Stress-Induced Inflammatory Responses in Women: Effects of Race and Pregnancy

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Objective: African Americans experience preterm birth at nearly twice the rate of whites. Chronic stress associated with minority status is implicated in this disparity. Inflammation is a key biological pathway by which stress may affect birth outcomes. This study examined the effects of race and pregnancy on stress-induced inflammatory responses. **Methods:** Thirty-nine women in the second trimester of pregnancy (19 African American, 20 white) and 39 demographically similar nonpregnant women completed an acute stressor (Trier Social Stress Test). Psychosocial characteristics, health behaviors, and affective responses were assessed. Serum interleukin (IL)-6 was measured at baseline, 45 minutes, and 120 minutes poststressor. **Results:** IL-6 responses at 120 minutes poststressor were 46% higher in African Americans versus whites (95% confidence interval = 8%–81%, $t(72) = 3.51$, $p = .001$). This effect was present in pregnancy and nonpregnancy. IL-6 responses at 120 minutes poststressor tended to be lower (15%) in pregnant versus nonpregnant women (95% confidence interval = −5%–32%, $p = .14$). Racial differences in inflammatory responses were not accounted for by demographics, psychological characteristics, health behaviors, or differences in salivary cortisol. Pregnant whites showed lower negative affective responses than did nonpregnant women of either race (p values ≤ .007). **Conclusions:** This study provides novel evidence that stress-induced inflammatory responses are more robust among African American women versus whites during pregnancy and nonpregnancy. The ultimate impact of stress on health is a function of stressor exposure and physiological responses. Individual differences in stress-induced inflammatory responses represent a clear target for continued research efforts in racial disparities in health during pregnancy and nonpregnancy. **Key words:** pregnancy, racial disparities, inflammatory response, interleukin-6, acute stress, affective response.

IL-6 = interleukin-6; **TNF-α** = tumor necrosis factor α; **TSST** = Trier Social Stress Test; **PANAS** = Positive and Negative Affect Scale; **CES-D** = Center for Epidemiological Studies Depression Scale; **STAI** = State-Trait Anxiety Inventory; **PSS** = Perceived Stress Scale; **CTQ-SF** = Childhood Trauma Questionnaire–Short Form; **EOD** = Experiences of Discrimination Scale; **MSPSS** = Multidimensional Scale of Perceived Social Support; **AUC** = area under the curve.

INTRODUCTION

Preterm birth affects 12% to 13% of births in the United States and is a leading cause of infant mortality (1,2). The estimated societal economic burden is at least US $26.2 billion per year, or US $51,600 per preterm infant (1). In the United States, the preterm birth rate is approximately 18% among African American women and 10.5% to 11.5% among non-Hispanic white, Asian, and Hispanic women (3). Although numerous explanations have been forwarded, demographic characteristics and health behaviors do not adequately account for this racial disparity. Associations between race and preterm birth, as well as low birth weight, and infant mortality remain after accounting for indicators of socioeconomic status including educational attainment, income, and occupational status (4–8).

Because traditional explanations have failed to adequately explain the racial disparity in preterm birth, theories have increasingly focused on understanding the health implications of chronic stress associated with minority status (1,9–13). Supporting the conceptualization of minority status as a chronic stressor, perceived racial discrimination has repeatedly been linked to an increased risk of preterm delivery and low birth weight (12,14–18). These relationships remain after accounting for traditional behavioral risk factors, suggesting a role for more direct physiological links between stress and preterm birth.

Inflammation is a key biological pathway by which psychological stress may affect birth outcomes. Available data suggest that healthy pregnancy elicits mild elevations in both pro inflammatory and anti-inflammatory serum cytokine levels and that exaggerated increases in circulating inflammatory markers are predictive of a greater risk of spontaneous preterm delivery (19–24). Moreover, successful pregnancy in humans has been associated with attenuated proinflammatory cytokine production in response to in vitro immune challenges, with the most marked changes in the third trimester (25–29). This adaptation may be critical in preventing rejection of the fetus by the maternal immune system and protecting the fetus from excessive maternal inflammatory responses to infectious agents (30,31). Failure to demonstrate the attenuation of inflammatory responses has been reported among women who subsequently experience miscarriage or deliver small for gestational-age babies (27) as well as in nonpregnant women with a history of recurrent spontaneous miscarriage versus women with a history of successful pregnancy (32). Thus, factors influencing appropriate adaptation of inflammatory responses may affect the risk of adverse pregnancy outcomes.

Psychosocial factors including perceived stress, stressful life events, depressive symptoms, and trauma have been associated with higher circulating inflammatory markers including interleukin (IL)-6, tumor necrosis factor (TNF) α, and IL-1 receptor antagonist (33–38) as well as exaggerated inflammatory responses to in vivo and in vitro immune challenges in pregnant women (39,40). Linking such effects to birth outcomes, in a

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study of 173 women followed up across pregnancy, an association between prenatal stress and gestational age at birth was mediated by levels of circulating inflammatory markers (41).

Notably, we know of no studies examining inflammatory responses to acute psychological stress in pregnancy, or the extent to which psychosocial factors modify this response. Data in nonpregnant adults show that IL-6 responses to acute stressors are delayed and extended as compared with responses of the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. Increases in IL-6 from baseline have been observed beginning at 30 to 45 minutes poststressor, with continuing increases at final follow-up time points of up to 2 hours poststressor (42,43). Thus, exposure to elevated inflammatory markers may be considerable in duration among individuals who experience frequent stressors in daily life.

Prior studies show that African American race and greater exposure to racial discrimination predict greater cardiovascular reactivity to a variety of acute stressors (44–48). In addition, stressors of a racially provocative nature elicit stronger cardiovascular responses among African Americans than do stressors that are racially neutral (49). Thus, race is a factor that may affect not only frequency of stressor exposure (particularly racial discrimination) but also the magnitude of physiological response to stressors. The extent to which race modifies inflammatory responses to acute stress is not known. However, other sociodemographic factors indicative of chronic stress including low socioeconomic status and clinical depression have been associated with exaggerated stress-induced IL-6 responses (43,50,51).

In sum, given the growing literature linking both stressor exposure and inflammation to racial disparities in adverse pregnancy outcomes, examination of effects of race and pregnancy on stress-induced inflammatory responses is warranted. The first goal of this study was to examine the effect of race on stress-induced inflammatory responses. It was hypothesized that during pregnancy and nonpregnancy, African American women would exhibit more robust stress-induced IL-6 responses than their white counterparts. The second goal of this study was to examine the effect of pregnancy on stress-induced inflammatory responses. It was hypothesized that pregnant women would show attenuation of IL-6 responses as compared with nonpregnant women and that this attenuation would be more notable among whites versus African Americans. Finally, in exploratory analyses, we examined the associations between cortisol and inflammatory responses to determine whether pregnancy or race-related differences in cortisol may mediate the hypothesized differences in IL-6 response.

METHODS

Participants

Participants included 40 pregnant women (20 African American, 20 white) who were assessed during the second trimester of pregnancy (21–24 weeks' gestation) and 40 nonpregnant control participants matched for age, race, parity, and income. Study visits were conducted between August 2009 and November 2011. The study was approved by the Ohio State University Biomedical Sciences institutional review board. Women were recruited from the Ohio State University Wexner Medical Center General Prenatal Clinic, which serves a racially diverse group of primarily socioeconomically disadvantaged women. In addition, women were recruited from the general community of Columbus, Ohio. Blood sampling was unsuccessful for two women (one nonpregnant African American, one pregnant African American). Thus, the final sample included 78 women (39 pregnant and 39 nonpregnant).

For the trimester of assessment, we focused on the second trimester rather than the first trimester because a primary goal was to examine differences in stress reactivity caused by pregnancy status; more significant adaptations in cardiovascular, neuroendocrine and immune function are evidenced by the second trimester than in the first trimester. We chose to focus on the second rather than third trimester for two reasons. First, increasing evidence suggests that stressors that occur earlier in pregnancy are more likely to have detrimental effects (e.g., Glynn et al. (52)). However, research to date has focused almost exclusively on stress reactivity during the third trimester (53). Second, assessment in the second trimester avoids systematic exclusion of women who may go on to deliver preterm during the third trimester.

Women were ineligible if they reported current tobacco use or chronic health problems, which affect immune, endocrine, or cardiovascular function including cancer, diabetes, chronic hypertension, gestational hypertension, preeclampsia, or anemia at the time of screening. In addition, women were excluded if they were taking antidepressants, antianxiety medications, or mood stabilizers. If a woman reported antibiotic use, she was scheduled at least 2 weeks after usage.

Women were excluded if they reported consuming more than 300 mg of caffeine per day. Women reporting use of any recreational drugs (e.g., marijuana, cocaine, and methamphetamines) in the previous 6 months were excluded. Women were excluded if they were obese, defined as a prepregnancy (if pregnant) or current (if nonpregnant) body mass index of 30 kg/m^2 or greater. Because the racial disparity in preterm birth most clearly affects US-born African American women, women who were not US born were ineligible.

