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## RESEARCH ARTICLE

# Relationship between Hair Cortisol and Perceived Chronic Stress in a Diverse Sample

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### Abstract

Hair cortisol (CORT) is a biomarker of chronic stress via long-term alterations in hypothalamus–pituitary–adrenal axis activity. Relationships to perceived stress measures, however, have rarely been specifically investigated. A diverse sample of 135 adults participated in a study assessing relationships between chronic stress indicator CORT to perceived stress and health indicators. CORT was not correlated to single perceived domain indices but with a global stress composite. Differences in objective and subjective measures were found for sociodemographics: racial/ethnic identity, sex and socioeconomic status (SES). Race by SES interactions predicted both CORT and perceived stress, but produced a complex and partially unanticipated pattern of results. For minorities, low and high SES showed the highest CORT, with mid-SES showing the lowest CORT; there was little change in perceived stress at all levels of SES. For non-minorities, mid-SES showed the highest CORT, with decreases in both CORT and perceived stress in high SES. The unanticipated findings of deleterious outcomes for high SES minorities highlight the importance of investigating potential stressors and moderators, including perceived discrimination and social identity. Moreover, these results suggest that CORT may not always correlate with single stress indices but may provide a global assessment of chronic stress, with implications for the allostatic load literature. Copyright © 2012 John Wiley & Sons, Ltd.

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### Keywords

hair cortisol; chronic stress; perceived stress; health disparities; social identity

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## Introduction

Cortisol (CORT) measurement in hair has recently been recognized as a reliable index of chronic stress, across the life span and in clinical and non-clinical biopsychosocial research (e.g. Gow, 2010; Yamada, 2007; DeSantis et al., 2007). Since hair grows approximately 1 cm per month, it has been posited that 3 cm can measure CORT remnants reflecting the past 3 months (Davenport, Tiefenbacher, Lutz, Novak, & Meyer, 2006). This report provides the first data on the relationship between this long-term biomarker and subjective stress reports in a diverse sample. Understanding these complex associations is critical for understanding the multi-domain effects of chronic stress on physical and psychological health outcomes.

Hair CORT is a compelling instrument to assess chronic stress. The extant research using CORT, however, has only examined specific populations—individuals with post traumatic stress disorder (PTSD) (Steudte et al., 2011a), shift workers (Manenshijn, van Kruysbergen, de Jong, Koper, & van Rossum, 2011), hospitalized

neonates (Yamada, 2007) and pregnant women in their third trimester (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009)—which may not generalize to other populations under chronic and cumulative stress. Moreover, since CORT is a relatively new biomarker, there are inconsistencies in the literature whether this long-term measure of circulating CORT is correlated with acute stress, as indexed via salivary CORT, urine or other assays (e.g. Steudte, et al., 2011a; Steudte, et al., 2011b).

### Biological and self-reported stress

In prior psychophysiological research, acute stress measures often circumvent well-known limitations for self-reported indices, such as memory recall biases or impression management biases (e.g. Guglielmi, 1999). Furthermore, acute biological stress measures and reported stress measures have often been unrelated (e.g. Mendes, 2002). It is not yet known if CORT will be similarly dissociated from self-reported experiences of chronic stress.

Recent studies including both CORT and measures of perceived stress have yielded inconsistent findings.

For example, Kalra, Einarson, Karaskov, Van Uum, and Koren (2007) investigated maternal stress and anxiety on fetal development. Using the Perceived Stress Scale (PSS; Cohen & Williamson, 1988), they found positive associations between maternal hair CORT and the PSS in 25 pregnant women ( $r=0.47, p < 0.05$ ). Alternatively, in a study of long-term unemployment, Dettenborn, Tietze, Bruckner, and Kirschbaum (2010) did not find a significant association between their measure of perceived chronic stress and CORT in a sample of 59 adults (31 unemployed). Finally, Van Uum et al. (2008) examined perceived stress (PSS) and hair CORT levels in 15 patients who suffered from chronic pain compared with 39 healthy controls. Those in the chronic pain group were significantly higher in both CORT and perceived stress ( $p < 0.001$ ), but the overall association between CORT and perceived stress did not quite reach significance ( $r=0.24, p=0.08$ ).

