outcomes (Cheng & Pickler, 2010; Entringer, 2011). Four studies did find a relationship between elevated perceived stress and a smaller infant size at birth (D'Anna-Hernandez et al., 2012; Hoffman, 2016; Stewart et al., 2015; Su, 2015).

Cortisol and Perceived Stress. Five of the studies described earlier found no relationship between perceived stress and cortisol (Baibazarova et al., 2013; Bolten et al., 2011; Goedhart et al., 2010; Kramer et al., 2013; R. C. Stewart et al., 2015). In the other half of the studies, a relationship was found between elevated perceived stress and cortisol outside of anticipated norms (Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Hoffman et al., 2016; Su et al., 2015; Valladares et al., 2009).

Perceived Stress and Cortisol as Predictors of Birth Outcome. Although four studies above show a positive relationship between self-reported stress and adverse birth outcomes when analyzed without cortisol, different findings emerge when perceived stress and cortisol are examined together as predictors. When considered together, research shows that cortisol trajectories or cortisol means are more predictive of low birthweight and shorter gestation than responses on self-administered, perceived stress questionnaires (Baibazarova et al., 2013; Bolten et al., 2011; Cheng & Pickler, 2010; Entringer et al., 2011; Hoffman et al., 2016; Kivlighan et al., 2008) In these studies, perceived stress is not a strong a predictor of birth outcomes. Self-appraisal of stress and a woman's cortisol measurement did show a relationship in one other study (Valladares et al., 2009). High perceived stress was associated with elevated levels of cortisol in the afternoon (Valladares et al., 2009).

Quality Assessment and Analysis

Scores from the STROBE Checklist can range from 0-22, with higher scores indicating that studies provided all necessary information for replication, were transparent regarding statistical analysis, and effectively addressed potential sources of bias. Four of the studies received scores greater than or equal to 19 (Baibazarova et al., 2013; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Stewart et al., 2015). Two received a score of 17 (Bolten et al., 2013; Valladares et al., 2009). Finally, three studies received a score of 16 (Cheng & Pickler, 2010; Hoffman et al., 2016; Su, 2015). The range of scores demonstrates that not all of the evidence from the studies is consistently reported. Studies with lower scores are challenging to meaningfully interpret, since key elements may be lacking that influence validity and would allow future researchers to replicate their study.

The main issues of concern with studies relate to methodology. This is important for replicability and generalizability. When issues arise with methodology, the potential for error is introduced. Three studies did not include information about the study design in the study title or abstract (Bolten et al., 2013; D'Anna-Hernandez et al., 2012; Entringer et al., 2011).

Designs. Most of the studies included in this review were cohort studies. There was one randomized control trial (RCT) and two case-control studies. Three studies collected samples from women multiple times during their pregnancy (Bolten et al., 2013; Hoffman et al., 2016; Stewart, Umar, Gleadow-Ware, Creed, & Bristow, 2015). One study collected samples across all three trimesters (Hoffman et al., 2016). Seven studies collected samples during the second trimester (Baibazarova et al., 2013; D'Anna-Hernandez et al., 2012; Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013; Valladares, Pena, Ellsberg, Persson, & Hogberg, 2009). Among the studies that sampled

women during the second trimester, three captured samples around 16 weeks' gestation (Baibazarova et al., 2013; Hoffman et al., 2016; Valladares et al., 2009), and four captured samples between 22-26 weeks' gestation (Entringer et al., 2011; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013). Seven studies sampled women during their third trimester (weeks 27-40+ gestation) (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Hoffman et al., 2016; Stewart et al., 2015; Su et al., 2015; Valladares et al., 2009). Two studies collected samples at 28 weeks gestation (Hoffman et al., 2016; Stewart et al., 2015). Four sampled women between 32-37 weeks gestation (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Hoffman et al., 2016). Four studies sampled women at 40 weeks of gestation (Hoffman et al., 2016; Stewart et al., 2015; Su et al., 2015; Valladares et al., 2009). These multiple variations make comparison across studies extremely difficult.

In addition to the timing during pregnancy that samples were collected, the time of day was considered for samples collected from serum or saliva, as they are susceptible to changes across the day. Five of the studies that used salivary cortisol included multiple samples across the day, allowing the opportunity to examine the dynamic changes of cortisol across the day (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Valladares et al., 2009).

Settings. The studies varied in the location of where data collection occurred. The method of cortisol measurement impacted the location of where data was collected. Some data were collected where women received perinatal care, and cortisol was measured in serum, saliva, and hair (Baibazarova et al., 2013; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013; Su et al., 2015). The studies that captured cortisol in a clinic setting were generally limited in being able to describe the women's pattern of cortisol secretion since they were limited to a

single time point measure. However, Hoffman et al. (2016) were able to capture chronic cortisol, by sampling hair in the clinic setting.

In studies that measured salivary cortisol, a naturalistic approach was most often used, with data collection occurring in whatever setting a woman interacts with during the day (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Stewart et al., 2015; Valladares et al., 2009). Data collection in two studies occurred with research staff present in the women's home (Stewart, 2015; Valladares et al., 2009).

Participants. There were 5,573 women included across all the studies, with sample sizes ranging between 25 and 2,810 (standard deviation: 860; median: 142). Two studies reported that they determined sample size via power analysis (D'Anna-Hernandez et al., 2012; Stewart et al., 2015). Many of the studies did not clearly describe the method of sample size estimation or the number of women who were identified as potential participants in their original recruitment plan (Baibazarova et al., 2013; Bolten et al., 2010; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Hoffman et al., 2016; Kramer, 2013; Stewart, 2015; Su et al., 2015; Valladares et al., 2009). Two of the studies addressed how many potentially eligible participants were approached and the final sample size (Entringer et al., 2011; Goedhart et al., 2010). Two studies had sample sizes on an epidemiological scale ($n = 1,372 - 2,810$) (Goedhart et al., 2010; Stewart, 2015).

The characteristics of the women were heterogeneous in ages and parity status across studies. Women over age 35 were included in about half of the publications reviewed (Baibazarova et al., 2013; Hoffman et al., 2016; Stewart et al., 2015; Valladares et al., 2009). Su et al. (2015) only included primiparous women, while Entringer et al. (2011) only included nulliparous women. The other studies were mixed with parity status, and mostly had equal representation of nulliparous and primiparous women.

Women varied in educational attainment. Some had women who completed high school or less (Bolten et al., 2013; Cheng & Pickler, 2010; Hoffman et al., 2016; Stewart et al., 2015). Others included mostly women with a college degree or beyond (Baibazarova et al., 2013; Entringer et al., 2011; Kramer et al., 2013).

The samples included participants from different socioeconomic statuses across the studies. Among the seven studies that reported on income (Bolten et al., 2013; D'Anna-Hernandez et al., 2012; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013; Su et al., 2015; Valladares et al., 2009), four included women who were mostly high or middle income (Bolten et al., 2013; Hoffman et al., 2016; Kramer et al., 2013; Su et al., 2015). Two studies included women who were mostly low income (D'Anna-Hernandez et al., 2012; Valladares et al., 2009), and one had women in a rural community in a developing country (Stewart et al., 2015).

The women across the studies identified with different races and ethnicities. Among the seven studies that reported on race and ethnicity (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013), two included samples that were representative of their countries demographic trends (Entringer et al., 2011; Goedhart et al., 2010). Four studies featured samples with traditionally underrepresented groups as the majority (Bolten et al., 2013; Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Hoffman et al., 2016). There was a mix between developed and developing countries among the studies who did not report race and ethnicity (Baibazarova et al., 2013; Stewart et al., 2015; Valladares et al., 2009). One of the studies occurred in developed countries (Baibazarova et al., 2013;), and two were in developing countries (Stewart et al., 2015; Valladares et al., 2009).

There were differences among women and their living situations. Partnership status, including being married or cohabitation with a partner, was collected in nearly half of the studies (Cheng & Pickler, 2010; D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Hoffman et al., 2016; Kramer et al., 2013). In the studies that reported partnership, the overwhelming majority of women were either living with or cohabitating with a partner (D'Anna-Hernandez et al., 2012; Entringer et al., 2011; Hoffman et al., 2016; Kramer et al., 2013). In one study, a quarter of the women were neither partnered or cohabitating (Cheng & Pickler, 2010).

All studies, except three, excluded women who had pregnancy complications or risks factors with their pregnancy (D'Anna-Hernandez et al., 2012; Kramer et al., 2013; Valladares et al., 2009). Five studies included women who smoked cigarettes during pregnancy (Bolten et al., 2013; D'Anna-Hernandez et al., 2012; Goedhart et al., 2010; Hoffman et al., 2016; Kramer et al., 2013). About half of the studies excluded women with an endocrine disorder (Baibazarova et al., 2013; Bolten et al., 2013; Entringer et al., 2011; Goedhart et al., 2010; Kramer et al., 2013; Su et al., 2015).

Analysis of Results. Across studies, multiple statistical tests were performed; however, few used sophisticated techniques. Only one study used structural equation modeling (Baibazarova et al., 2013). Four studies used ANOVA analyses to investigate their main study questions (Bolten et al., 2013; Hoffman et al., 2016; Stewart, 2015; Su et al., 2015). There was inconsistency with controls for potential confounders (refer to **Table 2.3**), and it was not always clear if the confounders actually were related to the outcome of interest.

Internal Validity. Internal validity is threatened when there are methodological issues within the study. When key information about the validity and reliability of different study variables is not included, it remains unclear if the study adequately answered the research

question correctly. When measures are not adequately capturing the concept of interest, the risk of a type II error increases. Four of the studies did not include the interassay and intrassay coefficients of variability for cortisol, threatening the validity of the study's findings (Cheng & Pickler, 2010; Kramer, 2013; Su et al., 2015; Valladares, 2009). Further details regarding internal validity for each study are presented in **Table 2.2**.

The secretion of cortisol across the day follows a circadian pattern. Serum samples can be sensitive to the time of day, as the diurnal rhythm of cortisol impacts serum cortisol concentration. One study did not include information on the length of time since waking from sleep occurred when their early morning samples were collected (Goedhart, 2010). The ability to detect a change from waking is dependent on the time sampling occurred after sleep, and cortisol decreases from 45 minutes after waking until around 12 pm daily. Therefore, information on the time of day and time since waking from sleep is needed for the cortisol data to be interpreted in a meaningful way. Another study asked their participants to rise at 7 am to collect their samples (Bolten, 2013). Altering wake time from normal may introduce a deviation in salivary cortisol patterns that would be captured if provided during a normal waking time. Additionally, waking up when there is little light exposure can result in lower cortisol values in the morning, and in Trier, Germany, where Bolten et al.'s study occurred, the sun rises after 7 am in 5 months out of the year (October-February). It was not clear what time of year the women provided their samples and if they controlled for season.

Medical complications can alter normal cortisol parameters, making it an important factor to consider when investigating this biomarker. One study controlled for most pregnancy complications, except preeclampsia (Goedhart, 2010), while the others did not include women with pregnancy complications (Baibazarova et al., 2013; Bolten, 2013; Cheng & Pickler, 2010;

Entringer et al., 2011; Hoffman, 2016; Stewart, 2015; Su, 2015). It was not clear if preeclampsia was controlled for in the analyses, and it could have potentially confounded the results. Not controlling for potential confounders could impact the results of an analysis.

In addition to medical and clinical features affecting cortisol values, not all the values reported across the studies have the same degree of precision. The inter- and intra-assay coefficients of variability indicate the quality of the assays from the lab on the cortisol sample as a whole and on the differences within each cortisol value reported. The intra-assay coefficient of variation represents the variation in all of the values obtained from an assay. Coefficients <10% are acceptable for the inter-assay coefficient of variability. Cortisol values are derived from multiple runs of the same sample, with an average between the runs being reported as the cortisol value. The differences in values obtained for a single sample from multiple runs is the inter-assay coefficient of variability (Hanneman et al., 2011). Coefficients <15% are acceptable for the intra-assay coefficient of variability. Four studies did not provide data on either their inter-assay or intra-assay coefficients of variability (Cheng & Pickler, 2010; Kramer, 2013; Su et al., 2015; Valladares, 2009). Among the studies that reported these coefficients, all except one (Entringer et al., 2011), reported an inter-assay coefficient of variability < 6.1% (D'Anna-Hernandez et al., 2012; Stewart, 2015). Entringer et al. (2011) reported <9% inter-assay coefficient of variability.

Control for Confounders. There is also inconsistency across studies in the potential confounders that were controlled for in analyses. Some studies controlled for maternal age (Bolten, 2013; Goedhart, 2010; Hoffman, 2016; Stewart, 2015; Su, 2015; Valladares et al., 2009). Age did not influence any variables in two of the studies (Stewart, 2015; Valladares et al., 2009). Increased maternal age did show a relationship with lower cortisol values in one of the studies (Goedhart, 2010).

