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Original article

Racial/Ethnic Differences in Cortisol Diurnal Rhythms in a Community Sample of Adolescents

Amy S. DeSantis, M.S.W.[a,*], Emma K. Adam, Ph.D.[a], Leah D. Doane, M.A.[a], Susan Mineka, Ph.D.[b], Richard E. Zinbarg, Ph.D.[b], and Michelle G. Craske, Ph.D.[c]

[a]*Program on Human Development and Social Policy, School of Education and Social Policy and the Cells to Society Center, Institute for Policy Research, Northwestern University, Evanston, Illinois*

[b]*Department of Psychology, Northwestern University, Evanston, Illinois*

[c]*Department of Psychology, University of California at Los Angeles, Los Angeles, California*

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Abstract

Purpose: To identify potential physiological pathways to racial disparities in health outcomes, this study uses cortisol data collected from a community sample of 255 adolescents to examine whether there are racial/ethnic differences in cortisol slopes and levels across the waking day in naturalistic settings.

Methods: This study uses salivary cortisol data (sampled five times per day over 3 days) to examine racial/ethnic differences in diurnal cortisol rhythms, while covarying the presence of major depressive disorder and chronic and episodic life stress (assessed by structured interviews), momentary negative emotion (reported in diaries completed with cortisol samples), and socioeconomic status, sleep, and health variables (assessed by questionnaire) previously found to be associated with cortisol levels.

Results: African-American and Hispanic youth were found to have flatter cortisol slopes across the waking day than their Caucasian counterparts. Differences are due to higher bedtime cortisol levels among Hispanics and to both lower wakeup and higher bedtime levels among African-Americans. Although higher levels of negative emotion were associated with flatter diurnal rhythms, the socioenvironmental factors examined failed to explain the observed racial/ethnic differences in diurnal cortisol rhythms.

Conclusions: Significantly flatter diurnal cortisol slopes were found among African American and Hispanic adolescents, a pattern which has been related to negative health consequences. Further research is needed to examine how early these differences emerge and to identify their developmental origins. Although genetic contributions are possible, greater prenatal stress exposure, low birth weight, adverse early childhood experiences, experiences with racism or discrimination, and lifetime history of chronic stress are all reasonable psychosocial hypotheses to pursue.

Keywords: Cortisol diurnal rhythms; Racial/ethnic differences; HPA activity; Stress; Health disparities; Adolescents

In recent years, there has been an increasing concern about racial and ethnic disparities in health in the United States. Minorities are at increased risk for a wide variety of different illnesses including asthma, obesity, low birth weight, diabetes, and cardiovascular disease [1]. Moreover, minorities, especially African-Americans, have higher mortality rates and lower life expectancies, and health outcomes are especially poor among minorities of low socioeconomic status [2]. Although many possible explanations for these disparities have been examined [3], researchers have begun to focus on the role of the social environment, in particular psychosocial stress [3,4], as a potential contributor to observed differences in morbidity and mortality rates.

*Address correspondence to: Amy S. DeSantis, 2120 Campus Drive, Northwestern University, Human Development and Social Policy, Evanston, IL 60208.

E-mail address: a-desantis3@northwestern.edu

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Stress and health: physiological pathways

Stress is a potentially important mediator between race/ethnicity and health because racial/ethnic minorities tend to be exposed to greater psychosocial stressors, including episodic life events, chronic stress, and racism [4], and because stress has important physiological implications. There are two major biological systems through which perception of stress causes changes in the body: (1) the sympathetic–adrenal–medullary (SAM) system, and (2) the hypothalamic–pituitary–adrenal (HPA) axis. These systems influence many physiological processes relevant to health, including metabolic regulation, cardiovascular activity, and blood pressure, as well as immune and inflammatory functioning [5]. Thus these systems provide a potential pathway by which socioenvironmental stressors can influence physiological processes and disease outcomes.

Most past research on racial/ethnic differences in physiological stress and health has focused on the SAM, specifically high blood pressure and cardiovascular reactivity [6,7]. Surprisingly, little research has addressed racial/ethnic differences in HPA axis activity, despite the fact that cortisol, one of its primary products, is thought to play a role in many disease processes [5].

Overview of hypothalamic–pituitary–adrenocortical axis activity

Reactivity. When faced with psychological or physical stressors, the human body undergoes a complex physiological response intended to help the individual cope with immediate threat [8]. The hypothalamic–pituitary–adrenocortical (HPA) axis in particular undergoes a cascade of reactions including the release of corticotrophin releasing hormone (CRH) from the hypothalamus, causing the release of adrenocorticotrophic hormone (ACTH) by the anterior pituitary, and ultimately the release of cortisol by the adrenal cortex into the bloodstream. Cortisol increases occur in response to both laboratory stressors [9] and momentary and daily changes in negative emotion and stress in naturalistic environments [10]. Although periodic activations of the HPA axis are necessary to cope with acute, time-limited stressors, mounting evidence indicates that frequent or chronic HPA axis activation may have deleterious effects on emotional and physical health [5].