The present research was conducted to assess relationships between biological chronic stress as measured by CORT and perceived stress indices. Furthermore, this research examines potential differences in biological and perceived stress by racial/ethnic identity, SES, sex and age. We obtained domain-specific indices of stress (i.e. personal perceived stress, chaos in the home and neighborhood assessments) and examined associations between CORT, subjective stress and health indicators [blood pressure and waist-to-hip ratio (WHR)]. Finally, we also investigated the interactions of well-known factors associated with health disparities: racial/ethnic identity and SES with both hair CORT and the perceived stress indices as the dependent variables.

## Method

### Participants and recruitment

This study took place at the University of Massachusetts Boston, recognized by the National Institutes of Health (NIH) as a minority-serving institution, with a population including 60% female and 39% minority. We recruited 135 adults (18–66 years, 65% female, 48% minority), including students, staff and faculty. There were no prescreening criteria. Posters and tables with handouts were on display in high traffic areas on campus, and recruitment was opportunistic.

### Measures

#### Hair cortisol

Samples were obtained by cutting approximately 100 strands (3 cm) from the posterior vertex of the head using surgical scissors (Davenport et al., 2006). Samples were folded into a 4 × 4-in. foil, the scalp end marked, and placed in plastic bags with the subject identifier for later assay preparation.

#### Anthropometric measures

Measurements of WHR and resting blood pressure, associated with overall health, allostatic load (McEwen

& Seeman, 2003) and metabolic syndrome (Juster, McEwen, & Lupien, 2009), were obtained.

### Psychosocial parameters

#### Perceived Stress Scale (Cohen & Williamson, 1988)

The PSS is a standard validated subjective stress measure (Cohen & Williamson, 1988), which was modified to assess perceived stress over the past 3 months. Respondents answer on a Likert scale from 1 to 5, where 1 = never and 5 = very often. The possible range is 10–50 ( $\alpha=0.78$ ).

#### Chaos, Hubbub, and Order scale (Matheny, Wachs, Ludwig, & Phillips, 1995)

This reliable and validated measure assesses chaos/order of the home environment. For example, 'You can't hear yourself think in our home.' Respondents answer on a Likert scale from 1 to 4, where 1 = strongly agree and 4 = strongly disagree. The possible range is 15–120 ( $\alpha=0.79$ ).

#### City Stress index (Ewart & Suchday, 2002)

This reliable and validated measure assesses neighborhood risk factors in the past year. For example, how often were there 'Drug dealers near my home.' Respondents answer on a Likert scale from 1 to 4, where 1 = never and 4 = often. The possible range is 18–72 ( $\alpha=0.72$ ).

### Total subjective stress

The individual stress scales (Perceived, Chaos and City) were standardized (*z*-scored), and the mean was derived to create a Total subjective stress scale ( $\alpha=0.73$ ). Tertiles were constructed for statistical analyses by group ( $M=2.06, SD=0.82$ ).

### Hair treatment

This item included six questions on the frequency of hair washing and treatments.

### Medications

Six questions asked about medications that potentially alter hypothalamus–pituitary–adrenal axis activity (e.g. corticosteroids and beta-blockers), as well as depression, anxiety and hormones (e.g. birth control).

### Sociodemographic

#### Objective social status (socioeconomic status)

Income and education were standardized (*z*-scored), and the mean was derived ( $\alpha=0.71$ ). Tertiles of SES were constructed to examine group level differences ( $M=2.03, SD=0.82$ ).

