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Associations of salivary cortisol levels with inflammatory markers: the Multi-Ethnic Study of Atherosclerosis

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Abstract

Socioeconomic and psychosocial factors have been found to be associated with systemic inflammation. Although stress is often proposed as a contributor to these associations, no population studies have investigated the links between inflammation and biomarkers of stress. The current study examines associations between daily cortisol profiles and inflammatory markers interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor (TNF-a) in a population-based sample of 869 adults with repeat measures of cortisol over multiple days. Persons with higher levels of IL-6 had a less pronounced cortisol awakening response, a less steep daily decline, and higher cortisol area under the curve for the day with associations persisting after controls for risk factors and other cytokines. Persons with higher levels of TNF-a had lower cortisol levels upon waking, and flatter daily decline, although associations with decline were attenuated when controlling for inflammatory risk factors. Higher levels of IL-10 were associated with marginally flatter daily cortisol decline ($p < .10$). This study is the first to identify associations of basal cortisol activity and inflammatory markers in a population-based sample. Findings are consistent with the possibility that HPA axis activity may mediate associations between psychosocial stressors and inflammatory processes. Additional prospective data are necessary to clarify the directionality of associations between cortisol and inflammatory markers.

Keywords

HPA Axis; cortisol; inflammation; cytokines

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Contributors Author contributions: ASD drafted the paper and conducted all analyses; ADR supervised the work and assisted with the writing of the paper; AH assisted with analyses and interpretation of results; Aiello, Golden, Jenny, Seeman, and Shea provided critical feedback on successive drafts and are listed in alphabetical order.

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There is a growing body of evidence linking socioeconomic and psychosocial stress to inflammation and immune function (Black, 2003; Kiecolt-Glaser et al., 2002; Segerstrom & Miller, 2004). The physiologic mechanisms underlying these associations have not been fully explained, but chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis, a stress-responsive biological system, has been proposed as a potential contributor to observed links between adverse psychosocial influences and immune or inflammatory processes.

The HPA axis is responsible for mobilizing the body's resources when an individual encounters psychological or physical stressors (Sapolsky et al., 1986). Cortisol, a hormone produced by the HPA axis, increases in response to stress in both naturalistic and laboratory settings (Adam et al., 2006; Dickerson & Kemeny, 2004) and varies according to a circadian rhythm in which levels are typically high upon awakening, increase sharply within 30 to 40 minutes of waking (a phenomenon referred to as the cortisol awakening response or CAR) (Pruessner, 1997), and decline across the remainder of the waking day (Kirschbaum & Hellhammer, 1989). Because the HPA axis is known to interact in complex ways with immune system activity (Petrovsky et al., 1998; Sapolsky et al., 2000; Sternberg, 2001), it is reasonable to expect that HPA axis activity could contribute to immune system or inflammatory alterations in the face of psychosocial stress.

Elevated levels of inflammation-related cytokines, including interleukin-6 (IL-6), have been associated with increased levels of chronic and acute stress and anxiety levels (Kiecolt-Glaser et al., 2003; Maes et al., 1999; Maes et al., 1998). Higher levels of pro-inflammatory cytokines have been found among racial/ethnic minorities in the US and individuals of low socioeconomic status in the US and UK (Gimeno et al., 2007; Koster et al., 2006; Ramsay et al., 2008; Ranjit et al., 2007). A parallel body of work has also linked racial/ethnic minority status, socioeconomic disadvantage, and adverse psychosocial profiles to alteration of the levels and circadian rhythm of cortisol (Cohen et al., 2006; DeSantis et al., 2007; Hajat et al., 2010). Taken together, these results suggest that alteration of the levels or the circadian rhythm of cortisol may serve as a mechanism underlying associations between psychosocial stress and inflammation. However, few if any studies have examined the direct links between HPA axis activity and systemic levels of inflammatory markers in population-based samples.

Associations between cortisol levels and inflammation-related cytokines are likely to be complex and bidirectional: pro-inflammatory cytokines stimulate activity in the HPA axis, leading to increases in cortisol, but cortisol and other glucocorticoids (GCs) typically act as immuno-suppressants (Riechlin, 1993). Much early research focused on the role of GCs as immuno-suppressants (Chrousos, 1995; Elenkov & Chrousos, 1999; Petrovsky, 1998). However, recent research has increasingly found that GCs are immuno-modulatory, rather than simply immunosuppressive (Elenkov, 2008; Elenkov & Chrousos, 2002; McEwen, 1997). Although in the short-term, cortisol acts as an immuno-suppressant (Chrousos, 1995; Elenkov, 2002), long-term chronic activation of the HPA axis has been hypothesized to contribute to inflammation (Chrousos, 1995; Elenkov & Chrousos, 2002; Elenkov & Chrousos, 1999; Miller et al., 2002; DeRijk et al., 1997). For example, there is some evidence that white blood cells may respond to excessive exposure to GCs by downregulating the expression and/or function of GC receptors (Miller et al., 2002). These results suggest that chronic stress may impair the immune system's capacity to respond to hormonal signals that terminate inflammation, and that this pathway could be one of the mechanisms through which chronic stress contributes to inflammation-related conditions (Ibid., 2002).