Diurnal rhythm. In addition to responding to stressful events, the HPA system also follows a strong circadian rhythm [11,12]. Typically, cortisol levels are high upon waking, reach a peak about 30–40 minutes after waking, then decline throughout the remainder of the day, reaching a nadir around midnight [12,13]. Deviations from the typical diurnal rhythm may have important implications for health. Flattened diurnal rhythms have been found among individuals with greater difficulties in interpersonal relationships [11] and exposure to stressful life events and trauma [14]. They have also been associated with negative health outcomes including decreased life expectancy among cancer patients, decreased natural killer (NK) cells [15], and higher levels of risk factors for cardiovascular disease and diabetes [16]. One study of cancer patients found that mortality rates 7 years later were higher among individuals with flatter slopes (77%) compared with those with steeper slopes (60%), and that individuals with steeper slopes lived longer on average than those with flatter slopes (4.5 years vs. 3.2 years) [15].

Race, socioeconomic status, and cortisol: recent findings

There has been very little research on socioeconomic status (SES), race/ethnicity, and cortisol. Lupien et al [17] found differences in morning cortisol levels according to SES among young children, although differences decreased to non-significance by adolescence, and racial/ethnic disparities in cortisol were not examined. To our knowledge, only one prior study has examined racial/ethnic differences in cortisol diurnal rhythms in the U.S., finding that African-American adults (aged 33–45 years) have flatter diurnal cortisol slopes from waking to bedtime than Caucasian adults, with differences primarily due to higher bedtime cortisol levels [18]. These differences remained after covarying income and educational attainment. The authors noted that additional research was needed to determine at what age racial/ethnic differences emerge and to what degree they are mediated by daily experiences of negative emotion.

Current study. The current study builds upon prior research by examining diurnal rhythms in a diverse group of adolescents, helping to establish the extent to which racial/ethnic differences are present before adulthood. We examined differences in diurnal cortisol slopes among African-American, Hispanic, Asian-American, Caucasian, and multiracial youth, while covarying other factors previously shown to be associated with cortisol, including: age, sex [19], oral contraceptive use [20], nicotine use [18], sleep timing [21], and major depressive disorder [22].

We also examined potential mediators of the association between race/ethnicity and cortisol rhythms, including chronic and episodic stress, negative emotion on the days of testing, and socioeconomic status. These factors have been associated both with race/ethnicity and cortisol levels in prior literature [10,14,18] and may help to account for associations between race/ethnicity and diurnal cortisol slopes.

Methods

Participants

Participants were 255 adolescents, ages 16–18 years (mean 17.1 years), from two racially diverse high schools, one in suburban Chicago and one in the greater Los Angeles

area. All juniors in these schools were asked to complete a questionnaire designed to identify those at high risk of developing emotional disorders, as determined by levels of neuroticism, a known risk factor for mood and anxiety disorders [23]. Students high on neuroticism, based on scores on the Eysenck Personality Questionnaire-Revised (EPQ-R) [24], were oversampled such that 60% of the sample scored in the top third of the neuroticism distribution. In the final sample selected, there were no differences in levels of neuroticism according to sex and/or race/ethnicity; however, there were more females chosen to participate in the study because of higher levels of neuroticism in our initial screening. The sample was recruited in three cohorts. The current analyses use data from the first two cohorts; a smaller third cohort is not yet available and contains few minority youth. Of the 1977 students screened for the first two cohorts, 923 were invited to participate in the longitudinal study, 520 consented, and 491 completed a set of initial diagnostic and questionnaire procedures, the former using the Structured Clinical Interview for DSM-IV-TR (SCID). Of these, a random subsample of 375 participants (76.4%) were invited to participate in additional procedures involving salivary cortisol sampling and momentary diary reports; 278 (74%) completed these procedures. Participants received $40 for completing the first set of interviews and questionnaires and $10 for the cortisol protocol and momentary diary reports.

Exclusion criteria and group classification. Participants taking medications containing corticosteroids (N = 12) or meeting criteria for psychotic disorders (N = 2) were excluded from this study, as were two participants who were extremely noncompliant with the requested sample timings. Participants were required to have completed at least one wakeup and one bedtime cortisol sample and at least five total cortisol data points. Nine participants failing to meet these criteria were excluded; the final sample includes 255 adolescents.

Procedures

Six types of measures are used in this study: (a) a demographic questionnaire; (b) the Structured Clinical Interview for DSM-IV-TR (SCID) [25]; (c) a Life Stress Interview [26]; (d) salivary cortisol samples, collected six times per day on 3 consecutive weekdays during the school year; (e) momentary diary reports of negative emotion at the time of cortisol sampling; and (f) health questionnaires. Demographic questionnaires, SCID interviews and Life Stress Interviews were completed at study entry, and cortisol samples, momentary diary reports, and health questionnaires were completed together between 0 to 9 months later (mean delay 1.96 months; median 1.56 months). There were no significant differences by race/ethnicity in the length of time between the initial assessment and completion of the cortisol task.

Measures collected upon study entry

Racial/ethnic categorization. Participants were classified into racial/ethnic categories based upon their responses on the demographic questionnaire. Response options included African-American/black, Hispanic/Latino, Asian, Pacific Islander, Native American/American Indian, Caucasian/white, multiracial, and other. For the purposes of these analyses, Pacific Islanders were grouped with Asian-Americans (a similar grouping convention is used in the U.S. Centers for Disease Control and National Center for Health Statistics' annual reports on morbidity and mortality rates), and multiracial youth were combined with those who indicated "other."

The racial/ethnic breakdown of the final analytic sample was as follows: 27 African-American (21 female); 54 Hispanic (45 female); 12 Asian and Pacific Islander (6 female); 43 multiracial (32 female); and 121 Caucasian (87 female) (Table 1). The greater number of females in the sample is consistent with previous research showing that females tend to score higher on neuroticism measures than males [27]. In addition, they were more likely to accept participation in the study.

