

traditionally been termed *racial health disparities*, potentially via psychoneuroimmunologic mechanisms (Larrabee Sonderlund et al., 2021). For example, Black American women are at greater risk for hypertensive disorders of pregnancy and preterm birth than White American women (Thompson & Suter, 2020), which are major contributors to the racial disparities witnessed in maternal and infant mortality (Chambers et al., 2019; Gyamfi-Bannerman et al., 2020).

One aspect of the maternal stress experience that remains understudied is that of interpersonal racial discrimination and how this unique form of stress interacts with alternative forms of stress (e.g., interpersonal loss, physical danger, humiliation, entrapment, and role disruption; Slavich & Shields, 2018) to shape physiological and psychological health during pregnancy. Multiple frameworks in the stress sciences suggest that wear and tear occurs when stressor exposures are many (Geronimus, 1996; McEwen, 1998), suggesting that increasing levels of each form of stress may potentiate the effects of others. When stress is prolonged or anticipated frequently, such as with racial discrimination, physiological mechanisms meant to protect the body from repeated activation of stress systems (e.g., decreased sensitivity to glucocorticoids) have also been known to inadvertently introduce alternative forms of risk (e.g., decreased ability of glucocorticoids to dampen inflammation; Barnes & Adcock, 2009). Indeed, racial discrimination has been linked to perinatal glucocorticoid resistance and systemic inflammation (Gillespie & Anderson, 2018; Giurgescu et al., 2016). During chronic stress, psychological coping mechanisms may also reach capacity, increasing risk and depressed mood, which has been noted during pregnancy (reviewed by Traylor et al., 2020).

To advance our understanding of these important topics, we examined associations among interpersonal racial discrimination, stress, and their interaction across the life course and inflammation, perceived stress, and depressive symptoms during pregnancy among Black American women. We also completed subscale analyses in which stress ratings were partitioned into chronic versus acute stress to determine whether patterns of associations depended upon the nature of the stress experience. The a priori hypotheses of this work were that associations among interpersonal racial discrimination and prenatal parameters would be stronger under conditions of significant life course stress, signifying a progressive toll on physiological and psychological coping capacity. We hypothesized that greater chronic stress (as opposed to acute stress), combined with more experiences of interpersonal racial discrimination, would be particularly deleterious.

METHODS

Design

A prospective cohort of Black American women were enrolled into the PATH Study (Pathways to Shortened Gestation Among

Black Women) during their early third trimester of pregnancy. The primary aims of the parent study were to examine associations among psychological stress, genetic variation, functional regulation of the interleukin-1 β (IL-1 β) signaling pathway, and birth timing. Here, we have conducted a series of secondary analyses focusing on racial discrimination and markers of systemic inflammation, perceived stress, and depressive symptoms during pregnancy. Although the study was powered to address the primary aims of the parent study, post hoc power analysis suggests that our sample size of 93 achieves 0.82 power to detect moderate associations (effect size $f^2 = 0.15$) using multivariate linear regression with the predictors, interactions, and covariates under study. The study was approved by The Ohio State University and OhioHealth Institutional Review Boards, with participants completing written informed consent prior to data collection and receiving \$50 in gift cards at the study visit.

Participants

Women were eligible for the PATH Study if they self-identified as non-Hispanic, Black, and U.S.-born. Eligible women were also required to be aged 18–34 years, report nonsmoking status and no illicit drug use by the second trimester, and have prepregnancy body mass index of 18.5–39.9. Upon screening for eligibility, women were also asked to self-report if they had been told that their pregnancy was “high risk” for any reason or that they had a pregnancy-related complication or condition. Women who reported chronic immune-related conditions (e.g., diabetes mellitus, chronic hypertension, heart disease, cancer, human immunodeficiency virus, hepatitis, syphilis, rheumatoid arthritis, and sickle cell disease) were deemed ineligible, though postbirth medical record review later identified chronic hypertension among several women. This was relatively rare ($n = 6$), and these individuals were retained in the sample. Although mental health conditions have also been considered chronic immune-related conditions, because stress and its correlates were a primary interest of this study, women with diagnoses of depression or anxiety remained eligible. Similarly, although women self-reporting gestational diabetes, a gestational hypertensive disorder, or fetal anomaly before the third trimester were deemed ineligible, several women were later diagnosed with a gestational hypertensive disorder ($n = 12$) and were retained in the sample. Women with regular medication use with immune implications (e.g., corticosteroids and albuterol more than once weekly) were not eligible. Women were also required to have a dating ultrasound at less than or equal to 15 weeks of pregnancy.