A number of clinical and experimental studies have analyzed associations between cytokine activity and GCs (DeRijk et al., 1997; Elenkov & Chrousos, 2002; Miller et al., 2002;

Riechlin, 1993; Steensburg et al., 2003). However, the extent to which cortisol activity in naturalistic settings (e.g., levels at various points in the day, rates of decline across the waking day, and cortisol responses to awakening) is related to cytokine activity in non-clinical population-based samples has not been extensively investigated. We used data from a large population based sample to investigate associations between daily cortisol levels and circulating levels of three cytokines, IL-6, interleukin-10 (IL-10), and tumor necrosis factor-α (TNF–α).

We investigated these three cytokines because each participates in a different aspect of the inflammatory process. IL-6 is increasingly recognized as a pleiotropic cytokine that serves both to activate as well as suppress inflammatory activity. It is an important mediator of the acute phase response (Feghali & Wright, 1997) and has been found to be particularly sensitive to exogenous corticosteroids in experimental research (DeRijk et al., 1997). The principal function of IL-10 appears to be limiting and terminating inflammatory responses (Moore et al., 2001). Because levels of IL-10 been found to increase in response to psychological stress, IL-10 has been proposed as a potential key mediator of stress-induced immune-suppression (Curtin et al., 2009). TNF-α has pro-inflammatory properties and plays a key role in the initial stages of the APR (Feghali & Wright, 1997). It stimulates the synthesis of several other pro-inflammatory cytokines, as well as the release of corticotropin releasing hormone (CRH) from the hypothalamus. Through its effects on the release of CRH, TNF-α plays a key role in modulating the immuno-suppressive response of the HPA axis.

Prior work from this sample has examined associations of socioeconomic and psychosocial factors with inflammatory markers and with HPA axis activity (Ranjit et al., 2007; Ranjit et al., 2009; Hajat et al., 2010). This report focuses on direct associations between cortisol activity and inflammatory markers.

Specifically, we address the following questions:

1. Is there an association between diurnal cortisol rhythms (wake-up levels, the cortisol awakening response (CAR), or the decline in cortisol over the day) and IL-6, IL-10, or TNF-α levels?
2. Is there an association between total cortisol output measured by area under the curve (AUC) and IL-6, IL-10, or TNF-α levels?

Specifically, we hypothesized that higher levels of IL-6 and TNF-α would be related to higher cortisol AUC, flatter cortisol decline and higher bedtime cortisol levels. In contrast, we hypothesized that higher levels of IL-10 would be related to lower cortisol AUC, more pronounced declines, and lower bedtime values.

Method

The data utilized in these analyses come from an ancillary study to the Multi-Ethnic Study of Atherosclerosis (MESA), the MESA Stress Study. MESA is a longitudinal study designed to prospectively examine risk factors for subclinical cardiovascular disease and its progression to clinical disease. The main study included 6814 participants between 45 and 84 years old without clinical cardiovascular disease from six cites at baseline. Participants were selected through various population-based approaches, including sampling from lists of area residents, area residents enrolled in union health plans, and lists from the Centers for Medicare and Medicaid Services for those 65 and older, as well as random digit dialing (Bild et al., 2002).

The MESA Stress study was initiated in 2003, in conjunction with exams three and four at two of the MESA study sites (New York and Los Angeles) (Hajat et al., 2010). Participants were enrolled in the ancillary study in the order in which they completed the follow-up exams for the main study until approximately 500 participants were enrolled at each site and resulted in an approximately random sample of Black, White, and Hispanic participants in each site. Relative to the larger parent study, the MESA Stress study included slightly fewer persons in the oldest age category (75+ years old) (18.2% in the parent study vs. 12.1% in the Stress study). There were also slightly more males (47.6% versus 44.7%) and more participants with some college education (29.7% versus 23.9%) in the ancillary study.

Cortisol

Participants were asked to provide six salivary cortisol samples per day over three weekdays. Saliva samples were to be provided immediately upon awakening, 30 minutes after awakening, and at 10 AM, 12 PM (or before lunch in the event that lunch occurred before noon), 6 PM, and at bedtime. All samples were collected using Salivette collection tubes and stored at -20 degrees Celsius until being prepared for assay. Saliva samples were thawed and centrifuged at 3000 rpm for 3 minutes before cortisol levels were determined using a chemiluminescence assay with a high sensitivity of 0.16 ng/mL (IBL-Hamburg, Germany). Intra- and inter-assay coefficients of variation (CVs) were < 8%. Cortisol values in nmol/L were logarithmically transformed before analyses to adjust for a positive skew in the distribution, and analytical procedures adjusted for clustering of multiple cortisol values within person when appropriate.

The precise timing of samples was determined using time tracking devices (trackcaps) placed in the caps of small containers from which cotton swabs were extracted. Prior research has indicated that use of such devices is associated with increased compliance with sampling protocols (Kudielka et al., 2003), and all participants were informed that this tracking device was being utilized.

