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Perceived Discrimination is Associated with the Inflammatory Response to Acute Laboratory Stress in Women at Risk for Cardiovascular Disease

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Abstract

Cardiovascular disease (CVD) is the leading cause of death in the United States and exacts a disproportionate toll on minorities. Growing evidence demonstrates that perceived discrimination is a significant contributing factor to psychological distress, chronic low-grade inflammation, and cardiovascular health. However, little is known regarding the extent to which perceived discrimination contributes to the inflammatory response to acute stress. Therefore, the purpose of this study was to examine the influence of perceived discrimination on the inflammatory response to a laboratory acute stress paradigm in women at risk for CVD. A cross-sectional sample of 99 postmenopausal women (50 African American and 49 non-Hispanic White) (mean age 60.2 years) with at least two risk factors for CVD underwent the Trier Social Stress Test (TSST). Subjects completed the Detroit Area Study Discrimination Scale (DAS-DS) Everyday Discrimination subscale and provided blood and saliva samples prior to the TSST and every 15 minutes up to 90 minutes post-TSST to measure a pro-inflammatory cytokine, interleukin-6 (IL-6). Perceived discrimination was significantly associated with the salivary IL-6 response to the TSST ($b = 0.49$,

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SE = 0.13, $p = <0.001$) controlling for age, race, marital status, household income, BMI, statin use, childhood maltreatment, depressive symptoms, and subjective social status. Women who reported higher levels of perceived discrimination had higher levels of salivary IL-6 at baseline and following the TSST as compared to women who reported lower levels of perceived discrimination. Results suggest that higher levels of perceived discrimination, regardless of race and socioeconomic status, may heighten levels of inflammation, prior to and following an acute stress exposure. The circulating IL-6 response was associated with BMI only and did not correlate with salivary IL-6. These data suggest that perceived discrimination may contribute to the salivary-IL-6 acute stress response. However, more research is needed to help clarify the complex relationships among stress and salivary proinflammatory cytokines.

Keywords

Perceived discrimination; IL-6; TSST; CVD

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States¹ and exacts a disproportionate toll on minorities.² For example, the CV death rate for African Americans (AA) is 37% higher than for Whites and the risk for having a first-time stroke is almost two times greater for AAs than for Whites.² Cardiovascular health disparities emerge by middle age and exist across all socioeconomic levels. Traditional risk factors such as obesity, hypertension, and diabetes do not fully explain these disparities.^{3–5} Growing evidence demonstrates that perceived discrimination is a significant contributing factor to psychological distress, chronic low-grade inflammation, and cardiovascular disease.^{6–12} A recent epidemiological study found that frequent perceived discrimination was associated with nearly twice the odds of having a chronic illness.¹³

Racism and discrimination are pervasive, insidious, and distressing realities of everyday life for many individuals¹⁴ regardless of color (colorism), socioeconomic status, or residence.¹⁵ Furthermore, AA women experience greater perceived discrimination than AA men.¹⁶ Despite progress in reducing racism in the U.S., there is strong evidence for its persistence.¹⁷ A 2012 national U.S survey revealed that 67% of non-Hispanic Whites (NHW) and 90% of AAs agreed that AAs and Hispanics currently experience discrimination and 74% of AAs and 31 % of Whites report that they had personally experienced racial discrimination.¹⁸

Discrimination

Discrimination refers to unfair treatment based upon personal characteristics such as age, race, sex, religion, or sexual orientation¹⁹ and is considered to be a chronic stressor. Strong evidence demonstrates that perceived discrimination is associated with negative mental and physical health consequences,¹⁹ such as depression²⁰, sleep disturbance,²¹ poor self-rated health²², cardiovascular disease,^{12,23} and morality.²⁴ African American women who reported race-related chronic stress had significantly greater carotid artery plaque than those who did not report racial discrimination.²⁵ In addition, a significant association has been demonstrated between perceived discrimination and hypertension, particularly among AAs.

²⁶ It has been posited that perceived discrimination may be an important underlying factor explaining health disparities among AAs that cannot be accounted for by sociodemographic variables.^{23,27}