#### Race

Participants selected among 13 race/ethnic categories including African-American/Black, Caribbean, Arab/

Middle Eastern, Asian, Brazilian/Portuguese, Dominican, European/White, Haitian, Hawaiian, Latino-Hispanic, Indian, Native-American, Pacific Islander and Other as an open response. Category *N*s were not equal and were collapsed into two race categories: (1) minorities, which included African-Cuban, Afro-Cuban, Asian, Brazilian, Indian, Latino-Hispanic and Pacific Islander ( $N=67$ ), and (2) non-minorities, including European and/or White American ( $N=68$ ).

### Age

Age was positively skewed and recoded into three tertiles of adult age (ranges: young = 18–21 years,  $N=44$ ; middle = 22–29 years,  $N=45$ ; older = 30–66 years,  $N=43$ ).

### Procedure

Interested participants were informed of all aspects of the study and gave verbal consent. Upon completion, participants were entered into a raffle for a bookstore gift certificate (\$80) or a grand prize, an Apple iPad. The gloved experimenter clipped 3 cm of hair from the posterior vertex, along a diagonal to minimize any obvious change in hairstyle. Scissors were washed in Barbicide® (Barbicide, Milwaukee, IL, USA) for 10 min and dried between each use. Health indicators were then obtained by measuring waist-and-hip circumference and resting blood pressure values. The participant then completed the questionnaires online with Psychdata (Psychdata, LLC, State College, CA, USA), and data were downloaded into SPSS (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. USA).

### Hair cortisol assay process

Taking note of the scalp end, we washed the samples twice for 3 min each time in 5.0 mL of isopropanol to remove contamination from sweat and sebum. After drying, the samples were weighed in pre-tared 2.0 polypropylene microcentrifuge tubes specially reinforced for tissue grinding (BioSpec Products, Bartlesville, OK, USA; catalog # 330TX). Weights ranged from 10.8 to 71.3 mg. Three 3.2-mm diameter chrome steel beads were added to each tube, and hair was ground for at least 2 min using a Mini-Beadbeater-16 (BioSpec). Next, 1.5 mL of high-performance liquid chromatography (HPLC)-grade methanol was added to each tube, and the CORT was extracted overnight using slow rotation. Tubes were centrifuged at 10,000 RPM for 5 min to pellet the powdered hair, after which 1.0 mL of the methanol extract was removed and transferred to a fresh 1.5-mL microcentrifuge tube. The methanol was evaporated using a Savant DNA110 SpeedVac. The extract was reconstituted in 200  $\mu$ L of assay buffer and analyzed by means of a sensitive and specific enzyme immunoassay (Salimetrics, LLC, State College, PA, USA; catalog #1-3002). Intra-assay and inter-assay coefficients of variation were <10%. Values were converted to pg/mg hair for analyses and interpretation.

## Results

### Primary analyses

During an initial examination of the data, CORT values (pg/mg) were found to be positively skewed and were log-transformed (from range 0–164 pg/mg,  $M=14.47$ ,  $SD=19.13$  to range 0.54–2.22,  $M=1.04$ ,  $SD=0.28$ ). This range was consistent with recent research on clinical and non-clinical populations.

Associations between levels of CORT, health indicators, self-reported indices and sociodemographic factors were conducted with Pearson product-moment correlations ( $r$ ). Analyses of variance were conducted to assess main effects of SES (3) and age (3);  $t$ -tests examined mean differences in objective and subjective stress indices by race (2) and sex (2). For  $t$ -tests examining differences by sex, we used unweighted means to correct for unequal cell sizes (65% female). There were no significant differences in hair treatment or medication use on CORT levels (all  $ps$  n.s.), and these were not included as covariates in the remaining models.

### Hair cortisol correlations

Table I shows the means, standard deviations and zero-order correlations for dependent and independent measures. CORT was not significantly associated with individual self-report indices of domain-specific stress (all  $ps > 0.05$ ) but was positively associated with the Total subjective stress score ( $r=0.19$ ,  $p < 0.05$ ; Figure 1). CORT was positively related to only one of the health indicators, systolic blood pressure ( $r=0.25$ ,  $p < 0.01$ ).