Six studies controlled for parity (Bolten, 2013; Cheng & Pickler, 2010; Goedhart, 2010; Hoffman, 2016; Stewart, 2015; Valladares, 2009), and three did not find an influence on parity on any of the study outcomes (Cheng & Pickler, 2010; Stewart, 2015; Valladares, 2009). One study did find a relationship with lower cortisol levels among women who had previously delivered a child (Goedhart, 2010). Disparate findings across the studies could be related to whether certain confounds were addressed or not in analysis, and variability in ages and parity across the samples. This measurement concern could lead to challenges with generalizability and hinder the ability to replicate studies.

External Validity. Many of the studies excluded women who delivered preterm or who had pregnancy complications, making the results from some of the studies only generalizable to a healthy population, and to women in general during pregnancy. The studies that only included healthy samples have limited external validity and potential selection bias (Baibazarova, 2013; Bolten, 2013; Cheng & Pickler, 2010; Entringer, 2011; Hoffman, 2016; Stewart, 2015; Su, 2015). Including a diverse sample with some medical risk may be more representative of the population experiencing stress.

Since many of the studies included homogenous samples, they may have limited external generalizability. Less variability may occur in a homogenous sample. Potentially, the studies that did not find a relationship with cortisol and birth outcomes were subject to issues with external validity (Goedhart, 2010; Kramer, 2013). In both of these studies, cortisol was only collected once and both were serum samples.

Response Bias. Response bias could be an issue with reporting perceived stress. In some cultures and depending on the desirability of the pregnancy, it may not be socially acceptable or desirable for women during pregnancy to endorse feelings of stress (Goedhart, 2010). Two

studies had the women complete the measure of psychological stress with a researcher (Stewart, 2015; Valladares, 2009). This bias may be especially influential if a woman was having a questionnaire read to her by study staff, rather than completing it with some degree of privacy.

Discussion

This integrative review demonstrated that both the relationships between perceived stress and cortisol, and how they relate to birth outcomes remain unclear. Results of the studies were not consistent for either set of relationships. The studies that did establish a relationship of cortisol to either perceived stress or birth outcomes, sampled salivary cortisol, allowing for a circadian pattern to be examined. These studies that captured salivary cortisol were able to test relationships between cortisol values across various times of the day (i.e. CAR, diurnal, afternoon decline) and birth outcomes. In contrast, studies that did not show a relationship of cortisol to perceived stress or birth outcomes were more likely to collect a single sample of cortisol via blood draw and not control for the time when the sample was acquired. There was also inconsistency across studies on the covariates or confounds that were included in order to adjust for their effects. In addition, moderators of the relationships between stress or cortisol and birth outcomes were not addressed in the research, including infant sex or maternal mental health during pregnancy. Few studies found a relationship between self-reported stress and cortisol or between self-reported stress and birth outcomes. Cortisol was a stronger predictor of birth outcomes than self-report of stress.

This review extends the contributions of previous literature reviews in the field. The six previous reviews did not address a number of issues identified in this review (Cherak et al., 2018; Duthie & Reynolds, 2013; Entringer, Buss, & Wadhwa, 2015; Graignic-Philippe et al., 2014; Shapiro et al., 2013; Zijlmans, 2015). First, there was inconsistency with how

psychological, perceived stress was defined and measured (Shapiro et al., 2013). Some studies reported exposure to life events, without an exploration of how an individual responds and evaluates the stressor (Graignic-Philippe et al., 2014; Shapiro et al., 2013). One review erroneously reported that psychological stress is positively correlated with salivary cortisol, and this statement was made without supporting literature (Shapiro et al., 2013). However, our review found that psychological stress has not consistently been related to cortisol in studies to date. Similar to previous literature reviews, we found variation across studies with their inclusion and exclusion criteria. Additionally, we, like other reviews, found that some studies had a limited range in variation of birth outcomes, reducing their ability to detect effects of perceived stress or cortisol.

Current Gaps in the Field

A clear relationship with cortisol and birth outcomes across the course of pregnancy has yet to be established. Increased sampling of self-reported stress and cortisol across different stages of pregnancy, and clarifying when or how cortisol and perceived stress may be related to one another will enhance the ability to understand the relationships of these factors to birth outcomes. One study found a relationship of hair cortisol during the second trimester to birth outcome, suggesting that hair sampling may be a promising method of capturing chronic stress. However, previous research indicates that salivary cortisol seems to be most sensitive approach to detecting relationships. With saliva sampling also being the least invasive, it may be a highly useful method for researchers to use. Studies that utilize a single serum marker do not appear to capture the same cortisol profile as salivary cortisol samples. To date, use of inconsistent methodologies and a lack of robust findings have limited what is known about these relationships.

Previous research has not adequately studied women with multiple stressors in their lives and those at greater risk for pregnancy complications. In addition, assessing stress and stress hormones across multiple times during pregnancy may improve the ability to detect effects and target underlying mechanisms that may contribute to decreased growth of the fetus and dysregulated timing of parturition. Careful consideration of the relationship of potential covariates to both cortisol and perceived stress needs to be considered, as well as potential confounders that may influence birth outcomes.

Although most of the studies discussed conceptual or mechanistic underpinnings related to the changing HPA-axis across pregnancy as a basis for their research, others did not address potential pathways that link cortisol, psychological stress, and birth outcomes (Baibazarova, 2013; Cheng & Pickler, 2010; Su, 2015; Stewart, 2015). In addition, there were differences in whether conceptual models considered mediating or moderating effects (Baibazarova, 2013; Bolten, 2010; Entringer, 2011; Kramer, 2013; Su, 2015; Valladares, 2009). The underlying etiology predicting growth during gestation and timing of parturition remains unclear. Examining potential pathways, along with mediators and moderators may help to clarify processes that are still not clearly understood.

Limitations

Some studies have demonstrated differences in stress or cortisol between women who are of minority status or experiencing scarce resources and those who are not (Borders et al., 2010; Corwin et al., 2013; Premji, 2014; Wallace & Harville, 2013). However, social and demographic characteristics were not described for all studies in this review, making it impossible to effectively examine the role of these factors. These characteristics may be moderating some of the mechanisms around either the perception of stress or the expression of cortisol.

It is important to note that the studies in this review include samples from various countries. The different countries may vary substantially in income, access to health care, exposure to air pollution, pesticides, or other health risks, as well as support for time to care for and bond with a newborn (e.g. maternity leave, paternity leave, Family Medical Leave of Absence). Having systems in place either through the work place or through the government, that allow adequate bonding time, with financial support, could help mitigate stress, and have beneficial impacts on the quality of the relationship between a woman and her child or partner.

Prematurity varies across the different countries where the studies occurred, ranging from 6.7% in Nicaragua (Vogel, Lee, & Souza, 2014) to 12% in China and Sub-Saharan Africa (Chawanpaiboon et al., 2019). The PTB rate in the US is 9.4% (Martin et al., 2018), but varies even across States. Together, these data suggest that the location that an infant is born may come with varying levels of risk, meaning infants born in some parts of our world, or even our own country are at an increased risk of being born with an adverse birth outcome. These factors could not be adjusted for in this review.

Conclusion

Analysis of studies to date suggests that future research should include both self-reports of stress and multiple sources of cortisol when assessments occur and that these assessments should take place several times during pregnancy. Without this integrated, longitudinal approach, it is unlikely that adequate knowledge will be developed regarding the relationship between self-reports of stress and cortisol, or their impact on birth outcomes. Uncovering the nuances of stress across gestation will help clinicians determine when women may experience the greatest perceived stress or hormonal effects from it, and how to best mitigate women's stress in order to reduce their risk of adverse birth outcomes.

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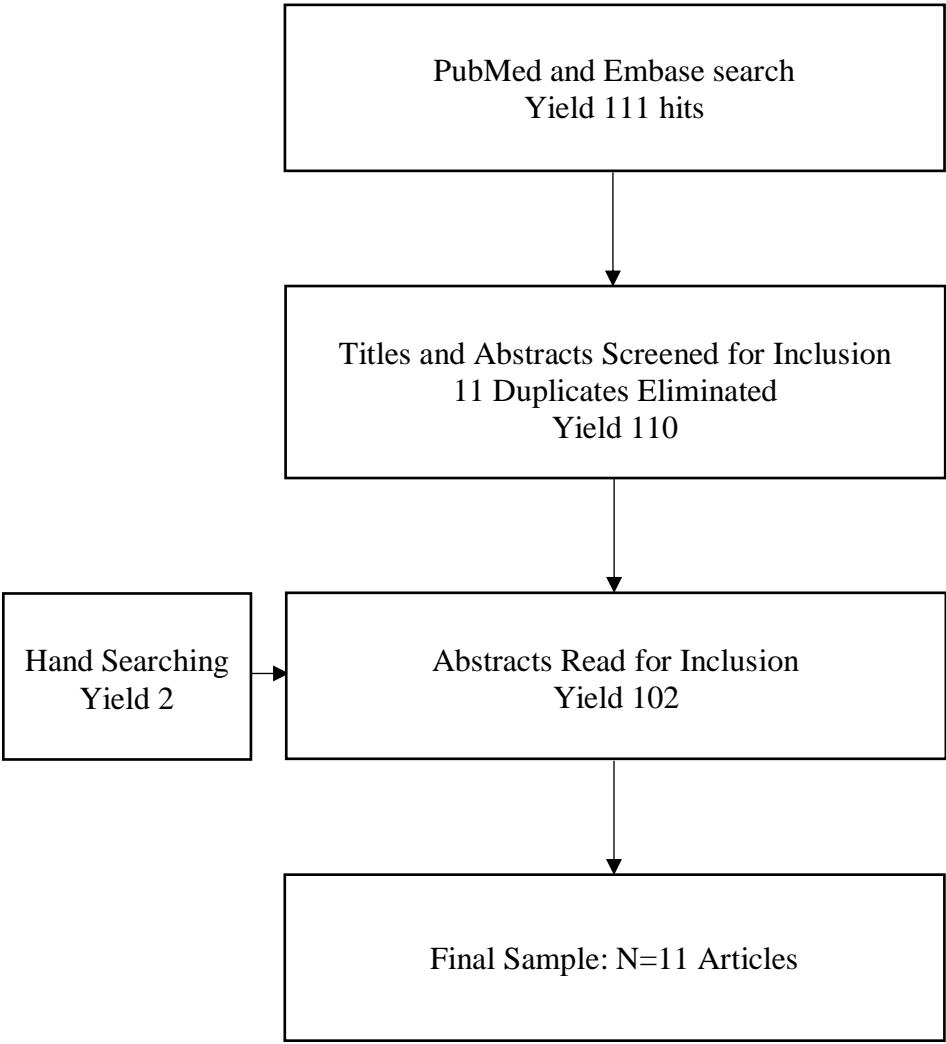


Figure 2.1 Search Strategy

Table 2.1 Birth Outcome Data

Gestational age: in weeks, with standard deviation and range when provided
 Gestational weight: in grams, with standard deviation and range when provided

Author	Gestational Age	Preterm Birth (%)	Gestational Weight
Baibazarova et al. (2013)	39.8 ± 1.7 (30.7-42.7)	Not reported	3483.8 ± 551.8 (1435-4772)
Bolten et. al (2013)	39.3 ± 1.8	0%	3,421 ± 551.5
Cheng & Pickler (2010)	39.21 ± 1.2	0%	3226.34 ± 479.91
Danna-Hernandez et al. (2012)	All >38 weeks	0%	Not reported
Entringer et al. (2011)	39.26 ± 1.96 (36.5-42)	Not reported	Not reported
Goedhart et al. (2010)	39.7	Not reported	Not reported
Hoffman (2016)	Term: 39.5 ± 1.2 Preterm: 32.6 ± 3.9	12%	Term: 3,289 ± 423 Preterm: 1,967 ± 805
Kramer (2013)	Not reported	32%	Not reported
Stewart et al. (2015)	39.4 ± 2.1	0%	2,971 ± 445
Su Q et al. (2015)	39	0%	Not reported
Valladares et al. (2009)	Not reported	4%	Not reported

Table 2.2 Internal Validity

Study	Power Analysis	Intraassay Coefficient of Variability	Interassay Coefficient of Variability
Baibazarova et al. (2013)	No	Not reported	6%
Bolten et. al (2013)	No	Not reported	3.9% [high] 7.1% [low]
Cheng & Pickler (2010)	No	Not reported	Not reported
Danna-Hernandez et al. (2012)	Yes	<3%	<7.5%
Entringer et al. (2011)	No	<9%	<9%
Goedhart et al. (2010)	No	Not reported	4.9% [high] 10.2% [low]
Hoffman (2016)	No	Not reported	Not reported
Kramer (2013)	No	2.8%	9.2%
Stewart et al. (2015)	Yes	3.5%	5.1%
Su et al. (2015)	No	Not reported	Not reported
Valladares et al. (2009)	No	Not reported	Not reported

Table 2.3 Control for Confounders

Age: in years with standard deviation when provided

* Difference when controlled in analysis

** No difference when controlled in analysis

Study	Primiparous	Control for Parity	Age	Control for age	Uncomplicated Pregnancy
Baibazarova et al. (2013)	33.5%	No	37.6	No	Yes
Bolten et. al (2013)	37.2%	Yes **	31.4 ± 5.3	No	Yes
Cheng & Pickler (2010)	Not reported	Yes **	26.9 ± 6.4	No	Yes
Danna-Hernandez et al. (2012)	5%	No	28 ± 6	No	No
Entringer et al. (2011)	0%	No	29.45 ± 5.9	No	Yes
Goedhart et al. (2010)	57.5%	Yes *	31.4	Yes *	No
Hoffman (2016)	63%	No	≈ 30.3	No	Yes
Kramer (2013)	≈58.4%	Yes	20-34	Yes	No
Stewart et al. (2015)	≈19.6%	No	15-49	Yes **	Yes
Su et al. (2015)	100 %	No	20-35	Yes **	Yes
Valladares et al. (2009)	40%	Yes **	14-40	Yes **	No

Associations between Women's Perceived Stress and Cortisol Parameters during Pregnancy

Abstract

Objective: Previous research suggests that self-reported stress and cortisol values are not associated during pregnancy. The purpose of this study was to conduct a detailed assessment of different salivary cortisol parameters and their relationship to perceived stress during pregnancy.