Women were not eligible as nonpregnant control participants if they had given birth within the past 6 months or were currently breast-feeding. Among the pregnant participants, women were excluded if they had multifetal gestation or known fetal anomaly. Because previous pregnancy has been associated with more significant physiological changes in subsequent pregnancy, pregnant participants with at least one previous live birth were targeted. Pregnancy timing in terms of maternal age varies considerably with sociodemographic factors such as income and marital status. Thus, to provide ideal demographic matching, nonpregnant women with a prior live birth were also targeted. In the final sample, 76 (97.4%) of 78 women had a prior live birth (38 pregnant and 38 nonpregnant). Two nulliparous women were included because of mistaken endorsement of a prior live birth by one nonpregnant woman at the time of screening who was subsequently matched with a pregnant nulliparous woman.

Study Visit Overview

All study sessions were conducted in the afternoon, beginning at 12:00 PM. Upon arrival at the Ohio State University Clinical Research Center, participants provided informed consent and were given a standardized lunch to ensure a euglycemic state. After lunch, a catheter was inserted into an antecubital vein to allow for serial blood sampling and baseline questionnaires were completed assessing mood. After a 20-minute acclimation/rest period, baseline blood samples were obtained. Next, the Trier Social Stress Test (TSST) was initiated. Serum samples were collected at 45 and 120 minutes after the conclusion of the TSST for assessment of circulating IL-6. Across the course of the 4.5-hour study protocol, a total of 122 ml of blood was drawn. Saliva samples were collected by salivette at 25 minutes before stressor initiation and at 0, 15, 30, 45, 60, and 90 minutes poststressor.

Laboratory Stressor: The TSST

This commonly used and well-validated laboratory stressor reliably evokes physiological reactivity and increases in self-reported stress (54–57). The TSST is a 20-minute task that requires participants to make a speech and perform mental arithmetic in front of an "audience" of two to three evaluators. For this study, the audience was composed of two female evaluators, one African American and one white. The participant was told to imagine that she had applied for a job and been invited to an interview by the selection committee.

She was informed that she would be given 10 minutes to prepare a speech about why she would be best for the job and 5 minutes to talk with the committee, followed by a second experimental task. After the speech, the participant completed a 5-minute mental arithmetic task involving serial subtraction. The difficulty of the subtraction task was adjusted each minute based on the participant's performance during the previous minute to improve the equivalence of the stress task across participants, as described elsewhere (42,58). To enhance the evaluative aspect of the task, the speech and math tasks were videotaped and participants were informed that these would be used for later "behavioral analysis."

Psychosocial Measures

Questionnaires were used to assess various psychological constructs and subjective responses to the stressor to determine the similarity between groups on these factors. All questionnaires, with the exception of the baseline Positive and Negative Affect Scale (PANAS), were completed poststressor. The Experiences of Discrimination Scale (EOD) was administered after the final blood draw to ensure that racial differences in recall of potentially stressful events did not differentially affect inflammatory responses.

The *PANAS* was administered three times to measure transient affective responses to the stressor: at the conclusion of the acclimation/rest period, immediately upon conclusion of the stressor, and at 120 minutes poststressor. With excellent norms and strong reliability and validity, this is an excellent self-report measure of transient affective states (59).

Depressive symptoms were assessed with the *Center for Epidemiological Studies Depression Scale (CES-D)*. This measure is brief and shows good reliability and validity (60,61). Furthermore, CES-D scores during pregnancy are associated with negative outcomes including restricted fetal growth (62), spontaneous preterm birth (63), and impaired neuromotor performance among neonates (64). A cutoff of 16 or higher is commonly used to indicate clinically meaningful depressive symptoms.

The state subscale of the *State-Trait Anxiety Inventory (STAI)* includes 20 items that measure state anxiety (65). This measure shows good internal consistency and test-retest reliability (66).

The 14-item version of the *Perceived Stress Scale (PSS)*, also widely used and well validated, was used to measure the subjective experiences of stress and coping with stress during the past month (67). The PSS measures a construct that is independent of depressive symptoms (67). Demonstrating predictive validity in pregnant populations, the PSS has been associated with maternal neuroendocrine function (68,69) and risk of bacterial vaginosis (70).

The *Cook-Medley Hostility Scale* is a 50-item set of true-false items that sum to yield a hostility score (71). A subset of 13 items measure cynical hostility. The scale has good internal consistency and test-retest reliability (72). Hostility tends to be positively correlated with perceived racism (73,74) and has been associated with stronger cardiovascular reactions to stress (75,76).

Social support was measured using the *Multidimensional Scale of Perceived Social Support (MSPSS)*. This 12-item measure assesses support from family, friends, and a significant other. It has been validated for use among pregnant women (77,78).

The short form of the *Childhood Trauma Questionnaire–Short Form (CTQ-SF)* is a 28-item self-report measure of childhood or adolescent abuse and neglect (79,80). This scale shows measurement invariance across diverse samples and good criterion-related validity among adolescents in relation to corroborative data (79). Standard cutoffs on the CTQ were used to classify women as having experienced or not experienced childhood abuse in the form of emotional abuse, physical abuse, and/or sexual abuse (81).

Sleep quality was assessed with the *Pittsburgh Sleep Quality Index*. This scale has good diagnostic sensitivity and specificity in distinguishing good and poor sleepers (82). A score higher than 5 is indicative of clinically disturbed sleep.

The *EOD* is a nine-item measure assessing the occurrence and frequency of discrimination caused by race/ethnicity. Specifically, participants indicate whether they have experienced discrimination over their lifetime (yes or no) in the following settings: a) at school; b) getting hired or getting a job; c) at work; d) getting housing; e) getting medical care; f) getting service in a store or restaurant; g) getting credit, bank loans, or a mortgage; h) on the street or in a public setting; and i) from the police or in the courts. For items endorsed, participants rate the frequency of this occurrence: once, two to three times, or four or more times. This scale has high test-retest reliability and predictive validity for health outcomes in black adults (83–85). Moreover, validation studies indicate that scores are not related to social desirability (83).

Health Behaviors

As described, women reporting tobacco use at the time of screening were excluded from participation. *Exercise* was operationalized as the frequency of engaging in vigorous physical activity long enough to build up a sweat with a range of "less than once per month" to "more than once per week." *Prenatal vitamin use* was defined as never, some days (1–3 d/wk), most days (4–6 d/wk), and every day (7 d/wk).

Interleukin-6

Serum was prepared from clotted blood samples from all participants at each of the three assessment time points (baseline, 45 minutes poststressor, and 120 minutes poststressor). Serum levels of IL-6 were measured using ultrasensitive kits from Meso Scale Discovery. All samples were batched and assayed on kits from the same lot. The lower limit of detection was 0.61 pg/ml. All samples were above the limit of detection. Interassay and intra-assay coefficients of variation were 10.65% and 10.66%, respectively.

Salivary Cortisol

Saliva was collected via salivette at seven time points: 25 minutes before the stressor (baseline), immediately upon completion of the stressor, and at 15, 30, 45, 60, and 90 minutes poststressor. Determinations are made using the Cortisol Coat-A-Count RIA kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra-assay coefficient of variation was 4.3%, and interassay coefficient of variation was 5.2%. The sensitivity of the assay was 0.025 μg/dl. Assays were counted and calculated on the Packard Cobra II Gamma Counter (PerkinElmer, Shelton, CT).

Statistical Analyses

Summary statistics were reported as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables. Participants were grouped into four categories based on race and pregnancy status. Demographic variables, health behaviors, and psychosocial assessments were compared using analysis of variance for continuous outcomes, χ^2 or Fisher exact tests for categorical outcomes, and the nonparametric Jonckheere-Terpstra (JT) test for ordered categorical outcomes. Differences by pregnancy and race overall and also between each pair of race/pregnancy subgroups were tested.

Linear-mixed models were used to test for differences in stress responses across race and pregnancy. Outcome variables were IL-6, salivary cortisol, and PANAS positive and negative affect. The distributions of IL-6 and salivary cortisol measures were right skewed; thus, IL-6 and salivary cortisol measures were log transformed to better satisfy the normality assumptions of the mixed-model analyses. For each model, the independent fixed effects were the fully saturated interactions and main effects for race, pregnancy, and time. For IL-6 and PANAS positive/negative, models were fit to the change scores at the two poststressor time points, controlling for the baseline value of the outcome variable by including it as a covariate. With the inclusion of the baseline covariate, this model yields equivalent p values whether postscores or change scores are modeled; the change score outcomes are preferred because they yield estimates directly indicating change from baseline. As a sensitivity analysis, we also evaluated IL-6, with the baseline level included as a dependent variable along with the two poststressor time points. For salivary cortisol, because there were six follow-up time points instead of two, the baseline value was included as a dependent variable at time = 0, and subsequent time points were tested against the baseline parameter estimate. A random-participant effect was also included in each model, accounting for the correlation across time within a participant. Parameter contrasts were set up in SAS PROC MIXED to test race and pregnancy effects at each time point. The contrasts produce a 1 degree-of-freedom test with a t statistic. Marginal distributions of race and pregnancy were used as the coefficients for interaction terms including race or pregnancy, as appropriate.

