Diurnal Cortisol Decline is Related to Coronary Calcification: CARDIA Study

KAREN MATTHEWS, PHD, JOSEPH SCHWARTZ, PHD, SHELDON COHEN, PHD, AND TERESA SEEMAN, PHD

Objective: Chronic stress may be a risk factor for coronary heart disease and is associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. We tested the hypotheses that two markers of HPA axis dysregulation, elevated average level (area under the curve, adjusted for time awake) and diurnal decline of salivary cortisol, were associated with presence of coronary calcification (CaC). **Method:** Seven hundred eighteen black and white middle-aged adults enrolled in an ancillary study of Coronary Artery Risk Development in Young Adults provided six salivary cortisol samples throughout one full day and had measurements of CaC. **Results:** The prevalence of any calcification was low, 8.1% in the participants of the ancillary study, with white men having the highest proportion. Average cortisol did not differentiate groups, means = 2.15 and 2.08. Those with any CaC declined approximately 6% per hour in cortisol over the course of the day, whereas those with no CaC declined more than 8% per hour ($p < .003$). Those persons with slope scores in the flattest quartile had a greater likelihood of any CaC than did those in the remaining quartiles adjusted for sex–race group, age, smoking, treatment for diabetes, systolic blood pressure, logged triglycerides, average cortisol, and educational attainment (odds ratio = 2.58; 95% confidence interval = 1.26–5.30). **Conclusions:** Our results are consistent with the hypothesis that HPA axis dysregulation may affect risk for atherosclerosis. **Key words:** coronary calcification, neuroendocrine, stress, cortisol.

CHD = coronary heart disease; **HPA** = hypothalamic–pituitary–adrenal; **CARDIA** = Coronary Artery Risk Development in Young Adults; **CaC** = coronary artery calcification; **BMI** = body mass index; **LDL-C** = low-density lipoprotein cholesterol; **HU** = Hounsfield unit; **BP** = blood pressure; **HDL** = high-density lipoprotein; **AUC** = area under the curve; **SBP** = systolic blood pressure.

INTRODUCTION

Cumulative exposure to chronic stressors may be a risk factor for coronary heart disease (CHD (1)). Individuals who are exposed to high levels of demands and low levels of control at work, to distressing marriages, and to low social support are more likely to have incident CHD than their less stressed counterparts (2–4). Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is one of the pathways through which chronic stress may affect CHD risk. In healthy individuals, cortisol has a distinct diurnal pattern with the peak cortisol occurring in the early morning, declining throughout the day, and reaching a nadir around 2 or 3 AM (5). Cortisol also exhibits sizable, short-term increases during the first hour after awakening and in response to a lunch meal or a threat-provoking stressor. Aging is associated with increased mean 24-hour plasma cortisol, but the diurnal variation is preserved (5). Dysregulation can take the form of altered overall levels of cortisol or a smaller decline in cortisol throughout the day and evening, i.e., flatter slope.

The literature clearly supports that chronic stress in both animal and human studies alters the ability of the HPA axis to recover from challenges (6). Elevated cortisol levels are associated with depressive symptoms (7), anxiety (8), caregiver stress (9), unemployment (10), and overall negative affect (11). Elevated evening cortisol, resulting from a smaller cortisol decline throughout the day, is associated with unhappy marriages (12), low positive affect (men only (11)) and depressive symptoms, low network diversity, support, feelings of control, and low socioeconomic status (13). No epidemiologic studies have tested the association between cortisol level and pattern and risk for CHD, although a higher level of morning cortisol has been observed among men with moderate to severe coronary atherosclerosis than those with no detectable disease (14). In the ongoing epidemiologic study of young and middle-aged adults called the Coronary Artery Risk Development in Young Adults (CARDIA (15)), we tested the hypothesis that coronary calcification (CaC) is associated with average awake cortisol level and diurnal pattern, i.e., the average rate of decline throughout the day.

METHODS

Participants

CARDIA is an ongoing prospective, multicenter study of the natural history of cardiovascular risk development starting in young adulthood. Participants were initially recruited in 1985 to 1986 to achieve a balance at each of four sites by race (black, white), sex, and education (high school degree or less vs. more than a high school degree). Detailed description of the original sampling procedure is available elsewhere (15). Participants were examined at study entry and years 2, 5, 7, 10, and 15 with the reexamination rate among surviving cohort members at year 15 of 73.5%. Comparisons of CARDIA participants in the year 15 examination with nonparticipants showed that nonparticipants were more likely to be black, younger, less educated, and smokers (16). Site institutional review committee approval and informed consent were obtained for each examination. The CARDIA Publications Committee approved this article.