Methods: Fifty eight women in a cohort study were recruited from obstetric clinics during their third trimester of pregnancy. They were ethnically diverse and ranged in age from 23 to 47. Women completed the Perceived Stress Scale and provided 8 saliva samples from morning wake time until nocturnal sleep time across 2 days. Samples were assayed and parameters were calculated for average cortisol levels, cortisol awakening response (CAR), diurnal slope, and total cortisol secretion across the day (AUC_G).

Results: On average, women reported a moderate amount of perceived stress. For the total sample, women's perceived stress was not related to any cortisol parameters. However, there were significant associations between perceived stress and mean cortisol, CAR, and AUC_G for women between 26-29 weeks of gestation. These relationships did not persist beyond 29 weeks of gestation.

Conclusions: Results for women ≥ 30 weeks gestation support the majority of prior research, suggesting that self-reported stress and salivary cortisol parameters are not correlated during pregnancy and may be measuring different facets of stress. However, elevations of CAR and AUC_G in response to higher perceived stress have been reported in other populations, congruent with our findings for women ≤ 29 weeks gestation. Findings suggest the need for

further research on HPA axis response at different pregnancy stages, as well as inclusion of self-report and different cortisol parameters when assessing stress.

Keywords: prenatal, perceived stress, cortisol, third trimester, gestation, pregnancy

Introduction

The prevalence of adverse birth outcomes in the U.S. (i.e., preterm birth, small for gestational age (SGA) status, and low birth weight (LBW)) remains a public health concern. Of the 3.95 million infants born in the U.S. recently, 389,075 infants were preterm (Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). However, for 50% of these births the reasons for prematurity remain unknown (Martin et al., 2018).

Current evidence suggests a link between stress during pregnancy and adverse birth outcomes (Lilliecreutz, Larén, Sydsjö, & Josefsson, 2016; McEwen, 2007; Wadhwa, Entringer, Buss, & Lu, 2011). Findings indicate that high maternal perceived stress may shorten the length of gestation, and increase the risk of an infant being SGA or LBW (Gavin, Nurius, & Logan-Greene, 2012; Kane, Dunkel Schetter, Glynn, Hobel, & Sandman, 2014; Lau, 2013; Sable & Wilkinson, 2000). However, some research shows that stress biomarkers, such as cortisol trajectories or cortisol means are more predictive of LBW and shorter gestation than responses on self-reports of perceived stress (Baibazarova et al., 2013; Bolten et al., 2011; Cheng & Pickler, 2010; Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011; Hoffman, Mazzoni, Wagner, Laudenslager, & Ross, 2016; Kivlighan, DiPietro, Costigan, & Laudenslager, 2008). Such studies suggest that self-reported stress and biological measures of stress may assess different rather than equivalent characteristics of stress.

It is interesting to note that studies which explore both a self-evaluation of stress and cortisol during pregnancy find that self-appraisal of stress is *not* always predictive of cortisol values. To

our knowledge, 12 published studies have explored the relationship between cortisol and perceived stress during pregnancy. Six of these studies measured plasma cortisol, four studies measured salivary cortisol, and two studies measured cortisol in hair.

Research using plasma cortisol has shown no relationship between cortisol and perceived stress among pregnant women. In women who were assessed during the second trimester, three studies found no relationship between cortisol levels and perceived stress (Baibazarova et al., 2013; Ghaemmaghami, Dainese, La Marca, Zimmermann, & Ehlert, 2014; Kramer et al., 2013). Similarly, when women provided samples during their third trimester, two additional studies found no relationship between the women's perceived stress and cortisol levels (Salacz, Csukly, Haller, & Valent, 2012; Shaikh et al., 2011). Harville and colleagues (2009) identified no relationship between self-reported stress and cortisol during either the second or third trimester. Among non-pregnant, healthy people, research also indicates that plasma cortisol is not correlated with perceived stress (Ebrecht et al., 2004; Smyth et al., 1997).

Some variation in findings emerged from the studies evaluating whether salivary cortisol parameters were associated with perceived stress. Bolten et al. (2011) examined the cortisol awakening response (CAR) during the second and third trimesters of pregnancy, and reported no relationship with women's self-report of stress during either time point. Cheng & Pickler (2010) assessed CAR at 36 weeks of gestation or later and also found no significant relationship to perceived stress. They also evaluated diurnal rhythm at 36 weeks of gestation and reported no relationship to perceived stress. In a secondary analysis of women at 36 weeks of gestation, Kivlighan et al (2008) found no association between salivary cortisol trajectories over the day and perceived stress. However, Valladeres et al (2009) did report higher levels of perceived

stress were associated with higher salivary cortisol levels in the afternoon among women assessed during the second and third trimesters.

Using hair cortisol assays, Hoffman et al (2016) explored cortisol concentration across all three trimesters. In this study, women with high perceived stress had higher mean hair cortisol levels, but only during the second trimester of pregnancy. These findings were consistent with other research among women towards the end of the first trimester or early second trimester, who demonstrated a relationship between elevated levels of perceived stress and hair cortisol levels (Kalra, Einarson, Karaskov, Van Uum, & Koren, 2007).

In sum, most of the research using blood and salivary measures of cortisol suggests that self-reported stress and cortisol values may not measure equivalent, correlated phenomena. Similar results emerged in these studies regardless of whether women were multiparous or primiparous, and across countries. However, the two studies using retrospective assay of hair cortisol did find a significant relationship between cortisol levels and perceived stress among women either late in their first trimester or early in their second trimester. In addition, one of the saliva studies found an association between higher perceived stress and lower cortisol levels in the afternoon. Normally, salivary cortisol values decrease significantly in the afternoon.

The purpose of this study was to investigate the association between perceived stress during the last trimester of pregnancy and salivary cortisol parameters in women who were at risk of preterm birth. The specific aims were to determine if there were relationships between women's self-reported stress and four salivary cortisol measures: 1) overall mean cortisol level, 2) Cortisol Awakening Response (CAR), 3) Cortisol Diurnal Trajectory, and 4) Cortisol Area Under the Curve (AUC_G).

Methods

Sample and Recruitment

Women were recruited from two medical centers in the San Francisco Bay Area that are affiliated with the UCSF Health System for the primary study, which was investigating the stress response of infants. A Clinical Research Coordinator at each site identified potential participants from the clinical roster who were between 24 and 34 weeks gestation and were reported to be likely candidates for preterm delivery by their clinicians. Preterm risk was based on their obstetric health and/or a medical history or status associated with early parturition. Potential participants were approached by a research assistant (RA), who provided a study flier, gave a brief overview of the study activities, answered questions, and obtained signed informed consent from interested women.

In addition to their gestational status, women were included if they met the following criteria: at least 18 years of age, able to speak and read in English or Spanish, and resided within one hour of the study catchment area. Women were not included if they were too psychologically or physically ill to participate, had a cognitive impairment, had an adrenal or endocrine disorder, smoked or used steroid medication (oral, inhalation, or topical). The study was approved by the UCSF's Internal Review Board [14-13516].

Procedures

Women were asked to complete a self-report questionnaire on perceived stress, a demographic questionnaire, and to provide 4 saliva samples each day across 2 consecutive days during their third trimester of pregnancy. After participants provided signed informed consent, the RA described how to collect saliva samples using the passive drool method (Granger et al., 2007).

A packet was given to the woman which included a copy of the Perceived Stress Scale-10 (PSS-10), a sociodemographic questionnaire, 8 pre-labeled saliva collection tubes, 8 cotton chewing rolls to aid in collecting passive drool, and a stamped envelope addressed to the study team. Participants were instructed to avoid collecting their saliva on days when their routine was expected to change during the night or early morning. They were told to provide the sample before eating, doing any physical exercise, or using alcohol, caffeine, or taking any medication or drug. They also received a brief instruction sheet on how to collect their saliva at specific times across the two days, and a saliva checklist to identify timing of their samples and factors that might affect the cortisol assays. The mailed materials were supported by a phone call or text to remind the women about key points made at enrollment in the study and to offer availability of the research team if any questions arose.

Women rinsed their mouth with water 10 minutes before collection of the sample, allowed saliva to pool in the mouth and drool down into a cryovial until 1 ml was in the vial. They were instructed to collect their first sample upon waking, their second sample 45 minutes after waking, then around 4pm, and prior to sleep at night. The women stored their samples in their home freezer after each saliva collection. Once women completed their two days of saliva collection and PSS-10, the materials were mailed back to the study staff. The saliva was brought to UCSF's CTSI lab for storage until samples were assayed at the biochemical laboratory of Salimetrics® (Carlsbad, CA, USA). Study data were collected and managed using REDCap electronic data capture. Lastly, the electronic medical record of each woman was reviewed at delivery to extract information needed to complete an index of prenatal risks, identify gestational week when saliva samples were acquired, and determine whether women were administered

antenatal corticosteroids as part of clinical care. These data were collected to control for their potential confounding effects on the analysis.

Measures

Sociodemographic. Data on participants' age, occupational status, race, ethnicity, and receipt of varied types of governmental assistance (as an indicator of economic disadvantage) was gathered through a brief sociodemographic questionnaire at recruitment. Age and governmental assistance were examined as potential confounds in the analysis. Other demographic data was used to describe the sample.

Prenatal Risk. To control for the influence of medical risk factors, a prenatal medical risk score was calculated from information in the medical record using items from the Obstetric Medical Risk Index as the basis for extracting data (Lobel et al., 2008). Scores range from 0-37, with higher scores indicating more medical risk. The domains of the index include: unusual features of pregnancy, gynecology and obstetrics history, complications of past pregnancies, family history, patient history, current pregnancy complaints, and other risk factors specific to preterm birth. Content validity of the items stems from data acquired through meta-analysis of key risks for adverse birth outcomes. The creators of the index gathered clinician consensus ratings. In addition, the Obstetric Medical Risk Index has demonstrated excellent predictive validity in relation to adverse birth outcomes (Lobel, DeVincent, Kaminer, & Meyer, 2000).

Perceived Stress Scale. The Perceived Stress Scale was used to measure stress (Cohen, Kamarck, & Mermelstein, 1983). It is a brief, 10-item questionnaire that provides a global assessment of perceived stress over the past month. Each item is rated on a 5-point Likert scale, with four positive items that get reverse scored, and six negative items. The total score represents a sum of all 10-items, with scores ranging from 0 to 40. Higher scores are indicative

of more perceived stress (Cohen, 1983; Taylor, 2015). Scores between 0-13 indicate low perceived stress, 14-26 represent moderately high perceived stress, and 27-40 indicates high perceived stress (Cohen, 1988).

The PSS-10 has been widely utilized in pregnant and postpartum populations across many countries, and has been translated into several languages (Andreou et al., 2011; D'Anna-Hernandez, Aleman, & Flores, 2015; Lau, 2013; Lesage, Berjot, & Deschamps, 2012; Remor, 2006). Internal consistency of the PSS-10 was also good in a diverse Canadian sample of 3,283 pregnant and postpartum women (Cronbach's $\alpha = .85$) (Robinson, Benzies, Cairns, Fung, & Tough, 2016). It has demonstrated predictive validity with preterm birth in women who had medical risks for obstetric complications in a sample of 581 pregnant women (Lau, 2013).