Parental socioeconomic status/family structure. Participants reported the highest level of education attained by each parent; whether their parents received public assistance, and whether their parents lived together. Parental education was coded on a scale of 1–7 (1 = 8th grade or less; 2 = some high school; 3 = high school graduate; 4 = technical/vocational school; 5 = some college; 6 = college graduate; 7 = graduate school education; 8 = unknown). The average of the two parents' education levels was used as our Parental Education variable; if data for only one parent were provided, it was substituted for the average. When education data were missing for both parents (N = 11), missing data points were replaced using multiple imputation for chained equations [28]. We also created dummy variables and used logistic regression–based multiple imputation to replace missing data for parents' public assistance status (N = 12) and marital/residential situation (N = 4).

Presence of psychiatric disorder. Participants were interviewed for the presence of mood and anxiety disorders using the Structured Clinical Interview for DSM-IV-TR (SCID) [25]. Interviews were administered and scored by highly trained graduate students and B.A.-level research assistants under the supervision of Ph.D.-level clinical psychologists. Inter-rater reliability ranged from .72 to .94 for mood and anxiety disorder diagnoses. Because prior research has suggested associations between clinical depression, post-traumatic stress disorder (PTSD), and basal cortisol levels [22], a dummy variable for this was included as a covariate in all analyses for the 15 participants who met the clinical criteria for major depressive disorder (MDD). There were insufficient PTSD cases (N = 1) to include a covariate for this diagnosis. Exclusion of the single PTSD case and the MDD cases had no effect on the results. Thus

Table 1
Descriptive statistics for variables included in these analyses

	Mean (%)	SD	Minimum	Maximum
Socioeconomic variables				
Parents residing together (N = 152)	0.60			
Parents on welfare (N = 17)	0.07			
Parental education, by race				
Parents' education (total)	5.12	1.73	1	7
Parents' education (African-American)	4.41	1.77	1	7
Parents' education (Asian–Pacific Islander)	5.66	1.12	4	7
Parents' education (Caucasian)	5.96	1.09	1	7
Parents' education (Hispanic)	3.25	1.86	1	7
Parents' education (multiracial/other)	5.22	1.23	2	7
Sleep and health variables				
Major depressive disorder (N = 15)	0.06			
Birth control (among females) (N = 21)	0.11			
Nicotine use	0.01	0.06	0.00	0.72
Negative emotion and stress exposure				
Mean chronic life stress rating	2.28	0.36	1.45	3.50
Number of episodic events*severity level	2.54	2.85	0	15.2
Negative emotion average	−0.01	0.67	−1.00	2.35
Negative emotion morning	0.55	0.44	0	2.07
Negative emotion evening	0.62	0.49	0	2.03
Hours of sleep	7.19	0.89	4.00	10.00
Time of awakening	6:49 AM	0.64	4:37 AM	9:52 AM
Time of bedtime cortisol sample	11:08 PM	0.99	8:34 PM	4:30 AM
Dependent variables				
Cortisol slopes across the day	−.0189	.01167	−.09	.01
Number of cortisol data points	12.12	1.52	5	15
Wakeup cortisol levels[a]	.4442	.23399	.04	2.00
Bedtime cortisol levels[a]	.1003	.12652	.01	1.10

[a] Cortisol values indicated are raw scores; those used in the regression analyses were natural log transformed, as is the convention in salivary cortisol research.

we decided to retain these participants to maintain greater statistical power for analyses.

Chronic stress. Participants also participated in semi-structured interviews that assessed their ongoing life stress and satisfaction across nine domains (e.g., intimate relationships, close friendships, social relations with family members, academic performance) over the past year [26]. In each domain, interviewers rated participants on the severity of chronic stress using a scale ranging from 1 (exceptionally good functioning/no stress) to 5 (extreme adversity/impairment). For our analyses, we calculated the average level of chronic stress across all domains.

Episodic life stress. In addition, semi-structured episodic life stress interviews were administered as part of the same interview session [29], yielding information on the number and severity of episodic events experienced in the past 18 months, with severity scores ranging from 1.5 (mild) to 5 (severe). Our measure of episodic stress represents the sum of the severity scores for episodic events rated greater than 2 (mild) in severity.

Cortisol, diary, and health measures

Cortisol data collection. Participants were asked to provide six samples of saliva per day for 3 days. Sampling was scheduled with respect to participants' self-reported wake times. Saliva samples were requested: immediately after waking, 40 minutes after waking, immediately before going to sleep, and at three semi-random times across the day and early evening. Mid-day and early evening samples were prompted by a specially programmed watch (Casio DBC150-1 150-PG Databank) at approximately 2.5, 8, and 12 hours after waking (varying within (±) 30 minutes of these times each day, so as to minimize anticipation of beeps). Saliva was collected by passive drool without use of stimulants; participants expelled saliva through a small straw into a 2-mL polypropylene vial and recorded the exact time on a preprinted label. Participants were instructed not to eat, drink, or brush their teeth in the 30 minutes before sampling; when such events did occur, they were indicated in a diary report accompanying each sample. On average, participants included in these analyses provided 12 of the 15 cortisol samples across the 3 days of testing. Of the participants, 94% had at least 10 samples.