Inflammatory markers

Cytokine data are fasting morning levels obtained from plasma. The assays for all three cytokines were performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). Concentrations of interleukin-6 (IL-6) were measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). The laboratory average analytical CV was 6.3%. IL-10 was measured using the Milliplex MAP Human Cardiovascular Disease Panel 3 (Millipore Corporation; Billerica, MA) and run as a single-plex assay; average analytical 8.1%. TNF-α was measured using the LINCOplex Human Cardiovascular Disease Panel 3 kit (Millipore Corporation, St. Charles, MO) run as a single-plex assay; average analytical CV 10.3%. MESA research staff drew fasting blood samples from participants at the time of exam, between 7:30 and 10:30 am. Serum was stored at -70 °C, until being shipped on dry ice to the University of Vermont Central Blood Analysis Laboratory, where they were stored in the laboratory freezer until the time of assay.

Covariates

Factors that have previously been shown to be associated with cortisol and/or inflammatory markers in MESA and other studies and could therefore confound the cortisol-inflammation association were included as covariates. Age, gender, wake-up time, study day, site, and medication use were included as covariates in all analyses. Use of non-steroidal anti-inflammatory medications was derived from questionnaires. Consideration of lipid lowering medications did not appreciably change the results. In addition, socioeconomic status and psychosocial factors were also investigated as covariates. On one hand, HPA axis activity

may be part of the mediating mechanisms though which SES and psychosocial factors affect level of inflammatory markers. However, because they may also relate to inflammation through mechanisms that do not involve HPA axis activity, they may confound estimates of the causal associations between HPA axis activity and inflammation. Because these analyses focused determining whether HPA axis activity and levels of inflammatory markers are independently associated, we adjusted for SES, race/ethnicity, and selected psychosocial factors in analyses.

Race/ethnicity was categorized as Black/African-American, Hispanic/Latino, and non-Hispanic White. Participants were asked to report on both their income and their wealth. The wealth index reflected a count of the number of specific assets that participants owned among the following: one or more cars, a home or paying mortgage on a home, land, or an investment (e.g., stocks, bonds, mutual funds, or retirement investments). These were combined to create a 5-point wealth index in which participants received one point for ownership of each of the assets listed above. Total family income was reported as one of 13 income categories. These were combined into an income-wealth scale that has been used in prior analyses of MESA Stress data (Hajat et al., 2010). Because prior studies have revealed associations of feelings of hostility and cynicism with cortisol activity, this was also included as a covariate in all analyses (Ranjit et al., 2009). This was assessed using an eight-item subscale (cynical distrust) taken from the Cook-Medley Hostility Scale (Barefoot et al., 1989). Other psychosocial factors (chronic burden (a measure of chronic stress) (Bromberger & Matthews, 1996), depressive symptoms (Center for Epidemiologic Studies Depression Scale) (Radloff, 1977), social support (ENRICHED, 2000)) were investigated in sensitivity analyses.

Selected analyses were also adjusted for other risk factors for inflammation that could confound the cortisol inflammation association including health behaviors (physical activity and smoking) as well as body mass index, diabetes and hypertension status (Badrick, Kirschbaum, & Kumari, 2007; Agnatosis et al., 2009). Physical activity was assessed by questionnaire. Each day of the cortisol sampling, participants were asked to report on whether or not they had engaged in vigorous exercise (yes/no) as well as the amount of time (in minutes) they spent doing so in daily diaries. The number of minutes was converted to hours before being included as a covariate in analyses.

Smoking was assessed by questionnaire and participants were categorized as current, former, or non-smokers. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and modeled as standard categories: normal (BMI less than 25 kg/m^2), overweight (BMI between 25 kg/m^2 and 29.9 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2). Diabetes status was determined based on American Diabetes Association criteria (participants with fasting glucose levels $\geq$ 126 mg/dL or who were taking hypoglycemic medications were considered diabetic). Hypertension was defined as: systolic blood pressure (BP) $\geq$ 140 mm Hg and diastolic BP $\geq$ 90 mm Hg, taking anti-hypertensive medications, and/or physician diagnosis of hypertension. Non-steroidal anti-inflammatory medication use was also included as a covariate.

Analyses

Levels of IL-6, IL-10, and TNF-α were analyzed in relation to various cortisol parameters, including: the CAR (considered a measure of reactivity and believed to be influenced by mechanisms distinct from those that regulate the rest of the diurnal cycle) (Clow et al., 2004), which is measured in these analyses as the deviation of “wake up + 30 minutes” value from the wakeup-to-bedtime slope; the slope (or hourly rate of decline between wakeup and bedtime excluding the second “wakeup + 30 minutes” sample); the total cortisol

output (area under the curve or AUC); and time-specific cortisol levels (wakeup and bedtime levels).

