

Prenatal Stress and the Cortisol Awakening Response in African-American and Caucasian Women in the Third Trimester of Pregnancy

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Published online: 8 July 2016
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Abstract *Objectives* Prior studies have shown significant racial disparities in psychosocial stressors for pregnant women. One physiological mechanism by which prenatal stress is expressed is via the stress-sensitive hormone cortisol, which itself differs by race. In this study, we examine differences in cortisol awakening response (CAR) for African-American and Caucasian pregnant women during late pregnancy, particularly whether racial disparities are evident after accounting for measures of psychosocial stress. *Methods* During their third trimester of pregnancy (32–40 weeks of gestation), we asked women to self-collect salivary samples at home over 2 days. We then measured salivary cortisol across the day for 30 pregnant women (18 Caucasian; 12 African-American) to examine the CAR by race and by multiple measures of self-reported psychosocial stress, including perceived discrimination. *Results* Although the women in our sample showed

normative cortisol diurnal rhythms (high on waking, peak 30 min post-waking, lowest at bedtime), we found that African-American women had blunted (smaller) awakening responses compared to Caucasian women ($p < 0.05$). The CAR was significantly larger in Caucasian women compared to African-American women even after accounting for covariates in a multivariate equation. However, when we added measures of psychosocial stress to the multivariate equation, higher levels of stress were significantly associated with a smaller CAR ($p < 0.05$), and the association between maternal race and CAR was no longer significant. *Conclusions* Our results add to a growing body of evidence that racial differences in the activity of the hypothalamic–pituitary–adrenal axis are associated with psychosocial stress during pregnancy.

Keywords Cortisol · Racial disparities · Pregnancy · Stress · Discrimination

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Significance

Our study is the first to show ethnic disparities in late pregnancy cortisol awakening responses that are partly accounted for by psychosocial stressors.

Introduction

Prenatal stress has been linked to a variety of poor psychosocial, birth and developmental outcomes for mothers and offspring [4]. One physiological mechanism by which stress is expressed is through alterations of the hypothalamic–pituitary–adrenal (HPA) axis, which produces the stress hormone cortisol. Cortisol levels increase in response to stress,

and also follow a strong diurnal rhythm with levels being high on waking, increasing in the 30–45 min after waking, and declining across the day until bedtime. The typical 50–60 % increase in the first half hour of waking is called the cortisol awakening response (CAR) [24].

A limited number of prior studies have examined the CAR during pregnancy, showing a dampening of the CAR (a smaller increase in cortisol upon awakening) from the 17th to the 31st week of pregnancy [6], yet a similar relative CAR during late pregnancy (32nd week) compared to post-partum [13]. Cortisol levels increase during pregnancy, including at wakeup, with plasma levels tripled during late pregnancy [13]. During pregnancy, there is mixed evidence that the CAR is linked to psychosocial measures such as depression [29] and childhood adversity [17], with more evidence for links to obstetric outcomes, including between smaller decreases in the CAR over pregnancy and shorter gestational length [6]. In non-pregnant populations, the CAR has been associated with depressive symptoms, perceived stress, inflammation, and chronic fatigue [15]; however, disparate psychosocial factors are associated with changes in the size of the awakening response. In one meta-analysis, general life and job stress was associated with a larger CAR, while exhaustion and presence of posttraumatic stress syndrome was associated with a smaller CAR [8]. These studies further suggest links between chronic stress and the CAR, yet the direction of effects is unclear.

Racial Disparities and Psychosocial Correlates of the Cortisol Awakening Response

Prior studies have found racial/ethnic disparities in the cortisol diurnal rhythm [11]. For African-American women, lower cortisol levels across pregnancy are thought to reflect both an increased presence of chronic stress, as well as differences in physiologic vulnerability to stress [34]. One contributor to chronic stress is self-reported discrimination based on racial or ethnic status, which is associated with alterations in the cortisol diurnal rhythm [3, 16], longitudinally associated with a lower CAR [3] and experienced more often for ethnic minorities [31]. Among non-pregnant individuals, measures of self-reported discrimination among racial minorities have been associated with flatter, lower cortisol rhythms [3].