Assay procedures. Completed samples and diaries were returned to the two university-based laboratories by way of a drop box in the school or regular mail. At the laboratories, samples were refrigerated at −20°C until they were sent by

courier to Trier, Germany, to be assayed. Cortisol values are not significantly affected by transport over a period of several days without refrigeration [30]. Samples were assayed in duplicate using a time-resolved immunoassay with fluorometric detection (DELFIA) [31]. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the interassay coefficient of variation was between 7.1% and 9.0%.

Cortisol values were natural logarithmically transformed before analysis to correct a strong positive skew in the cortisol distribution. Slope coefficients were calculated by regressing, for each individual, their natural log-transformed cortisol values on the times of day the samples were collected. The coefficient for the effect of time of day on cortisol level served as an estimate of each individual's diurnal cortisol slope. The second sample each day (i.e., 40 minutes after waking) was excluded from the slope calculation, logic prior work in this area [18], and because prior evidence suggests that the immediate post-awakening cortisol increase may be under a different regulatory influence than the rest of the diurnal cortisol profile [32]. (An additional rationale for this exclusion is that slopes including the 30 minute post awakening sample are highly correlated with, and as such confounded with, the size of the cortisol wakening response [CAR], whereas wakeup to bedtime slopes measures aspects of basal HPA axis activity more independent of the CAR response. These analyses are intended to focus on basal cortisol rhythms.) Slopes were only modestly correlated across days ($r = .200$ to $.270$, $p < .01$), suggesting the presence of substantial day-to-day variation. (These slope estimates correlate .97 with slopes estimated separately each day and then averaged together; slopes estimated through all 3 days of data are used, as they are more reliable and slightly more robust to the effects of missing data.) To aid interpretation, our log-transformed outcome variable was standardized such that a one-unit change in our independent variable represents a one standard deviation change in cortisol slope.

Momentary negative emotion. To provide a measure of experiences of negative emotion on the days of testing, participants completed diary entries six times per day over 3 days at times coinciding with the cortisol sampling, using a modified Experience Sampling Method (ESM) protocol [33]. For each diary entry, participants rated themselves on 12 different mood state adjectives using a three-point Likert scale: nervous, lonely, frustrated, worried, irritable, stressed, sad, happy, active, alert, relaxed, and cheerful. Principal axis factor analysis with an oblimin rotation indicated that the logic negative mood states loaded together onto a single factor: nervous, lonely, frustrated, worried, irritable, stressed, and sad. (A parallel analysis of randomly generated data produced two factors for which the random eigenvalues generated were lower than the corresponding eigenvalues using the actual data. Thus, we determined that there were two true factors, the first of which was used to create the negative emotion/stress variable in these analyses.) Values on these variables were averaged to form a negative emotion composite variable for each sampling point ($\alpha = .83$, $p < .001$); negative emotion stress scores were then averaged across all points available for each person.

Health questionnaire. Variables on the health questionnaire included: age (in years), gender, hours of sleep, use of nicotine, use of oral contraceptive use and/or other medications, and presence of physical health problems. As noted above, participants using steroid-based medications were excluded from these analyses. The impact of other health behaviors and/or medications on cortisol was tested; use of oral contraceptives and nicotine were both related to cortisol levels and were retained as covariates in all models. Sleep behaviors including sleep timing and hours of sleep were also used as covariates.

Data analysis. We first examined whether there were racial/ethnic differences in cortisol slopes across the waking day, using hierarchical multiple regression analyses predicting the cortisol slope coefficients previously calculated for each individual. The effects of potential health confounds were covaried by entering them in the regression model simultaneously with the race/ethnicity dummy variables. We then tested whether any observed racial/ethnic differences in diurnal cortisol slopes were mediated by chronic or episodic life stress or momentary negative emotion, by adding these to the model, and examining changes in the race/ethnicity coefficients. Next, we added parental education, residential status, and welfare receipt to the model to examine whether racial/ethnic differences were attributable to differences in these variables. Finally, we added race by gender interactions to assess whether associations between race and cortisol were moderated by gender. All variables were centered at their mean. Variables with arbitrary scaling were standardized with a mean of 0 and an SD of 1 for ease of interpretation. In a set of follow-up analyses, we regressed our racial/ethnic dummies and full set of covariates on cortisol levels at each of the five measurement points, to identify which points of day were significant contributors to any overall racial/ethnic differences in diurnal cortisol rhythms.

Results

Descriptive statistics

On average, participants experienced declines of $-.02$ μg/dL (raw units) in cortisol per hour between time of awakening and bedtime (Table 1, bottom). Twenty participants did not experience the expected decreases in cortisol across the day: of these, five experienced slight increases; whereas 15 experienced no change (slope = 0). Participants had wakeup cortisol levels averaging .44 μg/dL and ranging from .04 μg/dL to 2 μg/dL. Cortisol levels at bedtime ranged from .01 and 1.1 μg/dL and averaged .10 μg/dL.