Salivary Cortisol. Upon receipt by the study staff, saliva samples were visually inspected for quality, and stored at -20°C in a lab at UCSF before shipment for assay. Samples were assayed at the Salimetrics® SalivaLab (Carlsbad, CA). All samples were assayed in duplicate using high sensitivity salivary cortisol enzyme immunoassay (ELISA) and were run in multiple batches. Samples were brought to room temperature, vortexed, and centrifuged for 15 minutes at 3,000 RPM prior to assay. The assay had a lower limit of sensitivity of $.007 \mu\text{g/dl}$, a standard curve range from $0.012\text{-}3.0 \mu\text{g/dl}$, an average intra-assay coefficient of variation of 4.6% and an average inter-assay coefficient of variation of 6%. The following 4 parameters were calculated from the saliva samples: overall mean cortisol level, Cortisol Awakening Response (CAR), the diurnal trajectory or decline (slope) from awakening to bedtime, and total cortisol secretion across the day (area under the curve; AUC_G). These parameters were examined because they provide measures of the overall amount or exposure to cortisol (mean and AUC_G) as well as the expected or typical circadian pattern of cortisol which occurs under normal circumstances. After

awakening, there is a typical boost of cortisol that occurs within the next 30-45 minutes (CAR) (Chida & Steptoe, 2009). Following the CAR, there is a natural decline in cortisol level throughout the day (diurnal trajectory or slope) (Adam et al., 2017).

The mean of each parameter across the two days was used as a woman's level for that particular value. Cortisol scores were adjusted for week of the woman's pregnancy to account for natural increases in endogenous cortisol that occur prior to parturition.

The four cortisol parameters - mean cortisol level, CAR, Diurnal Trajectory/Slope, and AUC_G- were operationally defined as follows. The mean cortisol level was derived by averaging the total cortisol concentration of the 8 samples across the 2 days of sampling. The CAR score was the difference between cortisol level from wake time to 45 minutes following wake time (Alder et al., 2011). The slope was calculated as the linear degree of change in cortisol levels across the day from initial waking to evening, excluding the second sampling (morning awakening response). AUC_G measured total cortisol secretion across the day, considering the difference of single measurements from one another and the time between each sampling period (Khoury, 2015). The trapezoidal formula developed by Pruessner, et al. (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) was used to calculate AUC_G.

Data Analysis

All variables were tested for linearity and normality of their distributions. Normality was assessed by examining histograms and identifying outliers. Skewness and kurtosis were also examined. Log transformations were computed to address skew for both CAR and diurnal slope. Multiple imputation procedures were employed to address data that were missing for cortisol parameters (Stradler et al. 2016).

Means, standard deviations, medians, and ranges were calculated for continuous variables (cortisol parameters, perceived stress, prenatal risk, age, week of gestation). Frequencies and percentages were reported for categorical variables (parity status, partnership status, occupational status, educational attainment, recipient of government assistance, race, and ethnicity).

Pearson partial correlations were used to examine the relationships between the Perceived Stress Scale scores and the 4 cortisol parameters (overall mean, CAR, diurnal slope, AUC_G), controlling for week of gestation when the cortisol sample was acquired. All tests were calculated as two-sided with $p < .05$. Analyses were computed using SPSS 25.

Results

Demographic and Clinical Characteristics

Demographic data on the women are presented in **Table 3.1**. The sample included fifty-eight women who were racially diverse: 64% were white, 17% Asian, 4% Black, and 16% reported other racial group. Nineteen percent indicated Hispanic ethnicity. Most were in a partnered relationship (86.3%) and more than half (58.8%) were full-time employed and nulliparous (58.8%). Approximately 25% of the women were recipients of government support.

Women in the study ranged from 26 to 40 weeks of gestation. Approximately 44% of the women were ≤ 29 weeks gestation ($n=26$) and the remaining 56% were ≥ 30 weeks' gestation ($n=32$). Overall, they experienced a low level of prenatal medical risk ($M=3.5$ out of a possible 37 risks). Few women ($n=9$) received corticosteroids as a prophylactic treatment during their pregnancy, with the average being receipt at 30.7 weeks of gestation ($SD \pm 8.5$). No differences were found between women who received antenatal corticosteroids to reduce infant complications at birth and those who did not on any of the variables; so it was not controlled for

in the analysis. Similarly, prenatal risk was not related to cortisol values or perceived stress so there was no adjustment for it in the correlational analyses.

Stress and Cortisol Parameters

Women reported a moderate amount of perceived stress ($M=14.86, \pm.88$, possible scores 0-40). About 22% experienced moderately high or high stress based on their scores.

Saliva samples were collected for 57 women, and less than 5% of the saliva samples were missing. Values for all cortisol parameters are presented in **Table 3.2**. As shown in **Figure 3.1**, cortisol rose after awakening and followed an expected decline, with a steeper fall from wake time to noon and dampened decline between 4pm and prior to the hour of sleep.

The Relationship between Stress and Cortisol

For the total sample, women's perceived stress was not related to any of the cortisol parameters (See **Table 3.3**). Because cortisol levels increase as delivery nears (Glynn et al., 2001), we examined women who were in the earlier stage of their third trimester and those closer to parturition as separate groups. Significant findings emerged when data were analyzed based on the woman's week of gestation (See **Table 3.4**). For women whose cortisol was sampled before 29 weeks of gestation, a negative correlation was found between perceived stress and overall mean cortisol level ($r=-.37, p<.05$). Women who had higher self-reported stress had lower overall mean cortisol values. Additionally, among the women sampled earlier in the third trimester, positive correlations between perceived stress and both CAR ($r=.37, p<.05$) and AUC_G ($r=.42, p<.05$) were found. When cortisol was measured later in the third trimester, inverse but non-significant relationships were found between self-reported stress and mean cortisol level, CAR, and AUC_G. No significant relationships were found between diurnal slope and self-reported stress at either time point during pregnancy (See **Table 3.4**).

Discussion

Summary of Key Findings

For the total group, women's perceived stress was not related to their average salivary overall mean cortisol, CAR, cortisol diurnal slope, or AUC_G. However, when examining differences in these associations based on women's specific stage of gestation, there were a number of significant relationships between cortisol values and perceived stress for women in the early third trimester. Women at or before 29 weeks of gestation had lower cortisol levels when experiencing greater perceived stress. They also demonstrated a steeper CAR when experiencing greater perceived stress. This finding was the opposite of the cortisol and stress relationships we found for women in later gestation (**Table 3.4**). Women sampled before 29 weeks of gestation also showed an elevated AUC_G with greater perceived stress.

The Relationship of Findings to Previous Research

Our findings for the total sample support previous research on the relationship between salivary cortisol and perceived stress. Results for women who were 30 weeks gestation or greater support the majority of research to date, suggesting that self-reported stress and cortisol parameters are not correlated during pregnancy and may be measuring different facets of the stress experience. However, elevations of CAR and AUC_G in response to higher perceived stress have been reported as normative responses in other healthy populations (Chaumette et al., 2016; Chida & Steptoe, 2009; Hellhammer et al., 2007; Pruessner, Cullen, Aas, & Walker, 2017; Stalder et al., 2016; Zorn et al., 2017). These responses are congruent with our findings for women at 29 weeks gestation or less. An individual's ability to mount an adequate HPA-axis response to external demands or stressors has been described as adaptive under most conditions (Herman et al., 2016; Herman, McKlveen, Solomon, Carvalho-Netto, & Myers, 2012; Jacobson,

2014; Vashist & Schneider, 2014). As part of this response, a cascade of hormones is released, including cortisol. It is possible that the dynamic fluctuations in various hormones that occur during pregnancy may mask or modulate more typical relationships between cortisol values and perceived stress that are observed in other populations.

Three previous studies found no association between cortisol level and perceived stress during the third trimester of pregnancy (Harville, Savitz, Dole, Herring, & Thorp, 2009; Salacz et al., 2012; Shaikh et al., 2011). However, Valladares et al.'s (2009) cross-sectional study of Nicaraguan women during the second and third trimester found that higher perceived stress was associated with higher means levels of cortisol in the afternoon (Valladares, Pena, Ellsberg, Persson, & Hogberg, 2009). Findings in the Valladares et al. study combined second and third trimester data and did not represent mean level of cortisol across times of the day and across days (as did ours) so comparison with their results is not ideal.

Our findings also support previous research regarding CAR and perceived stress. In Bolten et al.'s (2011) prospective study of women in Germany during their third trimester, neither stress specifically related to pregnancy fears or generalized perceived stress were associated with CAR. Similarly, in a repeated measures study of women in the U.S., no significant relationship between CAR and perceived stress was found during the third trimester (Cheng & Pickler, 2010).

For diurnal cortisol slope, only one study was available for comparison; but it was also congruent with our findings. For U.S. women at 36 weeks gestation, Kivlighan et al. (2008) also found that normal decline in cortisol level across the day was not affected by perceived stress.

We identified only one other study that examined AUC_G and perceived stress during the third trimester. In a cohort of diverse women from the Southern U.S., Harville et al (Harville et al., 2007) reported no correlation between AUC_G and perceived stress for women sampled at 24-29

weeks of gestation. Our findings do not support this research. For women in our study who were sampled at this same gestational time point, we found a strong, positive relationship between higher AUC_G and greater perceived stress.

Implications: Accounting for Week of Gestation in Understanding Pregnancy Stress

The different associations between cortisol values and perceived stress that we found for women at different stages of the third trimester may be influenced by natural changes in cortisol levels that occur as gestation proceeds. These changes affect other hormones such as estrogen, as well as inflammatory markers that may in turn influence perceptions of stress. It has been posited that there is an intricate system of different clocks, driven by hormones, that determines the onset of parturition (Menon, Bonney, Condon, Mesiano, & Taylor, 2016). Down-regulation of cortisol parameters when experiencing perceived stress may serve a protective role for women in later gestation by enabling the pregnancy to progress closer to the desired 40 weeks of gestation. If cortisol responded to perceived stress in a robust way after 29 weeks of gestation, the cascade of hormones that initiate parturition could be released. This interpretation supports other findings that the hormonal milieu changes just before an infant's arrival. For instance, there is evidence that cortisol levels increase preceding the onset of parturition (Busada & Cidlowski, 2017) and that the CAR is attenuated as gestation progresses (Buss et al., 2009; Entringer, Buss, & Wadhwa, 2010). The maternal stress response system may be programmed to respond differently to perceived stress as delivery becomes more imminent.

For women earlier in the third trimester, perceived stress appears to be more closely aligned with their cortisol awakening response (CAR) and their total cortisol secretion across the day (AUC_G) than with diurnal slope.

In addition, decreased mean levels of cortisol were associated with greater self-reported stress for these women. These findings indicate that higher cortisol levels per se are not necessarily an objective and reliable indicator of the stress experienced by women. In contrast, higher AUC_G was strongly associated with higher perceived stress. AUC_G is a measure of total cortisol output rather than the average level of cortisol secretion (Pruessner et al., 2003). This marker may provide a more significant indicator of the intensity of overall hormonal exposure experienced by a woman than simply averaging levels of secretion. The rationale for not seeing these relationships in the latter part of the third trimester could stem from increased efforts of the negative feedback mechanism of the HPA axis to dampen overall cortisol output in response to perceived stress. Such a response may serve a protective function as delivery nears, by preventing rising cortisol concentrations that could stimulate labor. Future research needs to examine this hypothesis.

These findings should be interpreted cautiously, as the sample is small and may not be representative of all women's experiences during pregnancy. In particular, the women were well-educated and almost entirely living with a partner. However, the sample was diverse and included women who were both employed and unemployed as well as those who were primiparous and multiparous. Although it has been documented that there are differences among women based on their racial and ethnic identity (Corwin et al., 2013; Harville, 2009), the limited sample size did not permit for meaningful comparisons between the different races and ethnicities represented in the study. In addition, we were interested in the relationship of cortisol to general life stress. It is possible that measures of pregnancy-specific stress would show different relationships to cortisol parameters. Lastly, it is important to note that salivary cortisol provides information about immediate cortisol secretion by the HPA axis whereas our measure

of perceived stress asked for the woman's retrospective account of her stress over the last month. The different time periods assessed by these measures could diminish the potential for significant associations (Weckesser et al., 2019). A measure of hair cortisol would help to reduce the difference in time periods by reflecting past cortisol exposure.

Implications for Further Research

Our findings suggest the importance of examining both perceived stress and cortisol values when studying stress during pregnancy. Results indicate that these measures may provide different information at different times during pregnancy and likely measure different facets of stress. Different cortisol parameters appear to have unique relationships to self-reported stress that help in understanding a more comprehensive picture of the stress response during pregnancy. In particular, limiting cortisol assays to the average level of cortisol may underestimate and restrict our understanding of cortisol's relationship with perceived stress. This concern is supported by our findings that CAR and AUC_G showed more significant and opposite associations with perceived stress than average cortisol level for women in the early stage of their third trimester.