Racial disparities in overall levels of psychosocial stress are thought to play a role in the pronounced disparity in birth outcomes [22], developmental outcomes, and maternal health [26]. Psychosocial stress may result in adverse health outcomes through alterations in HPA-axis activity. Empirical evidence supports this hypothesis; racial/ethnic differences in cortisol rhythms have been found in non-

pregnant samples [11] and in late pregnancy samples [18]. At least one prior study has provided evidence of lower CAR in pregnant women with higher cumulative stress [32]; however, the time period for CAR collection was not within the recommended norm, nor did that study compare African-American to Caucasian women. To date, no study has examined the connection between the CAR, race, psychosocial functioning, and discrimination among African American and Caucasian pregnant women during their third trimester of pregnancy.

Current Study

In this study, we examined the cortisol awakening response over 2 days for urban African-American and Caucasian pregnant women in their third-trimester (32–40 weeks of gestation). Our study aims to examine racial differences in the CAR, while disentangling the relationship between a variety of psychosocial factors and the cortisol awakening response during pregnancy.

Given the current and historically higher rates of perceived racial discrimination for African Americans, the connections between (a) discrimination and higher levels of perceived stress and (b) perceived stress and CAR, we expect to find racial disparities in CAR for 3rd trimester pregnant women. Examining this research question is important for better understanding how racial disparities, including those related to perceived stress, are connected to measures of physiological stress during pregnancy.

Methods

Participants

This study is a supplementary pilot study to the Stress in Pregnancy Study (SIPS), designed to clarify the relationship between maternal stress and pregnancy outcomes [5.] During the SIPS study, 114 women (56 African-American and 58 Caucasian pregnant women, equally divided between those with private health insurance and those with Medicaid, from two prenatal clinics associated with Northwestern Memorial Hospital, were enrolled between May 2008 and July 2009. In order to be eligible to participate, pregnant women were English speakers, at least 18 years old, and less than 23 weeks of gestation at enrollment. Women with multiple gestations, prior cervical surgery, prolapsed amniotic membranes, current or planned cervical cerclage, progesterone treatment during the current pregnancy, congenital Mullerian abnormality of the uterus, or a chronic disease known to increase the risk of preterm birth were excluded.

Procedure

Participants were recruited from women receiving prenatal care in the Prentice Ambulatory Care Clinic and Winfield Moody Health Clinic, clinics affiliated with Prentice Women's Hospital. They were consented and then privately interviewed in-person by a trained research assistant, answering questions about life stress, overall health, and pregnancy stress. Participants were interviewed in a consulting room in the prenatal clinics either before or after the participant's regularly scheduled prenatal appointment at two time points of collection: the time of enrollment and again in the 3rd trimester of pregnancy. Upon completion of the 3rd trimester interview, as part of the supplementary study, participants were provided a home salivary collection kit and asked to collect six saliva samples over the following 2 days, as well as complete four diaries over the 2 days of collection (2 morning diaries and 2 evening diaries) which included questions on health behavior, sleep, and mood states. Participants were paid \$10 to complete the saliva collection.

Thirty-eight women were asked to participate; of these, 1 (African-American) refused and 36 agreed; 4 African-American and 2 Caucasian women did not return completed kits prior to birth, and one woman did not self-identify her racial/ethnic status. Thus, 30 SIPS participants (18 Caucasian women, 12 African-American women) in their 3rd trimester (32–40 weeks gestation) of pregnancy returned salivary kits, comprising the analytic sample for this study.

Psychosocial Measures of Self-reported Stress

All participants answered questions from validated scales encompassing the following categories of self-reported extrinsic measures of stress: [4] external stress; [24] depressive symptoms; [6] buffers against stress; and [13] perceived stress, which includes perceived discrimination. The administered stress scales represent a comprehensive model of chronic stress and were selected based on review of the literature and expert opinion [7].

External Stressors

The Home Hardship Scale, the Stressful Life Events Scale, and the USDA Food Security Scale were used to assess external stress. The 22-item Home Hardships Scale was used in the Survey of Income and Program Participation conducted by the U.S. Census Bureau. It also was utilized in the Illinois Family Study to examine associations between prenatal stress and low birth weight [33]. All items in the Home Hardship Scale are on a binary scale and include items such as affordability of basic health resources and daily living. The Stressful Life Events Scale lists 13 events grouped into

the following four categories: emotional, financial, partner-related, and traumatic. The Food Security Scale was comprised of the US household survey food security module, a structured measure utilized in numerous national surveys conducted by the US Department of Agriculture. This measure included questions aimed at determining household ability to afford needed food. Cronbach alpha levels for our sample are: Home Hardship $\alpha = 0.79$, Stressful Life Events $\alpha = 0.83$. The Food Security Scale had no variability and was therefore not included in any analysis.