Table 2
Correlations among independent variables

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) age	1.00						
(2) waketime	0.00	1.00					
(3) hours of sleep	0.06	−0.01	1.00				
(4) major depressive disorder	−0.06	−0.04	0.02	1.00			
(5) male	−0.01	0.10	0.07	0.08	1.00		
(6) nicotine use	0.03	0.09	−0.08	−0.03	−0.03	1.00	
(7) oral contraceptives	0.04	0.06	0.10	−0.07	−0.18**	0.03	1.00
(8) mean chronic stress	−0.08	−0.22**	−0.08	0.35**	0.01	0.15*	−0.04
(9) average negative mood	0.03	0.01	−0.09	0.28**	−0.16**	0.07	0.20**
(10) morning negative mood	0.07	0.06	−0.06	0.25**	−0.13*	0.02	0.18**
(11) evening negative mood	0.03	−0.00	−0.11^{+}	0.18**	−0.15*	0.07	0.23**
(12) episodic stress	0.07	−0.10	−0.04	0.16**	−0.14*	0.17**	0.15*
(13) parents reside together	0.06	0.11^{+}	−0.03	−0.12^{+}	−0.00	−0.10	−0.06
(14) public assistance	−0.01	−0.01	−0.01	−0.01	0.00	0.11^{+}	−0.02
(15) parental education	0.14*	0.14*	−0.02	−0.13*	0.03	0.06	0.09
(16) bedtime	0.01	0.20**	−0.52**	−0.06	−0.01	0.08	−0.09
(17) African-American	0.04	−0.06	−0.07	0.02	−0.03	−0.05	−0.10
(18) Asian Pacific Islander	−0.03	0.16*	−0.21**	−0.06	0.12^{+}	0.09	−0.07
(19) Hispanic	−0.07	−0.14*	0.13*	0.20*	−0.11^{+}	−0.01	−0.16*
(20) multiracial/other	−0.07	0.02	−0.05	−0.07	−0.00	−0.05	0.17**
(21) Caucasian	0.10^{+}	0.07	0.06	−0.10	0.06	0.03	0.09

$^{+}$ $p < .10$.
* $p < .05$.
** $p < 01$.
*** $p < .001$.

Correlations among predictor variables

Before examining associations between race/ethnicity and diurnal cortisol, we first examined how race/ethnicity was correlated with self-reported chronic stress, episodic events, momentary emotion, and other health and demographic factors (Table 2). Hispanic and African-American youth had higher levels of chronic stress and had parents with lower levels of education. African-Americans were less likely to have parents that were married and more likely to be on welfare. Asian youth had later bedtimes, later waketimes, and fewer overall hours of sleep than other youth.

Diurnal slopes across the day

Model 1. In the first regression model (Table 3), diurnal cortisol slopes were found to be significantly flatter for African-Americans and Hispanics as compared with Caucasians, with age, gender, presence of MDD, nicotine use, and sleep hours and time included as covariates (Figure 1). Note that because diurnal slopes typically decline from waketime to bedtime, a higher coefficient corresponds to a flatter slope. There were no significant differences in cortisol slopes for Asian/Pacific Islander and multiracial/other youth. In addition to racial/ethnic differences in cortisol slopes, nicotine use (smoking) predicted significantly flatter diurnal rhythms, in accordance with prior research [18]. The first model, including only race/ethnicity and sleep and medical covariates, explains 14% of the total variance in cortisol slopes. (We also conducted a repeated-measures analysis of covariance, with time of sampling as a within-person factor, and race/ethnicity and other covariates as between-person factors. As expected, this revealed a significant main effect for sampling occasion [Wilks' lambda $F(4, 226) = 2.7$, $p < .05$], and a significant race/ethnicity by sampling occasion interaction [Wilks' lambda $F(6, 691) = 2.8$, $p < .001$], supporting our regression results indicating that associations between time of sampling and cortisol vary by race/ethnicity.)

Model 2. In the next model, we examined whether racial/ethnic differences in cortisol slopes were mediated by levels of chronic or episodic stress and/or momentary negative emotion. After including these variables as covariates (Model 2 in Table 3), the differences between African-Americans and Hispanics, relative to Caucasians, remained nearly identical to those in Model 1. Formal tests of mediation failed to provide evidence that levels of negative emotion and stress explained racial/ethnic differences in cortisol slopes [34]. (Mediation was tested using an SPSS macro that examines the direct and indirect of race/ethnicity, stress exposure, and socioeconomic status variables [34], while including health and sleep variables as covariates. It uses bootstrapping to allow for asymptotic distributions and is believed to be more effective at identifying mediation in samples of small to moderate size.) Participants who reported higher levels of negative emotion on the days of testing did, however, have flatter ($p < .05$) cortisol

(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1.00								
0.13*	1.00							
0.10	0.86**	1.00						
0.14*	0.85**	0.68**	1.00					
0.30**	0.22**	0.23**	0.21**	1.00				
−0.38**	−0.04	−0.06	−0.05	−0.12^{+}	1.00			
0.18**	−0.07	−0.10	−0.05	0.02	−0.11^{+}	1.00		
−0.28**	0.15*	0.18**	0.13*	−0.03	0.14*	−0.09	1.00	
−0.07	0.02	0.04	0.11^{+}	0.04	0.12^{+}	0.02	−0.11^{+}	1.00
0.18**	−0.01	0.03	−0.01	0.04	−0.20**	0.19**	0.13*	0.09
0.03	−0.06	−0.05	0.01	−0.00	0.07	0.08	−0.11^{+}	0.18**
0.17**	−0.12*	−0.15*	−0.16**	0.02	0.01	−0.01	0.49**	−0.14*
0.05	0.02	0.09	0.02	0.08	−0.07	0.05	−0.11^{+}	−0.00
−0.30**	0.12	0.06	0.12*	−0.10	0.14*	−0.18**	−0.35**	−0.01

slopes, even after accounting for the effects of race/ethnicity. Including negative emotion and episodic and chronic stress covariates increased the proportion of the variance explained from 14% to 16%.

