Psychosocial and Behavioral Predictors of Inflammation in Middle-Aged and Older Adults: The Chicago Health, Aging, and Social Relations Study

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Objective: C-reactive protein (CRP) is emerging as an important predictor of cardiovascular disease (CVD), and chronic inflammation may be a mechanism through which stress affects disease risk. We investigated the contribution of behavioral and psychosocial factors to variation in CRP concentrations in a population-based sample of middle-aged and older adults. **Methods:** A high sensitivity enzyme-linked immunosorbent assay (ELISA) validated for use with dried blood spot samples was used to determine CRP concentrations in a representative sample of 188 52- to 70-year-olds. Demographic (gender, ethnicity, socioeconomic status), anthropometric (height, weight, waist circumference, percent body fat), behavioral (alcohol consumption, smoking, sleep quality, dietary quality), and psychosocial data (perceived stress, chronic stress, depressive symptoms, loneliness, perceived social support) were collected on the same day as blood samples. Psychosocial variables collected the year before were also used to investigate the impact of changing psychosocial environments. Log-transformed CRP concentrations were examined in a series of nested multivariate regression models. **Results:** African American and female participants were found to have higher CRP concentrations, as did individuals with lower levels of education. However, ethnic differences disappeared after the addition of behavioral and psychosocial variables. Waist circumference, latency to sleep, smoking, and perceived stress were independently associated with increased concentrations of CRP. **Conclusions:** Psychosocial stress, as well as health behaviors, are important predictors of inflammatory activity in a population-based sample and should be considered in future research on inflammation and CVD.
Key words: C-reactive protein, perceived stress, inflammation, psychoimmunology, cardiovascular disease.

BMI = body mass index; **CRP** = C-reactive protein; **CVD** = cardiovascular disease; **ISEL** = Interpersonal Support Evaluation List; **PSS** = Perceived Stress Scale.

INTRODUCTION

Recent research has highlighted the contribution of inflammatory processes to the development of cardiovascular diseases (CVD). Several clinical, as well as population-based, studies demonstrate strong, independent associations between inflammatory markers and CVD morbidity and/or mortality (1,2), leading to recommendations for the assessment of inflammation in clinical practice (3,4). Although current research has established that markers of inflammation are related to traditional CVD risk factors such as smoking and obesity, the contribution of broader behavioral and psychosocial factors is not known.

Psychosocial stress is a well-established risk factor for CVD (5). Stress has direct physiological effects, primarily through activation of sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal pathways, as well as indirect effects through the modification of health-related behaviors (e.g., poor diet, smoking, sleep quality) (6,7). Similarly, stress is an important modulator of human immune function, including processes related to inflammation (8–10). Recently, chronic stressors, acute experimental stressors, and symptoms of depression have been associated with increased production of proinflammatory cytokines (11–14). In addition, two recent studies suggest that psychosocial stress may also upregulate C-reactive protein (CRP) production (15,16). As such, inflammation may represent an important pathway through which psychosocial environments shape CVD risk, as well as risk for other inflammatory conditions (17).

In this study we focus on CRP as a primary biomarker of inflammation. This acute phase protein is produced by hepatocytes in response to proinflammatory cytokines, including IL-6, IL-1, and TNFα (3,18). CRP is an important component of innate immunity involved in opsonizing pathogens and activating phagocytes and complement and may contribute to the progression of CVD by inducing proatherosclerotic activities in vascular endothelial cells (19,20).

CRP is currently the most intensively investigated biomarker of inflammation. The recent development of highly sensitive CRP assays (21–23) has led to the discovery that slight elevations of CRP—in the range of what was previously considered normal—are predictive of CVD independent of traditional risk factors (1,3,24,25). These associations have been reported in middle-aged, as well as elderly, populations and include a wide range of cardiovascular outcomes. In addition, elevated CRP has been prospectively associated with increased mortality risk in a healthy elderly population for both cardiovascular, as well as noncardiovascular, causes of death (14).

Our main objective is to investigate the contribution of behavioral and psychosocial factors to CRP production in a longitudinal, population-based sample of middle-aged and older adults. Although prior epidemiological research on CRP has focused primarily on traditional CVD risk factors, we set out to evaluate the relative contributions of a broader range of lifestyle and stress-related variables. We find that perceived stress is an important predictor of CRP, along with a number of demographic factors and health behaviors.