### Main effects

#### Socioeconomic status

Table II presents the analysis of variance summaries for SES and age group on CORT, Total subjective stress and health indicators.

Cortisol was not significantly different by SES, although examination of other health indicators showed significant differences in WHR. Unexpectedly, higher SES was associated with higher WHR. Significant differences for SES were also found for the single indices of Perceived Stress (PSS), Chaos and the composite of Total subjective stress. All of the subjective stress reports were lower for those with high SES.

#### Age group

Cortisol was not significantly different by age group. Significant differences were found for WHR and systolic blood pressure such that the middle and older groups were higher in each measure. Among perceived stress measures, significant differences were found for Chaos and Total stress, with older adults reporting the lowest subjective stress.

**Table 1.** Means, standards deviations and zero-order correlations for independent and dependent variables

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11	12
1. Age	30.26	12.80	—											
2. Sex	1.65	0.50	-0.06	—										
3. Race	0.50	0.50	0.32***	0.14	—									
4. Hair cortisol (pg/mg)	14.47	19.13	0.07	-0.24**	-0.09	—								
5. City Stress index (CSI)	25.99	7.38	-0.14	0.01	-0.02	0.03	—							
6. Personal stress (PSS)	27.73	6.23	-0.18*	0.11	0.13	0.07	0.21	—						
7. Chaos (home stress)	31.52	6.12	-0.36***	-0.02	0.22	0.04	0.46***	0.40***	—					
8. Total subjective stress	0.00	0.75	-0.32**	0.04	0.20*	0.19*	0.74**	0.71**	0.82**	—				
9. Objective socioeconomic status	0.03	0.89	0.63***	0.03	-0.27**	0.02	-0.11	-0.17*	-0.34***	0.18*	—			
10. Waist-to-hip ratio	0.84	0.09	0.23**	-0.18*	0.07	-0.03	0.09	0.04	0.16†	0.13	0.16†	—		
11. Systolic blood pressure	119.14	14.99	0.27**	-0.43***	-0.17†	0.25**	0.06	-0.01	-0.02	0.05	0.16†	-0.14	—	
12. Diastolic blood pressure	79.55	9.89	0.20*	-0.19*	0.11	0.08	0.06	-0.01	-0.08	-0.03	0.1	0.01	0.33***	—

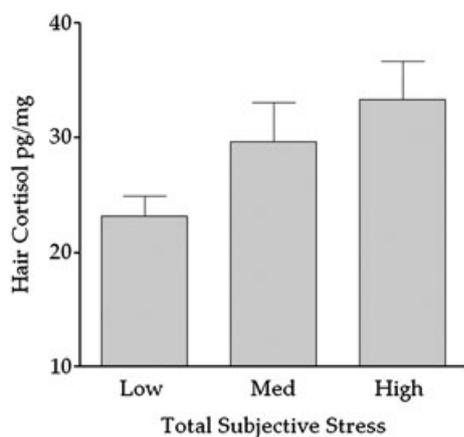
Note. N = 135.

\**p* < 0.05.

\*\**p* < 0.01.

\*\*\**p* < 0.001.

†*p* < 0.10.



**Figure 1.** Cortisol (in pg/mg) as Objective Stress measure correlation with Total subjective stress measure ( $N = 135$ )

### Race

Cortisol was not significantly different by race, nor were any of the health indicators significantly different (all  $ps > 0.05$ ). Domain-specific stress indices, however, showed significant differences by race: Chaos,  $t(134) = 2.70$ ,  $p < 0.001$ , Confidence Interval (CI) =  $-4.91$  to  $-0.83$ , and City Stress,  $t(134) = 1.15$ ,  $p < 0.001$ ,  $CI = -3.96$  to  $-1.05$ . Total subjective stress was also significantly different,  $t(134) = 2.40$ ,  $p < 0.05$ ,  $CI = -0.56$  to  $-0.05$ . Further examination found that the minority group reported significantly higher Chaos, City Stress and Total subjective stress, compared with the non-minority group.