Further research is needed to confirm these findings with a larger sample and using additional measures of both stress and stress-related biomarkers. If our results are replicated with larger samples, they may have ultimate implications for more accurate and precise assessment of stress experienced by pregnant women. Such assessments could then be used to better inform the need for interventions to reduce or manage stress.

The relationships between symptoms of stress experienced by women and their stress biomarkers remains unclear, with the changing hormonal milieu during pregnancy introducing challenges to meaningful interpretation. There is also a need to examine potential moderators,

such as sleep time and fatigue, chronic stress, and depression since these have been associated with stress response in previous research (Adam et al., 2017; Dedovic & Ngiam, 2015; Epstein et al., 2019; Fries et al., 2009; Vargas & Lopez-Durand, 2017). Most importantly, our findings emphasize the need to better understand the complex and changing dynamics of stress during pregnancy. While we found significant associations between perceived stress and varied cortisol parameters for women between 26-29 weeks of gestation, these relationships did not persist beyond 29 weeks of gestation. Dynamic fluctuations in various hormones during pregnancy may modulate cortisol values and how they respond to perceived stress in unique and important ways.

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Table 3.1 Demographic, Clinical and Stress Descriptors for the Sample (n=58)

Sample characteristic	Mean \pm SD or % (n)
Age	35.5 \pm 4.8
Parity status	
Nulliparous	55.2% (32)
Multiparous	44.8% (26)
Partnership status	
Partnered	93.1% (54)
Single	6.9% (4)
Occupational status	
Full time employed	58.6% (34)
Homemaker/unemployed	20.7% (12)
Part-time/occasionally employed	19% (11)
Educational attainment	
Elementary or high school	12.1% (7)
Some college/associates degree	13.7% (8)
College graduate	31% (18)
Graduate degree	43.1% (25)
Recipient of government assistance	
Multiple forms of assistance	15.5% (9)
One form of assistance	10.3% (6)
No assistance	74.1% (43)
Race (n=52)	
White	63.5% (33)
Asian	17.3% (9)
Black	3.8% (2)
Other	15.4% (8)
Ethnicity	
Hispanic	19% (11)
Non-Hispanic	81% (47)
Perceived Stress score	14.88 \pm 6.48
Prenatal Risk Score	3.54 \pm 1.67
Antenatal Corticosteroids	15.5% (9)
Week of Gestation Received	30.75 \pm 3.0

Table 3.2 Salivary Cortisol Parameters (n=53)

Note: All parameters measured in $\mu\text{g/dl}$

*n=52

Cortisol Index	Mean \pm SD	Range
Overall mean cortisol value	.23 \pm .10	.02-.55
Cortisol awakening response	.07 \pm .14	-.41-.29
Diurnal cortisol slope	.28 \pm .18	-.02-.78
Area under the curve *	-126.43 \pm 94.74	-460.97-14.28

Table 3.3 Pearson Correlations between Women's Perceived Stress and their Salivary Cortisol Parameters (n=58)

Note: Correlations controlled for week of gestation when samples were acquired and were based on log transformed scores for CAR and diurnal slope.

Cortisol Index	r
Overall mean cortisol level	-.16
Cortisol awakening response	.05
Diurnal cortisol slope	-.16
Area under the curve	.22

Table 3.4 Pearson Correlations between Women's Perceived Stress and their Salivary Cortisol Parameters by Stage of 3rd Trimester

* p<.05

Cortisol Parameters	Early 3 rd Trimester	Late 3 rd Trimester
	\bar{r} <u>n=26</u>	\bar{r} <u>n= 32</u>
Overall mean cortisol level	-.37 *	.14
Cortisol awakening response	.37*	-.29
Diurnal cortisol slope	-.14	-.24
Area under the curve	.42*	-.15

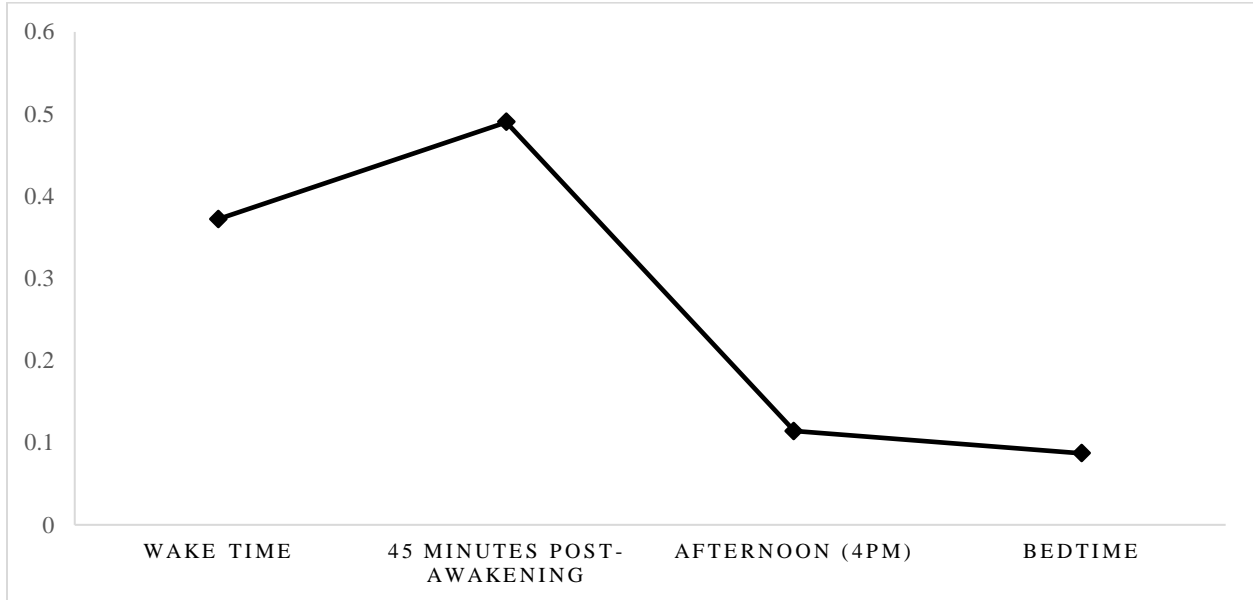


Figure 3.1 Salivary Cortisol Values across the Day

Note: Cortisol measured in µg/dl

Cortisol and Self-Reported Stress during the Third Trimester of Pregnancy: Interactions with Fetal Sex in Predicting Birth Outcomes

Abstract

Objective: Birth outcomes such as prematurity and low birth weight are often associated with neonatal morbidity and mortality. Stress of women during pregnancy has been linked to adverse birth outcomes. However, there have been inconsistent findings on whether self-reported stress or stress-related hormones may best predict birth outcomes and little attention to the role of fetal sex as a potential moderator of these relationships. The purpose of this study was to determine if cortisol or perceived stress best predict birth outcomes, and if fetal sex moderates the relationship between stress or cortisol and birth outcomes.

Methods: The sample included 58 women who were recruited in obstetric clinics during their third trimester of pregnancy. They ranged in age from 23 to 47 years and were between 26 and 40 weeks gestation. Women completed the Perceived Stress Scale and provided 4 saliva samples each day across 2 days from the time they awoke until bedtime. Salivary samples were used to determine average cortisol level, cortisol awakening response (CAR), diurnal slope, and total cortisol secreted throughout the day (AUC_G). Electronic medical records were used to derive a measure of perinatal risk and acquire data on infant gestational age, weight, and sex at birth. Logistic regression models were computed to determine the odds of infants being born preterm or low birth weight as a result of their mothers' self-reported stress and cortisol parameters. The moderating role of fetal sex in the relationship between stress or cortisol and birth outcomes was also examined.

Results: Average gestational age was 38.36 weeks (± 2.12). Infants weighed 3,055 grams on average (± 690.16). Regression models showed that neither women's perceived stress nor

most of the cortisol parameters predicted adverse birth outcomes. However, male infants whose mothers had higher salivary CAR during the third trimester of pregnancy had greater odds of being born low birth weight (OR=1.36, p=.04).

Conclusions: Findings suggest that a woman's elevated CAR during the 3rd trimester could serve as an indicator of risk for low birth weight male infants. Research is needed to identify steroidal and/or epigenetic mechanisms that may contribute to greater vulnerability to maternal stress for the male fetus.

Background

Adverse birth outcomes such as preterm birth and low birth weight threaten both children's survival and their health (World Health Organization, 2015). Later cardiovascular disease, cognitive delays, autism, and diabetes are common in infants who are born prematurely (Saigal & Doyle, 2008). Children with low birth weight frequently have behavioral problems as well (Debattista, Huffman, Alkon, Cooper, & Weiss, 2015; Weiss, 2005; Weiss, Wilson, & Morrison, 2004). In addition to the challenges such problems create for children and their families, hospitalizations during the first year of life for low birth weight and preterm infants cost billions of dollars each year (Russell et al., 2007).

Of the nearly four million U.S. infants born in 2016, 389,075 infants were preterm. For 50% of these births the reasons for prematurity remain unknown (Martin, Hamilton, Osterman, Driscoll, & Drake, 2018). Evidence suggests that stress during pregnancy may play a role in adverse birth outcomes (Lilliecreutz, Larén, Sydsjö, & Josefsson, 2016; McEwen, 2007; Wadhwa, Entringer, Buss, & Lu, 2011). Previous research indicates that high maternal perceived stress may shorten the length of gestation, and increase the risk of an infant being small for gestational age (SGA) or low birth weight (LBW) (Gavin, Nurius, & Logan-Greene, 2012; Kane,

Dunkel Schetter, Glynn, Hobel, & Sandman, 2014; Lau, 2013; Sable & Wilkinson, 2000).

However, findings are not consistent across studies. Results of five studies indicate that women who report greater stress during pregnancy are more likely to deliver a low birth weight infant and have an infant born early (Gavin et al., 2012; Heaman et al., 2013; Hoffman, Mazzoni, Wagner, Laudenslager, & Ross, 2016; Lau, 2013; Sable & Wilkinson, 2000). Two studies found no relationship between perceived stress and birth outcomes (Ghaemmaghmi et al., 2013; Goedhart et al., 2010).

In addition, studies indicate that women's cortisol, the down-stream hormone of the hypothalamic-pituitary-adrenal axis, may be a more accurate predictor of low birth weight and shorter gestation than self-report regarding their stress (Baibazarova et al., 2013; Bolten et al., 2011; Cheng & Pickler, 2010; S. Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011; Hoffman et al., 2016; Kivlighan, DiPietro, Costigan, & Laudenslager, 2008). But not all studies support these findings. Three studies that examined cortisol did not identify a relationship with birth outcomes (Ghaemmaghmi, Dainese, La Marca, Zimmermann, & Ehlert, 2014; Goedhart et al., 2010; Shaikh et al., 2011).

When perceived stress and cortisol have been assessed together, most research studies have found that cortisol is more predictive of low birth weight and shorter gestation than self-reported perceived stress using standard questionnaires. Seven studies reported that women whose cortisol profiles were different from expected cortisol norms (i.e., high overall cortisol values, elevated cortisol awakening response (CAR), dampened afternoon trajectory) had greater risk for delivery of infants who were lower birth weight and born at an earlier gestation (Baibazarova et al., 2013; Bolten et al., 2011; Buss et al., 2009; D'Anna-Hernandez et al., 2012; S. Entringer et al., 2011; Ghaemmaghmi et al., 2014; Hoffman et al., 2016). However, these studies differ in the specific

cortisol parameters that were found to be predictive, including overall cortisol level, cortisol awakening response (CAR), and the decline in cortisol secretion throughout the day. To some extent, these differences may relate to the timing of cortisol sampling during pregnancy. Cortisol patterns change across pregnancy, with an increase in cortisol levels being greatest as parturition approaches (Busada & Cidlowski, 2017) and CAR becoming attenuated (Buss et al., 2009; Entringer, Buss, & Wadhwa, 2010).

It is also important to note that a number of studies show differences in how stress of the pregnant woman or exposure to stressors may have differential effects on birth outcomes, depending on whether the fetus is male or female (Chason et al., 2012; Goedhart et al., 2010; Graignic-Philippe, Dayan, Chokron, Jacquet, & Tordjman, 2014). For example, following natural disasters and intentional mass casualty events, the ratio of male to female births changes, with more females being born than males (Torche & Kleinhaus, 2012; Tourikis & Beratis, 2013). Women who experience childhood trauma and have symptoms of post-traumatic stress are also less likely to have a male infant (Kaitz, Rokem, Mankuta, Davidov, & Faraone, 2014).