At the year 15 examination conducted in 2000 to 2001, participants at the Oakland and Chicago sites living within 50 miles of the clinic were invited to participate in an ancillary study of socioeconomic status, which included assessment of salivary cortisol. Informed consent was obtained separately for participation in the ancillary study. Of the 1336 participants who were eligible for the ancillary study, 836 (62.6%) agreed to participate and 808 returned salivettes containing saliva. Excluded from analysis were two because of inadequate data on the times that samples were collected, 25 because they awoke after 11 AM and had a distinctly different cortisol pattern from the remainder of the sample, one who reported having had a heart attack, and 62 who did not have CaC measures. The final sample was 151 and 235 black men and women, respectively, and 154 and 178 white men and women, respectively. Among those who participated in the CaC protocol at the two sites, participants in the ancillary study had higher body mass index (BMI), less education, and were more likely to be black compared with nonparticipants in the ancillary study at the two sites; they were similar in age, blood pressure, low-density lipoprotein cholesterol (LDL-C), and percentage of women, current smokers, or those having any calcification.

From the Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania (K.M.); the Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, New York (J.S.); the Department of Psychology, Carnegie Mellon University, Pittsburgh, Pennsylvania (S.C.); and the Division of Geriatrics, UCLA School of Medicine, Los Angeles, California (T.S.).

Address correspondence and reprint requests to Karen Matthews, PhD, Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, Pittsburgh, PA 15213. E-mail: matthewska@upmc.edu

Received for publication August 25, 2006; revision received May 30, 2006.
DOI: 10.1097/01.psy.0000244071.42939.0e

0033-3174/06/6805-0657

Coronary Calcification

Coronary calcification was measured by using an Imatron C-150 electron beam scanner. A committee of cardiologists, radiologists, and a physicist developed a scanning protocol to standardize scan acquisition, which included two 2.5- to 3-mm thick electrocardiogram-gated scans completed within 100 to 520 ms on each participant using a hydroxyapatite phantom to allow standardization of image brightness. With the help of specialized image processing software, trained readers identified the presence of CaC in each scan (17). A total CaC score was calculated for each scan by multiplying the area of the focus by a coefficient ranging from 1 to 4 based on the peak density in the focus (1 = 130–200 Hounsfield units [HU], 2 = 201–300 HU, 3 = 301–400 HU, and 4 = 401+ HU) according to the method described by Agatston (18). All readers were blind to participant characteristics. Both between- and within-reader reproducibility were high (17).

Other Relevant Measures

Standardized questionnaires were used to assess age, race, sex, treatment for diabetes, and years of education. At each examination, three seated blood pressure (BP) measurements at 1-minute intervals were taken on the right arm using a Hawksley random zero sphygmomanometer (WA Baum Co., Copaigue, NY) after a 5-minute rest. Systolic BP and diastolic BP were recorded as phase I and phase V Korotkoff sounds, and the latter two measures were averaged. BMI was calculated as measured weight (in kilograms) divided by height squared (in meters). Smoking status was categorized as currently smoking at least five cigarettes per week, yes/no. A fasting blood draw was conducted and total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were measured (19) with LDL-C calculated by the Friedewald equation.

Cortisol Protocol and Data Reduction

Cortisol was measured in saliva as opposed to plasma because of the epidemiologic and ambulatory nature of the study. Salivary "free" cortisol levels track closely with plasma levels, correlations ranging from 0.70 to 0.90 (20) showing a 1- to 2-minute lag, and have been previously used to monitor cortisol activity in both ambulatory and laboratory challenge paradigms (21–22). Participants were given materials and instructed regarding the collection of the saliva samples at the conclusion of their year 15 follow-up CARDIA clinic visit. Samples were collected from participants on a single weekday, in most cases the Monday after a Friday or Saturday clinic visit. They provided six saliva samples over the course of the day: at awakening ("when your eyes open and you are ready to get up"), 45 minutes, 2.5 hours, 8 hours, and 12 hours after awakening, and at bedtime ("right before getting into bed"). Participants were instructed to record the time they woke up in a log and were provided with alarm watches (preset to their regular wakeup time) to remind them to collect samples; they were also given a form that allowed them to easily recalculate the desired sample times if they woke up at a different time than anticipated.