Discrimination, Stress, and inflammation

Despite abundant evidence linking perceived discrimination to poor health, the mechanism whereby perceived discrimination results in negative health outcomes, such as CVD, is unknown. It is established, however, that acute and chronic stress exposure is associated with increased proinflammatory cytokines, such as interleukin-6 (IL-6).^{28,29} This has been demonstrated in situations of naturalistic stress, including stress associated with providing informal care to chronically ill family members,³⁰ job burnout,³¹ and low socioeconomic status.³² As well, laboratory paradigms simulating acute stress also lead to elevations in proinflammatory cytokines, particularly IL-6.³³ For example, individuals subjected to social evaluative stress [(i.e., Trier Social Stress Test (TSST))] exhibit elevations in both circulating IL-6^{33,34} as well as salivary IL-6.^{28,35} Sex differences in the inflammatory response to stress have also been demonstrated.³⁶ Compared to men, postmenopausal women exhibit greater IL-6 reactivity in response to acute laboratory stress, which may intensify their risk for CVD as they age.³⁶ Stress-induced inflammation may also result from perceived discrimination. Using a stress and coping framework, evidence supports the idea that discrimination-related stress appraisal triggers emotion dysregulation that leads to chronic inflammation, increasing risk for CVD.³⁷ Uncontrolled and sustained generation of proinflammatory cytokines, like IL-6, can promote pathophysiologic processes that drive the development of CVD. In particular, IL-6 promotes the development of CVD by regulating fibrinogen synthesis³⁸ and was found to predict acute stroke survival,³⁹ as well as CVD.⁴⁰

Although less is known regarding the influence of perceived discrimination, a chronic stressor, on inflammation, there is some evidence that perceived discrimination is associated with inflammatory biomarkers.^{9,23,41,42} For example, an evaluation of men and women participating in the Survey of Midlife in the U.S study, revealed that lifetime perceived discrimination was related to higher levels of not only IL-6, but also fibrinogen and E-selectin independent of socioeconomic status and demographic variables.⁴² In women, the perception of everyday discrimination has been linked with elevated circulating levels C-reactive protein (CRP).⁴¹ In a large population study⁴¹ of racially diverse midlife women, investigators found that non-obese women who perceived higher levels of everyday discrimination exhibited higher CRP levels over the 7-year observation period. It is notable that these findings persisted after controlling for demographic, negative affect, biomedical, and behavioral confounds. An evaluation of a large multiethnic sample of men and women revealed associations among discrimination and inflammatory markers, using three measures of discrimination: everyday discrimination, lifetime discrimination due to any attribution, and lifetime discrimination attributed to race/ethnicity.⁹ For women, all three measures of discrimination were related to higher IL-6,⁹ which is linked to a greater risk for CVD.^{43,44} In men, only everyday discrimination was associated with higher IL-6. Of note among women, following adjustment for body mass index (BMI), there was no significant association between IL-6 and measure of discrimination suggesting that BMI accounted for

the relationships between IL-6 and discrimination. Discrimination and CRP were not associated in either men or women.

Although the association between perceived discrimination and inflammation is fairly well established, only a few studies have evaluated linkages among perceived discrimination and the inflammatory response to acute stress. In a study⁴⁵ of AA men and women (N=85), salivary CRP, salivary cortisol, dehydroepiandrosterone-sulfate, and salivary alpha-amylase were measured before and after the TSST, a laboratory social-evaluative acute stressor.⁴⁶

Findings demonstrated that in subjects who reported strong racial identity, higher levels of perceived discrimination predicted higher levels of inflammation following the TSST. In another study,⁴⁷ comparing the effects of race and pregnancy on the inflammatory response following the TSST, AA women, both pregnant and nonpregnant, demonstrated a greater inflammatory response as measured by circulating IL-6 to the acute stressor as compared to NHW women. Although AA women reported higher levels of discrimination than NHW women did, the racial difference in inflammatory response did not change when controlling for discrimination. However, these studies did not consider subjective social status, depressive symptoms, or early life adversity in relationship to the inflammatory response. In addition, IL-6 response to the acute stressor was not evaluated in terms of perceived discrimination in either study. Evidence demonstrates chronic stressors over the lifespan, such as early life adversity,^{33,35,48} depressive symptoms⁴⁹, and lower social subjective status³⁴ intensify the proinflammatory response to an acute laboratory stressor.

In summary, although evidence demonstrates that perceived discrimination is linked to inflammation and inflammatory disease, little is known regarding the extent to which perceived discrimination contributes to the inflammatory response to acute stress. Therefore, the purpose of this study was to examine the influence of perceived discrimination on the inflammatory response (IL-6) in blood and saliva to a laboratory acute stressor in women at risk for CVD.