### Sex

Cortisol levels were significantly different by sex,  $t(129) = 2.91$ ,  $p < 0.01$ ,  $CI = 0.14$  to  $0.72$ , with males slightly higher than females. Health indicators WHR and systolic blood pressure were also significantly different by sex [ $t(127) = 2.13$ ,  $p < 0.05$ ,  $CI = 0.02$  to  $0.07$ ;  $t(113) = 4.95$ ,  $p < 0.001$ ,  $CI = 7.82$  to  $18.25$ , respectively]. Women were significantly lower on all measures. There were no significant differences in single domain or Total subjective stress measures by sex (all  $ps > 0.05$ ).

### Interactions: Race and socioeconomic status on objective and subjective stress

To assess the usefulness of CORT for chronic stress associated with health disparities, we explored the interaction of Race and SES, which are well-established social determinants of health (e.g. Omojokun & Fine, 2008). Analyses of covariance (ANCOVAs) were conducted, covarying sex, age and WHR to control for baseline differences. The first ANCOVA produced a significant interaction of Race  $\times$  SES,  $F(2, 122) = 3.26$ ,  $p < 0.05$ ,  $\eta^2 = 0.16$ , on CORT. Figure 2 (left panel) reveals that minorities were significantly higher in CORT at both low and high SES than at mid-SES. Non-minorities exhibited their highest levels of CORT at mid-SES and lowest levels at high SES. Higher CORT values for non-minorities at mid-SES were similar to those of the low-SES or high-SES minorities.

The next ANCOVA also produced a significant interaction of Race  $\times$  SES on Total subjective stress,  $F(2, 125) = 4.28$ ,  $p < 0.05$ ,  $\eta^2 = 0.07$ . Figure 2 (right panel)

**Table II.** Analysis of variance summary table of SES and age group differences in biological and subjective stress measures

Source	Sum of squares	<i>df</i>	Mean square	<i>F</i>	$\eta^2$	<i>p</i> value
SES						
CORT		n.s.				—
WHR	0.057	(2,128)	0.028	3.49*	0.05	<0.05
SBP		n.s.				—
Total stress	5.571	(2,134)	90.96	5.18**	0.08	<0.01
City		n.s.				—
Chaos	467.35	(2,134)	233.676	6.82**	0.10	<0.01
Perceived	298.63	(2,134)	149.32	4.03*	0.06	<0.05
Age group						
CORT		n.s.				—
WHR	0.043	(2,130)	0.021	2.61 <sup>†</sup>	0.04	<0.10
SBP	1424.82	(2,111)	712.41	3.50*	0.06	<0.05
DBP		n.s.				—
Total stress	5.09	(2,131)	2.55	4.85**	0.08	<0.01
City		n.s.				—
Chaos	672.55	(2,131)	336.28	10.59***	0.15	<0.001
Perceived		n.s.				—

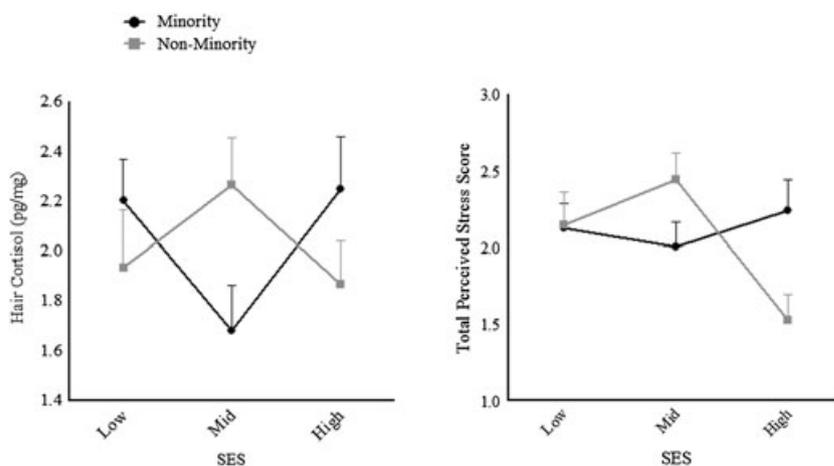
Note.  $N = 135$ . SES: socioeconomic status; CORT: cortisol; WHR: waist-to-hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.