Model 3. Next, we examined whether the observed racial/ethnic differences could be explained by differences in socioeconomic status. Although 83% of parents were high school graduates, only 43% had college degrees. The addition of parents' average levels of education, marital status, and public assistance receipt did not significantly change the slope differences for African-Americans or Hispanics, relative to Caucasians. Formal tests of mediation failed to provide evidence that these SES variables mediated associations between race and cortisol slopes [6]. The inclusion of these factors increased the variance explained from 16% to 17%.

Model 4. Finally, when race by sex interactions (created by multiplying each race dummy by gender) were included, African-American males were found to have significantly flatter slopes than same-race females by 1.21 SD ($p < .05$). In contrast, Caucasian males did not differ significantly from their same-race female counterparts, and if anything, they may have steeper slopes (−0.06 SD). The sample of African-American males was very small (N = 6); this race-by-gender interaction should therefore be considered with caution and replicated in a larger sample. Overall, the final model explained 20% of the total variance in cortisol slopes.

To help interpret the racial/ethnic differences in slopes, we investigated whether the slope differences for African-Americans and Hispanics were attributable to differences in wakeup, late morning, mid-afternoon, evening, or bedtime cortisol levels There were no significant racial/ethnic differences in the midday cortisol points. African-Americans showed significantly lower cortisol levels by −.46 SD ($p < .05$) upon waking and significantly higher cortisol levels at bedtime by .53 SD ($p < .05$), relative to Caucasians, covarying exposure to life events, mean chronic stress, negative emotion/stress, and socioeconomic variables, a finding which is consistent with earlier research by Cohen et al [18] (Figure 2). Hispanic participants also had significantly higher bedtime cortisol levels than Caucasians by .39 SD ($p < .05$), including health and stress exposure variables as covariates, although these differences were reduced to nonsignificance when SES factors were included as covariates (.28 SD flatter, $p > .10$). Formal tests of mediation failed to provide evidence that the SES variables included here mediated associations between race and cortisol slopes [6].

African Americans and Hispanics were also overrepresented among the group of individuals with flat or positive profiles as compared with those with the more typically declining cortisol rhythm: 20% of those with flat or positive profiles were African-American, as compared with 10% of those with normal rhythms; 35% of the flat or positive group were Hispanic, whereas Hispanics comprise only 20% of the normal rhythm group.

Table 3
Cortisol slopes from wake time to bedtime regressed on race/ethnicity (N = 255)

	(1)		(2)		(3)		(4)	
	B	SE	*B*	SE	*B*	SE	*B*	SE
Covariates								
Bedtime sample time	0.17*	(0.07)	0.17*	(0.07)	0.17*	(0.08)	0.19*	(0.08)
Wakeup sample time	−0.15	(0.10)	−0.14	(0.10)	−0.14	(0.10)	−0.19*	(0.10)
Hours of sleep	−0.04	(0.08)	−0.02	(0.08)	−0.02	(0.08)	−0.01	(0.08)
Major depressive disorder	0.38	(0.26)	0.12	(0.29)	0.10	(0.29)	0.06	(0.29)
Nicotine[a]	3.14†	(0.95)	2.90†	(.98)	2.93†	(0.99)	2.57	(0.99)
Age	0.16	(0.16)	0.16	(0.16)	0.15	(0.16)	0.15	(0.16)
Male	0.01	(0.14)	0.05	(0.15)	0.05	(0.15)	−0.06	(0.15)
Racial/ethnic background								
African-American	0.64†	(0.21)	0.62†	(0.22)	0.68†	(0.23)	0.67†	(0.23)
Hispanic	0.39*	(0.17)	0.44*	(0.18)	0.49*	(0.20)	0.49†	(0.20)
Asian–Pacific Islander	0.01	(0.31)	0.04	(0.31)	0.08	(0.31)	0.14	(0.34)
Multi-racial/other	0.06	(0.17)	0.06	(0.18)	0.08	(0.18)	0.06	(0.18)
Negative emotion/stress								
Mean chronic stress			0.07	(0.07)	0.07	(0.08)	0.09	(0.08)
Negative emotion			0.14*	(0.07)	0.13+	(0.07)	0.11‡	(0.07)
Episodic stress			−0.02	(0.07)	0.03	(0.07)	0.01	(0.02)
Parental socioeconomic status								
Parent education					0.03	(0.05)	0.04	(0.04)
Parents reside together					−0.09	(0.14)	−0.09	(0.14)
Parents on welfare					−0.32	(0.25)	−0.27	(0.25)
Race by gender interactions								
African-American male							1.21*	(0.49)
Hispanic male							0.37	(0.40)
Asian-American male							1.03‡	(0.59)
Multi-racial male							−0.13	(0.39)
R^2	0.14		0.16		0.17		0.20	

Model 1 includes race and health/sleep covariates. Model 2 adds stress exposure and negative emotion. Model 3 adds SES factors. Model 4 adds race-male interaction terms. Reference category for male is female. Reference category for all racial/ethnic groups is Caucasian. Reference group for residential status is residing apart. Models also include study site (NS) and oral contraceptive use (NS).

* $p < .05$.

† $p < 01$.

‡ $p < .10$.

[a] Nicotine refers to the percentage of diary entries in which participants indicated that they had smoked during the previous hour.