In order to estimate associations of inflammatory markers with wake-up levels, the CAR and the decline over the day, multilevel models were fit to repeat measures of log-transformed cortisol, where repeat measures of cortisol are nested within persons. Log-transformed cortisol levels were modeled as a function of time since wake and a dummy variable to indicate the second sample with the second sample being defined as one that was taken between 15 and 60 minutes of waking (in most cases and per protocol, 30 minutes after waking). Random intercepts were used to account for within-subject correlations. Levels of inflammatory markers as main effects and in interaction with the second sample dummy and with time since wake up were included to assess associations of inflammatory markers with the CAR (interaction with second sample dummy) and with daily decline (interaction with time since wake up), respectively. Sets of covariates (see below) were included as main effects and in interaction with the second sample dummy or with time since wake up to adjust for the effects of these covariates on these parameters. In sensitivity analyses we also examined the robustness of results when using spline models that pool across all available cortisol measures and include knots at 30 minutes and 2 hours after wake up (Hajat et al., 2010).

In order to estimate associations of inflammatory markers with area under the curve (AUC) and bedtime cortisol levels, both measures were modeled as a function of inflammatory markers and covariates in separate models. Multi-level models with measures nested within person (and a random intercept for each person) were used to account for correlations between the three daily measures within a person. The AUC was determined by measuring the size of a series of “polygons” between each set of time points based on the following schedule: 0 to 30 minutes post-awakening; 30 minutes post-awakening and 10 AM; 10 and 12 PM (or before lunch in the event that lunch occurred before noon); between 12 and 6 PM; and between 6 PM and 16 hours post-awakening.

The control variables included in each series of models were identical for analyses of all parameters:

Model 1: included sociodemographic controls: age, sex, race/ethnicity, wakeup time, study day, site, non-steroidal anti-inflammatory medications, and income/wealth as well as cynical distrust.

Model 2: added behavioral factors (smoking and physical activity)

Model 3: added other inflammation risk factors (BMI, diabetes, hypertension).

All models were fit with each inflammatory marker in separate models as well as all three in the same model in order to estimate their independent associations with cortisol.

Exclusionary criteria

A total of 1002 participants were enrolled in the MESA Stress Study. We excluded days with insufficient track cap data on sampling times (n = 127), days without saliva samples (n = 15) or with no data on wake up times (n = 5). We also excluded participants taking corticosteroid medications (n = 35) and participants who failed to provide at least 12 or more valid cortisol measurements over the three days of the study period and at least one wakeup, bedtime, and CAR (wakeup + 30 minutes) sample (n = 31). Participants who had valid data on at least one cytokine measure were included in the analyses for that particular cytokine. The final analytical sample included 869 participants who provided 15,123 samples over 2622 days. In total, 843 participants had data on IL-6 levels; 866 had valid data on IL-10 levels; and 862 had valid data for TNF-α levels.

Results

The 869 participants included 236 Blacks (27%), 466 Hispanics (54%), and 167 non-Hispanic whites (19%) (Table 1). There were 425 males (49%) and 444 females (51%) who ranged in age from 48 to 89 years old, with a median age of 65 years. There were 365 (42%) overweight and 303 (35%) obese participants. In total, 80 participants (9%) were current smokers, and an additional 345 (40%) were former smokers. There were 150 participants (17%) with diabetes and 443 (51%) with hypertension. Twelve percent of participants exercised for at least a half hour per day on average, and 69% did not engage in any recreational exercise on the study days. Average levels of cytokines were 2.8 pg/mL (SD = 1.92) for IL-6, 8.3 pg/mL (SD = 14.0) for IL-10, and 4.5 pg/ml (SD = 6.6) for TNF-α. Inflammatory markers had low correlations: IL-6 and IL-10 ($r = 0.07$, $p < 0.01$); IL-6 and TNF-α ($r = 0.04$, $p < 0.05$); and TNF-α and IL-10 ($r = 0.13$, $p < 0.01$). Cortisol levels increased an average of 39% between the waking up and 30 minutes later and decreased by 11% per hour when the second (CAR) sample was excluded.

Cortisol-IL-6 associations

There were significant associations between IL-6 levels and multiple cortisol parameters (Table 2). Participants with higher levels of IL-6 had lower cortisol awakening responses (CAR). This association was consistent across models that included only basic controls, SES, race/ethnicity, cynical distrust, and waketime (3.3% lower, $p < 0.01$), as well as those that included smoking status and physical activity (3.7% lower, $p < 0.01$) and other inflammation risk factors (3.3% lower, $p < 0.01$) as covariates. The rate of hourly decline between wakeup and bedtime also differed significantly according to IL-6 levels. For each one SD increase in IL-6 level, hourly rates of declines were reduced by 0.5% ($p < 0.01$), 0.5% ($p < 0.01$), and 0.4% ($p < 0.05$), in models controlling for demographics and sleep, health behaviors, and inflammation risk factors, respectively (Table 2). (Note that because cortisol levels typically decline across the day, a positive coefficient reflects a reduced decline or a “flatter” slope).