Data Collection

Participants completed a single study visit during the early third trimester of pregnancy. Demographic, behavioral, and clinical data were collected using standardized questionnaires, and the psychosocial instruments discussed below were

administered. At the conclusion of the visit, venipuncture was performed for collection of whole blood. Participants were then prospectively followed to birth, and additional clinical data were manually abstracted during a detailed review of prenatal, labor and delivery, and newborn medical records that occurred during the postpartum period and covered the entire course of the pregnancy and perinatal period.

Measures

Racial Discrimination The Experiences of Discrimination Scale measures self-reported experiences of discrimination on the basis of race, ethnicity, or color across the life course, up to the time of assessment (Krieger, 1990; Krieger & Sidney, 1996). Participants are asked if they have experienced discrimination in the context of one or more situation out of nine assessed situations (e.g., getting service in a store or restaurant, at school, and at work). The frequency of an unendorsed situation is scored as zero. For each endorsed situation, the frequency is scored as one (once), two and one half (2–3 times), or five (≥ 4 times). To produce a total frequency, the frequency scores are summed. Test–retest reliability was .7, and Cronbach's α was .86 for the Experiences of Discrimination Scale among Black samples (Krieger et al., 2005).

Life Course Stress The Stress and Adversity Inventory (STRAIN) was created to allow for the systematic, computer-based query of experiences with 96 stressors that might be encountered across the life course (e.g., chronic stressors such as relationship problems and acute stressors such as deaths of relatives; Slavich & Shields, 2018). Its development was guided by tools such as the Life Events and Difficulties Schedule (an extensive, time-intensive interview and panel rating approach; Brown & Harris, 1978). The STRAIN is now a National Institute of Mental Health Research Domain Criteria-recommended instrument. The STRAIN uses extensive branching logic and can be administered by interview (as was done for the current study) or completed independently by the participant. Questions that are not pertinent to the participant are not asked. Endorsed stressors are probed further to determine the timing and duration of the stressor and how stressful or threatening the experience was perceived to be by the participant. The STRAIN produces multiple indices of life course stress, including total life course stress up to the point of assessment. Total life course stress can also be partitioned into chronic versus acute stress. The current study examined average ratings of total (for primary analyses), chronic, and acute (for subscale analyses) life course stress. Greater life course stress, as estimated by the STRAIN, correlates positively with perceived stress and depressive symptoms in adulthood and negatively with healthy prenatal behaviors and ratings of wellbeing (McLoughlin et al., 2021; Smith et al., 2020). STRAIN indices are not significantly associated with personality traits or social desirability and test–retest

reliability over a period of 2–4 weeks is excellent ($r \geq .9$; Slavich & Shields, 2018).

Prenatal Perceived Stress The Perceived Stress Scale (PSS-14) is a widely used stress assessment tool guided by the notion that negative emotional states arise when events are appraised as threatening or overwhelming coping resources (Cohen et al., 1995). Participants are asked to reflect on the past month and indicate if they have *never*, *almost never*, *sometimes*, *fairly often*, or *very often* had each of the possible 14 experiences (e.g., been upset because of something that happened unexpectedly). Each item is scored from 0 to 4 (with seven items reverse-scored). Higher scores indicate greater perceived stress, with a possible range of 0–56. The PSS-14 has been validated in representative samples across various age and racial groups, including during pregnancy. PSS scores correlate positively with depressive symptoms and negatively with physical and mental health-related quality of life among pregnant women (Zhang et al., 2019).

Prenatal Depressive Symptoms The Center for Epidemiological Studies Depression Scale (CES-D) was developed to measure depressive symptoms among the general population (Radloff, 1977). Participants are asked to reflect on the past week and indicate if they *rarely*, *some of the time*, *occasionally*, or *most of the time* experienced feelings consistent with each of the 20 statements (e.g., I felt I was just as good as other people). Each item is scored from 0 to 3 (with four items reverse-scored). Higher scores indicate more depressive symptoms, with a possible range of 0–60. The CES-D does not appear to demonstrate racial/ethnic bias and demonstrates an internal validity of .88–.92 during pregnancy (Canady et al., 2009; Maloni et al., 2005). The CES-D also shows predictive validity for Diagnostic and Statistical Manual of Mental Disorders-determined minor (CES-D ≥ 16) and major (CES-D ≥ 24) prenatal depression among Black American women (Tandon et al., 2012).