METHODS

Participants and Data Collection Protocol

This study is part of a larger longitudinal investigation of social isolation and health. Details regarding sampling design and recruitment strategies are reported elsewhere (26). A population-based sample of 229 50- to 67-year-old

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Cook County residents was recruited, including 81 self-identified African Americans, 66 Latino Americans, and 82 European Americans. Participants had to be ambulatory and able to speak English; no other exclusionary criteria were imposed. Data were collected from all participants at baseline (completed in 2002), as well as at annual follow-ups in year 2 (2003) and year 3 (2004). During each round of data collection, participants arrived at the laboratory between 8:00 and 9:00 AM, provided informed consent, and then began a day of assessments that included standard psychological surveys, interviews, lunch, and a cardiovascular protocol. All procedures were performed as approved by the University of Chicago institutional review board. The sample was relatively healthy at baseline, with approximately 10% reporting diagnosed CVD (prior stroke, heart attack, coronary artery disease, or heart failure).

This study reports on results from year 3, when 192 participants were interviewed and 188 provided a finger-prick blood sample for the analysis of CRP (see below). Compared with those providing a blood sample in year 3, attriters (including those not providing blood) had significantly lower household income (8.3 versus 7.0 on a 12-point scale; analysis of variance $F(1,215) = 9.07$, $p = .029$) and had lower educational attainment (3.0 versus 2.4 on 5 point scale, $F(1,227) = 6.29$, $p = .013$). Ethnicity was not significantly associated with dropout (Pearson $\chi^2 = 2.16$, $p = .34$).

Demographic, Anthropometric, Behavioral, and Psychosocial Variables

Table 1 presents the distribution of the independent variables considered in this analysis. Demographic covariates included gender, ethnicity, age, education (five categories: less than high school, high school graduate/GED, some college, undergraduate degree, graduate school), and household income (12 categories, ranging from less than $5,000 to more than $200,000; see Hughes et al. (26)). Gender- and ethnicity-specific means were imputed for 13 individuals with missing income data.

Participants were weighed and measured on a standard medical scale (Detecto, Pro-Med Products, Atlanta, GA). Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Waist circumference was measured with a flexible tape placed in a horizontal plane around the abdomen above the iliac crest. Body composition was measured using an electrical impedance system manufactured by RJL Systems Fluid & Nutrition System (RJL Systems, Clinton Township, MI) that calculates body cell mass, intracellular water, extracellular water, and extracellular mass. Fat mass is calculated as the sum of body cell mass and extracellular mass.

TABLE 1. Distribution of Demographic, Anthropometric, Behavioral, and Psychosocial Variables[a]

Female (%)	55.3
Ethnicity (%)	
European American	37.8
African American	33.5
Latino American	28.7
Age (yr)	59.5 (4.3)
Married/living with partner (%)	60.5
Education (1–5)	3.0 (1.3)
Household income (1–12)[b]	8.2 (2.3)
Body mass index (kg/cm^2)	31.4 (6.3)
Waist circumference (cm)	101.7 (14.9)
Percent body fat	35.2 (12.9)
Taking anti-inflammatory medication (%)	13.3
Current smoker (%)	13.3
Usual no. hours sleep/night, past month	6.6 (1.1)
Minutes to fall asleep	23.3 (32.1)
Frequency of sleep disturbances (0–3)	1.3 (0.6)
Percent calories from alcohol	3.4 (5.9)
Percent calories from fat	35.2 (7.8)
CESD score	9.5 (8.6)
UCLA loneliness score	35.7 (9.4)
Chronic stress (0–3)	1.4 (1.0)
Perceived Stress Scale	11.9 (6.0)
Evaluation of interpersonal support	7.0 (1.9)

[a] Mean (SD) values are presented for continuous variables.
[b] 1 = <$5,000/year, 12 = >$200,000; 8 = $40,001–$50,000.