\* $p < 0.05$ .

\*\* $p < 0.01$ .

\*\*\* $p < 0.001$ .

<sup>†</sup> $p < 0.10$ .



**Figure 2.** The left panel illustrates that the minority group with low or high SES resulted in significantly higher cortisol than the low-socioeconomic status (SES) or high-SES non-minority group (in pg/mg). The mid-SES non-minority group shows cortisol values similar to the high-SES minority group ( $N = 122$ ). The right panel illustrates that high-SES non-minorities reported less overall perceived stress, whereas the minority group reported similar levels of perceived stress, regardless of SES level. At mid-SES, the non-minority group shows greater perceived stress than the mid-SES minority group. At low SES, there were no significant differences in perceived stress between the minority and non-minority groups ( $N = 125$ )

illustrates that high-SES non-minorities reported less perceived stress compared with high-SES minorities. However, mid-SES non-minorities reported greater perceived stress than the mid-SES minorities. At low SES, there were no significant differences in perceived stress between the minority and non-minority groups.

## Discussion

We found that hair CORT, as a biomarker of chronic stress, was positively and moderately associated with total subjective stress ( $r = 0.19$ ,  $p < 0.05$ ; Table I) but not the domain-specific stress reports. Specifically, the analyses showed that the personal, home and environmental stress domains were not significantly correlated with CORT. The global subjective stress score, however, was significantly and positively associated (Figure 1). One interpretation may be that single domain indices do not also capture cumulative stress from other domains. For example, if an individual is experiencing high stress in only one domain (e.g. work is stressful, but home is not), this may not create the demands that produce long-term CORT release. This implies that CORT may provide a similar global measure of chronic stress but may not disambiguate specific sources of stress. Although similar research has also found that single domain indices were not always correlated with CORT, a novel finding in these data is that the combination of single indices became associated.

Interestingly, CORT was associated with only one of the other biomarkers of stress, higher systolic blood pressure. Waist and WHR were not significantly associated with CORT in these data. This was unexpected, since WHR is a well-known indicator of allostatic load

and has been positively associated with CORT in other research (e.g. Manenschijn, et al., 2011). The relationship between CORT and health risk indicators needs further investigation. It is not yet known whether CORT will be consistently associated with chronic health issues and reliably predictive of chronic diseases associated with allostatic load.

We also found significant differences in CORT by sex, where males were higher (17 versus 12 pg/mg). There is a substantial literature examining acute stress reactivity differences and hypothalamus–pituitary–adrenal axis reactivity by sex (for a review, see Kudielka & Kirschbaum, 2005); whether CORT will be consistently different by sex to assess chronic stress bears further investigation and replication.

We found significant interactions between factors associated with health disparities and chronic stress, but the results were complicated and partially unexpected. The direction of the slopes in Figure 2 suggests that in contrast with non-minorities, the minority group did not benefit from higher SES in either diminished CORT or decreased subjective stress. This high-SES finding is similar to findings on low-birth weight for high-SES African-American women (e.g. Foster, Wu, Bracken, Semanya, & Thomas, 2000; Colen, Geronimus, Bound, & James, 2006; Lu & Halfon, 2003). Several studies have found that although there are decreases in low-birth weight across generations with upward social mobility, African-Americans do not gain as much of a decrease as their Caucasian counterparts, suggesting that high SES may not be serving similar protective functions across racial/ethnic populations. This body of research on the race by SES interaction suggests that historical, institutional and individual experiences of discrimination