To assess the association of cortisol with change in IL-6, we calculated the area under the curve (AUC) for salivary cortisol from 25 minutes prestressor to 90 minutes poststressor for each participant by using the trapezoidal rule.

TABLE 1. Demographic Characteristics

	Nonpregnant White (n = 20)	Nonpregnant African American (n = 19)	Pregnant White (n = 20)	Pregnant African American (n = 19)
Age, M (SD), y[a]	24.85 (3.10)	23.16 (4.20)	23.90 (3.21)	23.68 (3.56)
Marital status, n (%)[b,c]				
Married	5 (25)	1 (5)	11 (55)	7 (37)
In a relationship	11 (55)	7 (37)	8 (40)	12 (63)
Single	4 (20)	11 (58)	1 (5)	0 (0)
Education, n (%)[c]				
High school graduate or less	6 (30)	9 (47)	9 (45)	5 (25)
Some college	6 (30)	7 (37)	7 (35)	9 (47)
College degree (2 or 4 y)	8 (40)	2 (11)	4 (20)	4 (21)
Income, n (%)[d]				
<US$15,000	7 (35)	8 (42)	8 (40)	9 (47)
US$15,000–29,999	7 (35)	6 (32)	6 (30)	6 (35)
≥US$30,000	6 (30)	5 (26)	6 (30)	4 (21)
Nulliparous[e], n (%)[c]	0 (0)	1 (5)	0 (0)	1 (5)
BMI[f], M (SD)[a], kg/m^2	24.13 (3.88)	23.57 (3.48)	24.32 (2.72)	23.57 (3.70)

M = mean; SD = standard deviation; BMI = body mass index.
[a] Analysis of variance, two tailed.
[b] Nonpregnant African American women were significantly less likely to be married than pregnant white ($\chi^2(1) = 11.3$, $p = .001$) or pregnant African American women ($\chi^2(1) = 5.7$, $p = .017$).
[c] χ^2 Test.
[d] Jonckheere-Terpstra test, two tailed.
[e] Women with prior pregnancies were targeted for recruitment; see "Methods."
[f] Based on prepregnancy weight for pregnant women; obese women (BMI ≥30 kg/m^2) were excluded from participation.

Correlations between cortisol AUC and IL-6 change from baseline at 45 and 120 minutes were calculated.

The study was powered based on enrolling 40 pregnant and 40 nonpregnant women (50% African American and 50% white), which would yield greater than 90% power to detect the expected effect sizes of 1 SD in terms of Cohen *d* for pregnancy and race effects on IL-6 responses.

RESULTS

Demographic Characteristics

Women did not significantly differ by race or pregnancy status in age, education, income, nulliparity, or body mass index (based on prepregnancy weight for pregnant women; Table 1). Nonpregnant women were less likely to be married than pregnant women ($\chi^2(1) = 8.67$, $p = .003$). This effect was driven by nonpregnant African Americans who were less likely to be married than pregnant white ($\chi^2(1) = 11.3$, $p = .001$) or pregnant African American women ($\chi^2(1) = 5.7$, $p = .017$).

Inflammatory Responses to Acute Stress

Baseline IL-6 levels were significantly lower in pregnant white versus nonpregnant white women ($t(74) = 2.54$, $p = .013$). Linear-mixed model results demonstrated that, controlling for baseline IL-6, African American women exhibited significantly greater IL-6 increases at 120 minutes poststressor as compared with whites ($t(72) = 3.51$, $p = .001$). Controlling for baseline, IL-6 levels at 120 minutes poststressor were 46% higher among African American women (95% confidence interval [CI] = 18%–81%; Fig. 1).

Model contrasts demonstrated that nonpregnant African Americans showed significantly greater inflammatory responses at 120 minutes poststressor than did either pregnant ($t(72) = 3.50$, $p = .001$) or nonpregnant ($t(72) = 2.26$, $p = .027$) whites. Pregnant African American women had significantly greater inflammatory responses than did pregnant whites ($t(72) = 2.67$, $p = .009$). Thus,

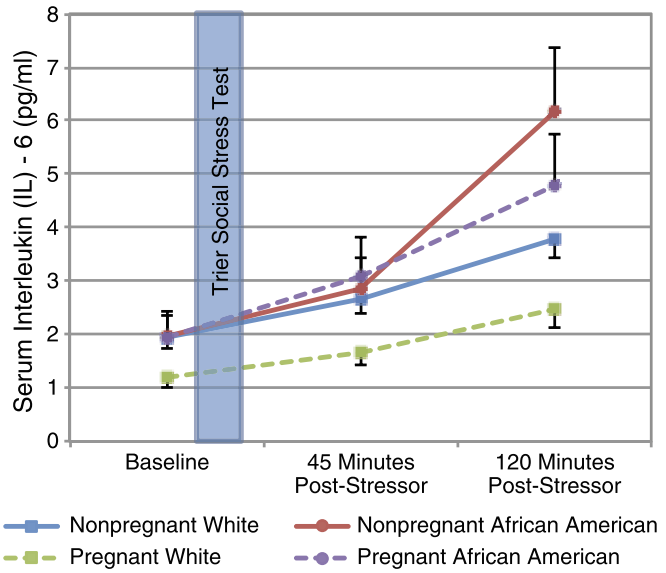

Figure 1. Inflammatory responses to the Trier Social Stress Test in pregnant and nonpregnant women. Controlling for baseline, IL-6 levels at 120 minutes poststressor were 46% higher in African Americans than in whites (95% confidence interval = 18%–81%, $t(72) = 3.51$, $p = .001$). This effect of race was significant during pregnancy and nonpregnancy. Note: data are pictured in raw values. Analyses were conducted using log-transformed values. To view image in color, please visit: *www.psychosomaticmedicine.org*.

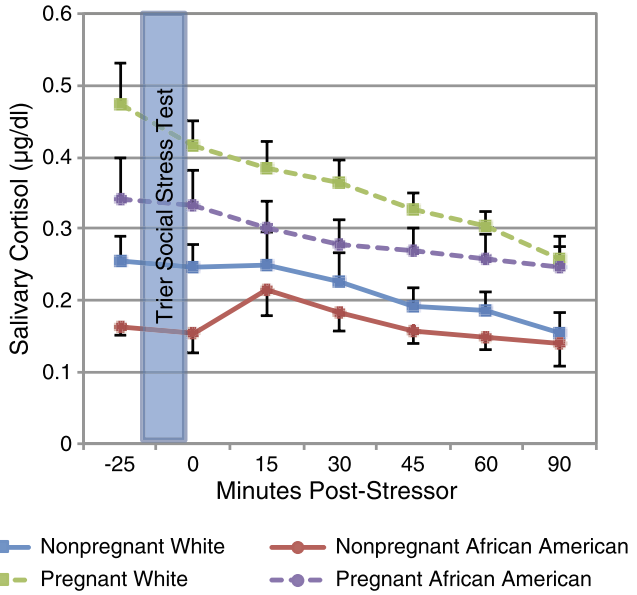

Figure 2. Cortisol responses to the Trier Social Stress Test in pregnant and nonpregnant women. At baseline, cortisol was significantly higher in pregnant women than in nonpregnant women ($t(72) = 15.30$, $p < .001$). A nonsignificant trend was seen for lower baseline cortisol among African Americans compared with whites ($t(72) = 2.65$, $p = .11$). The area under the curve of cortisol across the study session was significantly higher among pregnant that among nonpregnant women ($t(75) = 4.45$, $p < .001$) and marginally lower among African Americans that among whites ($t(75) = 1.77$, $p = .08$). Note: data are pictured in raw values. Analyses were conducted using log-transformed values. To view image in color, please visit: *www.psychosomaticmedicine.org*.

effects of race were observed in pregnancy and nonpregnancy. In a sensitivity analysis modeling IL-6 at baseline, 45 minutes, and 120 minutes, the race by time interaction was significant ($F(2,146) = 5.15$, $p = .007$). For pregnancy effects, controlling for baseline, IL-6 levels at 120 minutes poststressor tended to be lower (15%) in pregnant versus nonpregnant women (95% CI = −5% to 32%, $p = .14$).