2. Methods

2.1 Study Design and Participants

Participants included 99 postmenopausal women (50 AA and 49 NHW) between the ages of 50 and 75 years who were at risk for CVD. Participants were recruited from the Chicagoland community and several area healthcare facilities via recruitment flyers, media announcements, and letters. Women were included in the study if they reported at least two of the following CVD risk factors: (1) body mass index (BMI) ≥ 25 , (2) total cholesterol ≥ 240 mg/dL, (3) diabetes mellitus, (4) systolic blood pressure ≥ 120 mm Hg, or diagnosis of hypertension, and/or (5) parental history of myocardial infarction (MI) prior to age 60. In addition, participants were required to have had a negative cardiac stress test within the past 12 months. Women were excluded from the study if they reported a history of MI, heart disease or angina, left ventricular hypertrophy, ischemic stroke, or currently had systolic blood pressure > 175 mm Hg, and/or resting heart rate > 120 beats per minute. Participants were excluded if they reported currently having cancer, an active infection, an immune-related disorder such as lupus, active substance abuse, taking immune altering drugs such as

glucocorticoids, smoked within past 3 months, had a dental procedure within the past 72 hours, or had bleeding gums or periodontal disease. The study was approved by the sponsoring institution Institutional Review Board (IRB) and all participants provided informed consent prior to beginning the study. Gift cards were provided immediately after informed consent and before the start of the TSST.⁴⁶

2.2 Procedures

Eligible participants were scheduled for the TSST between the hours of 10 am and noon to control for the circadian rhythm of cortisol and IL-6. Prior to testing, participants were instructed to refrain from caffeine for 4 hours, alcohol and exercise for 12 hours and over the counter medications for 24 hours. Upon arrival and after resting quietly for 20 minutes, the participant's blood pressure and heart rate were assessed using a Welch AllynProB3400® electronic blood pressure monitor. An indwelling intravenous (IV) catheter for blood collection was inserted in the participant's arm. Saliva samples were collected using salivettes (Sarstedt, Nümbrecht, Germany).

Following collection of baseline blood and saliva samples, the standard protocol for the TSST was administered.⁴⁶ Briefly, participants were given 2-min to prepare for a mock job interview, and then underwent a 4-min mock job interview, followed by a 4-min mental arithmetic task. Two evaluative judges, who wore white lab coats, conducted the TSST. In addition, in order to enhance the stressor, participants were told that they would be videotaped and a psychologist would later review the videotape to assess the participant's nonverbal behavior during the speech and mental arithmetic task. The TSST is a well-validated laboratory social evaluative.⁵⁰

Blood samples for determination of plasma IL-6 and saliva samples for measurement of IL-6 were obtained concurrently at baseline before the TSST, as well as at 15, 30, 45, 60, 75, and 90 minutes after cessation of the stressors. At the end of the blood and saliva sampling, participants were debriefed and told that the intervention was not videotaped.

2.3 Self-Report Measures

2.3.1 Demographic and Current Medication Questionnaire—Demographic information, including age, race, marital status, household income, educational level, employment status, and current medications were collected using a self-report questionnaire. Height and weight were measured and used to calculate BMI.

2.3.2 Perceived Discrimination—The Detroit Area Study Discrimination Scale (DAS-DS)⁵¹ Everyday Discrimination subscale was used to measure frequency of everyday discrimination. The instrument is comprised of nine items describing instances of everyday discrimination that respondents rate in terms of how often they experience each on a scale from 0 (*never*) to 5 (*almost every day*). Scores on the Everyday Discrimination subscale range from 0 to 45, with higher scores reflecting greater frequency of everyday discrimination. Construct validity has been demonstrated,⁵¹ and the measure has been validated in AA women.⁵² Cronbach's alpha for the Everyday Discrimination demonstrated excellent internal consistency at 0.90 for the current study.

2.3.3 Social Subjective Status—The MacArthur Scale of Subjective Social Status (SSS)- United States Ladder subscale was used to measure subjective social status.⁵³ The subscale depicts a 10- rung ladder representing social status or social standing relative to others. Participants were asked to place an “X” on the rung of the ladder on which they feel they stand in relationship to other people in the United States with the bottom of the ladder representing low social status and the top run indicating high social status. This instrument has been used with a variety of populations, including AA women,⁵⁴ and has demonstrated acceptable construct validity and test-retest reliability.⁵⁵

2.3.4 Childhood Maltreatment—The short form Childhood Trauma Questionnaire (CTQ-SF)⁵⁶ was used to measure adverse childhood events. The CTQ-SF is a 28-item questionnaire that generates a total score and five subscale scores for childhood adversity: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Total score ranges from 25 to 125 with higher scores indicating greater adversity. Cronbach’s alpha for total CTQ score for the current study was excellent at 0.83.

2.3.5 Depressive Symptoms—The Center for Epidemiologic Studies Depression Scale (CES-D)⁵⁷; was used to measure the frequency and duration of depressive symptoms. The CES-D is a 20-item instrument measuring respondent’s level of depressive symptoms during the past week using a 4-point Likert-type scale, with responses ranging from 0 (*none*) to 3 (*most of the time*). A total score is obtained by summing each item, with higher numbers indicating more prevalent depressive symptoms. A total score of 16 or greater is considered to indicate a high level of depressive symptoms.⁵⁸ The CES-D has demonstrated validity for assessing depression in healthy adults.⁵⁹ Cronbach’s alpha for the CES-D in this study was excellent at 0.88.