Additionally, cortisol has differential effects based on fetal sex. Among women sampled prior to conception, those with elevated salivary cortisol were less likely to give birth to a male infant (Chason et al., 2012). Further, it has been found that male infants are more likely to be born SGA and low birth weight when the pregnant woman has elevated salivary cortisol (Frith, Naved, Persson, & Frongillo, 2015; Khashan et al., 2014). In addition, when women have elevated salivary cortisol during pregnancy, only their male infants are more likely to have altered stress reactivity and delayed neurocognitive growth (Braithwaite et al., 2017; Ellman et al., 2008). These findings suggest an increased vulnerability of the male fetus to elevated maternal stress, including stress experienced during both the preconception and pregnancy time

periods. However, not all studies have been able to replicate these findings. Two studies found no differences between male and female infants in relation to effects of maternal salivary cortisol parameters during pregnancy (Entringer et al., 2010; Shaikh et al., 2011).

The purpose of this study was to contribute new knowledge that may help clarify the conflicting findings from previous research. The two study aims were: 1) to determine whether women's self-reported stress or specific cortisol parameters during the third trimester of pregnancy are better predictors of delivering an infant preterm or LBW, and 2) to determine whether fetal sex moderates the relationship of either perceived stress or cortisol parameters to preterm birth or LBW. We hypothesized that women's cortisol parameters would be a more significant predictor of PTB and LBW than their self-reported stress. We also proposed that male fetuses may be more vulnerable to altered cortisol parameters or elevated perceived stress of their mothers during pregnancy, resulting in stronger relationships of these predictors to preterm birth and LBW for males than females.

Methods

Sample and Recruitment

This study included a subsample of women and infants who are part of a larger cohort study in the San Francisco Bay Area (Weiss, R01 HD081188-05). The purpose of the parent study was to determine the effects of antenatal corticosteroids and maternal depression on stress regulation of infants over the first year of life. Recruitment of women involved review of clinical rosters at obstetric clinics of two university medical centers by a Clinical Research Coordinator, who then identified eligible participants at risk for preterm delivery. Women between 24 and 34 weeks gestation were invited to participate. Potential participants were approached by a research assistant (RA) who provided a brief verbal explanation of the study activities, answered

questions, and gave women an informative flier. Women interested in participating read, signed, and received a copy of their informed consent.

Women who enrolled were 18 years of age or older, were fluent English or Spanish speakers with the ability to read and write, and lived within one hour of the medical center. Exclusion criteria included being too psychologically or physically ill to participate, having a cognitive impairment, having an adrenal or endocrine disorder, or using a prescribed steroid medication (oral, inhalation, or topical). The research was approved by UCSF's Internal Review Board for Human Research Protection.

Procedures

The enrolled women received a questionnaire on perceived stress, a demographic questionnaire, and detailed instructions at the obstetric clinic visit on how to provide 4 saliva samples each day across 2 consecutive days. The RA taught the participants how to collect saliva samples using the passive drool method (Granger et al., 2007). Women were advised by the RA to provide their saliva samples on a day that didn't include any unexpected changes in their usual routines. They were also instructed to not consume food, engage in physical exercise, consume alcohol or caffeine, or take any medications or drugs an hour prior to their saliva sampling.

In addition to the RA's verbal teaching, women received detailed written and pictorial instructions on when and how to collect their saliva across the two sampling days. They also completed a brief form to document any factors that may have affected the quality of their saliva and the time they provided each of the 8 saliva samples. These printed materials were supplemented with phone calls or text messages from study staff to provide answers to any questions and facilitate collection of their samples. Saliva samples were obtained from 57 women, with less than 5% of the saliva samples missing.

The protocol for saliva data collection included rinsing their mouth with water 10 minutes prior to their sampling time. With the help of a cotton roll, they drooled into a cryovial until 1 ml of saliva was obtained. Samples were collected at their wake time, 45 minutes after waking, around 4pm, and just before sleep at night. Samples were stored in their home freezer before mailing them to study staff. After women completed their two days of saliva sampling and questionnaires, they mailed their materials to study staff. Upon receipt, staff brought samples to a lab in UCSF's Pediatric Clinical Research Center for storage, before being shipped to Salimetrics biochemical laboratory for analysis. Infant sex, gestational age, birth weight, variables indicative of prenatal risk (unusual features of pregnancy, gynecology and obstetrics history, complications of past pregnancies, family history, patient history, current pregnancy complaints), and other potential confounds were extracted from the electronic medical record.

Measures

Sociodemographic. Information was collected on participants' age, employment status, race, ethnicity, and level of government support on a sociodemographic questionnaire completed after enrollment. Sociodemographic data were used to describe the sample characteristics.

Prenatal Risk. Clinical risk factors that may contribute to adverse birth outcomes were collected from the electronic medical record and used to score the Obstetric Medical Risk Index (Lobel et al., 2008). Possible scores range between 0-37, with greater scores representing elevated risk. The index has the following domains: unusual features of pregnancy, gynecology and obstetrics history, complications of past pregnancies, family history, patient history, current pregnancy complaints, and other factors specific to timing of gestation. Items on the index were derived from a meta-analysis, along with an expert consensus, to establish content validity.

Previous research indicates the Obstetric Medical Risk Index can have excellent predictive validity with adverse birth outcomes (Lobel, DeVincent, Kaminer, & Meyer, 2000).

Perceived Stress Scale. Psychological stress was measured using Cohen's 10 item Perceived Stress Scale (PSS-10; Cohen et al., 1983). The PSS-10 captures a self-reported, global assessment of perceived stress over the past four weeks. It contains four positive questions that are reverse scored and six negative questions. Each response is rated on a 5-point Likert scale. The sum of the 10 questions (10-50) is used as a stress score, with higher scores indicating higher perceived stress (Taylor, 2015).

The PSS-10 has been administered to pregnant women across many countries and in several languages (Andreou et al., 2011; D'Anna-Hernandez, Aleman, & Flores, 2015; Lau, 2013; Lesage, Berjot, & Deschamps, 2012; Remor, 2006). It has demonstrated good internal consistency in a heterogeneous Canadian sample that included peripartum women (Cronbach's $\alpha = .85$) (Robinson, Benzies, Cairns, Fung, & Tough, 2016). Predictive validity has also been demonstrated in a sample of 581 pregnant women who had clinical risk factors for preterm birth (Lau, 2013).

Salivary Cortisol. High sensitivity salivary cortisol enzyme immunoassay (ELISA) was used to analyze samples in duplicate. The assay has a lower limit of sensitivity of .007 $\mu\text{g}/\text{dl}$, a standard curve ranging from 0.012-3.0 $\mu\text{g}/\text{dl}$, an average intra-assay coefficient of variation of 4.6, and an average inter-assay coefficient of variation 6%. Four cortisol parameters were derived from the cortisol values: overall mean cortisol level, Cortisol Awakening Response (CAR), the diurnal trajectory or decline (slope) from awakening to bedtime, and the area under the curve or pattern of total cortisol output across the day (AUC_G). The mean for the two days of samples was used to calculate each of the cortisol parameters.

The four cortisol parameters were operationalized as follows. Mean cortisol level was calculated by averaging the total cortisol concentration of the 8 samples from the 2 days of sampling. The CAR was the change in cortisol from wake time to 45 minutes following wake time (Alder et al., 2011). The diurnal trajectory or slope was the linear degree of change in cortisol values across the day from initial waking to the sample prior to sleep. AUC_G measured the pattern of total cortisol output across the day, considering the difference between individual cortisol samples and the time between each sampling period. The trapezoidal formula from Pruessner et al. was used (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Birth Outcomes. The infant's electronic medical record was reviewed after women gave birth to collect data on sex, gestational age and weight at birth. Infants born less than 37 weeks were classified as preterm. Infants born less than 2,500 grams were classified as LBW (Martin, Hamilton, Osterman, Driscoll, & Mathews, 2017).

Data Analysis

All variables were tested for linearity and normality of their distributions. Key variables showed adequate distributions and acceptable skew. Week of gestation when the salivary sample was acquired was correlated with all stress-related predictors as well as with infant birth weight and gestational age at birth to determine whether time of sampling should be included in the analyses. However, time of sampling had no relationship to any of the predictor or outcome variables. Partial bivariate correlations were calculated to examine the relationships of the predictors (PSS-10, mean cortisol, CAR, diurnal slope, AUC_G) to gestational age and weight at birth for the sample as a whole and by fetal sex.

Four multiple logistic regression models were computed on the two outcomes of interest: preterm birth and low birth weight. In the first step, perinatal risk was entered into the model. At

the second step, perceived stress, one cortisol parameter (i.e. mean cortisol, CAR, slope, or AUC_G), and fetal sex were entered. At a third step, two interaction terms were entered to test the moderating effect of fetal sex on the relationship of both perceived stress and the specific cortisol parameter being examined with preterm birth and LBW status.

Continuous variables (cortisol parameters, perceived stress, prenatal risk, age, week of gestation when cortisol was collected, gestational age at birth, and birth weight) were explored using descriptive statistics. Categorical data (parity status, partnership status, occupational status, educational attainment, government assistance, race, ethnicity, delivery method, infant sex, preterm birth status, LBW status, SGA status, and delivery method) were examined using frequencies and percentages. All tests were conducted as two-sided, with $p < .05$. Analyses were computed using SPSS 25.

Results

Characteristics of the Women

Sociodemographic characteristics of the enrolled women are listed in **Table 4.1**. Of the 58 women in the sample, 64% were white, 17% Asian, 4% Black, and 16% reported “other” race. Nineteen percent identified Hispanic ethnicity. The majority were in a partnered relationship (86%) and most (59%) were full-time employed and nulliparous (59%). Approximately a quarter (24%) of the participants received government support.

Women in the study ranged from 26 to 40 weeks of gestation. Overall, they experienced a low level of prenatal medical risk (mean=3.5 out of a possible 37 risks). Few women ($n=9$) received corticosteroids as a prophylactic treatment during their pregnancy, with the average being receipt at 30.67 weeks of gestation ($SD \pm 8.5$). No differences were found between women who received antenatal corticosteroids and those who did not for either of the outcomes, so it

was not controlled for in the analysis. The women experienced a moderate amount of perceived stress (mean=14.86, SD \pm .88, range 0-40). 22% experienced moderately high or high stress based on their scores. Salivary samples were collected on average at 31 weeks of gestation (range 26-40). The mean cortisol secreted was .23 μ g/dl (SD \pm .10, range .02-.55), the average CAR was .07 μ g/dl (SD \pm .14, range -.41-.29), the mean diurnal slope was .28 μ g/dl (SD \pm .18, range -.02-.78) and the mean AUC_G was -126.43 μ g/dl (SD \pm 94.74, range -460.97-14.28).

Infant Characteristics

The mean infant gestational age was 38.36 (SD \pm 2.12, range 29.1-41.3) and mean birthweight was 3,054.55 grams (SD \pm 690.16, range 1,250-4,400).16% of infants in the sample were born preterm and 22% were LBW. 41% of the sample were males (n= 24) born at 38.57 weeks gestation on average (SD \pm 1.69, range 35-40.5) and weighing an average of 3,206.87 grams (SD \pm 722.5, range 1925-4400). 59% of the sample were females (n=34) born at an average of 38.2 weeks gestation (SD \pm 2.40, range 29.1-41.3). The mean of the female's weight was 2,947.03 grams (SD \pm 655.84, range 1,250-4,285). There were 8 infants who were part of a twin set, only one twin was included per set of twins, and the infant who emerged first was selected for inclusion. We examined whether infants born as a twin differed from other infants in their birth weight or gestational age. Twins did not differ from other infants so this variable was not adjusted for in the regressions. Additional data on birth status are shown in **Table 4.2**.

Results of Logistic Regression Models

Aim 1. Perceived Stress and Cortisol as Predictors of Preterm Birth and LBW. Of the 8 logistic regression models that were computed, only one model showed any significant relationship of either self-reported stress or a cortisol parameter to preterm birth or LBW (see **Table 4.3**). This model examined predictors of LBW. As can be seen in **Table 4.3**, perceived

stress had no relationship to LBW; the odds ratio (OR) was very close to 1 (OR=.99, $p=.95$). This was the case for the relationship of perceived stress to preterm birth as well (OR= .84, $p=.47$). However, the relationship of CAR to LBW was significant (Wald Statistic =4.78) and the OR was .690 ($p=.03$; CI: .494, .962). Odds ratios for all other cortisol parameters that were tested were not significant, as evidenced by their Wald Statistics, ORs and confidence intervals.

Aim 2. The Moderating Effect of Fetal Sex. Results indicate that fetal sex had no moderating effect on the relationship between perceived stress and either preterm birth or LBW. The only significant interaction with fetal sex was on the relationship between CAR and low birth weight, also shown in **Table 4.3**. The OR was 1.36 (CI: 1.02, 1.82), $p=.04$. In order to better understand the meaning of this finding, we computed separate partial correlations for males and females to examine the relationship between CAR and birth weight, controlling for prenatal risk. These correlations are shown in **Table 4.4**, along with sex-specific correlations of birthweight and gestational age to other cortisol parameters and perceived stress. The correlation between CAR and birth weight for females was $r = .04$ while it was $r = -.26$ for males. Although the correlation for males did not reach significance, most likely due to small sample size, a coefficient of $-.26$ is approximately a moderate effect size (Cohen, 1988, 1992). These coefficients suggest that male infants whose mothers had higher CAR had lower birth weight than male infants whose mothers had lower CAR. In contrast, maternal CAR appeared to have no relationship to birth weight for females. This interaction indicated that the main effect we observed for the total sample may have resulted from an averaging of sex differences (weighted toward higher numbers of females in the sample) and a loss of the unique effects observed for males and females.