Salivary Cortisol

At baseline, cortisol was significantly higher in pregnant women than in nonpregnant women ($t(72) = 15.30$, $p < .001$; Fig. 2). A nonsignificant trend was seen for lower baseline cortisol among African Americans versus whites ($t(72) = 2.65$, $p = .11$). Across all participants combined, salivary cortisol was significantly lower than baseline immediately poststressor ($t(72) = 2.03$, $p = .046$) and at 30, 45, 60, and 90 minutes poststressor (30 minutes: $t(72) = 3.08$, $p = .003$; 45 minutes: $t(72) = 4.82$, $p < .001$; 60 minutes: ($t(72) = 5.99$, $p < .001$; 90 minutes: $t(72) = 9.70$, $p < .001$). There were no significant increases from baseline in cortisol in any of the groups. There were no significant differences in estimated slopes for linear change in salivary cortisol from 25 minutes prestressor to 90 minutes poststressor, with the exception of a flatter cortisol slope among nonpregnant African Americans compared with pregnant ($t(73) = 2.26$, $p = .027$) and nonpregnant ($t(73) = 2.05$, $p = .044$) whites.

The AUC of cortisol across the study session was marginally higher among whites versus African Americans ($t(75) = 1.77$, $p = .080$) and significantly higher among pregnant versus nonpregnant women ($t(75) = 4.45$, $p < .001$). In the overall sample, total salivary cortisol (AUC) was not associated with the change from baseline in IL-6 at 45 ($r = 0.14$, $p = .22$) or 120 ($r = -0.03$, $p = .81$) minutes poststressor. Moreover, after controlling for cortisol AUC, race remained significantly associated with change in IL-6 at 120 minutes poststressor ($t(71) = 3.95$, $p < .001$).

Subjective Responses to Acute Stress

At baseline (before stressor onset), pregnant women reported significantly less positive affect as measured by the PANAS as compared with nonpregnant women ($t(74) = 12.6$, $p < .001$). Controlling for baseline, positive affect did not differ based on race or pregnancy status immediately poststressor (race: $t(74) = 0.10$, $p = .92$; pregnancy: $t(74) = 0.22$, $p = .82$) or at 120 minutes poststressor (race: $t(74) = 1.86$, $p = .067$; pregnancy: $t(74) = 1.33$, $p = .19$; Fig. 3).

Women did not differ in negative affect at baseline based on either race or pregnancy status (race: $t(74) = 0.06$, $p = .95$; pregnancy: $t(74) = 0.98$, $p = .33$). Immediately poststressor, pregnant women had a significantly lower increase in negative affect than did nonpregnant women ($t(74) = 7.65$, $p = .007$; Fig. 4). This effect was driven by pregnant whites who had lower increases in negative affect than did both nonpregnant African Americans ($t(74) = 2.76$, $p = .007$) and nonpregnant whites ($t(74) = 2.94$, $p = .004$), whereas pregnant African Americans did not differ significantly from either nonpregnant African Americans ($t(74) = 1.11$, $p = .27$) or nonpregnant whites ($t(74) = 0.95$, $p = .35$). The race by pregnancy interaction was not significant immediately poststressor ($t(74) = 1.38$, $p = 0.17$). Women did not differ in negative affect at 120 minutes poststressor.

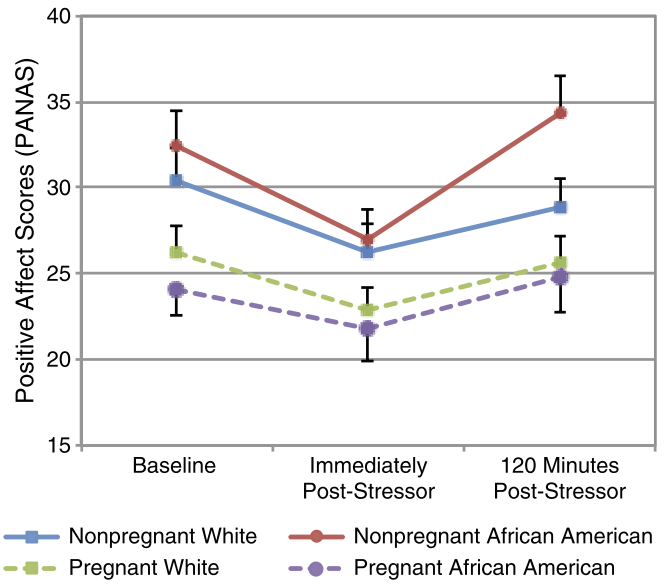

Figure 3. Changes in positive affect in response to the Trier Social Stress Test. Pregnant women reported significantly less positive affect at baseline as compared with nonpregnant women ($F(1,74) = 12.6$, $p < .001$). Controlling for baseline positive affect, positive affect immediately after stressor initiation or 120 minutes after stressor initiation did not differ based on race or pregnancy status (p values ≥ .06). To view image in color, please visit: *www.psychosomaticmedicine.org*.

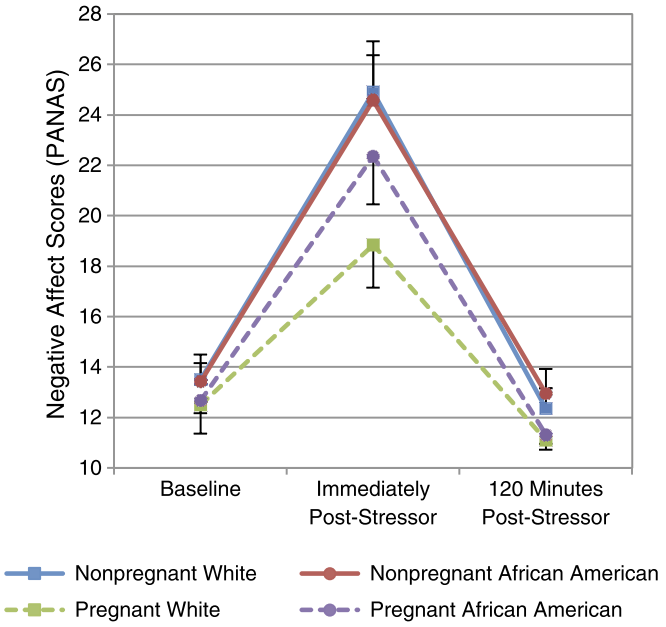

Figure 4. Changes in negative affect in response to the Trier Social Stress Test. Women did not differ in negative affect at baseline based on either race or pregnancy status. Immediately after stressor initiation, pregnant women had a significantly lower increase in negative affect than did nonpregnant women ($t(74) = 7.65$, $p = .007$). This effect was driven by pregnant whites who had lower increases in negative affect compared with either nonpregnant African Americans or nonpregnant whites (p values $\leq .01$). To view image in color, please visit: *www.psychosomaticmedicine.org*.

Health Behaviors

Health behaviors are presented in Table 2. Participation in vigorous activity was significantly greater among whites versus African Americans (JT, $Z = 2.35$; $p = .019$) and in nonpregnant versus pregnant women (JT, $Z = 3.20$; $p = .001$). These differences were driven by nonpregnant white women who reported taking part in vigorous activity significantly more frequently than each of the other three groups (p values $< .003$). Observed differences in inflammatory responses did not change after controlling for physical activity. Groups did not differ in smoking status or prenatal vitamin use (between pregnant groups).

Psychosocial Factors

For psychosocial factors, 26.9% of women overall scored at or above a clinical cutoff of 16 on the CES-D, indicating significant depressive symptoms. The mean state anxiety score (STAI) was 35.12, which is the 56th percentile for women in this age range (86). The mean (SD) perceived stress score (PSS) was 17.92 (6.52), which is also slightly higher than a mean (SD) of 16.14 (7.56) among women in a population-based sample of women (87). The overall mean (SD) total score on the social support measure (MSPSS) was 5.59 (1.06), which is slightly less than that reported in a prior study of pregnant women (mean [SD] = 6.01 [0.90]) (77). In the overall sample, 56.4% were classified as poor sleepers based on a score higher than 5 on the Pittsburgh Sleep Quality Index. In addition, 52.5% met the criteria for childhood abuse based on scores on the CTQ.

Analyses were conducted to psychosocial factors based on race and pregnancy status assessed (Table 3). Women did not differ by race or pregnancy status in the proportion of women with CES-D score higher than 16 or the proportion reporting childhood abuse (all $\chi^2(1) < 1.89$, p values $> .17$), or in state anxiety as measured by the STAI (both $t(74) > 0.53$, p values $> .60$). Perceived support in total ($t(74) = 2.26$, $p = .026$) and from a significant other ($t(74) = 2.67$, $p = .009$) was greater in

TABLE 2. Health Behaviors

	Nonpregnant White ($n = 20$)	Nonpregnant African American ($n = 19$)	Pregnant White ($n = 20$)	Pregnant African American ($n = 19$)
Smoking status[a]				
Current	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Past	8 (40%)	4 (21%)	9 (45%)	4 (21%)
Never	12 (60%)	15 (79%)	11 (55%)	15 (79%)
Prenatal vitamin use[b]	N/A	N/A		
Never			5 (26%)	8 (40%)
Some days (1–3/wk)			4 (20%)	2 (11%)
Most days (4–6/wk)			5 (25%)	2 (11%)
Every day (7 d/wk)			3 (15%)	10 (53%)
Exercise[b,c]				
Less than once per month	0 (0%)	3 (16%)	8 (40%)	9 (47%)
Once per month	1 (5%)	2 (11%)	0 (0%)	2 (11%)
2-3 times per month	2 (10%)	7 (37%)	5 (25%)	3 (16%)
Once per week	7 (35%)	4 (21%)	3 (15%)	2 (11%)
More than once per week	10 (50%)	3 (16%)	4 (20%)	3 (16%)

[a] χ^2 Test.
[b] Jonckheere-Terpstra test (JT), two tailed.
[c] Participation in vigorous activity was significantly greater among whites that among African Americans (JT, $p = .019$) and in nonpregnant that in pregnant women (JT, $p = .001$). These differences were driven by nonpregnant white women who reported taking part in vigorous activity significantly more frequently than each of the other three groups (p values $< .003$).