2.4 Biological Measures

2.4.1 IL-6—IL-6 was measured in both plasma and saliva. For assessment of IL-6 in plasma, blood was drawn from an indwelling peripheral vein in the arm and collected in BD Vacutainer Heparin Tubes (Becton-Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged immediately and plasma was aliquoted and stored at -20°C until batch processed. Blood samples were assayed in duplicate using a commercial high-sensitivity enzyme linked immunoassay (ELISA) (R&D Systems, Minneapolis MN) for IL-6. Saliva samples were assayed in duplicate using ELISA for high-sensitivity IL-6 (R&D Systems, Minneapolis, MN). The lower limit for detecting IL-6 in both blood and saliva was 0.156 pg/ml. The intra-assay coefficients of variation ranged from 1.6 to 4.2% and the inter-assay coefficients of variation had a range of 3.3 to 6.4%.⁶⁰

2.4.2 C-reactive Protein—High sensitivity CRP was measured at baseline in blood via the SYNCHRON® System(s). The CRPH reagent is based on the highly sensitive Near Infrared Particle Immunoassay rate methodology. An anti-CRP antibody-coated particle binds to CRP in the patient sample resulting in the formation of insoluble aggregates causing turbidity. The system monitors the change in absorbance at 940 nanometers. This change in absorbance is proportional to the concentration of hsCRP in the sample and is used by the

SYNCHRON System® to calculate and express hsCRP concentration based upon a single-point adjusted, pre-determined calibration curve.

2.5 Statistical Analysis

Participant characteristics were presented as counts and percentages, means and standard deviations, or medians and ranges as appropriate. A series of mixed effects linear regression models using SAS 9.4 (Cary, NC) were specified to explore the changes in natural log-transformed salivary and plasma IL-6 over time. The first model included time as a single fixed effect, with random intercepts for women to account for within-individual correlation. This model was used to calculate the intra-class correlation coefficient as a measure of the variation due to between-women differences. Perceived discrimination was added to Model 2 as a fixed effect. Adjustment variables were chosen *a priori* to account for demographic, clinical, and psychosocial confounders. Demographic variables (age, race, marital status, income) were added to Model 3, and Model 4 included BMI and statin use. Finally, Model 5 included Model 4 variables and the CTQ, CESD, and SSS scores as adjustment variables. All continuous variables were standardized prior to inclusion in models. Race, marital status, income and medication use were entered as dichotomous variables. Interaction terms were considered for Model 5 but not included when not statistically significant.

3. Results

3.1 Descriptive Characteristics of Participants

Demographic characteristics of the sample are presented in Table 1. Ninety-nine participants completed the study with approximately half being AA and half NHW. Participants were on average 60.2 years of age ($SD=6.7$) with approximately half reporting an annual household income of less than \$50,000. The majority of participants had completed at least some college and 46% were married or in a significant relationship. AA women reported significantly lower annual household income ($t(96)=1.41$, $p=0.000$) and less education ($t(95)=0.628$, $p=0.002$) than NHW women.

The median BMI was in the obese category at 32.8 kg/m^2 with 59% of the sample reporting being overweight, 24% having diabetes, 63% hypertension, 43% hypocholesteremia, 7% atrial fibrillation, and 10% parental history of MI before age of 60. Almost a third (32%) of participants reported taking a statin medication.

Descriptive data for the DAS-DS Everyday Discrimination subscale, MacArthur SSS subscale, CTQ total score and subscales, and CES-D are listed in Table 2. Overall, there were low levels of reported everyday discrimination for the sample ($M=8.4$, $SD=8.7$, range = 0–38) with AA women reporting higher levels of everyday discrimination ($M=12.0$, $SD=9.4$) than NHW women ($M=4.8$, $SD=6.0$) $F(1,94)=10.4$, $p=0.002$). However, there were no differences between those reporting higher levels of everyday discrimination (≥ 6) compared to those reporting lower levels of everyday discrimination (<6) in terms of household income ($p=0.19$) and educational level ($p=0.39$). Level of subjective social status was moderate in the sample ($M=5.7$, $SD=2.0$) with AA women reporting lower levels of ($M=5.2$, $SD=2.2$) compared to their NHW counterparts ($M=6.2$, $SD=1.6$), $F(1,96)=$

7.2, $p = 0.009$. The means observed for the CTQ scales were higher than those reported in older adults⁶¹ and there were no significant differences between AA and NHW for any of the CTQ subscales. The mean CES-D score ($M = 11.6$, $SD = 9.1$) was low with no differences between AA and NHW women in depressive symptoms.