Depressive Symptoms The Center for Epidemiologic Studies Depression Scale (CES-D) is a 20-item screening for depression, based on experiences in the past week. All questions have a 4-point response scale. The scale has a sensitivity of 0.75–0.84 and a specificity of 0.68–0.80 for detection of clinical depression [25]. In our sample, Cronbach alpha for the CES-D is 0.83.

Buffers of Stress To assess possible buffers of experienced stress, the following four scales were used: MOS Social Support Scale, the State Hope Scale, Rosenberg's Self-esteem Scale, and Pearlin's Mastery Scale. The Medical Outcomes Study-Social Support Survey (MOS-SSS) is a 20-item multidimensional measure of perceived functional support in the following five areas: emotional, informational, tangible, positive social interaction, and affectionate support. This self-report measure has Cronbach's α internal reliability ranges from 0.91 to 0.97 [30]. The State Hope Scale is a 6-item measure of agency, defined as an individual's capacity to both initiate and sustain action. As a measure of goal-directed thinking, it is both internally consistent and valid. Rosenberg's Self-Esteem Scale (SES) is a 10-item measure of an individual's global sense of self-worth [28]. All responses are on a four-point scale ranging from strongly agree to strongly disagree. This scale has demonstrated reliability and validity across multiple samples [27]. Pearlin's Mastery Scale, a 7-item measure of personal mastery, uses a four-point scale of agreement to assess perceived control of one's life [23]. When examined in analysis, these measures were reversed coded for consistency with the stress-based measures; higher scale values reflect lower support, agency, self-esteem, and mastery. Cronbach alpha levels for our sample are: MOS Social Support Scale $\alpha = 0.97$, State Hope Scale $\alpha = 0.90$, Rosenberg's Self-esteem Scale $\alpha = 0.94$, and Pearlin's Mastery Scale $\alpha = 0.88$.

Perceived Stressors

Four separate scales were included to measure perceived stress: Krieger Perceived Discrimination Scale, Misra Stress Scale, Cohen's Perceived Stress Scale, and the Prenatal

Distress Questionnaire. The Krieger scale measures perceptions of discrimination within the past year and responses to this unfair treatment [21]. This 20-item scale includes dichotomous responses regarding experiences of discrimination based on race, gender, and social standing. The Misra Stress Scale, or Prenatal Psychosocial Profile Hassles Scale, is a 12-item scale psychometrically validated [12] and modified from a longer scale of prenatal stress. This scale includes concerns about money, neighborhood crime, and problems with family, and uses a four-point scale of stress with options ranging from none to severe for each of the 12 items. A prior study using the scale to predict preterm birth had a Cronbach's $\alpha = 0.80$ [12]. Cohen's Perceived Stress Scale (PSS) is a 10-item measure aimed at assessing stressful life feelings and thoughts within the last few months. Questions have 5 response categories, and have past study Cronbach's α coefficients ranging from 0.84 to 0.86, with relative psychometric invariance by sex, race, and education [10]. Finally, the Prenatal Distress Questionnaire (PDQ) is a 12-item measure of pregnancy and childbirth stress [35]. Cronbach alpha levels for our sample are: Krieger Perceived Discrimination Scale $\alpha = 0.72$, MISRA Stress Scale $\alpha = 0.71$, Cohen's PSS $\alpha = 0.70$, PDQ $\alpha = 0.73$. To aid in interpretation of effects, scales were recoded where necessary such that psychosocial stress and lower support were always at the higher end of the scale. To create the cumulative risk score, we summed the standardized individual stress scales (PSS, MISRA, State Hope and Home Hardship) that were significantly ($p < 0.05$) or marginally ($p < 0.10$) correlated with the CAR. Therefore, participants with higher cumulative risk scores represent those with increased psychosocial stress over multiple domains.

Demographic and Socioeconomic Status Variables

Information on maternal age, parity, household income, and obesity status ($\text{BMI} > 30 \text{ kg/m}^2$) were collected and retained for final models shown. Due to the small sample size and to create a more parsimonious model, maternal education, gestational age at delivery, infant birth weight, lifestyle factors (alcohol use, smoking, caffeine use), and sleep variables were not included in final models. None of these variables impacted final results shown and none were significantly ($p < 0.05$) correlated with CAR.