Prenatal Inflammatory Markers Prenatal venous whole-blood samples were stored on ice after collection, returned to the laboratory within an hour of venipuncture, and processed immediately. Plasma was isolated from heparinized whole blood by centrifugation at 1,200g for 10 minutes at 15°C. Plasma was aliquoted and frozen at –80°C until thawed in batches for multiplexed (simultaneous) quantification of IL-6, IL-8, tumor necrosis factor- α (TNF- α), and IL- β using the Meso Scale Discovery ProInflammatory 4-Plex Kit and Sector Imager 2400 (Meso Scale Discovery, Gaithersburg, MD). This method applies the principles of sandwich immunoassay through the use of electrochemiluminescence. All cytokines were quantified upon the sample's first thaw. For the PATH Study sample, inter- and intra-assay coefficients of variations were 4.0% and 7.1% for IL-6, 3.6% and 4.0% for IL-8, 7.2% and 3.9% for TNF- α , and 2.3% and 15.5% for IL-1 β , respectively.

Statistical Analyses

First, participant characteristics were described, and primary variable descriptive statistics were produced. Next, bivariate associations among untransformed continuous variables of interest were examined according to Spearman rank-order correlations. Next, variables were examined for normality. Transformations were applied to plasma cytokine values (log, 1/square root, inverse, and log for IL-6, IL-8, TNF- α , and IL-1 β , respectively) to address the significant positive skew of the cytokine distributions, as is common for systemic inflammatory markers. This approach allows a linear model to be fit by addressing nonnormality and heteroscedasticity of model error terms while also maintaining power, maximizing precision, and minimizing risk for Type I error (Knief & Forstmeier, 2021). For models in which a transformation with an element of inversion was applied to the dependent variable (i.e., IL-8 and TNF- α), directions of associations are reversed in the results text to assist the reader with interpretation.

Next, separate multivariate linear regression models were built with racial discrimination tertile (X_1), average life course stress rating (X_2), and their interaction ($X_1 \times X_2$) serving as predictors and prenatal cytokine levels, perceived stress, and depressive symptoms as outcomes (Y). All models included the following covariates: maternal age, marital status, health insurance, smoking status, prepregnancy body mass index, sleep quality (assessed using the Pittsburgh Sleep Quality Index; Buysse et al., 1989), maternal parity (nulliparous vs. multiparous), fetal gender, gestational age at sampling, and time of day of sampling. Based on our initial hypotheses, frequency

of exposures to racial discrimination and average ratings of life course stress were each included as continuous predictors. Upon noting multiple quadratic trends when discrimination served as the predictor, the discrimination variable was recoded as tertiles to aid in the interpretation of the results.

RESULTS

Participant Characteristics

Participant characteristics for the analytical sample ($n = 93$) are shown in Table 1. The PATH Study enrolled 96 women, with a target enrollment and data collection window of 28 weeks 0 days to 32 weeks 6 days of pregnancy. Ninety-five (98.96%) participants were enrolled within the target window, and one (1.04%) participant was enrolled at 19 weeks 4 days because of an incorrectly reported due date. This participant was retained in the analytical sample, and gestational age at sampling was included as a covariate. Three women were excluded from analyses because of unsuccessful venipuncture ($n = 2$) or inability to obtain prenatal and birth records, prohibiting determination of key descriptive and control variables ($n = 1$). Therefore, the final analytical sample included 93 non-Hispanic Black American women assessed at a mean of 30 weeks 2.3 days of pregnancy ($SD = 1$ week 5.91 days).