TABLE 3. Psychological Functioning

	Nonpregnant White (n = 20)	Nonpregnant African American (n = 19)	Pregnant White (n = 20)	Pregnant African American (n = 19)
Depressive symptoms[a]				
CES-D ≥16, n (%)	6 (30)	7 (36.8)	3 (15.0)	5 (26.3)
Anxiety[b]				
State anxiety (STAI), M (SD)	35.25 (13.14)	36.26 (10.40)	34.80 (8.82)	34.17 (9.46)
Perceived stress[b]				
PSS score, M (SD)	17.79 (7.6)	19.72 (6.24)	17.10 (6.56)	17.21 (5.66)
Impaired sleep quality[a]				
PSQI score >5, n (%)	11 (55)	13 (68)	11 (55)	9 (45)
Social support, M (SD)[b,c]				
Total MSPSS	5.22 (1.02)	5.44 (1.16)	5.81 (1.14)	5.92 (0.83)
Family support	5.40 (1.64)	5.43 (1.20)	5.66 (1.59)	5.75 (1.59)
Friend support	4.94 (1.24)	5.55 (1.27)	5.52 (1.37)	5.74 (1.07)
Significant other support	5.33 (1.84)	5.33 (1.98)	6.25 (1.22)	6.28 (0.91)
Hostility[b]				
Cook-Medley score, M (SD)	19.40 (7.44)	22.05 (7.74)	20.10 (7.91)	20.42 (6.69)
Childhood trauma[a]				
Abused, n (%)	10 (50)	12 (63.2)	8 (40)	11 (57.9)
Racial discrimination[d,e]				
EOD score situations, n (%)				
0	12 (60)	6 (32)	11 (55)	7 (37)
1–2	7 (35)	7 (37)	7 (35)	5 (26)
>2	1 (5)	6 (32)	2 (10)	7 (37)
EOD score frequency, n (%)				
0	12 (60)	6 (32)	11 (55)	7 (37)
1–5	6 (30)	7 (37)	7 (35)	4 (21)
6–10	1 (5)	2 (11)	0 (0)	7 (37)
>10	1 (5)	4 (21)	2 (10)	1 (5)

CES-D = Center for Epidemiological Studies Depression Scale; M = mean; SD = standard deviation; STAI = State-Trait Anxiety Inventory; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index; MSPSS = Multidimensional Scale of Perceived Social Support; EOD = Experiences of Discrimination Scale.

[a] χ^2 Test.

[b] Analysis of variance, two tailed.

[c] Perceived support was significantly greater among pregnant versus nonpregnant women in total ($t(74) = 2.26$, $p = .026$) and from a significant other ($t(74) = 2.67$, $p = .009$).

[d] Jonckheere-Terpstra test, two tailed.

[e] Racial discrimination was significantly greater among African Americans that among whites in terms of both the number of situations (Jonckheere-Terpstra (JT), $p = .007$) and total frequency of discrimination (JT, $p = .012$), as reported on the EOD.

pregnant than in nonpregnant women. African American women reported greater racial discrimination both in the number of situations (JT, $Z = 2.70$; $p = .007$) and in total frequency (JT, $Z = 2.52$; $p = .012$). Among African American women, greater racial discrimination was not significantly related to scores on the CES-D, STAI, or MSPSS (p values ≥ .39). The correlation between the PSS and EOD scores in number of situations ($r = 0.26$, $p = .11$) and total frequency ($r = 0.32$, $p = .056$) approached statistical significance. Observed racial differences in inflammatory responses did not change when controlling for social support or racial discrimination in separate models.

DISCUSSION

The first goal of this study was to examine the effects of race on inflammatory responses to acute stress. As hypothesized, African American women exhibited greater serum IL-6 responses to the stressor as compared with whites. Model contrasts demonstrated that this effect of race was significant during both pregnancy and nonpregnancy. This effect was not accounted for by demographic variables or health behaviors.

Notably, African Americans and whites were similar in all psychosocial variables measured, with the exception of greater reported experiences of discrimination among African Americans. The relationship between race and inflammatory responses remained after controlling for perceived racial discrimination, indicating that exposure to racism as measured by this scale did not account for observed racial differences. Despite this result, the potential role for racial discrimination in the observed effects cannot be discounted. It is of note that a relatively high percentage of white women in the current study (42.5%)

reported experiencing racial discrimination in one or more major life situations. However, the qualitative experience and social implications of racial discrimination among whites versus African Americans differ (88). Thus, a simple comparison or statistical control for frequency of exposures among whites versus African Americans may not meaningfully capture the psychosocial impact of such exposures. Moreover, this study did not have adequate statistical power to compare inflammatory responses among African Americans based on of the severity of perceived racial discrimination. Within-group variability may ultimately be more meaningful with regard to physical health effects of racial discrimination. For example, our prior work shows that among pregnant African Americans, those reporting greater racial discrimination have higher Epstein-Barr virus antibody titers, indicating poorer cellular immune function (89). Finally, it is also possible that a different measure of racial discrimination may provide more predictive value in this context.

These data have implications for understanding biological mechanisms by which racial minority status may confer increased risk of adverse birth outcomes. Most studies to date have focused on stressor exposure and/or subjective experiences of stress in predicting preterm birth. However, the ultimate physical impact of stress on health is a function of exposure and physiological response. As reviewed, inflammatory processes are implicated in adverse birth outcomes including preterm birth (9). Therefore, more robust and extended inflammatory responses may confer increased risk of adverse birth outcomes, particularly among women who experience more frequent acute stressor exposure. Moreover, prior data show that upon repeated exposures to an acute laboratory stressor, habituation of cortisol and blood pressure reactivity is evidenced, but stress-induced elevations in IL-6 remain similar (42). Therefore, the experience of continued or repeated psychosocial stressors may confer considerable exposure to inflammatory markers.

An exploratory aim of this study was to examine the correspondence between cortisol and stress-induced IL-6 responses. Baseline and cortisol AUC was higher among pregnant versus nonpregnant women, as expected. Compared with whites, African Americans showed a trend toward lower baseline cortisol ($p = .11$) and lower cortisol AUC ($p = .080$). The study protocol was conducted in the early afternoon. Prior data show that, compared with whites, African Americans have lower cortisol levels in the morning/early afternoon and higher levels in the evening, resulting in a flatter diurnal slope (90,91). Thus, these data are consistent with prior studies. Cortisol AUC was not associated with IL-6 responses in the overall sample, and the noted racial differences in IL-6 responses were not modified by inclusion of cortisol AUC in the model.

Some prior data suggest that activity of the HPA axis is inversely associated with IL-6 responses to acute stress (92), although other studies have found this relationship to be inconsistent (42). It is important to consider that although cortisol has robust anti-inflammatory effects on cytokine-producing cells, extended exposure to elevated levels of glucocorticoids (GCs), such as that seen in conditions of repeated or chronic stress, may produce GC insensitivity at the level of both cytokine-producing cells and the HPA axis (93,94). GC insensitivity is marked by a diminished ability of the HPA axis and cytokine-producing cells to respond to cortisol, resulting in more sustained HPA axis responses and greater production of inflammatory markers. Thus, in the context of GC resistance, the expected inverse relationship between cortisol and inflammatory markers may be diminished or eliminated. Therefore, without knowledge of glucocorticoid receptor function or typical cortisol exposure (i.e., measurement of the complete diurnal slope), the lack of association between IL-6 and salivary cortisol responses in the current study is difficult to interpret.

Of note, in this study, no significant cortisol increases were seen in response to the stressor. Owing to a typical diurnal decline in cortisol, effects of stress on cortisol responses are less apparent without the use of a nonstressor control day, particularly when measurement occurs in the afternoon (95). In the absence of stress-induced cortisol increases, stressor exposure may slow the downward diurnal cortisol slope, but this effect within participants is not quantifiable without comparison with a nonstress day. In addition, prior studies show that cortisol reactivity to acute stressors is less robust among women than men (96). Thus, these factors may explain the lack of increase in cortisol in response to the stressor.