are robust factors in maintaining the level of health inequity (e.g. Colen et al., 2006; Lu & Halfon, 2003). Indeed, there is ample evidence that minorities with higher SES groups have greater daily social interactions within majority group settings, in which race becomes a more salient factor (e.g. Schaafsma, 2011; Brody et al., 2006), and consequentially, they may experience greater stress and vigilance associated with social identity (e.g. Kaiser, Vick, & Major, 2006).

The findings of greater CORT and perceived stress for non-minorities in mid-SES in contrast with minorities in our sample were also unanticipated and anomalous to the literature. It may be that SES carries different meanings for minority and non-minorities. As one possible scenario, an improvement in SES to a mid-level may reduce stress by alleviating poverty while also introducing new stressors associated with discrimination for some groups. In other groups, the 'same' mid-SES level may introduce new responsibilities, such as increased expectations associated with education, which may confer greater stress. If these race and SES interactions are replicated, they suggest research questions that bear further investigation.

These findings illustrate the critical need for additional questions that are suggested yet cannot be answered with these current data. For example, for high-SES minority individuals, what are the underlying psychosocial factors that operate as mechanisms for the increase in both their objective and subjective stress? What are the potential moderators of discrimination or stigmatized identity group, which may be operative in high-SES environments (e.g. Schaafsma, 2011; Crabtree, Haslam, Postmes, & Haslam, 2010)? Were the student participants primarily the first generation in higher education? These questions would be useful to examine.

### Limitations

The present study has several limitations that warrant discussion. Because our recruitment process was opportunistic in a diverse campus setting rather than planned, we did not have equal groups amongst all of the racial/ethnic categories (originally 13 groups). Thus, the final race variable, coded as minority and non-minority, cannot adequately or accurately capture the breadth of unique social and psychological stress experiences for each cultural/heritage group. In addition, the present study did not measure the occurrence or frequency of acute and chronic illnesses associated with chronic stress. It would be of inestimable value to assess the specific relationships between CORT and well-known chronic health disparities, including cardiovascular disease, diabetes, the incidences of low-infant birth weight, and psychological and mental health inequities (e.g. Nepomnyaschy, 2009).

### Future directions

Although unanticipated in the present study, the race by SES results give specific and critical direction for

several future studies. Discrimination is a well-known factor influencing minority health and stress-related illness (Williams, Neighbors, & Jackson, 2008; Williams & Mohammed, 2009); thus, the inclusion of measures that capture experiences of daily and lifetime discrimination at all levels of SES would provide valuable insight. In addition, other psychosocial factors, such as assessments of social identity and the extent to which a minority individual identifies with their heritage or majority group, can be examined as modifiers of discrimination experiences in high-SES environments, potentially leading to decreases in CORT and lower perceived stress for high-SES minorities. Also valuable, measures of heightened sense of social threat (e.g. vigilance) can give insight into biological ramifications of cumulative psychological threat. Finally, a series of studies could also add detailed assessments of physical and psychological health outcomes with CORT as a potential predictor of the long-term deleterious influences of chronic stress on health.

### Summary

This research was conducted with the primary goals of investigating whether CORT, as a long-term chronic stress measure, would be correlated with subjective stress and whether there would be differences by sociodemographic and health indicators, including SES, age, race, sex, WHR and systolic blood pressure. We found that CORT was not significantly associated with perceived measures of stress across single domains but a composite of subjective stress. This suggests that CORT may be a better measure of global and non-specific stress.

These findings suggest future studies with the following additions: subjective stress measures that assess a wider array of psychosocial risk factors associated with health disparities, including perceived discrimination, threats to social identity, social exclusion or other stressors historically linked to social, economic and/or environmental disadvantage.

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