Discussion

This study replicates the findings of Cohen et al [18] using the CARDIA dataset of flatter diurnal cortisol rhythms among African-Americans, relative to Caucasians, driven by both lower wakeup and higher bedtime cortisol levels. It also extends this work in important ways. First, we find that racial differences in cortisol patterns are also present for Hispanics, and begin to emerge at least as early as late adolescence. We find that differences in slopes are moderated by gender among African-American adolescents, with slopes being flatter among African-American males than females. This latter effect must, however, be interpreted with caution in light of the fact that the sample of African-American males is extremely small (N = 6). In addition to replicating the finding that socioeconomic status and current life stress were not significant mediators of racial/ethnic differences in cortisol slopes [18], we also tested the role of negative emotion on the days of testing. Although greater negative emotion predicted flatter diurnal cortisol slopes, there was no evidence that this accounted for associations between race/ethnicity and cortisol.

Interpretation of the observed diurnal cortisol patterns

Given the correlational nature of our data, there is no way to determine whether the observed racial/ethnic differences in diurnal cortisol slopes are environmental or genetic in origin. Nonetheless, it seems likely that these differences are at least partially environmental. Flatter slopes among Hispanics are due solely to higher bedtime cortisol levels, and higher bedtime levels also contribute to the differences between African-Americans and Caucasians. Prior research indicates that bedtime levels are more strongly influenced by social factors; although morning levels have higher heritability quotients [35].

Interestingly, however, the social-contextual variables in our study (e.g., SES, chronic and episodic life events, and

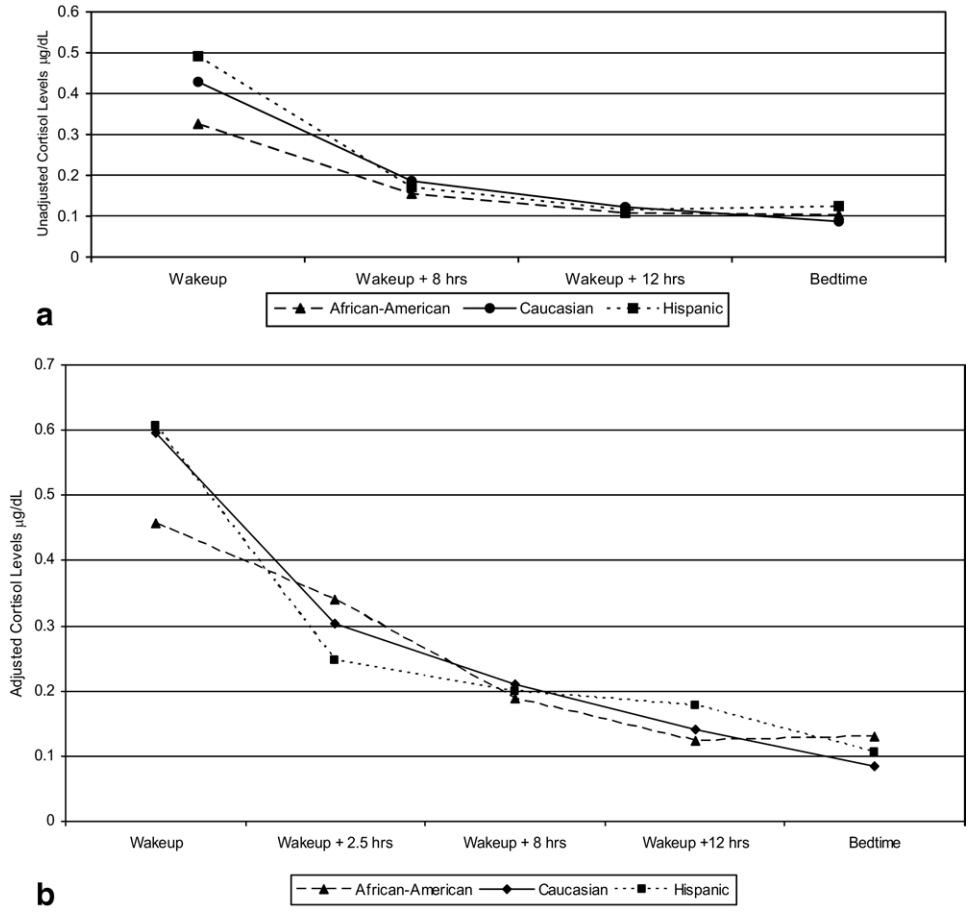

Figure 1. (a) Racial/ethnic differences in cortisol slopes across the waking day (raw values). (b) Racial/ethnic differences in cortisol slopes (adjusted values including covariates).

negative emotion) failed to explain the racial/ethnic differences in cortisol slopes. For both African-Americans and Hispanics, it seems likely that the measures of chronic and episodic stress used failed to capture certain aspects of the experience of life as a minority in the U.S., such as experiences of discrimination (although discrimination did not mediate racial/ethnic differences in the CARDIA study [18]). The challenges in accurately measuring concepts such as discrimination and social and economic disadvantage are enormous. Moreover, prior research indicates that African-Americans and Hispanics are more prone to socially desirable reporting biases, such that they underreport negative emotion and undesirable events [36]. Such biases would hinder our ability to examine accurately whether current life stress and levels of negative emotion account for racial/ethnic differences in cortisol levels.