We considered the following behavioral variables as predictors of CRP concentration: alcohol consumption, use of anti-inflammatory medication, smoking, sleep quality, and dietary quality. Sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI), a 19-question self-report questionnaire that assesses the sleep quality over a 1-month time frame (27). Seven component scores, all ranging from zero (no problems in area) to 3 (high problems), are calculated using scoring instructions given in (26). For the purposes of this study, we employed component scores for subjective sleep quality, sleep latency (minutes to fall asleep), sleep duration (usual number of hours of sleep per night), and sleep disturbances (frequency per night).

Diet was assessed using the Brief Block 2000 Nutritional Questionnaire, a commercially available nutrition questionnaire used to obtain comprehensive information about participants' dietary habits (Block Dietary Data Systems, Berkeley, CA). The Brief Nutritional Questionnaire asks about usual eating habits in the past year, and poses two kinds of questions about each food: First, participants are asked how often, on average, they ate the food during the past year; and second, how much of the food they typically ate each time. Responses are used to generate measures of daily caloric intake and percentage of calories from various nutrient categories. In this study we evaluated percentage of calories from fat, alcohol, protein, carbohydrates, and sweets.

We considered the following psychosocial factors: perceived stress, chronic stress, symptoms of depression, loneliness, and perceived social support. The Perceived Stress Scale (PSS; (28)) is a 10-item self-report questionnaire that asks participants to indicate how often they felt or thought a certain way during the past week. Responses to each item were recorded using a 5-point Likert scale that ranged from 0 (never) to 4 (very often). Scale scores for each participant were calculated by summing the responses to all items, yielding a scale range of 0 (low perceived stress) to 40 (high perceived stress).

The Chronic Stress Questionnaire (CSQ; (29)) is a 51-item instrument used to measure levels of chronic stress in eight domains: general stress, financial matters, employment, love and marriage, family and children, social life and recreation, residence, and health of self and close others. Respondents are asked to rate each statement on a scale where 0 is "not true," 1 is "somewhat true or very true." For the purposes of the present study, we employed the three-item general stress subscale (e.g., "You're trying to take on too many things at once," and "Too much is expected of you by others").

Depressive feelings and behaviors were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D) (30). This scale, which has been used extensively to gauge depression in epidemiological studies, consists of 20 items such as "I felt depressed" and "I enjoyed life." Participants were asked to rate how often they felt the way described by the items during the past week on a scale ranging from 0 (rarely or none of the time) to 3 (most or all of the time). After specific items were reverse scored, all of the items (except those asking about loneliness) were summed to calculate depressed affect scores for each participant, with higher scores indicating higher levels of depressive symptoms.

We used the R-UCLA Loneliness Scale as a measure of general loneliness and degree of satisfaction with one's social network (31). Examples of the items are "I lack companionship" and "I feel in tune with the people around me." Each of the 20 items is rated on a scale of 1 (never), 2 (rarely), 3 (sometimes), and 4 (often). After reverse scoring appropriate items, loneliness scores were calculated by summing all items. The range of possible scores was 20 to 80, with higher scores signifying greater loneliness.

Perceived social support was evaluated with the Interpersonal Support Evaluation List (ISEL). The ISEL consists of 12 statements for which participants are asked to rate on a 4-point Likert scale how truly each item reflects their own feelings (28,32). After reverse scoring appropriate items, subscale scores were calculated for appraisal support (e.g., "There is someone I can turn to for advice about handling problems with my family"), belonging support (e.g., "If I wanted to have lunch with someone, I could easily find

someone to join me"), and tangible support (e.g., "If I were sick, I could easily find someone to help me with my daily chores"). For the purposes of this study, an overall social support score (range = 4–16) was computed by averaging the subscale scores.

Measurement of CRP

At least one drop of free-flowing capillary blood was collected on filter paper for analysis of CRP. Each participant's finger was cleaned with alcohol, and a sterile, disposable microlancet was used to deliver a controlled, uniform puncture. Whole blood was placed directly on standardized filter paper commonly used for neonatal screening (#903, Schleicher and Schuell, Keene, NH). This relatively noninvasive blood collection protocol minimizes pain and inconvenience to the participants and has facilitated the collection of blood samples in a number of community-based studies (33,34). After collection, samples were covered, allowed to dry overnight, and stored frozen at −30°C until analysis.