To provide a saliva sample, participants placed a roll of cotton in their mouths, chewed on it for approximately 30 seconds or until it became saturated, and placed it in a tube called a salivette (Sarstedt, Rommelsdorft, Germany). They then filled in the time of the sample on the tube label. The investigators collected all six salivettes the next morning. Salivettes were refrigerated at each site until they were assayed. Cortisol level was determined by time-resolved immunoassay with fluorometric end point detection (23). Nine samples with levels below the minimum detectable level (0.7 nmol/L) for this assay were assigned values of 0.5 nmol/L. Intra- and interassay variabilities were each less than 12%.

Not every cortisol sample was taken precisely at the intended time. For each of the six targeted times, a window relative to time since awakening was established for considering the sample collected at the appropriate time. This window was narrowest for the morning sample as a result of the typical pattern of a rapid increase in cortisol during the first 30 to 60 minutes after awakening (the "morning rise") followed by a more gradual, but steady, decrease during the rest of the day. The targeted times and windows were as follows: +15 minutes for wakeup, +15 to +90 minutes for +45 minutes, +2 to +3.5 hours for +2.5 hours, +7 to +9 hours for +8 hours, +11 to +13 hours for +12 hours, and +12 to +20 hours for bedtime. The sixth sample was not targeted to a specific time. The window for each target time was determined from preliminary analyses documenting the interval over which there was no systematic tendency for cortisol levels to differ from those obtained within ±5 minutes of the target time. A sample provided outside the acceptable window was excluded from analyses of samples for that targeted time.

Analyses reported subsequently were performed separately on each of the six targeted cortisol samples after natural logarithmic transformation to reduce the positive skewness. However, the main focus was on two summary measures of cortisol: area under the curve (AUC) and slope. AUC, a time-adjusted measure of total cortisol exposure while awake, was calculated as the AUC defined by the plot of log-transformed cortisol values against collection times multiplied by 16 hours and divided by the duration, in hours, between the first and last sample. The AUC measure was computed only for those who had data for the first and/or second sample and a minimum of 12 hours between their first and last samples; 18 were excluded from AUC analyses. The slope was calculated for those who had at least the first or second sample and the fifth or sixth sample; 13 participants were therefore excluded from the slope analyses; six participants had slopes calculated based on the second cortisol sample rather than the first. The slope was estimated by fitting a linear regression line separately for each participant that predicted the log-transformed cortisol values from time (hours) since awakening. To minimize the impact of the morning rise on the estimation of slopes, the second saliva sample was excluded from the calculation of slopes for all but the six otherwise eligible participants who did not have a first cortisol sample collected within 60 minutes of awakening. Because the AUC and slope were based on real time, it was not necessary to exclude available data outside the targeted windows.

Statistical Analyses

Data are reported as means and standard deviations or proportions, and preliminary analyses by *t* tests were conducted on differences between those with and without any calcification. Because only a small minority of participants had any measurable calcification, we used logistic regression analysis to predict presence/absence of measurable calcification. (Preliminary Tobit conditional regression analysis, which applies linear regression to continuous data [CaC scores] did not reveal a graded relationship with extent of calcification.) Initial models adjusted for race–gender group, treatment for diabetes, and age; subsequent models also adjusted for risk factors that were associated with CaC ($p < .10$): smoking status, systolic blood pressure (SBP), logged triglycerides, and education attainment. To test whether the relationship between cortisol's diurnal pattern and CaC or AUC was linear or nonlinear, we collapsed the distribution of diurnal slopes into quartiles (using cut points of −0.11758, −0.8775, and −0.049955) and examined their relationship to CaC. To examine if the effect of slope was independent of the level of cortisol, we investigated whether the results changed when also adjusting for AUC.

RESULTS

On average, participants were 40 years old with almost 20% smokers (Table 1). Mean BMI of the total and four sex–race subgroups was within the overweight range, except for black women whose mean was in the obese range. The number of participants with measurable calcification was low in this relatively young sample. Men were more likely to have measurable coronary calcification than women with white men having the highest and white women the lowest prevalence. Participants with CaC did not differ in time since awakening at sample collections from those with no CaC (all $p > .15$).