Consistent with the study's recruitment strategies and eligibility criteria, most participants did not report private insurance ($n = 62$, 66.67%) and were employed at least part time during pregnancy ($n = 68$, 73.12%). No participants reported Type 1 or Type 2 diabetes or gestational diabetes, and six

TABLE 1. Participant Characteristics by Prenatal Self-Reported Depressive Symptoms ($n = 93$)

	Full analytical sample ($n = 93$) $M \pm SD$ or count (%)	Lack clinically significant depressive symptoms ($n = 65$) $M \pm SD$ or count (%)	Clinically significant depressive symptoms ($n = 28$) $M \pm SD$ or count (%)	p
Maternal age	26.31 \pm 4.47	26.77 \pm 4.37	25.25 \pm 4.60	.133
Married (yes)	22 (23.66%)	18 (27.69%)	4 (14.29%)	.193
Private insurance (yes)	31 (33.33%)	25 (38.46%)	6 (21.43%)	.110
Nonsmoker (yes)	71 (76.34%)	50 (76.92%)	21 (75.00%)	.841
Prepregnancy body mass index	28.37 \pm 5.70	28.31 \pm 5.64	28.51 \pm 5.94	.880
Sleep quality (PSQI global score)	7.47 \pm 3.43	6.68 \pm 3.15	9.32 \pm 3.40	.001
Nulliparity (yes)	29 (31.18%)	20 (30.77%)	9 (32.14%)	.896
Fetal gender (female)	51 (54.84%)	36 (55.38%)	15 (53.57%)	.872
Gestational age at sampling (days)	212.33 \pm 12.91	211.72 \pm 10.14	213.75 \pm 17.92	.490
Time of day of sampling	1300 hours \pm 93.6 min	1301 hours \pm 94.2 min	1256 hours \pm 91.2 min	.826
Prenatal perceived stress (PSS)	23.31 \pm 8.13	19.95 \pm 6.08	31.11 \pm 6.88	<.001
Prenatal depressive symptoms (CES-D)	13.57 \pm 10.65	7.77 \pm 4.32	27.04 \pm 8.54	<.001

Note. PSQI = Pittsburgh Sleep Quality Index; hours = hours (military time); min = minutes; PSS = Perceived Stress Scale; CES-D = Center for Epidemiological Studies Depression Scale; Lack clinically significant depressive symptoms = CES-D score < 16; Clinically significant depressive symptoms = CES-D score \geq 16.

(6.45%) participants were diagnosed with chronic hypertension before ($n = 3$) or during ($n = 3$) pregnancy per medical record review. Nine (9.68%) participants were diagnosed with gestational hypertension, and three (3.2%) participants were diagnosed with preeclampsia during the assessed pregnancy. No participants demonstrated abnormalities of amniotic fluid volume. Four participants (4.30%) developed chorioamnionitis. The mean gestational age at birth was 39 weeks 0.37 days ($SD = 1$ week 2.68 days).

Of the 93 assessed participants, 65 (69.90%) had a CES-D score of >16 , which represents clinically significant depressive symptoms. As shown in Table 1, participants with clinically significant depressive symptoms reported higher prenatal perceived stress, $t(91) = -7.800, p < .001$, and higher prenatal depressive symptoms, $t(91) = -14.458, p < .001$, compared to participants without clinically significant depressive symptoms.

Descriptive Statistics and Bivariate Associations

Women were divided into tertiles based on frequency of exposures to discrimination, with the low tertile encompassing those reporting no experiences ($n = 47, 50.5\%$), the midtertile encompassing those reporting one to three experiences ($n = 19, 20.5\%$), and the high tertile encompassing those reporting more than three experiences ($n = 27, 29.0\%$) of interpersonal racial discrimination. Participants reported experiencing, on average, three instances of individual racial discrimination ($SD = 4.94$). Participants also reported experiencing, on average, 21.9 life course stressors out of an assessed 96 potential life course stressors ($SD = 12.6$; 25% = 13, 50% = 21,

75% = 26). Average ratings of life course stress across these stressors ranged from 0 to 4.36 on a scale of 0–5, from *not at all* to *extremely stressful* ($M = 2.29, SD = 0.75$; 25th percentile = 1.85, 50th percentile = 2.28, 75th percentile = 2.73).