Samples were analyzed in the Laboratory for Human Biology Research at Northwestern University using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) protocol previously developed for use with blood spots (35). Calibrators for blood spots were prepared by diluting delipidated human serum enriched with CRP (standardized against the WHO International Reference Preparation; #X0923, Dako, Carpinteria, CA) with washed erythrocytes, followed by application onto filter paper. Blood spot calibrators, controls, and samples were included in all assays and were treated identically throughout the protocol.

Prior validation of assay performance indicates that the blood spot CRP method has good sensitivity, precision, and reliability and a high correlation between matched plasma and blood spot samples (Pearson $R = 0.96$, $N = 94$) (35). To monitor day-to-day variation across assays during the analysis of samples for this study, four control values were included with each run. Between-assay coefficients of variation (SD/mean) were less than 10%.

Data Analysis

All statistical analyses were conducted with Stata for Windows, version 8.0 (StataCorp, College Station, TX). The distribution of CRP was highly skewed and was therefore log-transformed before analysis. In addition, because we are using CRP as an indicator of chronic, low-grade inflammation that may contribute to CVD, we eliminated individuals with evidence of an acute inflammatory condition (e.g., infection). Concentrations of CRP increase dramatically as part of an acute inflammatory response, which may obscure the association between chronic low-grade CRP elevation and CVD. For this reason, a recent joint scientific statement issued by the American Heart Association and the Centers for Disease Control and Prevention recommends that plasma CRP concentrations above 10 mg/L be discarded (4). Using a conversion formula based on prior comparison of matched plasma/blood spot samples (35), this corresponds to a blood spot CRP concentration of 8.6 mg/L. We therefore removed individuals with blood-spot CRP greater than 8.6 mg/L from our analyses due to the likelihood that these high concentrations represented acute inflammation.

We pursued a nested modeling strategy, considering associations between CRP and demographic, behavioral, and psychosocial factors, in that order. The addition of psychosocial variables after the inclusion of demographic and behavioral variables represents a conservative test of the hypothesis that stress is related to CRP. All psychosocial variables were standardized before analysis. Our final model includes nine variables and loses 20 observations due to missing data, primarily from the PSS.

We pursued two strategies for investigating the potential impact of missing observations. First, we included all observations in models 1 and 2 but added a dummy variable to indicate the presence of an observation with incomplete data. In no case did this variable approach significance, suggesting that individuals with missing data did not differ with respect to CRP. Second, we evaluated models with complete cases only ($N = 153$) and compared results with models including median-imputed values for missing observations. Coefficients and standard errors for both sets of models were virtually identical. After these analyses were completed, we applied a series of regression diagnostic procedures to assess the validity of our final model. Tests for linearity, homoscedasticity, outliers, and collinearity did not reveal any deviations from the assumptions of multiple linear regression.

RESULTS

For the entire sample, the median blood-spot CRP concentration was 1.31 mg/L (25th percentile: 0.53; 75th percentile: 3.44). However, 15 individuals had values >8.6 mg/L and were therefore removed from subsequent analyses. Age, gender, and ethnicity were not associated with the likelihood of CRP >8.6 mg/L, although individuals with higher household incomes were marginally less likely to be in this group (OR = 0.58; 95% CI, 0.34–1.01).

We first considered demographic variables as predictors of CRP concentration. The distribution of CRP by gender across the three ethnic groups is presented in Table 2. In a regression model including age, gender, and ethnicity ($N = 173$), women had significantly higher CRP concentrations than men ($\beta = 0.238$, SE = 0.069, $p = .001$), and concentrations for both African Americans ($\beta = 0.224$, SE = 0.082, $p = .007$) and Latino Americans ($\beta = 0.179$, SE = 0.085, $p = .038$) were significantly elevated in comparison to European Americans. Age was marginally related to CRP, and there was no significant interaction between gender and ethnicity. The addition of education attenuated the associations between ethnicity and CRP, although concentrations for African Americans remained significantly elevated compared with European Americans (Table 3, model 1). Household income was not associated with CRP.