Salivary Cortisol

Saliva samples were collected over 2 days at the following times: wakeup, 30 min post waking, and bedtime [1.] All participants used a passive drool method to expel saliva through a straw into a 2 mL polypropylene tube. They then labeled the tubes with both the time and date of collection. Participants were asked to not eat, drink, or brush their teeth

30 min before each sample. The salivary kits with completed saliva samples and diaries were returned via mail to Northwestern University, where they were frozen at -20°C . Cortisol levels are stable at room temperature and unaffected with postal journey conditions [9]. At the completion of the study, samples were sent on dry ice to Biochemisches Laboratory in Trier, Germany, and assayed in duplicate for cortisol using a time-resolved immunoassay with fluorometric detection (DELFI) [14]. Intra-assay coefficients of variation (CVs) were between 4.0 and 6.7 % and inter-assay CVs ranged from 7.1 to 9.0 %. Although cortisol values are sometimes skewed, we found no such skew in our data and did not perform any transformations prior to analysis. CAR values were calculated by subtracting the second morning sample value (30 min post waking) from the first morning sample value (wakeup sample).

Statistical Analyses

We examined CAR by maternal race, demographic variables, and psychosocial measures using independent *t*-tests, and then conducted Pearson correlations between the CAR and psychosocial scores. We also ran OLS multivariate regressions to discern the independent associations between CAR and psychosocial measures, first using a model with no psychosocial measures (Model 1), and then individually (due to multicollinearity) adding each psychosocial scale that is associated with CAR (Models 2–5). Finally, as part of the OLS modeling, we also examined the cumulative risk score (Model 6) using these psychosocial scales that were independently correlated with CAR. All regressions controlled for age, parity, income, and BMI.

Results

Table 1 shows descriptive statistics for the analytic sample of 30 pregnant women. There were no significant differences by self-reported race in demographic data, including education, income, and age. Psychosocial data differed by race for perceived racism only, with African-American participants having a significantly greater report of perceived discrimination.

Figure 1 shows mean cortisol diurnal rhythms that, on average, follow the normal and expected pattern: high on waking, sharp increases after waking, with lowest values at bedtime; however, African-American participants have substantially lower second morning cortisol values, or blunted awakening responses. The CAR was significantly ($t = 2.25$, $p < 0.05$) larger in Caucasian as compared to African-American participants.

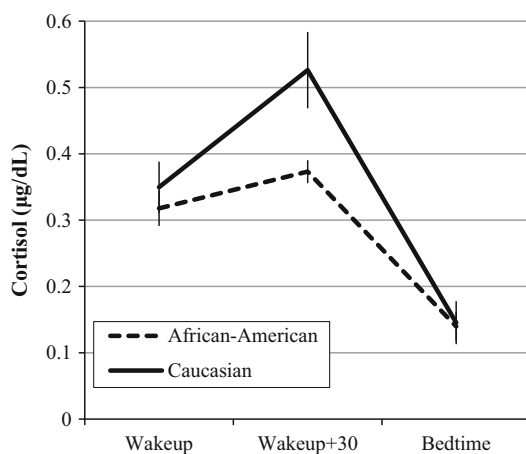
Table 2 shows univariate correlations between the CAR and psychosocial measures scores. Higher perceived stress

Table 1 Descriptive statistics

Variable	African-American (N = 12)		Caucasian (N = 18)		t
	Mean	SD	Mean	SD	
Body mass index (BMI)	28.45	5.78	26.66	4.91	0.91
College education	75.00 %	0.45	66.67 %	0.48	0.47
Household income >\$30,000	83.30 %	0.62	61.11 %	0.78	1.13
Multiparous	25.00 %	0.62	44.44 %	0.78	-1.07
Age	30.83	4.75	29.94	6.55	0.08
Gestational age at delivery	38.92	1.49	39.10	0.93	0.03
Cohen's Perceived Stress Scale (PSS)	28.00	7.42	26.67	5.90	0.55
Center for Epidemiology Scale of Depression (CES-D)	35.17	9.38	34.50	11.60	0.17
MISRA Stress Scale	20.17	5.17	18.44	3.03	1.15
Prenatal Distress Questionnaire (PDQ)	26.25	5.03	25.44	6.87	0.35
Pearlin's Mastery Scale	12.83	3.10	12.78	4.08	0.04
State Hope Scale	18.33	3.68	18.94	3.30	-0.48
Rosenberg Self-Esteem Scale	34.17	4.45	31.78	5.33	1.28
Home Hardship Scale	29.67	2.61	29.89	2.19	-0.25
Food Security Scale	13.50	2.07	13.28	1.97	0.30
Stressful Life Events	22.67	3.45	23.78	2.21	-1.08
MOS Social Support Survey	86.33	8.67	80.67	14.10	1.24
Krieger Perceived Racism Scale	35.08	4.03	38.11	2.00	-2.74*