The current study included 39 pregnant women, among whom only 2 (5.1%) delivered preterm (1 African American, 1 white). This preterm birth rate is lower than in the US population overall and reflects the exclusion of women with high-risk health conditions (e.g., hypertension) and health behaviors (e.g., smokers). Thus, this study was not appropriately powered to detect potential associations between inflammatory responses to acute stress and birth outcomes. This is a clear direction for future studies.

Although the focus of the current study was on pregnancy, the evidenced effect of race on inflammatory responses has implications for health outcomes well beyond pregnancy. In particular, there are substantial racial disparities in cardiovascular diseases (97). Black women and men experience greater prevalence, earlier disease onset, and greater cardiovascular mortality compared with white individuals of the same age (98). Inflammation is an established mechanistic factor in cardiovascular disease risk (99,100). Moreover, it has been demonstrated that stress-induced increases in IL-6 predict ambulatory blood pressure 3 years later (101). The current data provide novel evidence of racial differences in stress-induced inflammatory responses. Attention is needed regarding the potential role of inflammatory responses to acute stress in racial disparities in cardiovascular disease risk.

For pregnancy status, contrary to prediction, there was no significant difference between pregnant women, who were assessed in the second trimester of pregnancy, and nonpregnant women in terms of inflammatory responses, although there was a trend toward this effect ($p = .14$). IL-6 increases in response to the stressor were 15% lower in pregnant than in nonpregnant women (95% CI = −5% to 32%). This effect size translates to a Cohen d of 0.17, which is a small effect. Prior data in

humans and animals indicate that inflammatory responses to both in vivo and in vitro biological challenges (e.g., lipopolysaccharide) are attenuated in pregnancy (25–29). It has been postulated that this attenuation of inflammatory responses represents an adaptive mechanism by which the fetus is protected from excessive maternal responding. Following from this argument, adaptation of the inflammatory responses to psychological challenge may be similarly beneficial. Data from a larger cohort would provide greater statistical power to determine if this effect is present.

Prior data have shown that negative affective responses to a speech task are associated with the magnitude of inflammatory response (102). In the current study, there was no main effect of race on negative affective responses. However, pregnant white women reported significantly smaller increases in negative affect than did nonpregnant whites or nonpregnant blacks. Prior evidence suggests that stressors are experienced as less stressful during pregnancy than during nonpregnancy. For example, upon exposure to an earthquake, women rated the experience as more stressful in earlier than in later stages of pregnancy and showed increasingly shorter gestation upon earlier exposure (52). It is notable that in the current sample, pregnant African American women did not show attenuation of negative affective responses compared with nonpregnant African Americans.

The current study used the TSST. The TSST is mild stressor designed to be similar to stressors such as public speaking that most people encounter at some time in their daily life. It has previously been used with women during pregnancy and postpartum (e.g., Nierop et al. (103) and Redwine et al. (104)). However, most previous studies of reactivity to psychological stressors during pregnancy have used tasks such as mirror tracing, Stroop selective attention tasks, and cold pressor tasks (for review, see de Weerth and Buitelaar (53) and Christian (105)). Owing to its similarity to naturally occurring stressors, the TSST arguably provides a stronger approximation of reactivity to everyday psychological stressors than these other types of tasks. In addition, evidence suggests that pregnant women exhibit significantly higher reactivity to public speaking as compared with other types of acute stressors (106). Therefore, use of the TSST in this study provides both strong external validity and improved power to detect the effects of the stressor on physiological parameters of interest relative to other stressors.

This study included assessment of IL-6 at 45 and 120 minutes poststressor, given that inflammatory responses to laboratory stressors are delayed (107). Although these are appropriate sampling time points, longer follow-up would provide valuable data regarding the full duration and magnitude of inflammatory marker exposure in different groups. This should be considered in future studies. In addition, this study included assessment of pregnant women in the second trimester. It has been hypothesized that stress responsivity is progressively attenuated as pregnancy progresses (105). Longitudinal assessment during each trimester of pregnancy and/or cross-sectional studies with demographically matched groups of women during each trimester would provide valuable data regarding the adaptation of inflammatory responses across the course of gestation. Similarly, in the nonpregnant control group, effects of menstrual cycle phase were not assessed. Prior data indicate that menstrual cycle phase does not appreciably alter heart rate, blood pressure, catecholamine, lipid, or inflammatory cytokine responses to acute stress (108,109), but does alter cortisol responses (110).

Women with a previous live birth were targeted for recruitment and comprised 76 (97.4%) of 78 of the final sample. This strengthens the study design because available data suggest that physiological adaptation to pregnancy may be greater during subsequent than initial pregnancy (111). In addition, recruitment of nonpregnant controls with a prior live birth provided a sample with the best demographic equivalence to pregnant participants in terms of women experiencing motherhood at a given stage of life. Although this study design was chosen based on these empirical considerations, results may differ among women who are nulliparous.

This study included stringent inclusion/exclusion criteria. All women were normal weight, were generally healthy, and denied use of recreational drugs or smoking at the time of eligibility screening. However, we did not confirm drug or smoking status via objective measures (e.g., serum cotinine). Self-reported smoking likely underestimates true smoking behavior, particularly among pregnant women (112). These stringent inclusion/exclusion requirements increase homogeneity within groups, increasing statistical power. However, as noted earlier, these criteria also exclude women at greatest risk for adverse birth outcomes. Moving forward, future studies should endeavor to include women with more diverse health behaviors and demographic characteristics to provide power to examine inflammatory responses in relation to birth outcomes and typify the mediating and moderating role of the factors in predicting stress-induced inflammatory responses.

This study was powered to detect the effects of race and pregnancy status. Psychological data (e.g., hostility, depressive symptoms, etc) were collected to determine the comparability of groups in factors that may affect physiological responses to acute stress. However, this sample size did not permit analyses of meditational or moderation effects of these constructs (e.g., if hostility, sleep quality, or other factors differentially affected inflammatory responses by race or pregnancy status). In addition, we did not conduct clinical interviews to determine history of clinical depression. Future research should aim to examine such psychological mediators and moderators.

In sum, this study provides novel data regarding inflammatory responses to acute stress among women based on race and pregnancy status. These data represent a promising direction in delineating pathways by which stress may affect pregnancy outcome and fetal development. Although numerous studies demonstrate a link between various psychosocial stress exposures and pregnancy outcomes, comparatively few have focused on a) biological mechanisms underlying these links or b) individual differences in responses to stress. In addition, these results may possibly have relevance beyond pregnancy for racial disparities in diseases with an inflammatory component, particularly

cardiovascular diseases. Because the ultimate impact of stressor exposure is a function of not only the occurrence of the stressor but also the individual response to the stressor, focus on individual differences in stress-induced inflammatory responses represents a clear target for continued research efforts.