Although participants with calcification did not differ significantly from those without calcification on any of the six individual cortisol samples, there was a tendency for the last

TABLE 1. Sample Characteristics for Participants in the CARDIA Ancillary Study With Coronary Artery Calcification Measures, 2000 to 2001

Variable	Black Men (n = 151)	Black Women (n = 235)	White Men (n = 154)	White Women (n = 178)	Total (n = 718)
Mean age, years (SD)	39.5 (3.6)	39.9 (3.9)	40.1 (3.5)	40.9 (3.3)	40.1 (3.6)
Mean body mass index, kg/m^2 (SD)	29.6 (6.2)	32.3 (8.1)	27.5 (4.5)	26.4 (6.9)	29.2 (7.2)
Mean education, years (SD)	14.1 (2.1)	14.1 (2.2)	15.6 (2.6)	16.0 (2.3)	14.9 (2.4)
Mean systolic blood pressure, mm Hg (SD)	117.4 (13.7)	117.0 (15.4)	112.3 (11.0)	107.1 (13.4)	113.6 (14.3)
Mean high-density lipoprotein cholesterol, mg/dL (SD)	47.1 (13.4)	53.3 (12.8)	43.1 (11.1)	56.4 (13.7)	50.5 (13.8)
Mean low-density lipoprotein cholesterol, mg/dL (SD)	118.1 (34.0)	110.7 (30.9)	123.7 (34.5)	104.3 (26.1)	113.4 (32.0)
Mean triglycerides, mg/dL (SD)	106.3 (76.3)	83.9 (44.8)	153.6 (229.8)	93.8 (55.7)	106.3 (121.5)
Percent current smoker	27.2	25.2	11.0	11.8	19.2
Percent being treated for diabetes	2.6	3.8	1.9	0	2.2
Percent any coronary artery calcification	11.3	6.0	14.3	2.8	8.1

SD, standard deviation.

three samples to be higher in those with calcification (all $p <$ .20). As a result, those with calcification had flatter diurnal slopes of cortisol than did those without calcification, although there were no differences by group in cortisol AUC (Table 2). The mean slope of −0.061 for the group with any calcification means that, on average, (untransformed) cortisol decreased at a rate of approximately 6% per hour over the course of the day, whereas the mean slope of −0.084 means that on average cortisol decreased at a rate of more than 8% per hour over the course of the day.

The association between cortisol slopes (continuous) and having any calcification was significant in the logistic regression model adjusting for sex–race group, treatment for diabetes, and age (Table 3, row 1). The analysis based on the four quartiles of the cortisol slope showed that compared with the quartile with the steepest cortisol slopes, the quartile with the flattest slopes was approximately 3⅓ times more likely to have any calcification, whereas there were no differences among the remaining quartiles (all $p > .70$) (controlling for sex–race group, treatment for diabetes, and age; see Fig. 1). Compared with the three other quartiles combined, those in the quartile with the flattest slopes were markedly more likely to have any calcification. A similar analysis using AUC as the predictor variable did not show a nonlinear association with CaC.

TABLE 2. Mean (standard deviation) of Logged Salivary Cortisol Measures According to Calcification Group

Salivary Cortisol Level (nmol/L, logged) at	Coronary Calcification: Yes (N = 58)	Coronary Calcification: No (N = 660)	p Value[a]
Awakening	2.71 (0.71)	2.81 (0.60)	.23
+3/4 hour	3.04 (0.61)	3.11 (0.55)	.38
+2.5 hr	2.51 (0.55)	2.54 (0.59)	.70
+8 hr	2.25 (0.55)	2.11 (0.66)	.11
+12 hr	1.76 (0.78)	1.59 (0.73)	.12
Bedtime (mean = +16.8 hr)	1.67 (0.93)	1.51 (0.90)	.19
Area under curve (per 16 hr)	2.15 (0.47)	2.08 (0.49)	.30
Slope (change per hour)	−0.061 (0.064)	−0.084 (0.054)	.003

[a] Calculated with t test.

These associations remained statistically significant when adjusted for race–gender groups, age, current smoking, SBP, triglycerides, and educational attainment (Table 3, row 2). Additionally controlling for AUC, a time-weighted estimate of average cortisol during the day did not substantially alter the relationship.

The Hosmer and Lemeshow goodness-of-fit statistic was used to estimate the validity of the logistic regression models presented in Table 3. For none of these models was there evidence of a poor fit; the significance levels for this test ranged from p = .09 to .90 (mean p = .38), in which a nonsignificant result indicates an adequate or good fit.

DISCUSSION

The present article evaluated whether markers of HPA function, i.e., average cortisol or diurnal pattern throughout the day, were related to presence of coronary calcification in young and middle-aged black and European American men and women. Results showed that the flatter the cortisol slopes throughout the day, the greater the likelihood of any coronary calcification with the suggestion that the flatter slopes were the result of elevated afternoon and evening levels as opposed to lower morning levels. Further inspection of the data showed that the relationship was nonlinear. That is, the flattest quartile of cortisol slopes was associated with the presence of calcification. These associations remained statistically significant in models adjusting for sex–race group, age, treatment for diabetes, cigarette smoking, SBP, triglycerides, educational attainment, and average cortisol levels measured across the day. To our knowledge, this is the first epidemiologic study to show that the cortisol pattern is related to risk for coronary atherosclerosis.