We next considered a range of behavioral predictors of CRP, many of which are recognized risk factors for CVD (Table 3, model 2). As expected, and consistent with prior research (1), higher CRP concentrations were evident for current smokers. With this variable in the regression model, we then evaluated multiple measures of adiposity, including BMI, waist circumference, and body fat percentage as quantified by bioelectrical impedance. Each adiposity measure was significantly associated with higher CRP concentrations, but

TABLE 2. Median (Interquartile Range) Concentrations of CRP in Dried-Blood-Spot Samples by Gender and Ethnicity, Excluding Individuals With CRP >8.6 mg/L ($N = 173$)

	Female	Male	Total
European American	1.05 (0.44, 1.88)	0.59 (0.44, 1.50)	0.96 (0.44, 1.74)
African American	3.30 (1.39, 4.47)	1.07 (0.37, 1.70)	1.60 (0.55, 3.81)
Latino American	1.49 (0.78, 3.10)	1.00 (0.55, 1.65)	1.18 (0.69, 2.57)
Total	1.59 (0.63, 3.56)	0.89 (0.44, 1.65)	1.19 (0.50, 2.63)

TABLE 3. Results of Nested Multiple Linear Regression Models Predicting Log-Transformed CRP Concentration for Participants With Complete Data ($N = 153$)

	Model 1	Model 2	Model 3
Adjusted R^2	0.124	0.260	0.275
Age	0.016 (0.009)+	0.011 (0.008)	0.012 (0.008)
Gender[a]	0.253 (0.073)***	0.367 (0.072)***	0.349 (0.073)***
Ethnicity[b]			
African American	0.218 (0.090)*	0.115 (0.086)	0.091 (0.087)
Latino American	0.133 (0.096)	0.079 (0.089)	0.060 (0.089)
Education	−0.062 (0.031)*	−0.054 (0.029)+	−0.056 (0.029)+
Waist circumference		0.012 (0.003)***	0.012 (0.003)***
Current smoker		0.243 (0.099)*	0.226 (0.098)*
Time to fall asleep (>30 min)		0.183 (0.105)+	0.177 (0.105)+
PSS, baseline			−0.060 (0.040)
PSS, current			0.092 (0.041)*
Constant	−1.193 (0.574)*	−2.330 (0.577)***	−2.360 (0.573)***

$+p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$.
[a] Male is the omitted reference group.
[b] European American is the omitted reference group.

waist circumference was the strongest predictor and the only one that remained significant when adiposity measures were considered simultaneously.

Reported time to fall asleep was a marginally significant predictor of CRP. Due to the highly skewed distribution of this variable, we compared individuals reporting a time to fall asleep of more than 30 minutes (13.5% of the sample) with those reporting 30 minutes or less. We also considered individuals who report very short sleep latency, 5 minutes or less (22.5%). We found elevated concentrations of CRP among those taking longer to fall asleep. Additional measures of sleep, medication use, alcohol consumption, and diet were not significantly related to CRP, nor did they substantially modify the effects of other variables in the model.

Last, we considered the impact of psychosocial variables on CRP above and beyond these demographic and behavioral factors. In recognition of the fact that changing trajectories of cognitive/emotional well-being may be as meaningful, or possibly more meaningful, than contemporaneous measures, we modeled each psychosocial measure twice. First, we considered the cross-sectional association between each variable and CRP, both measured simultaneously. Next, we added a variable representing the value of each psychosocial measure from the year before, thereby allowing us to consider the association between CRP and the contemporaneous measure independent of the effects from the prior year. In effect, this approach controls for individual differences in stable traits that may be correlated with our psychosocial variables by modeling 1-year change in psychosocial status as a predictor of CRP.

Concurrent symptoms of depression were not significantly associated with CRP (B = 0.034, SE = 0.033, $p = .31$). However, there was a trend toward higher CRP with depressive symptoms after controlling for reported symptoms the year before (B = 0.086, SE = 0.045, $p = .057$).

We found a similar but more robust effect of perceived stress. Concurrent reports of perceived stress were marginally associated with CRP (B = 0.049, SE = 0.035, $p = .10$) and significantly associated after controlling for PSS the year before (Table 3, model 3). Symptoms of depression and PSS scores were highly correlated (Pearson $R = 0.66$, $p < .001$), and the association of depressive symptoms with CRP was attenuated with the addition of PSS (B = 0.063, SE = 0.052, $p = .226$). Measures of chronic stress, loneliness, and social support were not significantly predictive of CRP, nor did social support moderate the associations of other psychosocial variables.