All participants have health insurance, no differences by race in alcohol, caffeine, or cigarette use during pregnancy. All participants who returned salivary kits had full term, normal birth weight babies with high (8 or 9) 5-min APGAR scores. None of the lifestyle factors were associated with cortisol levels

~ $p \leq .10$; * $p < 0.05$

**Fig. 1** Mean cortisol diurnal rhythms stratified by race

and more home hardship were associated with a lower CAR, while higher MISRA stress scores and lower State Hope scores were marginally associated ($p < 0.10$) with a lower CAR. The rest of the scales were not significantly associated with CAR. All of the significant or marginally significant scales were negatively associated with CAR, in that more stress (or less buffering) is associated with a smaller CAR. Following the results of our Pearson

correlations between CAR and the individual stress scales, we created a cumulative risk scale with the four scales (Cohen's Perceived Stress Scale, MISRA Stress Scale, State Hope Scale, and the Home Hardship Scale) that were significant ($p < 0.05$) or marginally ($p < 0.10$) significant predictors of the cortisol awakening response.

Table 3 shows OLS regression results for maternal race, psychosocial scales, and demographic variables predicting the CAR. In Model 1, Caucasian mothers have significantly higher CAR ($\beta = 0.404$, $p < 0.05$) compared to African-American mothers, after controlling for age, parity, income, and body mass index (BMI). None of these covariates were significant ($p > 0.10$). In Model 2, the Perceived Stress scale (PSS) was added as an additional covariate and found to be inversely associated with CAR ($\beta = -0.439$, $p < 0.05$). Model 3 includes the recoded Home Hardship scale, which is significant ($\beta = -0.479$, $p < 0.05$) and inversely associated with the CAR. Models 4 and 5 include the MISRA and State Hope scales, respectively, which were marginally associated with CAR in univariable analysis (Table 2). In multivariable analysis, only the MISRA scale remained marginally associated with CAR ($\beta = -0.345$, $p < 0.10$), while State Hope was not significantly associated with CAR. Finally, Model 6 uses the cumulative stress scores, finding that more cumulative

Table 2 Correlations between cortisol awakening response and psychosocial measures

	Cortisol Awakening Response
Cohen’s Perceived Stress Scale	−0.43*
CES-Depression	−0.30
MISRA Stress Scale	−0.32 ~
Prenatal Distress Questionnaire	−0.07
Pearlin’s Mastery Scale	−0.08
State Hope Scale	−0.34 ~
Rosenberg Self-Esteem Scale	−0.12
Home Hardship Scale	−0.46*
Stressful Life Events	−0.17
MOS Social Support Survey	−0.17
Krieger Perceived Discrimination Scale	−0.27

All scales were recoded in analysis such that higher levels of stress or fewer stress buffers are coded at the higher end of the scale

~ $p \leq .10$; * $p \leq .05$

Table 3 OLS regression models for CAR

Variable	Model 1		Model 2		Model 3		Model 4		Model 6		Model 7	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Caucasian race	0.404*	0.061	0.309 ~	0.047	0.246	0.049	0.312	0.051	0.261	0.05	0.252	0.048
Age	0.095	0.005	0.05	0.004	−0.246	0.004	−0.081	0.005	−0.119	0.004	−0.091	0.004
Parity	0.099	0.059	0.289	0.046	0.193	0.047	0.232	0.049	0.303	0.049	0.267	0.046
Income	−0.172	0.041	−0.259	0.032	−0.144	0.035	−0.285	0.034	−0.367 ~	0.033	−0.243	0.032
BMI	0.172	0.006	0.137	0.005	0.09	0.005	0.059	0.005	0.017	0.005	0.097	0.004
PSS ^a			−0.439*	0.024								
Home Hardship					−0.479*	0.028						
State Hope							−0.265	0.025				
MISRA ^a									−0.345 ~	0.024		
Cumulative psychosocial average											−0.437*	0.029

~ $p \leq .10$; * $p \leq .05$

^a Distress recoded at the higher end of the scale. All psychosocial measures are therefore coded with distress at the higher end of the scale

stress is associated with a smaller CAR ($\beta = -0.437$, $p < 0.05$). Of particular note, when any psychosocial stress measure (models 2–6) is included in the multivariable regression, the coefficient for maternal race is reduced in size and no longer significant. This finding suggests that stress may at least partially explain the relationship between maternal race and CAR.