Means and standard deviations for IL-6 in plasma and saliva for baseline and at each of the six time points following the TSST are shown in Table 3. Participants ($N=99$) contributed $n=538$ blood and $n=674$ salivary IL-6 samples across all time points. Not all time points include the total sample size due to missing data. IL-6 in plasma and saliva were not correlated at any of the time points. In addition, AA and NHW women did not differ in plasma or salivary IL-6 at any time point.

Salivary IL-6 significantly increased from baseline to 30 minutes post TSST ($r=.306$, $p=.003$), however, plasma IL-6 did not significantly increase ($r=.120$, $p=.30$). Furthermore, plasma IL-6 levels had not returned to baseline levels at 90 minutes post TSST.

The mean hsCRP (drawn only at baseline) was 5.59 mg/L ($SD = 6.9$) and did not differ between AA and NHW women. However, hsCRP was positively associated with perceived discrimination ($r = 0.224$, $p = 0.041$), controlling for age, BMI, and statin use.

Higher levels of everyday perceived discrimination were associated with higher BMI ($r = 0.305$, $p = 0.003$), higher scores on the childhood maltreatment - subscale of emotional abuse ($r = 0.244$, $p = 0.017$), and greater levels of depressive symptoms ($r=.350$, $p = 0.001$). In addition, higher scores of perceived discrimination were associated with younger age ($r = -0.268$, $p = 0.009$) and lower subjective social status ($r = -0.278$, $p = 0.006$). AA women reported higher levels of perceived discrimination than NHW women ($t=-4.493$, $p=.000$).

3.2 Effect of Perceived Discrimination on TSST IL-6 Response

Perceived discrimination, demographic variables (age, race, marital status, and income), BMI, statin use, childhood maltreatment, depressive symptoms, and subjective social status were entered into statistical models in a sequential fashion (Table 4). Variables in which previous literature had reported an association with the IL-6 TSST response (childhood maltreatment³⁵, depressive symptoms⁶², and subjective social status³⁴) were retained in the model regardless of significance level. To note, the interaction term for discrimination and race was not statistically significant in a simple model without adjustment variables ($p=0.13$). It was also non-significant when added to model 5 presented in table 4 (page 17) ($p=0.11$) nor did it improve this model's fit statistics (e.g., $AIC=1171.3$ in model 5 versus $AIC=1170.7$ in model 5 with interaction). Participants with higher levels of reported perceived discrimination had higher levels of salivary IL-6 in response to the TSST than those with lower levels of reported perceived discrimination (Figure 1).

Only BMI was associated with plasma IL-6 response (Table 5). However, perceived discrimination was significantly associated with the salivary IL-6 response to the TSST as an unadjusted fixed effect ($b = 0.36$, $SE = 0.10$, $p<0.001$) and after full multivariable adjustment ($b = 0.49$, $SE = 0.13$, $p < 0.001$). Specifically, women who reported higher levels of perceived discrimination had higher levels of salivary IL-6 at baseline and following the

TSST as compared to women who reported lower levels of perceived discrimination; this relationship persisted after controlling for age, race, marital status, household income, BMI, statin use, childhood maltreatment, depressive symptoms, and subjective social status.

4. Discussion

This study examined the inflammatory (IL-6) response to the TSST in middle-aged AA women compared to NHW women. Participants who reported higher levels of perceived everyday discrimination had higher salivary IL-6 levels both prior to and during recovery from the TSST even after controlling for age, race, marital status, income, BMI, statin use, childhood maltreatment, depressive symptoms, and subjective social status. Notably, differences in inflammatory response to the TSST were not race-based but perception based. Furthermore, higher levels of baseline circulating hsCRP were correlated with greater perceived discrimination. To the best of our knowledge, only one previous study considered the inflammatory response to an acute stressor in relation to perceived discrimination.⁴⁵ Lucas et al.⁴⁵ demonstrated that higher levels of perceived discrimination were associated with higher levels of salivary CRP during recovery from the TSST in those who reported strong racial identity. That study did not measure IL-6 in response to the TSST. As noted by Lucas et al., salivary CRP has a long latency period (e.g. 120 minutes post-task) making it difficult to assess recovery post TSST.⁶³

Mean levels of salivary IL-6 ranged from 5.51 to 7.94 pg/ml. Although these levels were higher than found in a study of young AA men (mean age = 20.2), (IL-6 range 0.97 to 1.94 pg/ml)³⁵ and a study of police officers (mean age = 37) (IL-6 mean = 4.6 pg/ml),⁶⁴ the levels were lower than those in a study of healthy middle aged men and women (mean age = 58.6) (IL-6 mean = 14.47 pg/ml).⁶⁵ Differences in saliva collection methods⁶³, age,⁶⁶ sex,⁶⁷ and BMI⁶⁸ may explain differences in salivary IL-6 levels across studies. Salivary IL-6 increased by 22% from baseline to 15 minutes post TSST and returned to baseline levels 60 minutes after the TSST. Furthermore, salivary IL-6 levels continued to decline to below baseline levels at 75 minutes and 90 minutes post TSST suggesting that the pre-TSST (baseline) salivary level may have reflected anticipatory stress.