A few of the other correlations in **Table 4.4** also appear to be of moderate effect size, including CAR and gestational age for females ($r = .32$). For males, moderate effect sizes were found for gestational age as well, with both diurnal slope ($r = .33$) and AUC_G ($r = -.30$). However, these relationships were not significant in the logistic regression models, either for the group as a whole or for a sex-specific interaction.

Discussion

Summary of Key Findings

Perceived stress of women during pregnancy was not related to either preterm birth or LBW. Similarly, average cortisol level, diurnal slope and AUC_G were not related to either birth outcome. However, women who had elevated salivary CAR during the third trimester of pregnancy were more likely to deliver a LBW baby. This finding showed a moderating effect of fetal sex, with male fetuses having higher odds of being LBW. There were no other moderating effects of fetal sex for preterm birth or LBW. Although perinatal risk was entered into the model to control for its potential effect, it was not related to either PTB or LBW. This finding may be due to the fact that this sample of women had relatively low prenatal risk, with minimal variance in risk level.

The Relationship of Findings to Previous Research

Although only one cortisol parameter significantly predicted preterm birth or LBW, our findings are congruent with the majority of other studies which have shown that cortisol is more predictive of birth outcomes than perceived stress. Two of these studies specifically examined CAR in association with birth weight. Bolten et al.'s 2011 study of 75 women in Germany between 35-37 weeks' gestation found a similar result to what we found for males, reporting that elevated CAR was associated with lower birth weight while self-reported stress had no

relationship to birth outcome. However, Cheng and Pickler's 2010 study of 39 women in the U.S. included women who were sampled after 36 weeks of gestation and did not find a relationship of either CAR or perceived stress to birth weight.

Although fetal/infant sex is reported in many studies that have examined the impact of cortisol and perceived stress on birth outcomes (Bolten et al., 2011; Hoffman et al., 2016; Su et al., 2015), we found only one other study that examined the moderating effect of fetal sex on cortisol's relationship to birthweight. A study of 1,041 women in Bangladesh found results similar to those in our research for a different cortisol parameter. Male infants were more likely to weigh less at birth when their mothers had elevated morning salivary cortisol during 28-30 weeks gestation (Frith et al., 2015).

With the rise in cortisol expected across the course of pregnancy, it is important to consider the timing of when samples were collected during gestation. The women in our sample provided their samples on average, around 31 weeks of gestation, similar to the women in Frith et al.'s (2015) research. Women were sampled around 35 weeks of gestation in the other studies that examined how perceived stress and CAR relate to birth weight (Bolten et al., 2011; Cheng & Pickler, 2010). Entringer et al (2011) sampled women around 39 weeks of gestation. Since CAR is attenuated with advancing gestation (Buss et al., 2009; Entringer et al., 2010), its predictive value may be lessened in these later gestational groups. Longitudinal assessment over the course of gestation is needed to better understand specific points during pregnancy that may increase vulnerability of the fetus to cortisol elevations, including those specific to males or females.

In addition, our sample size was larger than Entringer et al. (2011) and Bolten (2011) which may have enhanced our ability to identify moderating effects. Frith et al.'s (2015) sample was very large, providing excellent power to identify moderating effects of fetal sex. The women in

our sample also experienced some degree of risk associated with their pregnancy, unlike other studies that only included women with uncomplicated pregnancies (Bolten, 2011; Entringer, 2011). Women with different degrees of risk are likely to respond differently to stress and elevated cortisol secretion than those with fewer challenges.

Lastly, the prevalence of preterm birth and LBW status was relatively higher in this sample than in other studies that have examined these outcomes. Our substantial proportion of infants with LBW and prematurity enabled us to more effectively assess the influence of perceived stress and cortisol on these outcomes.

Significance of the Findings

Although Frith et al. (2015) found a sex-specific moderating effect of elevated morning cortisol on low birthweight, to our knowledge, our study is the first to report a sex-specific moderating effect on the relationship between CAR and LBW status. Disparate findings on the relationships between cortisol and birth outcomes may be influenced by lack of attention to fetal sex and large enough samples to examine them. It would be valuable to study cortisol parameters both prior to conception and throughout the course of a pregnancy to understand the differential responsiveness of male and female fetuses to cortisol in women.

Males and female fetuses may have differences in the sensitivity of their developing HPA-axis to elevated cortisol during gestation, including differential glucocorticoid or mineralocorticoid receptor response, effectiveness of negative feedback mechanisms in shutting down hormonal secretion, or the ability of the placenta to serve as an effective barrier to excessive cortisol exposure. Differences in stress reactivity after birth have been noted in infants based on sex (Braithwaite et al., 2017). In addition, male infants are reported to have higher mean levels of cortisol, as compared to females after birth (Davis & Emory, 1995). Differences

in cortisol secretory patterns appear to persist through childhood between males and females (Hollanders, Van Der Voorn, Rotteveel, & Finken, 2017).

These results should be interpreted cautiously, as the sample size was limited, and may not be generalizable to either a pregnant group with no prenatal risk factors or a group with many prenatal risk factors. The women were also well educated and mostly living with a partner. Infants were mostly late preterm (between 34 and 37 weeks gestation), a group that has shown unique developmental challenges and thus may have unique responses to the gestational environment (McDonald et al., 2013 McIntire & Kenneth, 2008; Shapiro et al., 2006). Later preterm infants may have different mechanisms that underlie their birth risk.

Implications for Further Research and Practice

Findings suggest that CAR may be a better measure than perceived stress or other cortisol parameters for predicting the likelihood of having a low birth weight infant when assessed during the third trimester of pregnancy. More research is needed to specifically examine effects of CAR and other parameters on adverse birth outcomes. Not all of the cortisol parameters may be equally useful as predictors or may measure different aspects of HPA functioning.

Further research is needed to replicate these findings in a larger sample that has more heterogeneity and the power to examine moderating effects of fetal sex. It is important to include women who are experiencing complications associated with their pregnancy, since most studies that examine women during pregnancy either focus on women who are either expecting a healthy, uncomplicated pregnancy or who have many risk factors. Epigenetic studies are also needed to evaluate potential effects of elevated cortisol exposure on expression of stress-related genes such as the glucocorticoid receptor. High levels of cortisol may have differential effects on methylation of stress-related genes for the male and female fetus.

With salivary CAR being a non-invasive biomarker, it may be a valuable clinical tool in risk assessment of pregnant women. Currently, there is no specific laboratory test to effectively assess birth risk. If future findings support the role of CAR in predicting risk, salivary CAR sampling could be a valuable method to assist in mitigating women's risk for delivering an infant of small size and related morbidities.

Acknowledgements

Support for this study was provided by a grant from the National Institute of Child Health and Human Development (Weiss, R01 HD081188-05) and the National Institute of Nursing Research T32 Biobehavioral Research Training Program in Symptom Science (NR016920).

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Table 4.1 Demographic, Clinical, and Stress Descriptors for Women in the Sample (n=58)

Characteristic	Mean \pm SD or % (n)
Age	35.5 \pm 4.8
Parity status	
Nulliparous	55.2% (32)
Multiparous	44.8% (26)
Partnership status	
Partnered	93.1% (54)
Single	6.9% (4)
Occupational status	
Full time employed	58.6% (34)
Homemaker/unemployed	20.7% (12)
Part-time/occasionally employed	19% (11)
Educational attainment	
Elementary or high school	12.1% (7)
Some college/associates degree	13.7% (8)
College graduate	31% (18)
Graduate degree	43.1% (25)
Recipient of government assistance	
Multiple forms of assistance	15.5% (9)
One form of assistance	10.3% (6)
No assistance	74.1% (43)
Race (n=52)	
White	63.5% (33)
Asian	17.3% (9)
Black	3.8% (2)
Other	15.4% (8)
Ethnicity	
Hispanic	19% (11)
Non-Hispanic	81% (47)
Perceived Stress score	14.88 \pm 6.48
Prenatal Risk Index score	3.54 \pm 1.67
Antenatal Corticosteroids	15.5% (9)
Week of Gestation Received	30.75 \pm 3.0
Cesarean section	34.5% (20)
Vaginal delivery	65.5% (38)

Table 4.2 Clinical Descriptors for All Infants in the Sample and by Infant Sex (n=58)

Characteristic	Mean \pm SD or % (n)
Length of Gestation (weeks)	38.36 \pm 2.12 (58)
Males	38.57 \pm 1.69 (24)
Females	38.20 \pm 2.40 (34)
Preterm Birth (<37 weeks gestation)	15.5% (9)
Males	12.5% (3)
Females	17.6% (6)
Infant Weight (grams)	3,054.55 \pm 690.16
Males	3,206.87 \pm 722.5
Females	2,947.03 \pm 655.84
Low Birthweight (<2,500 grams)	22.4% (13)
Males	23.5% (8)
Females	20.8% (5)
Twins	13.8% (8)
Same sex	63% (5)
Opposite sex	27% (3)

Table 4.3 Logistic Regression for the Effects of Maternal Perceived Stress, Maternal Cortisol Awakening Response, and Fetal Sex on Delivery of a Low Birth Weight Infant

Chi Square for Model = 8.96 (df=6), p=.18

CAR = Cortisol Awakening Response

	β Coefficient	Standard Error	Wald Test	p-value	Odds Ratio	95% Confidence Interval
Prenatal Risk	.18	.25	.52	.47	1.19	(.74, 1.94)
Stress	-.01	.20	.00	.95	.99	(.67, 1.45)
CAR	-.37	.17	4.78	.03	.69	(.49, .96)
Fetal Sex	-.60	2.12	.08	.78	.55	(.01, 3.52)
Stress*FSex	.04	.13	.10	.75	1.04	(.81, 1.34)
CAR*FSex	.31	.15	4.27	.04	1.36	(1.02, 1.82)

Table 4.4 Pearson Correlations of Maternal Perceived Stress and Cortisol Parameters during Pregnancy with Infant Gestational Age and Birth Weight

Partial Correlations Adjusting for Prenatal Risk

AUC_G = Area Under the Curve

	Gestational Age		Birth Weight	
	Males	Females	Males	Females
Perceived Stress	-.03	-.05	.09	-.03
Average Cortisol	-.01	.09	.04	.18
Cortisol Awakening	-.14	.32	-.26	.04
Diurnal Slope	.33	-.10	.20	.01
AUC _G	-.30	.05	-.10	-.04

Discussion

Study Purpose and Key Findings

The overall purpose of this dissertation research was to advance knowledge regarding the association between women's perceived stress and cortisol values during pregnancy as well as the relationship of these variables to birth outcomes.

The specific aims were threefold:

- 1) To determine if there is a relationship between women's self-reported stress and four salivary cortisol values: 1) overall mean cortisol level, 2) Cortisol Awakening Response (CAR), 3) Cortisol Diurnal Trajectory, and 4) Cortisol Area Under the Curve (AUC_G);
- 2) To determine whether women's self-reported stress or their cortisol parameters during the third trimester of pregnancy best predict an infant being born preterm or low birth weight;
- 3) To examine the moderating role of fetal sex in the relationship of perceived stress and cortisol to preterm birth or low birth weight.

Review of previous research (described in Chapter 2) illustrated that cortisol has a more robust relationship with birth outcomes than perceived stress. Results of studies were conflicting in whether a relationship existed between perceived stress and cortisol. However, there was substantial heterogeneity in the characteristics of the women across samples, along with inconsistency in control for confounders. These differences created a challenge in synthesizing the body of literature on stress and birth outcomes as well as using the literature to inform hypotheses for the study.

For the sample as a whole, our a priori hypothesis was supported for Aim 1: none of the cortisol parameters were related to perceived stress. However, when women were analyzed based on their stage in pregnancy, we found significant relationships between two of the cortisol

parameters and perceived stress in the women sampled during the earlier stage of their third trimester. Women who were less than 29 weeks gestation during the time of their cortisol sampling had a positive correlation between elevated levels of perceived stress and both higher CAR and AUC_G. This same group of women (< 29 weeks gestation at sampling) also had lower overall mean cortisol values if their perceived stress was elevated. These moderate, significant correlations were not found in the women sampled after 29 weeks of gestation. Results indicate that stage of gestation may play an important role in understanding the relationship between stress and a women's cortisol response.