Discussion

This study adds to the current body of research that has shown ethnic disparities in cortisol for both non-pregnant [11] and pregnant [32] women, while extending these findings through examination of psychosocial stress. Specifically, we find that in their third trimester of

pregnancy, Caucasian women show a normative increase from awakening to 30 min post-waking (CAR), a physiological response associated with improved ability to cope with daily stressors [2], compared to African-American women, who show a dampened CAR.

Over the course of pregnancy, it has been reported that the diurnal rhythm of cortisol is maintained, [20], levels normatively increase, and CARs are approximately equal (based on relative, not absolute, increases) compared to the non-pregnant state [13], and there is a reduced physiological sensitivity to stressors [19]. In this study, pregnant women in their third trimester showed typical diurnal rhythms, with levels highest in the morning and lowest at bedtime. However, while cortisol levels typically rise 50–60 % rise in the 30 min after waking, this expected increase was only observed in the Caucasian women in our

study. Caucasian women show a normative 50.5 % rise in cortisol upon awakening, compared to the dampened 17.4 % increase for African-American women.

Our results provide additional explication of the possible pathways by which psychosocial distress can get “under the skin” during pregnancy. Additional research is needed to explore these pathways, including how psychological distress can translate into racial disparities in CAR. This study shows that the association between self-reported race and CAR in pregnant African-American and Caucasian women is no longer significant when psychosocial stressors are included in modeling and suggests that differences in psychosocial stress may be at the root of racial disparities in the CAR.

Studies of cortisol during pregnancy have indicated that prenatal stress and hardship are associated with blunted morning cortisol responses [20] and our results suggest that the “lower” CAR in our sample similarly represents a blunted response. Overall, although some studies in non-pregnant women have shown a heightened CAR in response to perceived stress, our findings suggest that a normatively rising CAR is associated with lower stress.

Several specific psychosocial stressors were associated with the CAR. The African-American women in this study reported more discrimination; however, we did not find any differences in the cortisol awakening responses by discrimination, unlike prior research [16]. Prior studies in pregnant women have found racial/ethnic cortisol disparities between Latina and African-American pregnant women, in which cumulative stress was associated with lower morning cortisol and flatter diurnal decline for African-American participants [32]. Our study compared Caucasians and African-Americans, and focused on differences in the cortisol awakening response, along with examination of individual chronic stress measures and a cumulative risk scale, but our small sample size may have precluded finding any differences by perceived discrimination.

We posit that the maintenance of a strong cortisol awakening response during pregnancy, despite overall elevations in the diurnal rhythm, may serve a preparatory role to assist women for daily demands. In our study, women who have increased perceived stress have smaller CARs, regardless of race, adding to the evidence that a flattened CAR during pregnancy may indeed reflect an undesirable or “risky” profile. Further identification and prevention of factors contributing to a low pregnancy CAR may be a promising direction for efforts to improve maternal prenatal health and wellbeing.

Our study benefits from collection of a comprehensive set of psychosocial stress measures. We collected cortisol data across 2 days, with three time points of collection, reducing the influence of day-to-day variability in cortisol

on our findings, while capturing the major elements of the cortisol diurnal rhythm [1]. Our limitations, however, are a small sample size and a corresponding inability to meaningfully examine health outcomes, and a lack of objective (electronic) monitoring of the exact timing of cortisol samples. Given the strong links between prenatal stress and birth outcomes [4], and some evidence with a non-African-American sample that a larger CAR is associated with poor birth outcomes [6], further studies should examine racial disparities and psychosocial stress in cortisol rhythms with a larger sample size to examine associations between stress hormones and birth outcomes. Additional research should study connections between racial disparities and biomarkers associated with chronic stress and pregnancy outcomes, including other hormonal markers, cytokines and measure of telomere length. While our results add to the small but growing body of literature showing ethnic disparities in cortisol rhythms, further research is needed to determine whether these results have clinical significance for pregnancy outcomes. Such research could examine how much experiences of discrimination, current perceived stress, and life course stressors [22] may explain or account for racial/ethnic disparities in health and developmental outcomes.

Acknowledgments This work was supported by National Institutes of Health/Eunice Kennedy Shriver National Institute of Child Health and Human Development grant # 1 K12 HD050121–02, Women’s Reproductive Health Research Program, and an Evergreen Invitational Women’s Health Grant Initiative Award, both awarded to Ann Borders. It was also supported by the NorthShore University HealthSystem Auxiliary Research Scholar Award and the NorthShore Research Career Development Award.

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