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REFERENCES

1. Committee on Understanding Premature Birth and Assuring Healthy Outcomes. In: Behrman RE, Butler AS, editors. Preterm Birth: Causes, Consequences, and Prevention. Washington, DC: National Academies Press; 2007.
2. Callaghan WM, MacDorman MF, Rasmussen SA, Qin C, Lackritz EM. The contribution of preterm birth to infant mortality rates in the United States. Pediatrics 2006;118:1566–73.
3. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S, Munson ML. Births: final data for 2005. Natl Vital Stat Rep 2007;56:1–103.
4. Mcgrady GA, Sung JFC, Rowley DL, Hogue CJR. Preterm delivery and low-birth-weight among 1st-born infants of black-and-white college graduates. Am J Epidemiol 1992;136:266–76.
5. Goldenberg RL, Cliver SP, Mulvihill FX, Hickey CA, Hoffman HJ, Klerman LV, Johnson MJ. Medical, psychosocial, and behavioral risk factors do not explain the increased risk for low birth weight among black women. Am J Obstet Gynecol 1996;175:1317–24.
6. Collins JW, Hawkes EK. Racial differences in post-neonatal mortality in Chicago: what risk factors explain the black infant's disadvantage? Ethn Health 1997;2:117–25.
7. Shiono PH, Rauh VA, Park M, Lederman SA, Zuskar D. Ethnic differences in birthweight: the role of lifestyle and other factors. Am J Public Health 1997;87:787–93.
8. Schoendorf KC, Hogue CJR, Kleinman JC, Rowley D. Mortality among infants of black as compared with white college-educated parents. N Engl J Med 1992;326:1522–6.
9. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75–84.
10. Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. Interleukin-6 promoter-174 polymorphism and spontaneous preterm birth. Am J Obstet Gynecol 2003;189:915–8.
11. Mulherin Engel SA, Erichsen HC, Savitz DA, Thorp J, Chanock SJ, Olshan AF. Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms. Epidemiology 2005; 16:469–77.
12. Giscombe CL, Lobel M. Explaining disproportionately high rates of adverse birth outcomes among African Americans: the impact of stress, racism, and related factors in pregnancy. Psychol Bull 2005;131:662–83.
13. Green NS, Damus K, Simpson JL, Iams J, Reece EA, Hobel CJ, Merkatz IR, Greene MF, Schwarz RH, Committee MDSA. Research agenda for preterm birth: recommendations from the March of Dimes. Am J Obstet Gynecol 2005;193:626–35.
14. Collins JW, David RJ, Handler A, Wall S, Andes S. Very low birthweight in African American infants: the role of maternal exposure to interpersonal racial discrimination. Am J Public Health 2004;94:2132–8.
15. Dole N, Savitz DA, Siega-Riz AM, Hertz-Picciotto I, McMahon MJ, Buekens P. Psychosocial factors and preterm birth among African American and White women in central North Carolina. Am J Public Health 2004;94:1358–65.
16. Mustillo S, Krieger N, Gunderson EP, Sidney S, McCreath H, Kiefe CI. Self-reported experiences of racial discrimination and Black-White differences in preterm and low-birthweight deliveries: the CARDIA study. Am J Public Health 2004;94:2125–31.
17. Dole N, Savitz DA, Hertz-Picciotto I, Siega-Riz AM, McMahon MJ, Buekens P. Maternal stress and preterm birth. Am J Epidemiol 2003; 157:14–24.
18. Rosenberg L, Palmer JR, Wise LA, Horton NJ, Corwin MJ. Perceptions of racial discrimination and the risk of preterm birth. Epidemiology 2002;13:646–52.
19. Curry AE, Vogel I, Skogstrand K, Drews C, Schendel DE, Flanders WD, Hougaard DM, Thorsen P. Maternal plasma cytokines in early- and mid-gestation of normal human pregnancy and their association with maternal factors. J Reprod Immunol 2008;77:152–60.
20. Vassiliadis S, Ranella A, Papadimitriou L, Makrygiannakis A, Athanassakis I. Serum levels of pro- and anti-inflammatory cytokines in nonpregnant women, during pregnancy, labour and abortion. Mediators Inflamm 1998; 7:69–72.
21. Austgulen R, Lien E, Liabakk NB, Jacobsen G, Arntzen KJ. Increased levels of cytokines and cytokine activity modifiers in normal-pregnancy. European Journal of Obstetrics, Eur J Obstet Gynecol Reprod Biol 1994;57:149–55.
22. Opsjon SL, Wathen NC, Tingulstad S, Wiedswang G, Sundan A, Waage A, Austgulen R. Tumor-necrosis-factor, interleukin-1, and interleukin-6 in normal human-pregnancy. Am J Obstet Gynecol 1993;169:397–404.
23. Makhseed M, Raghupathy R, Azizieh F, Farhat R, Hassan N, Bandar A. Circulating cytokines and CD30 in normal human pregnancy and recurrent spontaneous abortions. Hum Reprod 2000;15:2011–7.
24. Curry AE, Vogel I, Drews C, Schendel D, Skogstrand K, Flanders WD, Hougaard D, Olsen J, Thorsen P. Mid-pregnancy maternal plasma levels of interleukin 2, 6, and 12, tumor necrosis factor-alpha, interferon-gamma, and granulocyte-macrophage colony-stimulating factor and spontaneous preterm delivery. Acta Obstet Gynecol Scand 2007;86:1103–10.
25. Denney JM, Nelson EL, Wadhwa PD, Waters TP, Mathew L, Chung EK, Goldenberg RL, Culhane JF. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine 2011;53:170–7.
26. Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, Crane M, Kanik KS, Chrousos GP. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: Implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 2001;86:4933–8.
27. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E, Clerici M. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol 1996; 106:127–33.
28. Fofie AE, Fewell JE, Moore SL. Pregnancy influences the plasma cytokine response to intraperitoneal administration of bacterial endotoxin in rats. Exp Physiol 2004;90:95–101.
29. Aguilar-Valles A, Poole S, Mistry Y, Williams S, Luheshi GN. Attenuated fever in rats during late pregnancy is linked to suppressed interieukin-6 production after localized inflammation with turpentine. J Physiol (Lond) 2007;583:391–403.
30. Blackburn ST, Loper DL. Maternal, Fetal, and Neonatal Physiology: A Clinical Perspective. Philadelphia: WB Saunders; 1992.
31. Stables D. Physiology in Childbearing. Edinburgh: Bailliere Tindall; 1999.
32. Makhseed M, Raghupathy R, Azizieh F, Al-Azemi MMK, Hassan NA, Bandar A. Mitogen-induced cytokine responses of maternal peripheral blood lymphocytes indicate a differential Th-type bias in normal pregnancy and pregnancy failure. Am J Reprod Immunol 1999;42: 273–81.
33. Coussons-Read ME, Okun ML, Schmitt MP, Giese S. Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy. Psychosom Med 2005;67:625–31.

34. Christian LM, Franco A, Glaser R, Iams J. Depressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. Brain Behav Immun 2009;23:750–4.
35. Ruiz RJ, Stowe RP, Goluszko E, Clark MC, Tan A. The relationships among acculturation, body mass index, depression, and interleukin 1-receptor antagonist in Hispanic pregnant women. Ethn Dis 2007;17:338–43.
36. Blackmore ER, Moynihan JA, Rubinow DR, Pressman EK, Gilchrist M, O'Connor TG. Psychiatric symptoms and proinflammatory cytokines in pregnancy. Psychosom Med 2011;73:656–63.
37. Cassidy-Bushrow AE, Peters RM, Johnson DA, Templin TN. Association of depressive symptoms with inflammatory biomarkers among pregnant African-American women. J Reprod Immunol 2012;94:202–9.
38. Paul K, Boutain D, Agnew K, Thomas J, Hitti J. The relationship between racial identity, income, stress and C-reactive protein among parous women: implications for preterm birth disparity research. J Natl Med Assoc 2008;100:540–6.
39. Christian LM, Franco A, Iams JD, Sheridan J, Glaser R. Depressive symptoms predict exaggerated inflammatory response to in vivo immune challenge during human pregnancy. Brain Behav Immun 2010; 24:49–53.
40. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav Immun 2007;21:343–50.
41. Coussons-Read ME, Lobel M, Carey JC, Kreither MO, D'Anna K, Argys L, Ross RG, Brandt C, Cole S. The occurrence of preterm delivery is linked to pregnancy-specific distress and elevated inflammatory markers across gestation. Brain Behav Immun 2012;26:650–9.
42. von Kanel R, Kudielka BM, Preckel D, Hanebuth D, Fischer JE. Delayed response and lack of habituation in plasma interleukin-6 to acute mental stress in men. Brain Behav Immun 2006;20:40–8.
43. Brydon L, Edwards S, Mohamed-Ali V, Steptoe A. Socioeconomic status and stress-induced increases in interleukin-6. Brain Behav Immun 2004; 18:281–90.
44. Anderson NB, Lane JD, Monou H, Williams RB, Houseworth SJ. Racial-differences in cardiovascular reactivity to mental arithmetic. Int J Psychophysiol 1988;6:161–4.
45. Lepore SJ, Revenson TA, Weinberger SL, Weston P, Frisina PG, Robertson R, Portillo MM, Jones H, Cross W. Effects of social stressors on cardiovascular reactivity in black and white women. Ann Behav Med 2006;31:120–7.
46. Light KC, Sherwood A. Race, borderline hypertension, and hemodynamic responses to behavioral stress before and after beta-adrenergic blockade. Health Psychol 1989;8:577–95.
47. Hatch M, Berkowitz G, Janevic T, Sloan R, Lapinski R, James T, Barth WH. Race, cardiovascular reactivity, and preterm delivery among active-duty military women. Epidemiology 2006;17:178–82.
48. Light KC, Obrist PA, Sherwood A, James SA, Strogatz DS. Effects of race and marginally elevated blood pressure on responses to stress. Hypertension 1987;10:555–63.
49. Brondolo E, Rieppi R, Kelly KP, Gerin W. Perceived racism and blood pressure: a review of the literature and conceptual and methodological critique. Ann Behav Med 2003;25:55–65.
50. Pace TWW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, Heim CM. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am J Psychiatry 2006;163:1630–U1.
51. Fagundes CP, Glaser R, Hwang BS, Malarkey WB, Kiecolt-Glaser JK. Depressive symptoms enhance stress-induced inflammatory responses. Brain Behav Immun 2012.
52. Glynn LM, Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, Sandman CA. When stress happens matters: effects of earthquake timing on stress responsivity during pregnancy. Am J Obstet Gynecol 2001; 184:637–42.
53. de Weerth C, Buitelaar JK. Physiological stress reactivity in human pregnancy—a review. Neurosci Biobehav Rev 2005;29:295–312.
54. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 1993;28:76–81.
55. Kirschbaum C, Klauer T, Filipp SH, Hellhammer DH. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. Psychosom Med 1995;57:23–31.
56. Kirschbaum C, Bartussek D, Strasburger CJ. Cortisol responses to psychological stress and correlations with personality traits. Pers Individ Diff 1992;13:1353–7.
57. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 1994;19:313–33.
58. Schommer NC, Hellhammer DH, Kirschbaum C. Dissociation between reactivity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosom Med 2003;65:450–60.
59. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 1988;54:1063–70.
60. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Meas 1977;1:385–401.
61. Basco MR, Krebaum SR, Rush AJ. Outcome measures of depression. In: Strupp HH, Horowitz LM, Lambert MJ, editors. Measuring Patient Changes in Mood, Anxiety, and Personality Disorders. Washington, DC: American Psychological Association; 1997:207–45.
62. Hoffman S, Hatch MC. Depressive symptomatology during pregnancy: evidence for an association with decreased fetal growth in pregnancies of lower social class women. Health Psychology 2000;19:535–43.
63. Orr ST, James SA, Blackmore Prince C. Maternal prenatal depressive symptoms and spontaneous preterm births among African-American women in Baltimore, Maryland. Am J Epidemiol 2002;156:797–802.
64. Lundy BL, Jones NA, Field T, Nearing G, Davalos M, Pietro PA, Schanberg S, Kuhn C. Prenatal depression effects on neonates. Infant Behav Dev 1999;22:119–29.
65. Spielberger CD. State-Trait Anxiety Inventory: A Comprehensive Bibliography. 2nd ed. Palo Alto, CA: Consulting Psychologists Press; 1989:115.
66. Barnes LLB, Harp D, Jung WS. Reliability generalization of scores on the Spielberger State-Trait Anxiety Inventory. Educ Psychol Meas 2002; 62:603–18.
67. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24:385–96.
68. Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, Porto M, Sandman CA. Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. Psychosom Med 1996;58:432–46.
69. Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. Am J Obstet Gynecol 1999;180:S257–63.
70. Culhane JF, Rauh V, McCollum KF, Hogan VK, Agnew K, Wadhwa PD. Maternal stress is associated with bacterial vaginosis in human pregnancy. Matern Child Health J 2001;5:127–34.
71. Cook WW, Medley D.M.. Proposed hostility and pharisaic virtue scales for the MMPI. J Appl Psychol 1954;38:414–8.
72. Barefoot JC, Dodge KA, Peterson BL, Dahlstrom G, Williams RB Jr. The Cook-Medley Hostility Scale: item content and ability to predict survival. Psychosom Med 1989;51:46–57.
73. Brondolo E, Kelly KP, Coakley V, Gordon T, Thompson S, Levy E, Cassells A, Tobin JN, Sweeney M, Contrada RJ. The perceived ethnic discrimination questionnaire: development and preliminary validation of a community version. J Appl Soc Psychol 2005;35:335–65.
74. Branscombe NR, Schmitt MT, Harvey RD. Perceiving pervasive discrimination among African Americans: implications for group identification and well-being. J Pers Soc Psychol 1999;77:135–49.
75. Suls J, Wan CK. The relationship between trait hostility and cardiovascular reactivity: a quantitative review and analysis. Psychophysiology 1993;30:615–26.
76. Davis MC, Matthews KA, McGrath CE. Hostile attitudes predict elevated vascular resistance during interpersonal stress in men and women. Psychosom Med 2000;62:17–25.
77. Zimet GD, Powell SS, Farley GK, Werkman S, Berkoff KA. Psychometric characteristics of the Multidimensional Scale of Perceived Social Support. J Pers Assess 1990;55:610–7.
78. Zimet GD, Dahlem NW, Zimet SG, Farley GK. The Multidimensional Scale of Perceived Social Support. J Pers Assess 1988;52:30–41.
79. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, Stokes J, Handelsman L, Medrano M, Desmond D, Zule W. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl 2003;27:169–90.
80. Bernstein DP, Ahluvalia T, Pogge D, Handelsman L. Validity of the Childhood Trauma Questionnaire in an adolescent psychiatric population. J Am Acad Child Adolesc Psychiatry 1997;36:340–8.
81. Bernstein DP, Fink L. Childhood Trauma Questionnaire: A Retrospective Self-Report. San Antonio, TX: The Psychological Corporation; 1998.

82. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989;28:193–213.
83. Krieger N, Smith K, Naishadham D, Hartman C, Barbeau EM. Experiences of discrimination: validity and reliability of a self-report measure for population health research on racism and health. Soc Sci Med 2005;61:1576–96.
84. Krieger N. Racial and gender discrimination—risk-factors for high blood-pressure. Soc Sci Med 1990;30:1273–81.
85. Krieger N, Sidney S. Racial discrimination and blood pressure: the CARDIA study of young black and white adults. Am J Public Health 1996;86:1370–8.
86. Spielberger CD. State-Trait Anxiety Inventory for Adults: Manual, Instrument, and Scoring Guide. Palo Alto, CA: Consulting Psychologists Press, Inc.; 1983.
87. Cohen S, Janicki-Deverts D. Who's stressed? Distributions of psychological stress in the United States in probability samples from 1983, 2006, and 2009. J Appl Soc Psychol 2012;42:1320–34.
88. Byrd DR. Race/Ethnicity and self-reported levels of discrimination and psychological distress, California, 2005. Prev Chronic Dis 2012; 9:E156.
89. Christian LM, Iams JD, Porter K, Glaser R. Epstein-Barr virus reactivation during pregnancy and postpartum: effects of race and racial discrimination. Brain Behav Immun 2012;26:1280–7.
90. Cohen S, Schwartz JE, Epel E, Kirschbaum C, Sidney S, Seeman T. Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Psychosom Med 2006;68:41–50.
91. Skinner ML, Shirtcliff EA, Haggerty KP, Coe CL, Catalano RF. Allostasis model facilitates understanding race differences in the diurnal cortisol rhythm. Dev Psychopathol 2011;23:1167–86.
92. Kunz-Ebrecht SR, Mohamed-Ali V, Feldman PJ, Kirschbaum C, Steptoe A. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun 2003;17:373–83.
93. Spencer RL, Miller AH, Stein M, McEwen BS. Corticosterone regulation of Type I and Type II adrenal steroid receptors in brain, pituitary, and immune tissue. Brain Res 1991;549:236–46.
94. Sapolsky RM, McEwen BS. Down-regulation of neural corticosterone receptors by corticosterone and dexamethasone. Brain Res 1985;339:161–5.
95. Lovallo WR, Farag NH, Vincent AS. Use of a resting control day in measuring the cortisol response to mental stress: diurnal patterns, time of day, and gender effects. Psychoneuroendocrinology 2010;35:1253–8.
96. Kirschbaum C, Wust S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. Psychosom Med 1992;54:648–57.
97. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC Jr, Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women—2011 update: a guideline from the American Heart Association. J Am Coll Cardiol 2011;57:1404–23.
98. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 2012;125:e2–220.
99. Hansson GK. Mechanisms of disease—inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–95.
100. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
101. Brydon L, Steptoe A. Stress-induced increases in interleukin-6 and fibrinogen predict ambulatory blood pressure at 3-year follow-up. J Hypertens 2005;23:1001–7.
102. Carroll JE, Low CA, Prather AA, Cohen S, Fury JM, Ross DC, Marsland AL. Negative affective responses to a speech task predict changes in interleukin (IL)-6. Brain Behav Immun 2011;25:232–8.
103. Nierop A, Bratsikas A, Klinkenberg A, Nater UM, Zimmermann R, Ehlert U. Prolonged salivary cortisol recovery in second-trimester pregnant women and attenuated salivary alpha-amylase responses to psychosocial stress in human pregnancy. J Clin Endocrinol Metab 2006; 91:1329–35.
104. Redwine LS, Altemus M, Leong YM, Carter CS. Lymphocyte responses to stress in postpartum women: relationship to vagal tone. Psychoneuroendocrinology 2001;26:241–51.
105. Christian LM. Physiological reactivity to psychological stress in human pregnancy: current knowledge and future directions. Prog Neurobiol 2012;99:106–16.
106. De Weerth C, Wied CCGD, Jansen LMC, Buitelaar JK. Cardiovascular and cortisol responses to a psychological stressor during pregnancy. Acta Obstet Gynecol Scand 2007;86:1181–92.
107. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain Behav Immun 2007;21:901–12.
108. Stoney CM, Owens JF, Matthews KA, Davis MC, Caggiula A. Influences of the normal menstrual cycle on physiologic functioning during behavioral stress. Psychophysiology 1990;27:125–35.
109. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. Neuropsychopharmacology 2010;35:2617–23.
110. Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med 1999;61:154–62.
111. Clapp JF, Capeless E. Cardiovascular function before, during, and after the first and subsequent pregnancies. Am J Cardiol 1997;80:1469–73.
112. Shipton D, Tappin DM, Vadiveloo T, Crossley JA, Aitken DA, Chalmers J. Reliability of self reported smoking status by pregnant women for estimating smoking prevalence: a retrospective, cross sectional study. BMJ 2009;339:b4347.