Higher levels of IL-6 were also associated with greater values for the cortisol area under the curve (AUC) (Table 3). On average, AUCs were 6.5% higher ($p < 0.01$) for each one SD increase in IL-6, after controlling for demographic factors and 6.2% higher ($p < 0.05$) after controlling for behavioral factors. Including inflammatory risk factors as covariates in the model slightly increased this association (7.4% higher, $p < 0.01$). There were no significant differences in wakeup or bedtime cortisol levels (all p-values > 0.10) according to IL-6 levels (Tables 2 and 3, column 1).

Cortisol-IL-10 associations

Higher IL-10 levels were only marginally associated with a less pronounced decline over the course of the day (0.2% flatter, $p < 0.10$) in the model with basic controls. Including health behaviors and inflammation risk factors did not alter the magnitude of the association substantially, and the association remained very small and marginally significant ($p < 0.10$), and as such should be interpreted with caution and should be replicated to establish whether a consistent association of IL-10 with cortisol declines does indeed exist. There were no differences in the size of the CAR, AUC, or wakeup or bedtime cortisol levels according to IL-10 levels (Tables 2 and 3, column 2).

Cortisol-TNF-α associations

Increased levels of TNF-α were associated with lower wakeup cortisol levels and flatter declines. Each one SD increase in TNF-α was associated with a 2.5% decrease in wakeup cortisol levels ($p < 0.05$) when controlling for sociodemographic factors (Table 2). This

association did not change appreciably in models controlling for behaviors (2.7% lower, $p < 0.01$) and other inflammation risk factors (2.5% lower, $p < 0.01$). Each SD increase in TNF-α was also associated with a 0.3% flatter decline over the course of the day after controlling for sociodemographics and health behaviors ($p < 0.05$). This association was attenuated (0.3% flatter, $p < 0.10$) after controls for inflammation risk factors. There were no significant associations of TNF-α with CAR, AUC, or bedtime cortisol levels (Tables 2 and 3, column 3).

Models including all three cytokines

We next analyzed associations of cortisol with each of the three cytokines, while controlling for the others in order to determine whether associations of cytokines with cortisol parameters were independent of each other and to establish whether one cytokine in particular was driving the associations. IL-6 remained significantly associated with cortisol parameters after controlling for levels of the other cytokines. Participants with higher levels of IL-6 had lower CARs, higher AUC, and flatter rates of cortisol decline over the day after controlling for sociodemographics, behaviors, other inflammatory risk factors, and levels of IL-10 and TNF-α. Higher levels of TNF-α were significantly associated with lower wakeup cortisol levels ($p < 0.05$) controlling for levels of IL-6 and IL-10. There were no significant associations of IL-10 (all p-values > 0.10) with any of the cortisol parameters, when controlling for the other two cytokines. (Results not presented).

Additional adjustment for chronic burden (a measure of chronic stress) (Bromberger & Matthews, 1996), depressive symptoms (Center for Epidemiologic Studies Depression Scale) (Radloff, 1977), social support (ENRICHED, 2000), and recent illness (fever, cold/influenza, urinary or sinus infection, bronchitis, pneumonia, or arthritis flare up) during the past two weeks did not substantially affect the results (results not shown). In sensitivity analyses we examined whether results for the decline over the day were similar using the spline models used in prior work that separate the decline into two sections (Hajat et al., 2010). Results of these models also showed that higher levels of IL-6 were associated with flatter rates of decline, particularly the earlier part of the decline, between 30 minutes and two hours after waking. IL-10 was not significantly associated with any cortisol parameters, and higher levels of TNF-α were associated with lower wakeup cortisol levels in the spline models. We also investigated the robustness of our results to alternative parametizations to capture the CAR. Analyses that modeled the difference between the second and the first sample as a function of inflammatory markers and covariates showed very similar results with the exception of the minimally adjusted model in which the association between the CAR and IL-6 was positive, but not statistically significant ($\beta = 0.008$, $p = 0.61$).

Discussion

Although there have been several experimental and/or clinical studies relating various measure of cortisol or HPA axis function to cytokines activity (Chrousos, 1995; DeRijk et al., 1997; Elenkov & Chrousos, 2002; Elenkov & Chrousos, 1999; Miller et al., 2002; Nijm & Jonasson, 2009; Perry et al., 2000; Petrovsky, 1998), to our knowledge, our study is one of the first population-based studies to examine associations of levels of pro- and anti-inflammatory cytokines with daily cortisol levels in a non-clinical sample of participants in naturalistic settings In general, persons with higher levels of IL-6 had a less pronounced cortisol awakening response, a less steep daily decline, and higher cortisol area under the curve for the day with associations persisting after controls for risk factors and other cytokines. Persons with higher levels of TNF-α had lower cortisol levels upon waking, and flatter daily decline, although associations with decline were attenuated when controlling for inflammatory risk factors. Higher levels of IL-10 were associated with marginally flatter daily cortisol decline. While the magnitude of the differences in inflammatory markers

linked to cortisol measures was small, the physiological implications of long-term differences of this magnitude are not known but have the potential to be clinically meaningful.