Bivariate Spearman rank-order associations among the primary variables of interest are shown in Table 2. Participants reporting more instances of racial discrimination reported greater average ratings of life course chronic stress, prenatal perceived stress, and prenatal depressive symptoms ($\rho \geq .234$). Participants reporting greater average total life course stress reported greater prenatal perceived stress and prenatal depressive symptoms ($\rho \geq .22$). Greater average ratings of total life course stress were also associated with higher plasma IL-1 β levels ($\rho = .232, p = .025$). Greater average ratings of life course acute stress were associated with higher plasma IL-8 ($\rho = .242, p = .020$) and IL-1 β ($\rho = .295, p = .004$) levels.

Discrimination, Total Life Course Stress, and Prenatal Inflammatory Markers

Multivariate linear regression models revealed that associations among racial discrimination and plasma IL-8, TNF- α , and IL- β levels depended upon average ratings of total life course stress, adjusting for demographic, behavioral, and clinical covariates ($p \leq .034$; Figures 1a, 1b, and 1c, respectively). For each of the three models, orthogonal polynomial contrasts also revealed the presence of a quadratic trend when discrimination tertile served as the predictor, $F(1, 77)_{IL-8} = 8.91, p = .004$; $F(1, 77)_{TNF-\alpha} = 6.74, p = .011$; $F(1, 77)_{IL-\beta} = 5.67, p = .020$.

Specifically, participants reporting experiences of racial discrimination in the midtertile demonstrated significantly

TABLE 2. Spearman Associations Among Racial Discrimination, Life Course Stress, and Prenatal Parameters ($n = 93$)

	1.1	1.2	1.3	1.4	2.1	2.2	2.3	2.4	2.5	2.6
1. Life course parameters (ρ)										
1.1 Racial discrimination	1.0	—								
1.2 Life course stress (total)	.183	1.0	—							
1.3 Life course stress (chronic)	.234*	.712*	1.0	—						
1.4 Life course stress (acute)	.025	.758*	.187	1.0	—					
2. Prenatal parameters (ρ)										
2.1 Perceived stress	.257*	.267*	.364*	.003	1.0	—				
2.2 Depressive symptoms	.241*	.222*	.347*	-.073	.777*	1.0	—			
2.3 Plasma IL-6	-.087	.050	.138	-.050	.129	.092	1.0	—		
2.4 Plasma IL-8	.189	.193	.089	.242*	.118	.115	.016	1.0	—	
2.5 Plasma TNF- α	-.017	.033	.107	.037	.135	.178	.280*	.226*	1.0	—
2.6 Plasma IL-1 β	.017	.232*	.004	.295*	.058	.075	.054	.195	.169	1.0

Note. Life course stress is operationalized as average stress rating across stressors. For Spearman rank-order associations, variables are untransformed. IL = interleukin; TNF = tumor necrosis factor.

* $p \leq .05$.

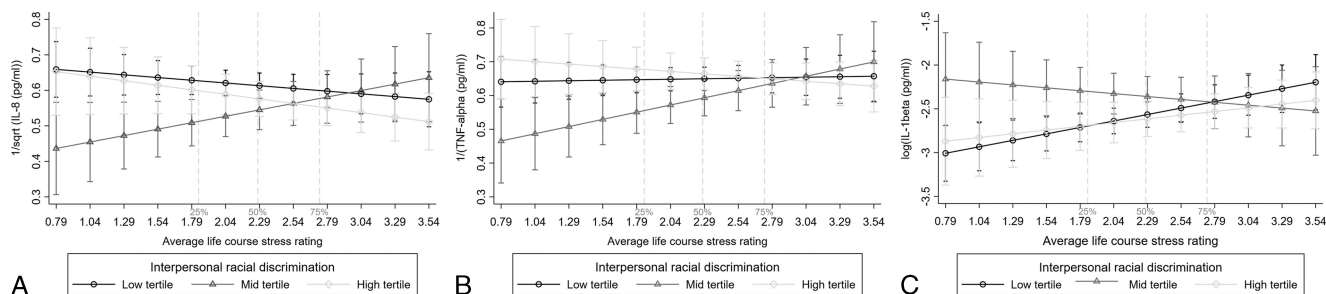


FIGURE 1. Associations among racial discrimination and prenatal inflammatory markers depend upon average ratings of life course stress. Associations among racial discrimination and (a) IL-8, (b) TNF- α , and (c) IL-1 β as a function of life course stress. Cytokine values are transformed. 95% confidence intervals are shown. Estimates are adjusted for maternal age, marital status, insurance, smoking status, prepregnancy body mass index, sleep quality, nulliparity, fetal gender, gestational age, and time of day at sampling. Dashed lines represent the cohort 25th, 50th, and 75th percentiles for average ratings of life course stress. IL = interleukin; TNF = tumor necrosis factor; sqrt = square root; pg = picogram; ml = milliliter.