DISCUSSION

Previous research has established that markers of inflammation are important predictors of CVD risk, and in our analyses we investigated CRP in relation to a wide range of behavioral and psychosocial variables in order to explore the lifestyle factors that may condition this risk. We found that perceived stress, as well as measures of adiposity, smoking, and latency to sleep, were positively associated with CRP. These results underscore the importance of psychosocial and behavioral processes to chronic inflammation, and suggest that inflammation may be an important mechanism through which these processes increase CVD risk.

Consistent with prior population-based research (36), we found higher CRP concentrations in women compared with men. We also found higher CRP in African American participants, although ethnic differences in CRP were not statistically significant once behavioral variables were added to our regression model. Although prior studies have reported ethnic differences in CRP concentration (36,37), our analyses highlight the importance of adequately considering lifestyle factors that may contribute to these differences.

We evaluated a number of behavioral predictors of CRP and found positive associations with adiposity and smoking. Although several studies have associated CRP with BMI (1,37,38), we found waist circumference to be a stronger

predictor. Waist circumference is a measure of central—as opposed to peripheral—fat deposition, and abdominal fat may be a particularly important source of pro-inflammatory cytokines such as IL-6 (39,40). Psychosocial stress may operate in part through this mechanism; recent research has suggested that stress-related cortisol exposure is associated with the accumulation of abdominal fat (41,42). However, we found no evidence for a mediating role of adiposity in our sample: the association between PSS and CRP was similar with or without waist circumference in the model.

The importance of adequate sleep to a number of health outcomes is becoming increasingly apparent: Observational and experimental studies have linked sleep duration with critical metabolic and endocrine processes, as well as aspects of immune function and inflammation (43–46). We found a trend toward higher CRP in individuals who take longer than 30 minutes to fall asleep but no association with sleep duration. Not surprisingly, these individuals also report higher levels of perceived stress (PSS = 13.6 versus 11.6; $p = .12$), suggesting that stress may be a partial mediator of this association. Recent research has associated experimental sleep deprivation with elevated CRP (45), further emphasizing that the effects of sleep duration and quality on inflammation deserve further investigation.

Psychosocial stress has direct effects on multiple aspects of immune function, including cytokine pathways associated with inflammation (8–10). Our finding of a positive association between perceived stress and CRP contributes to this literature and suggests that inflammation may be an important pathway through which psychosocial stress increases risk of CVD. Furthermore, by finding that changes in perceived stress over the preceding year are more strongly predictive of elevated CRP than individual differences in cross-sectional reports of perceived stress, we provide more convincing evidence for a direct causal link between perceived stress and inflammation. Proinflammatory cytokines are responsive to hypothalamic-pituitary-adrenal and sympathetic nervous system inputs and are therefore likely physiological mediators of this association (17). IL-6 in particular has been associated with a number of psychosocial stressors, including symptoms of depression, posttraumatic stress disorder, and caregiving for a spouse with dementia (12,14,47,48).

Limitations of this study include the relatively small sample and associated reductions in statistical power, although our sample is larger than many studies of psychosocial factors and CRP and we were able to replicate many of the known associations with inflammation (e.g., smoking, adiposity). An additional limitation is our use of a single CRP measure. Although individual CRP concentrations have been shown to be relatively stable across time (1), acute bouts of inflammation lead to dramatic increases in CRP that are not likely to be predictive of CVD risk. Our reliance on a single blood sample forced us to remove 15 individuals from our analyses, further reducing statistical power.

A strength of the study is the careful attention that was given to recruiting a highly representative, population-based sample of older adults. Our findings should therefore generalize beyond these analyses and suggest productive directions for future research with older adults, as well as younger populations, where obesity, sleep problems, and stress may be on the rise. An additional strength is the longitudinal design that allowed for multiple psychosocial measures across time.

The importance of considering behavioral and psychosocial predictors of CRP is underscored by our finding that a small fraction of the variance in CRP is accounted for by standard demographic factors. Explained variance more than doubles with the addition of behavioral and psychosocial variables. By considering psychosocial variables last, we present a conservative test of the hypothesis that stress is associated with inflammation since stress may act in part through behavioral processes (e.g., poor sleep, central fat deposition, smoking). Teasing out the direct and indirect pathways through which psychosocial stress affects inflammation is a significant challenge and remains as a goal for future population-based research.

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