To date, cortisol (and dysregulated HPA axis activity more generally) has not been the focus of investigation as a potential contributor to coronary atherosclerosis. The plausibility of a relationship between cortisol patterns and atherosclerosis is in accord with a number of recent findings. Cortisol abnor-

TABLE 3. Odds Ratio (95% confidence intervals) and *p* Values From Logistic Regression Models Testing the Relationship Between Diurnal Salivary Cortisol Slope and Presence/Absence of Any Coronary Calcification

Covariates	Continuous Slope		Quartiles of Slope: Flattest vs. Steepest Quartile		Flattest Slope Quartile vs. All Other Quartiles	
	Odds Ratio	*p*	Odds Ratio	*p*	Odds Ratio	*p*
Age, sex–race groups, and treatment for diabetes	2.54 (1.42–4.53)	0.002	3.36 (1.48–7.61)	0.004	3.12 (1.71–5.70)	0.0002
Above covariates and systolic blood pressure, current smoking status, (log) triglycerides, educational attainment	1.70 (0.91–3.17)	0.10	1.95 (0.81–4.67)	0.13	2.05 (1.06–3.97)	0.03
Above covariates and area under the curve cortisol	2.13 (1.06–4.29)	0.03	2.59 (0.99–6.73)	0.05	2.58 (1.26–5.30)	0.01

malities are associated with increased abdominal obesity (24,25). Pharmacologic inhibition of cortisol production prevents mental stress-induced endothelial function and baroreflex impairment, suggesting that cortisol may facilitate these phenomena (26). Growing evidence links dysregulation of the HPA axis with inflammatory processes (27), which then contribute to the pathogenesis of atherosclerosis (28). Finally, and most specific to calcification, animal data suggest that vascular lesions may be less affected by the antiproliferative/antifibrotic effects of cortisol during chronic stress, leading to excessive wound repair processes and calcification (29).

Several features of this study are noteworthy. First, the sample was small, relatively young, and very few had coronary calcification. Thus, the findings should be considered preliminary. Furthermore, the extent to which abnormal cortisol rhythms may be related to calcification in older populations with significant comorbidity and higher levels of calcification is unknown. Second, the study was cross-sectional in design. Although it is unlikely that coronary calcification alters HPA regulation, it is possible that there is a third correlated factor accounting for both. Indeed, we had reasoned that HPA dysregulation in response to stress would increase the risk for calcification. However, in the present sample, a flatter slope was not associated with number of chronic problems, although it was associated with greater depressive symptoms and lower emotional support and network diversity (13). Third, we did not expect average cortisol level summarized throughout the day to be unrelated to calcification. However, it should be noted that average cortisol was related to different and fewer psychosocial factors than was the slope in the same sample (13). Furthermore, some stress-related diseases, e.g., posttraumatic stress disorder, are associated with lower cortisol levels, and some theorists believe that either hyperresponsiveness or hyporesponsiveness of the HPA axis is deleterious (30). This notion is in keeping with the concept of allostatic load in which both excessive responding or the failure to mount an adequate response is considered to be health-damaging consequences of stress (6). Finally, the methods of salivary cortisol collection are demanding in ambulatory populations. Our data would suggest that compliance with the timing of collection, especially in the evening hours, is very important for hypothesis testing.

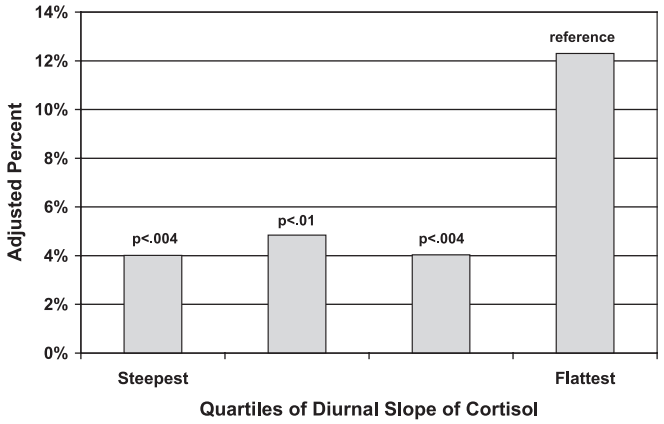

Figure 1. Probability of detectable coronary artery calcification by quartiles of diurnal slope of salivary cortisol adjusted for sex, race, treatment for diabetes, and age. *p* values refer to tests for whether the quartile group differs from the reference group.