Of the three cytokines investigated, IL-6 was the one most consistently related to daily cortisol profiles. Persons with higher levels of IL-6 had a less pronounced CAR, a less steep daily decline, and higher AUC. Less pronounced CARs (Hajat et al., 2010) and flatter cortisol declines have also been related to lower SES and other forms of chronic stress (Adam & Gunnar, 2001; Cohen et al, 2006; Gunnar & Vazquez, 2001). Our results indicate that this profile is in turn associated with higher levels of IL-6. The associations of IL-6 with all three cortisol parameters persisted when other cytokines were controlled for.

A notable finding is the positive association between IL-6 and AUC despite the known anti-inflammatory effects of cortisol. This finding is consistent with prior work showing that IL-6 is more “resistant” to the suppressive effects of GCs (DeRijk, 1997). More generally, it supports the notion that chronic dysregulation of the cortisol rhythm (of the type linked to chronic stress) could be associated with higher rather than lower levels of inflammation (McEwen, 1997). Because excessive exposure to GCs ultimately may cause white blood cells to downregulate the expression or function of GC receptors, chronic stress exposure may hinder the immune system’s ability to respond to hormonal cues to shut down inflammatory responses, thereby resulting in increased risk of inflammation (Miller et al., 2002). Studies from MESA and other samples have also linked less pronounced daily declines to socioeconomic adversity and psychosocial stress (Cohen et al., 2006; Ranjit et al, 2007; Hajat et al., 2010). We did not predict associations of higher levels of IL-6 with lower CARs. Given that we have only a single measure of IL-6, it is not possible to disentangle short-term bidirectional effects of IL-6 on waking levels or on the CAR. Delays in collecting the wake up sample are a major methodological limitation in estimating effects on the CAR. If omitted confounders associated with IL-6 levels were also associated with delays in the collection of the wake up sample, they could have contributed to biased estimates of associations of IL-6 with the CAR.

Higher levels of IL-10 and TNF-α were also marginally associated with a less steep daily decline but associations were less pronounced than for IL-6, and did not persist when other cytokines were adjusted for. No associations of IL-10 and TNF-α with the CAR or AUC were observed. Unfortunately, the nature of our data does not allow us to disentangle the dynamic relations between cortisol and inflammatory markers that may be occurring over the course of the day. Notably, associations of all three cytokines with features of the cortisol profile were similar in direction, though not in magnitude. Because IL-6 and TNF-α are pro-inflammatory cytokines that increase as part of the APR, it is not surprising that associations of these two cytokines with various cortisol parameters were similar and somewhat stronger; while associations with the anti-inflammatory cytokine were weaker.

These findings are limited by several factors. First, because the data are cross-sectional, we cannot determine the causal direction of this association. We cannot differentiate whether the cortisol pattern we identified as associated with higher IL-6 causes higher IL-6 levels or is itself caused by higher IL-6 levels. A second limitation pertains to the timing and number of samples. Although we had multiple measures of cortisol each day, we had only one morning measure of cytokines which was not obtained on the same day on which cortisol was measured. However, prior research indicates cytokine levels are relatively stable over time (Picotte et al., 2009). By pooling across multiple days, we attempted to capture relatively stable features of each individual’s daily cortisol pattern. Our analyses are therefore intended to capture associations between relatively stable cytokine level and

cortisol profile traits, rather than short-term dynamic relations between the two. The latter would necessitate a very different study design and data collection protocol.

A third limitation pertains to the observational nature of our analyses. Because all data were collected in naturalistic settings, rather than experimentally altering GC or cytokine levels, it is impossible to determine whether observed associations are truly causal in nature, or reflect the effects of additional "third" variables that influences both cortisol and cytokine levels. Although we have controlled for several potential confounders, such as SES, behaviors and other inflammation risk factors like diabetes, there undoubtedly remain several other potential confounds that we have not included in these analyses. However, key results were generally robust to adjustment for the measures we did have available, suggesting that string confounding is unlikely. We also did not have any direct measures of sympathetic nervous system activity, a known stimulator of IL-6 production and HPA axis activity which could also confound the associations we report.

Measurement error in the timing of the cortisol samples is always a challenge in population studies. Although the specific timing of samples was monitored with the use of electronic trackcaps and compliance was very good, we did not objectively measure wakeup times. Waketimes in these analyses were determined on the basis of trackcap information and coincide with the time of first sample. Significant delays between time of waking and providing first saliva samples each day could produce inaccurate (likely higher) estimates of wakeup cortisol levels and (likely reduced) CARs. However, prior analyses of self-reported wake up times and sample times indicate that compliance is generally very good, with 86% of participants providing samples within 15 minutes of the requested times (Hajat et. al, 2010). In addition, 59% of participants reported collecting the first sample within 5 minutes of waking; and 72% provided samples within 10 minutes. Moreover, delays between waking and providing the first saliva sample would strongly influence associations only in the event that compliance with sampling protocols varied systematically according to cytokine levels or omitted confounders associated with cytokine levels. Finally, because the MESA study systematically excluded persons with CVD and young adults as part of its initial recruitment process, it is unclear whether these associations would differ among individuals with CVD or younger individuals with fewer CVD risk factors.