Partial support for the hypothesis related to Aim 2 was found, as evidenced by a relationship between CAR and the odds of delivering a LBW infant. Although no other cortisol parameters were related to either birth outcome, perceived stress showed no relationship at all to either LBW or preterm birth. These findings suggest that cortisol is a better predictor of birth outcome than perceived stress and that CAR may warrant particular attention in future research as a useful marker of birth outcome, specifically for LBW.

The hypothesis for Aim 3 was also partially supported. Fetal sex had no moderating effect in the relationship between perceived stress and either LBW or preterm birth. However, fetal sex did moderate the relationship between CAR and LBW status. Male infants of women who had a higher CAR were more likely to be born LBW. In contrast, there was no relationship between CAR and LBW status for girls. Results indicate the importance of examining differential effects related to biological sex in understanding the potential impact of fetal exposure to stress hormones on birth outcome.

Significance

The Relationship between Perceived Stress and Cortisol

When considering findings for our entire sample, results support what has been found in most other studies that have examined women during the third trimester of pregnancy: women's cortisol is *not* related to their perceived stress. This finding was reported in three previous studies (Harville, Savitz, Dole, Herring, & Thorp, 2009; Salacz, Csukly, Haller, & Valent, 2012; Shaikh et al., 2011) and suggest that self-reported stress and cortisol parameters may be measuring different facets of the stress experience. However, we did find a relationship of CAR, AUC_G and cortisol level to perceived stress among women during the earlier stages of their third trimester. Elevations of CAR and AUC_G in response to higher perceived stress have been reported as normative responses in other healthy populations (Chaumette et al., 2016; Chida & Steptoe, 2009; Hellhammer et al., 2007; M. Pruessner, Cullen, Aas, & Walker, 2017; Stalder et al., 2016; Zorn et al., 2017). These responses are congruent with our findings for women at 29 weeks gestation or less. An individual's ability to mount an adequate HPA axis response to external demands or stressors has been described as adaptive under most conditions (Herman et al., 2016; Herman, McKlveen, Solomon, Carvalho-Netto, & Myers, 2012; Jacobson, 2014; Vashist & Schneider, 2014). As part of this response, a cascade of hormones is released, including cortisol. It is possible that the dynamic fluctuations in various hormones that occur during pregnancy may mask or modulate more typical relationships between cortisol values and perceived stress that are observed in other populations.

It is also possible that the different associations between cortisol values and perceived stress that we found for women at different stages of the 3rd trimester are influenced by natural changes in cortisol levels that occur as gestation proceeds. These changes affect other hormones

such as estrogen, as well as inflammatory markers that may in turn influence perceptions of stress. It has been posited that there is an intricate system of different clocks, driven by hormones, that determine the onset of parturition (Menon, Bonney, Condon, Mesiano, & Taylor, 2016). Down-regulation of cortisol parameters when experiencing perceived stress may serve a protective role for women in later gestation by enabling the pregnancy to progress closer to the desired 40 weeks of gestation. If cortisol responded to perceived stress in a robust way after 29 weeks of gestation, the cascade of hormones that initiate parturition could be released. This interpretation supports other findings that the hormonal milieu changes just before an infant's arrival. For instance, there is evidence that cortisol levels increase preceding the onset of parturition (Busada & Cidlowski, 2017) and that the CAR is attenuated as gestation progresses (Buss et al., 2009; Entringer, Buss, & Wadhwa, 2010). The maternal stress response system may be programmed to respond differently to perceived stress as delivery becomes more imminent.

The Role of Perceived Stress and Cortisol in Predicting Birth Outcomes

Findings from this dissertation are congruent with the majority of other studies which have shown that cortisol is more predictive of birth outcomes than perceived stress. Two of these studies specifically examined CAR in association with birth weight. Bolten et al's 2011 study of 75 women in Germany between 35-37 weeks' gestation found a similar result to what we found for males, reporting that elevated CAR was associated with lower birth weight while self-reported stress had no relationship to birth outcome. However, our findings were different from what was found in Cheng and Pickler's 2010 study of 39 women who were sampled after 36 weeks of gestation and did not find a relationship between CAR and birth weight.

Our results, in conjunction with those of Bolten et al (2011) suggest that CAR may be a better measure than perceived stress or other cortisol parameters for predicting the likelihood of

having a low birth weight infant when assessed during the third trimester of pregnancy. More research is needed to specifically examine effects of CAR and other parameters on birth outcomes. Not all of the cortisol parameters may be equally useful as predictors or may measure different aspects of HPA functioning.

The Moderating Role of Fetal Sex

Often when fetal sex is examined in research, it is included only as demographic data for description of the sample. But it is not examined as a potential covariate or moderator involved in the pathways that may influence birthweight or gestational age. We found no studies that examined the moderating role of fetal sex on the relationship between perceived stress and birth outcomes. We found only one study that examined the moderating effect of fetal sex on cortisol's relationship to birthweight or gestational age. A study of 1,041 women in Bangladesh found results similar to those in our research for a different cortisol parameter. Male infants weighed less at birth when exposed to elevated morning salivary cortisol of their mothers during 28-30 weeks gestation (Frith et al., 2015). This dissertation research is the first to report a sex-specific moderating effect on CAR at a similar time point in gestation as the Frith et al study. Males and female fetuses may have differences in the sensitivity of their developing HPA-axis to elevated cortisol during gestation, including differential glucocorticoid or mineralocorticoid receptor response, effectiveness of negative feedback mechanisms in shutting down hormonal secretion, or the ability of the placenta to serve as an effective barrier to excessive cortisol exposure. Differences in stress reactivity after birth have been noted in infants based on sex (Braithwaite et al., 2017). In addition, male infants are reported to have higher mean levels of cortisol, as compared to females after birth (Davis & Emory, 1995). Differences in cortisol

secretory patterns appear to persist through childhood between males and females (Hollanders, Van Der Voorn, Rotteveel, & Finken, 2017).

Limitations

The sample size in this study was relatively small and likely underpowered to detect significant differences. The small number of women made it difficult to analyze moderating effects such as gestational age, as women were not distributed evenly across the third trimester. In addition, although it was a strength to have a sample that had some prenatal risk factors, few women had multiple perinatal risks.

The women in the sample were older than many samples in previous research, making comparisons to findings from other studies challenging. Studies have found that cortisol decreases with age in women. However, age was examined in this sample and had no relationship to any of the cortisol values, perceived stress or birth outcomes. Women were also well-educated and almost entirely living with a partner. Thus, the sample may not be representative of all women's experiences during pregnancy.

As noted earlier, changing hormonal environments emerge as women get closer to delivery, including natural increases in CRH, cortisol, and oxytocin. These hormones work in synergistic patterns that can influence cortisol levels. Effects of this changing milieu and the potentially confounding effects of related hormones were not examined. Further exploration of how these hormones interact will be important.

In addition, effects of other potential covariates were not examined. For example, effects of physical activity, women's depressive symptoms, and the quality of prenatal care could have influential relationships to variables that were examined.

Lastly, this study may not be representative of the stress physiology or experiences of pregnant women who declined participation. Much of what we know about stress during pregnancy could be subject to non-response bias. Large pregnancy cohort studies have reported rates as low as 30% for enrollment (Nohr, Frydenberg, Henriksen, & Olsen, 2006). Women who felt the most stressed or had altered cortisol parameters that demonstrated risk may have been more likely to decline study participation because of their greater emotional or physical stress.

Implications for Nursing

A better understanding of how stress and stress-related hormones may predict birth outcomes will enable the development of more effective methods of screening and assessment by nurses. This knowledge may contribute ultimately to evidence-based and tailored interventions that can mitigate stress during pregnancy and reduce problematic consequences faced by high-risk infants.

Normal cortisol parameters for each week of gestation need to be established and adjusted for fetal sex. Once the patterns of cortisol are better understood across pregnancy, then nurses and other health professionals may have the ability to identify women at risk of an adverse birth outcome. If a specific cortisol parameter is identified as being a valid marker of risk, then this noninvasive measure could be incorporated into routine prenatal care. If women are identified as having the marker, nursing interventions could be implemented to reduce their stress or modify the cortisol parameter with medication.

Although it is a distal goal of this research, social and political change is needed in order to reach the greatest number of women who are pregnant, and especially those who may benefit most from stress mitigation. Nurses may be able to bridge the critical gap between government

policies and community needs. The findings from this study will contribute to a growing body of knowledge that can inform white papers and ultimately bring about policy changes.

Future Research

Findings from this dissertation research indicate the importance of examining both perceived stress and cortisol values when studying stress during pregnancy. These measures may provide different information at different times during pregnancy and likely measure different facets of stress. In addition, it seems important for investigators to study varied cortisol parameters rather than limiting cortisol assays to overall level or concentration, as is found in much of research. Different cortisol parameters appear to have unique relationships to self-reported stress and to birth outcomes.

Results suggest, in particular, that CAR may be a better measure than perceived stress or other cortisol parameters for predicting the likelihood of having a low birth weight infant. More research is needed to specifically examine effects of CAR and other parameters on birth outcomes. Not all of the cortisol parameters may be equally useful as markers of stress or risk.

Results also point to the need to better understand the complex and changing dynamics of stress throughout pregnancy. Findings differed even for women at different stages of the third trimester. It is likely that such differences exist for different trimesters of pregnancy as well.

Further research is needed to replicate findings of this study with a larger sample that has more heterogeneity and the power to examine moderating effects. It will be important to include women who are experiencing complications associated with their pregnancy, since most studies that examine women during pregnancy either focus on women who are expecting a healthy, uncomplicated pregnancy or who have many risk factors. In addition, results clearly implicate the importance of examining fetal/infant sex as a moderator in future research.

There is also a need to examine potential moderators and/or confounds such as genetic polymorphisms associated with stress and fetal development, race/ethnicity, patterns of sleep and fatigue, social or partner support, perinatal complications, life events and stressors, and chronic stress since these have been associated with stress response in previous research. Other methodologic moderating factors, including the time of day in which the sample was collected, consuming food, caffeine intake, exercise, and exposure to a stressful event prior to assessment should be accounted for in analyses. Consistency across studies on potential covariates that are controlled for would improve our understanding of prenatal stress. In addition, cortisol is often sampled in conjunction with procedures that a woman is undergoing. Research needs to examine the potential confounding effects of these procedures by increasing women's feelings of stress or physiologic responses to stress when samples are acquired. Results may provide biased values that are assumed to represent women's tonic or normative response.

Lastly, to better understand underlying mechanisms of our research findings, epigenetic studies are needed to evaluate potential effects of elevated cortisol exposure on expression of stress-related genes such as the glucocorticoid receptor, the enzyme involved in DNA methylation (DNMT3A), and the serotonin transporting gene (5HTT). Findings also indicate that dysregulated cortisol parameters may have differential effects on methylation of stress-related genes for the male and female fetus. Examining the sex-specific effects on methylation is essential to include in future research.

Conclusion

Results of this dissertation contribute to knowledge in a number of areas. First, stage of gestation appears to play an important role in understanding the relationship between stress and a women's cortisol response during pregnancy. While no relationships were found between

perceived stress and cortisol for women in the later stage of the third trimester, significant relationships emerged between three of the cortisol parameters and perceived stress in the women sampled during the earlier stage of their third trimester. Stage of gestation should be considered as a salient moderator in all future studies that assess relationships between self-reported stress and cortisol values.

Second, findings suggest that cortisol is a better predictor of birth outcome than perceived stress and that CAR may warrant particular attention in future research as a useful marker of birth outcome, specifically for LBW. With salivary CAR being a non-invasive biomarker, it may be a valuable clinical tool in risk assessment of pregnant women if future research supports the findings reported here. Currently, there is no specific laboratory test to effectively assess birth risk associated with stress.

Third, results indicate the importance of examining the moderating effects of fetal sex in order to fully understand the potential impact of fetal exposure to stress hormones on birth outcome. Male infants of women who had a higher CAR were more likely to be born LBW. In contrast, there was no relationship between CAR and LBW status for girls. For too long, research has ignored potential sex differences in sensitivity to the environment or in ways of adapting to stress. Findings of this research suggest that these sex differences are present in utero.

Finally, in conjunction with findings of previous research, results of this dissertation indicate that future research should include both self-reports of stress and multiple sources of cortisol when assessments occur; and that these assessments should take place several times during pregnancy. Without this integrated, longitudinal approach, it is unlikely that adequate knowledge will be developed regarding the relationship between self-reports of stress and cortisol, or their impact on birth outcomes. Uncovering the nuances of stress across gestation will

help clinicians determine when women may experience the greatest perceived stress or hormonal effects from it, and how to best mitigate women's stress in order to reduce their risk of preterm birth or low birth weight.

Acknowledgements

Support for this study was provided by a grant from the National Institute of Child Health and Human Development (Weiss, R01 HD081188-05) and the National Institute of Nursing Research T32 Biobehavioral Research Training Program in Symptom Science (NR016920).

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