higher IL-8 levels than participants reporting discrimination in the low and high tertiles, but only when average ratings of total life course stress were below approximately the cohort 50th and 25th percentiles, respectively (interaction: $t(77) = 2.16$, $p = .034$; $t(77) = 2.39$, $p = .019$, respectively). Participants reporting discrimination in the midtertile showed significantly higher TNF- α levels than women in the high tertile, but only when average ratings of total life course stress were below the cohort 25th percentile (interaction: $t(77) = 2.31$, $p = .024$). Participants reporting discrimination in the midtertile also showed significantly higher TNF- α levels than women in the low tertile (main: $\beta = 0.717$, $t(77) = 2.12$, $p = .038$). This association did not depend on average total life course stress ratings (interaction: $t(77) = -1.73$, $p = .087$). Participants reporting discrimination in the midtertile also showed significantly higher IL- β levels than the low tertile, but only when average total life course stress was below two, corresponding to the approximate 35th percentile (interaction: $t(77) = 2.20$, $p = .031$). Participants reporting discrimination in the high tertile did not demonstrate significantly different IL- β levels than those reporting discrimination in the low tertile or midtertile (main: $\beta = 0.200$, $t(77) = 0.53$, $p = .459$; $\beta = 0.703$, $t(77) = 1.43$, $p = .156$, respectively).

There was no evidence of a Discrimination \times Total Life Course Stress interaction in the prediction of maternal plasma IL-6 levels (p values $> .402$). Therefore, the interaction term was removed, and associations among racial discrimination, total life course stress, and IL-6 were examined. No significant associations were witnessed (p values $> .323$).

Chronic Versus Acute Life Course Stress In subscale analyses, the relationship between racial discrimination and plasma IL-8 levels depended upon average ratings of chronic life course stress ($p < .035$). Relationships among racial discrimination and plasma TNF- α and IL-1 β levels depended upon average ratings of acute life course stress ($p < .046$), again controlling for pertinent covariates.

Similar to the above, discrimination in the midtertile predicted significantly higher IL-8 levels than discrimination in the low and high tertiles but only when life course chronic stress was rated below roughly the 50th and 25th percentiles, respectively (interaction: $t(77) = 2.14$, $p = .035$; $t(77) = 2.68$, $p = .009$, respectively). Discrimination in the midtertile was associated with significantly higher TNF- α levels than discrimination in the high tertile, but only when average life course acute stress was rated below roughly the cohort 35th percentile (interaction: $t(77) = 2.12$, $p = .037$). Discrimination in the midtertile also predicted significantly higher IL- β levels than discrimination in the low and high tertiles, but only when average acute life course stress was rated below roughly the 50th and 35th percentiles, respectively (interaction: $t(77) = 3.06$, $p = .003$; $t(77) = 2.02$, $p = .046$, respectively). As above, neither discrimination, life course chronic stress, life course acute stress, nor their interactions predicted maternal plasma IL-6 levels ($p > .125$).

Discrimination, Life Course Stress, and Prenatal Perceived Stress and Depressive Symptoms

Multivariate linear regression models revealed that associations among racial discrimination and prenatal perceived stress and prenatal depressive symptoms did not depend on average ratings of total life course stress, adjusting for pertinent covariates ($p > .181$). Therefore, the interaction terms were removed. When examined together, greater racial discrimination but not greater total life course stress predicted higher prenatal perceived stress (main: $\beta_{\text{mid vs. low}} = 0.196$, $t(79) = 1.99$, $p = .050$; $\beta_{\text{high vs. low}} = 0.254$, $t(79) = 2.43$, $p = .017$). There was a linear trend to the data, $F(1, 79) = 5.92$, $p = .017$. Neither discrimination nor total life course stress predicted prenatal depressive symptoms ($p > .116$).

Chronic Versus Acute Life Course Stress In subscale analyses, the relationship between racial discrimination and prenatal perceived stress depended upon average acute life course stress ratings, controlling for pertinent covariates (Figure 2).