Prior analyses of these data have identified significant associations between lower levels of SES and racial/ethnic minority status and both cortisol patterns and cytokine levels (Hajat et al., 2010; Ranjit et al., 2007). The results of the current study are consistent with the possibility that flatter diurnal cortisol rhythms among low-income individuals and racial/ethnic minorities may either contribute to or result from increased levels of inflammation, and as such, may act as a mediator between psychosocial stress and health disparities in inflammation-related conditions.

Because psychosocial stress has also been related to flatter diurnal cortisol rhythms (Adam & Gunnar, 2001; Cohen et al., 2006; Gunnar & Vasquez, 2001), our results are also compatible with a role of altered cortisol profiles as a contributor to the observed associations between psychosocial factors and inflammation (Kiecolt-Glaser et al., 2002; Segerstrom & Miller, 2004). Conversely, it is possible that increased inflammation contributes to alterations in cortisol rhythms. If so, cortisol rhythms may represent a link between increased inflammation and a broad range of physical and mental health conditions possibly caused by alterations of cortisol rhythms, ranging from chronic fatigue syndrome to depression (Bower et al., 2005; Heim et al., 2008). Additional prospective or experimental data are necessary to clarify the directionality of associations between cortisol activity and inflammatory markers.

Although flatter cortisol patterns have previously been related to a number of adverse health conditions and outcomes (Kumari et al., 2009; Nater et al., 2008; Sephton et al., 2000), few studies have investigated associations between cortisol rhythms in naturalistic settings and cytokine levels in a non-clinical population-based sample. Understanding the reasons for these associations and the directionality and timing of the causal relationships that may be involved is beyond the scope of this observational study. However our findings do suggest that cortisol activity and levels of systemic inflammation are linked in population samples. Our results indicate that cortisol patterns and inflammatory cytokines are related; additional research on the causal direction and implications of this relationship would further enhance our understanding of associations among psychosocial factors, HPA axis activity, and inflammation.

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Table 1

Sample Characteristics and Inflammatory Markers by Demographic, Behavioral, and Risk Factor Characteristics: the MESA Stress Study (n = 869)

		Interleukin-6 pg/mL		Interleukin-10 pg/mL		TNF-α pg/mL	
	(%)	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Demographics							
Black	27%	2.77	1.98	7.13	16.68	4.56	3.88
Hispanic	54%	2.90	1.92	8.76	16.45	4.28	5.21
White	19%	2.40	1.79	9.07	17.94	4.54	10.86
[a]Race: *p*-value for differences in means		$p < 0.05$		$p > 0.10$		$p > 0.10$	
Male	49%	2.77	1.99	8.24	17.77	4.45	7.37
Female	51%	2.75	1.86	8.52	15.84	4.44	5.32
[a]Gender: *p*-value for differences in means		$p > 0.10$		$p > 0.10$		$p > 0.10$	
Age category							
48-54	17%	2.17	1.63	8.02	20.90	3.39	2.57
55-64	31%	2.44	1.54	9.65	19.80	4.77	10.06
65-74	33%	3.11	2.24	6.54	7.44	4.51	3.31
75-89	19%	3.21	2.02	9.84	18.71	4.87	4.68
[a]Age: *p*-value for differences in means		$p < 0.01$		$p < 0.05$		$p > 0.10$	
Income-Wealth							
index 1	6%	3.48	1.90	7.86	8.36	6.77	12.30
2	13%	2.80	1.82	7.42	9.00	4.42	3.84
3	16%	2.89	2.19	7.97	17.85	4.74	4.84
4	13%	2.95	1.91	10.81	29.20	4.05	2.68
5	12%	2.69	1.77	8.58	15.59	3.80	2.60
6	12%	2.60	1.73	6.45	6.36	3.93	2.60
7	12%	2.69	2.18	9.60	14.71	3.84	3.06
8	9%	2.51	1.88	7.57	11.02	6.13	15.19
9	6%	2.22	1.17	9.28	22.46	3.54	3.19
[a]Income-wealth: *p*-value for differences in means		$p < 0.10$		$p > 0.10$		$p < 0.10$	
Health Behaviors							