No studies were found that simultaneously examined both plasma and salivary IL-6 in relation to the TSST. Consistent with previous studies, plasma and salivary IL-6 did not correlate with one another.^{69–71} Furthermore, only the salivary IL-6 response to the TSST was associated with perceived discrimination. Several recent studies have reliably measured IL-6 from saliva and have demonstrated significant increases in salivary IL-6 in response to acute social stress.^{28,35,72,73} In a study of AA men, greater levels of early life adversity and neighborhood violence were associated with a greater TSST induced salivary IL-6 response.³⁵

Previous studies have found significant increases in plasma IL-6 following the TSST in relationship to other psychosocial variables.^{47,33} One potential explanation for our discrepant findings is that regulation of plasma IL-6 in response to stress may differ from that of salivary IL-6. Bosch⁷⁴ suggests that salivary IL-6 reflects compartmentalized oral immune activity and therefore does not correlate with circulating levels. A neural inflammatory

pathway in which inflammatory molecules within the mouth directly communicate with the brain has been demonstrated in a rodent model.⁷⁵ It has been suggested that inflammatory molecules within the environment of the mouth may trigger afferent trigeminal nerve activation to signal the brain and influence emotions and behavior.⁷⁶ Thus, elevation of salivary IL-6 in response to chronic stress, such as perceived discrimination, may lead to or perpetuate emotional distress. Local mucosal production of IL-6 may relate to activation of a stress pathway that may be dysregulated by the adversity of discrimination. Furthermore, higher levels of salivary IL-6 have been associated with carotid atherosclerosis as measured by intima-media thickness.⁷⁷ However, more investigation is needed in that the relationship between salivary IL-6 and perceived discrimination may be bidirectional. Furthermore, the relationship between IL-6 and perceived discrimination is likely to be complex such as local salivary production of IL-6 being affected by stress pathways that might be up or down regulated in the face of experiencing adversity, of which discrimination could be one.

As expected, based on previous literature,^{13,78} AA women reported higher levels of perceived discrimination and lower levels of subjective social status compared to NHW women. There were no differences between AA and NHW women in terms of childhood maltreatment. However, overall, participants reported higher levels of childhood maltreatment as compared to a community sample of older women.⁷⁹ In particular, scores on the sexual abuse subscale were higher than the 90th percentile of a community sample of older women⁷⁹ suggesting that the sample in this study experienced greater than average sexual abuse. Consistent with previous studies, higher levels of perceived discrimination were associated with younger age,⁸⁰ lower subjective social status⁵⁴, higher BMI,⁸¹ increased childhood maltreatment,⁸² and greater depressive symptoms.⁸³

This present study is one of the first to examine perceived discrimination and IL-6 in response to an acute laboratory stressor. We statistically controlled for other variables, such as race,⁴⁷ BMI,⁸⁴ childhood maltreatment,³⁵ depressive symptoms,⁶² and social subjective status³⁴ that have previously been demonstrated to contribute to the TSST inflammatory response. Limitations of this study include convenience sampling and a homogenous sample. In addition, our study was not powered to consider severity or type of discrimination. Furthermore, since women were recruited from the community, medical records were not available to confirm their self-reported medical history. Although participants were excluded, who reported periodontal disease or bleeding gums, an oral exam was not performed by a dentist. However, our mean salivary IL-6 levels are lower than that reported in a sample of healthy men and women without periodontal disease.⁸⁵ Future research may benefit from including an oral exam to confirm the absence of periodontal disease. In addition, it is possible that the 90-minute recovery following the TSST was not long enough to capture the plasma IL-6 rise as levels appeared to still be increasing at the 90-minute time point. Future studies may consider monitoring plasma IL-6 longer than 90 minutes following the TSST. Lastly, racial identity was not considered in this study. Findings from recent research suggest that racial identity may play an important role in moderating the stress response in relation to perceived discrimination.⁴⁵ However, a strength of our study is that the sample included 50% AA women.

5. Conclusion

This study is one of the first to demonstrate that perceived discrimination contributes to higher levels of salivary IL-6 in response to an acute laboratory stressor. Growing evidence demonstrates that perceived discrimination is a determinant of chronic disease and chronic disease risk factors.¹³ Therefore, it is important to clarify the mechanisms by which perceived discrimination contributes to inflammatory-related disease, particularly cardiovascular disease. Findings from this study may assist in developing, testing, and tailoring interventions to address chronic stress associated with perceived discrimination.