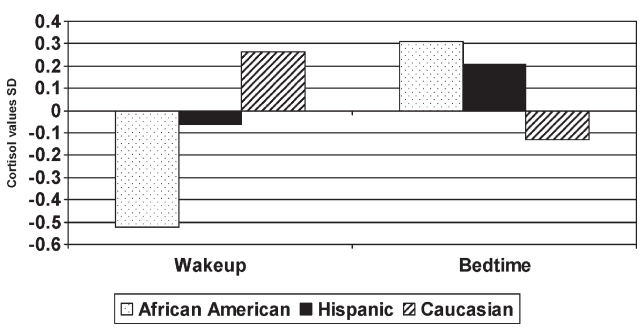

Figure 2. Standardized values of logged wakeup and bedtime cortisol levels by race/ethnicity (unadjusted).

Even if one were to measure current social conditions accurately, however, a lifetime of experience (beginning as early as the prenatal period) may have already modulated the functioning of the adolescent HPA axis, such that current differences may reflect organizational influences of prior experiences that are no longer present in current environments. Early stressful experiences, either independently or in interaction with current experiences, have been shown to be important influences on current HPA axis functioning [14,37]. It is thus possible that prior exposure to discrimination, economic strain, unsafe neighborhood conditions, parental depression, prenatal stress, low birth weight, and numerous other social disadvantages may help to account for the current differences. We call for an exam-

ination of racial/ethnic differences in HPA axis functioning at even younger ages and, more importantly, for longitudinal research on changes in diurnal cortisol slopes over time among the same individuals.

Limitations of the study

The generalizability of the results of these analyses is restricted by a number of factors. As these data were originally collected to examine risk for the development of psychopathology, adolescents at risk for developing psychopathology are overrepresented in the sample. Although significant racial/ethnic differences in cortisol rhythms remain when participants with MDD and/or PTSD are excluded from the analyses, it will be important to replicate these results with a normative or, better yet, nationally representative sample of adolescents. Our study includes adolescents from racially and socioeconomically diverse communities; unfortunately, however, there was limited socioeconomic diversity within each racial/ethnic group. In addition, the numbers of African-American and Asian American participants are relatively small, and there are also relatively few males in the sample. In addition, 97 of the participants asked to complete these procedures refused to participate, and we cannot know how they would have influenced these results. Finally, we do not currently have information about the participants' body mass indexes, which could help to explain a portion of race–cortisol associations. In future waves of data collection for this study, participants will be asked about their height and weight.

Implications of the observed diurnal cortisol patterns

The fact that we found higher bedtime cortisol levels among both African-American and Hispanic participants suggests either continued stress exposure into the evening hours or a failure to "turn off" the stress-response system in the evening. Such a phenomenon mirrors research on cardiovascular systems, in which African-Americans are more likely to fail to experience the expected decrease in resting blood pressure in the evening than Caucasians [38]. The reason and the physiological mechanism for lower waking cortisol levels is less clear, although it seems possible that reduced morning basal levels serve as a protective mechanism, as the body prepares itself for stress-related elevations throughout the rest of the day. It is also possible that, on a more acute day-to-day basis, higher bedtime cortisol levels caused by environmental demands during the day feed back directly to reduce cortisol levels the next morning.

Although the exact physiological mechanism by which loss of strong circadian rhythm in cortisol occurs is unclear, the association between this type of flattened profile and both adverse experiences and adverse health outcomes has become increasingly evident [15]. Thus this pattern of HPA axis activity could have implications for understanding the origins of health disparities between African-Americans, Hispanics, and Caucasians in the U.S. The fact that racial/ethnic differences in cortisol slopes are evident as early as adolescence, before the majority of stress-related disorders are clinically evident, suggests the possibility that cortisol differences could play an early role in the etiological pathway for the development of stress-related disorders. Beyond physical health effects, it is worth considering whether diurnal cortisol differences may have implications for disparities in emotional health, behavior, and achievement, given that dysregulation of HPA activity has also been implicated in depression [22], emotion regulation [39], and cognition [40].

The origin of racial/ethnic differences in HPA axis functioning, and whether these differences help to account for associations between race/ethnicity and physical and mental health, education, and/or behavioral and socioemotional outcomes, are crucial questions for future research. Reducing health disparities and closing the "achievement gap" have been proclaimed among the most important goals by the National Institutes of Health and the Department of Education, respectively [1]. Differences in HPA axis activity have not been the first factor to which researchers look to explain racial/ethnic differences in morbidity rates for nearly every health indicator or persistent gaps in educational achievement. They certainly are not the sole causes for such disparities. However, the existence of racial/ethnic differences in HPA axis activity patterns and the known impact of the HPA axis on emotional, cognitive, and physiological functioning suggest that this is a reasonable mechanism to consider. As such, the potential role of racial/ethnic differences in HPA axis activity, and the social-contextual factors contributing to these differences, merits further consideration by researchers and policymakers interested in improving our understanding of the pathways through which racial and ethnic inequalities operate to perpetuate disadvantage.

To our knowledge, this study is the first to suggest that racial/ethnic differences in cortisol rhythms emerge as early as late adolescence. In future research, it will be important to identify: (a) exactly how early in development these differences begin to emerge; (b) the extent to which these represent stable differences or temporary alterations in response to immediate experience; (c) the physiological systems that are affected by HPA activation; (d) whether differences in past and present social experiences such as individual experiences with racism, stressful life events, familial environments, or institution- or community-level factors play a role; and (e) the immediate and long-term impacts on health, cognition, and psychological well-being of flattened diurnal cortisol rhythms in adolescents. In addition, it will be important to explore possible mechanisms for intervention, which, if the origins of HPA axis differences are indeed environmental in origin, are likely to occur most effectively at a social rather than physiological level.

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