		Interleukin-6 pg/mL		Interleukin-10 pg/mL		TNF-α pg/mL	
	(%)	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Non-Smoker	51%	2.60	1.84	8.76	15.14	4.52	8.19
Former Smoker	40%	2.80	2.05	7.11	15.96	4.87	3.65
Current Smoker	9%	2.96	1.75	8.20	26.64	4.32	4.16
[a]Smoking: *p*-value for differences in means		$p < 0.05$		$p > 0.10$		$p > 0.10$	
Physically active[1]	16%	2.46	1.92	8.09	14.04	4.72	6.59
Physical inactive	16%	2.90	1.92	9.03	14.04	3.92	6.59
[a]Physical activity: *p*-value for differences in means		$p < 0.01$		$p > 0.10$		$p > 0.10$	
Risk factors							
Hypertensive	51%	3.07	2.01	7.77	19.33	4.37	3.83
Non-hypertensive	49%	2.45	1.76	8.98	13.72	4.57	8.27
[a]Hypertension: *p*-value for differences in means		$p < 0.01$		$p > 0.10$		$p > 0.10$	
Diabetic	18%	3.13	1.94	8.56	8.69	4.50	4.43
Non-diabetic	82%	2.68	1.91	7.53	18.06	4.35	6.72
[a]Diabetes: *p*-value for differences in means		$p < 0.10$		$p > 0.10$		$p > 0.10$	
Normal weight	23%	2.31	1.85	8.92	15.34	4.55	7.15
Overweight	42%	2.56	1.81	7.74	18.97	4.26	3.69
Obese	35%	3.30	1.99	8.82	14.89	4.68	8.17
[a]BMI: *p*-value for differences in means		$p < 0.01$		$p > 0.10$		$p > 0.10$	

Note: Physically active represents all participants who reported engaging in any form of intentional exercise on study days. Reference group for each categorical variable is all other categories.

[a]One-way analyses of variance were conducted; and *p*-values represent tests of differences in mean levels of cytokines for each covariate.

Table 2

Percent differences in selected features of the daily cortisol curve associated with a 1 standard deviation increase in inflammatory marker

	Interleukin-6 pg/mL		Interleukin-10 pg/mL		TNF-α pg/mL	
	% Differences	Confidence Intervals	% Differences	Confidence Intervals	% Differences	Confidence Intervals
Sociodemographic controls						
Wakeup level	-1.7	[-4.7, 1.1]	-2.2	[-6.0, 1.7]	-2.5*	[-4.6, -0.5]
CAR	-3.3*	[-6.3, -0.4]	-0.5	[-3.5, 2.4]	0.9	[-1.9, 3.8]
% decline/hour	0.5**	[0.2, 0.9]	0.2+	[-0.02, 0.4]	0.3*	[0.01, 0.5]
Behavioral Controls						
Wakeup level	-1.6	[-4.7, 1.3]	-2.3	[-6.2, 1.5]	-2.7**	[-4.7, -0.7]
CAR	-3.7*	[-6.7, -0.7]	-0.4	[-3.4, 2.5]	0.7	[-2.2, 3.6]
% decline/hour	0.5**	[0.2, 0.8]	0.2+	[-0.02, 0.4]	0.3*	[0.0, 0.5]
Other Inflammatory Risk Factors						
Wakeup level	0.0	[-2.9, 2.8]	-2.2	[-6.0, 1.5]	-2.5**	[-4.4, -0.7]
CAR	-3.3*	[-6.4, -0.2]	-0.3	[-3.2, 2.7]	0.7	[-2.1, 3.5]
% decline/hour	0.4*	[0.1, 0.7]	0.2+	[-0.03, 0.4]	0.3+	[0.0, 0.5]

Note: Demographic controls include: age, gender, race, waketime, study site, cynical-hostility, non-steroidal anti-inflammatory medications, and socioeconomic status. Behavioral controls include: smoking status and physical activity. Inflammatory risk factors include: diabetes, hypertension, and body mass index.

+ $p < .10$;

* $p < .05$;

** $p < .01$.

Table 3

Percent differences in Cortisol Area Under the Curve (AUC) and Bedtime Cortisol Levels associated with a 1 standard deviation increase in inflammatory markers

	Interleukin-6 pg/mL		Interleukin-10 pg/mL		TNF-α pg/mL	
	% Differences	Confidence Intervals	% Differences	Confidence Intervals	% Differences	Confidence Intervals
Demographic controls						
AUC	6.5*	[1.5, 11.6]	-3.0	[-9.0, 2.8]	-0.07	[-5.1, 5.0]
Bedtime Cortisol	3.8	[-1.7, 9.6]	2.3	[-1.7, 9.6]	0.8	[-3.4, 4.9]
Behavioral Controls						
AUC	6.2+	[1.1, 11.5]	-3.0	[-9.0, 2.7]	-0.2	[-5.1, 4.7]
Bedtime Cortisol	3.3	[-0.23, 9.5]	2.2	[-2.3, 9.1]	0.7	[-3.4, 4.8]
Inflammatory Risk Factors						
AUC	7.4*	[2.3, 12.8]	-3.0	[-8.9, 2.6]	-0.1	[-4.7, 4.5]
Bedtime Cortisol	3.5	[-2.2, 9.5]	2.1	[-1.9, 6.2]	0.7	[-3.3, 4.7]

Note: Demographic controls include age, gender, race, waketime, study day, site, cynical-hostility, non-steroidal anti-inflammatory medications, and socioeconomic status. Behavioral controls include smoking status and physical activity. Inflammatory risk factors include diabetes, hypertension, and body mass index.

+ $p < .10$;

* $p < .05$;

** $p < .01$.