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Highlights:

- Prior to TSST, greater discrimination was associated with greater salivary IL-6.
- During TSST, those with greater discrimination had higher salivary IL-6
- Relationships persisted after controlling for potential cofounders.

Figure 1.

(a -b): Graphs demonstrating unadjusted and untransformed plasma-IL-6 response (a) and salivary IL-6 response (b) to TSST at 15, 30, 45, 60, 75 and 90 minutes post TSST in relationship to higher levels of perceived discrimination (>6 on DAS-DS Everyday Discrimination subscale) and lower levels of perceived discrimination (< 6 on DAS-DS Everyday Discrimination subscale).

Table 1:**Demographic and Participant Characteristics (N=99)**

Age, mean (SD)	60.2 (6.7)
African American, n (%)	50 (50.5)
Income, n (%)	
< \$25,000	23 (23.5)
\$25,000 -\$50,000	23 (23.5)
\$50,000 -\$100,000	28 (28.6)
> \$100,000	24 (24.5)
Education, n (%)	
HS or less	11 (11.3)
Some college or technical school	44 (45.4)
College or post-college graduate	42 (43.3)
Marital status, n (%)	
Married or significant relationship	46 (47.4)
Divorced or separated	37 (38.1)
Single or never married	14 (14.4)
Body mass index (kg/m ²), median (range)	32.8 (27.0–40.6)
Cardiovascular risk factors, n (%)	
Overweight	59 (64.1)
Diabetes	24 (26.4)
High blood pressure	63 (67.7)
Cholesterol	43 (47.3)
Atrial fibrillation	7 (7.7)
Parental history of MI before age 60	19 (19.2)
Medications, n (%)	
Using a statin	32 (33.7)
Using an antihypertensive medication	43 (43.4)
Using a diabetes medication	14 (14.1)

SD-standard deviation; MI-myocardial infarction

N=4 missing medication use

Table 2:

Descriptive Findings for Perceived Discrimination, Subjective Social Status, Early Life Adversity, and Depressive Symptoms (N=99)

	Mean (SD)	Score Range
Perceived Discrimination	8.4 (8.7)	0–38
Subjective Social Status	5.7 (2.0)	1–10
Childhood Trauma Questionnaire (CTQ) Total	39.6 (17.2)	25–98
Emotional abuse subscale	8.5 (4.7)	4–25
Physical abuse subscale	7.3 (3.6)	5–23
Sexual abuse subscale	7.5 (5.5)	4–25
Emotional neglect subscale	8.9 (4.2)	4–22
Physical neglect subscale	6.8 (2.3)	5–19
Depressive Symptoms (CES-D)	11.6 (9.1)	0–47

CES-D is Center for Epidemiologic Studies Depression Scale

Table 3:

Descriptive Data for Plasma IL-6 and Salivary IL-6 at Baseline and After TSST

	Baseline	15 min post	30 min post	45 min post	60 min post	75 min post	90 min post
Plasma IL-6 pg/mL) Mean (SD)	1.75 (1.83)	1.86 (1.45)	1.76 (1.55)	1.81 (1.36)	1.92 (1.42)	1.88 (1.21)	2.19 (1.63)
Saliva IL-6 (pg/mL) Mean (SD)	6.49 (7.07)	7.94 (10.42)	7.76 (11.68)	7.23 (10.48)	6.41 (7.02)	5.92 (7.01)	5.61 (6.44)
Paired sample correlation	$r=.093$ $p=.387$	$r=-.047$ $p=.682$	$r=-.043$ $p=.705$	$r=-.067$ $p=.569$	$r=-.056$ $p=.643$	$r=-.114$ $p=.332$	$r=-.180$ $p=.136$

IL-6-interleukin-6, TSST-Trier Social Stress Test, SD-standard deviation

Table 4:

Linear Mixed Models / Predicting Salivary IL-6

	Model 1		Model 2		Model 3		Model 4		Model 5	
	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value
Fixed effects										
Intercept	1.323 (0.111)	<0.001	1.335 (0.107)	<0.001	1.292 (0.257)	<0.001	1.467 (0.288)	<0.001	1.444 (0.290)	<0.001
Time										
15 minutes	0.135 (0.070)	0.056	0.130 (0.072)	0.071	0.131 (0.072)	0.070	0.133 (0.074)	0.072	0.127 (0.076)	0.094
30 minutes	0.155 (0.071)	0.029	0.146 (0.072)	0.044	0.140 (0.072)	0.053	0.129 (0.074)	0.082	0.120 (0.076)	0.12
45 minutes	0.129 (0.071)	0.068	0.115 (0.072)	0.11	0.106 (0.072)	0.14	0.098 (0.074)	0.18	0.103 (0.076)	0.17
60 minutes	0.133 (0.072)	0.064	0.122 (0.073)	0.092	0.115 (0.073)	0.12	0.111 (0.076)	0.14	0.112 (0.076)	0.14
75 minutes	0.046 (0.071)	0.52	0.029 (0.072)	0.68	0.026 (0.072)	0.72	0.010 (0.074)	0.90	0.016 (0.076)	0.84
90 minutes	0.070 (0.072)	0.34	0.072 (0.073)	0.33	0.055 (0.074)	0.45	0.044 (0.075)	0.56	0.033 (0.077)	0.67
Discrimination ²			0.356 (0.097)	<0.001	0.357 (0.109)	0.002	0.398 (0.124)	0.002	0.494 (0.134)	<0.001
Age ²					0.005 (0.102)	0.96	-0.004 (0.109)	0.97	-0.023 (0.109)	0.84
African American race ³					-0.083 (0.231)	0.72	-0.179 (0.227)	0.43	-0.127 (0.237)	0.59
Married ³					0.049 (0.242)	0.84	0.014 (0.251)	0.96	0.048 (0.250)	0.85
Income < \$50,000 ³					0.123 (0.237)	0.60	0.103 (0.236)	0.66	0.093 (0.249)	0.71
Body mass index ²							0.038 (0.114)	0.74	0.018 (0.115)	0.87
Statin use ³							0.088 (0.231)	0.71	0.168 (0.234)	0.47
Antihypertensive ³							-0.372 (0.199)	0.064	-0.430 (0.200)	0.034
Diabetes medication ³							0.192 (0.306)	0.53	0.124 (0.311)	0.69
CTQ ²									0.197 (0.129)	0.13
CESD ²									-0.218 (0.119)	0.070
SSS ²									0.057 (0.117)	0.63
Variance components										

	Model 1		Model 2		Model 3		Model 4		Model 5	
	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value	Estimate (SE)	p-value
Level 1	0.242 (0.014)	<0.001	0.241 (0.015)	<0.001	0.240 (0.015)	<0.001	0.244 (0.015)	<0.001	0.245 (0.016)	<0.001
Intercept	0.976 (0.144)	<0.001	0.853 (0.130)	<0.001	0.851 (0.130)	<0.001	0.794 (0.124)	<0.001	0.761 (0.121)	<0.001

IL-6-interleukin-6, SE-standard error, Antihypertensive-Antihypertensive medication, CTQ- Childhood Trauma Questionnaire, CES-D-Center for Epidemiologic Studies Depression Scale, SS= Subjective Social Status Scale ICC=0.801

¹Linear mixed models with the natural log of salivary IL6 at each time point as the dependent variable. All models include random intercepts for individuals.

²Continuous predictors were standardized.

³Entered as dichotomous variables (yes/no).

Table 5:Linear Mixed Models¹ Predicting Plasma IL-6

Plasma IL-6		
	Estimate (SE)	p-value
Fixed effects		
Intercept	0.140 (0.226)	0.54
Time		
15 minutes	0.006 (0.050)	0.90
30 minutes	0.041 (0.050)	0.42
45 minutes	0.143 (0.051)	0.006
60 minutes	0.177 (0.052)	<0.001
75 minutes	0.203 (0.052)	<0.001
90 minutes	0.301 (0.052)	<0.001
Discrimination ²	−0.139 (0.098)	0.16
Age ²	0.129 (0.077)	0.099
African American race ³	0.029 (0.179)	0.87
Married ³	−0.149 (0.191)	0.44
Income < \$50,000 ³	0.148 (0.183)	0.42
Body mass index ²	0.323 (0.092)	<0.001
Statin use ³	−0.033 (0.173)	0.85
Antihypertensive ³	−0.087 (0.144)	0.55
Diabetes medication ³	0.225 (0.230)	0.33
CTQ ²	−0.012 (0.101)	0.90
CESD ²	0.042 (0.091)	0.64
SSS ²	0.028 (0.086)	0.74
Variance components		
Level 1	0.093 (0.007)	<0.001
Intercept	0.371 (0.063)	<0.001

IL-6-interleukin-6, SE-standard error, Antihypertensive-Antihypertensive medication, CTQ-Childhood Trauma Questionnaire, CES-D-Center for Epidemiologic Studies Depression Scale, SS= Subjective Social Status Scale

¹Linear mixed models with the natural log of the dependent variable at each time point. All models include random intercepts for individuals.

²Continuous predictors were standardized.

³Entered as dichotomous variables (yes/no).