In summary, we tested the hypothesis that cortisol level and diurnal patterns are related to the risk of any coronary calcification in a multiethnic sample. We found that a relatively flat diurnal pattern is associated with the risk of coronary calcification. These associations are independent of sociodemographic factors and established cardiovascular risk factors. Abnormal cortisol patterns may play a role in understanding why psychosocial factors are linked to risk for coronary disease. This is the first epidemiologic report of cortisol patterns being related to coronary atherosclerosis.

This work was supported by contracts University of Alabama at Birmingham, Coordinating Center, N01-HC-95095 University of Alabama at Birmingham, Field Center, N01-HC-48047 University of Minnesota, Field Center, N01-HC-48048 Northwestern University, Field Center, N01-HC-48049 Kaiser Foundation Research Institute, N01-HC-48050 University of California, Irvine, Echocardiography Reading Center, N01-HC-45134 Harbor-UCLA Research Education Institute, and Computed Tomography Reading Center, N01-HC-05187 from the National Heart, Lung and Blood Institute and by the MacArthur Research Network on SES and Health through grants from the John D. and Catherine T. MacArthur Foundation. Prepa-

ration of the manuscript was also facilitated by the Pittsburgh Mind-Body Center (HL65111 and HL65112).

REFERENCES

1. Rosengren A, Hawken S, Ôunpuu S, Sliwa K, Zubaid M, Almahmeed WA, Blackett KN, Sitthi-amorn C, Sato H, Yusuf S, for the INTERHEART investigators. Association of psychosocial risk factors with risk of acute myocardial infarction in 11,119 cases and 13,646 controls from 52 countries (the INTERHEART study): case–control study. Lancet 2004;364:953–62.
2. Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 1999;99:2192–217.
3. Hemingway H, Marmot M. Psychosocial factors in the aetiology and prognosis of coronary heart disease: systematic review of prospective cohort studies. BMJ 1999;318:1460–7.
4. Matthews KA, Gump BB. Chronic work stress and marital dissolution increase risk of posttrial mortality in men from the multiple risk factor intervention trial. Arch Intern Med 2002;162:309–15.
5. Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 1996;81:2468–73.
6. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338:171–9.
7. Schaeffer MA, Baum A. Adrenal cortical response to stress at Three Mile Island. Psychosom Med 1984;46:227–37.
8. van Eck M, Berkhof H, Nicolson N, Sulon J. The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. Psychsom Med 1996;58:447–58.
9. Vedhara K, Cox NK, Wilcock GK, Perks P, Hunt M, Anderson S, Lightman SL, Shanks NM. Chronic stress in elderly caregivers of dementia patients and antibody response to influenza vaccination. Lancet 1999;353:627–31.
10. Polk DE, Cohen S, Doyle WJ, Skoner DP, Kirschbaum C. State and trait affect as predictors of salivary cortisol in healthy adults. Psychoneuroendocrinology 2005;30:261–72.
11. Powell LH, Lovallo WR, Matthews KA, Meyer P, Midgley AR, Baum A, Stone AA, Underwood L, McCann JJ, Janikula Herro K, Ory MG. Physiologic markers of chronic stress in premenopausal, middle-aged women. Psychosom Med 2002;64:502–9.
12. Ochenfels MC, Porter L, Smyth J, Kirschbaum C, Hellhammer DH, Stone AA. Effect of chronic stress associated with unemployment on salivary cortisol: overall cortisol levels, diurnal rhythm, and acute stress reactivity. Psychosom Med 1995;57:460–7.
13. Cohen S, Schwartz JE, Epel E, Kirschbaum C, Sidney S, Seeman T. Socioeconomic status, race and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Psychosom Med 2006;68:41–50.
14. Troxler RG, Sprague EA, Albanese RA, Fuchs R, Thompson AJ. The association of elevated plasma cortisol and early atherosclerosis as demonstrated by coronary angiography. Atherosclerosis 1977;26:151–62.
15. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR Jr, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. J Clin Epidemiol 1988;41:1105–16.
16. Matthews KA, Katholi CR, McCreath H, Whooley MA, Williams DR, Zhu S, Markovitz JH. Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA Study. Circulation 2004;110:74–8.
17. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams D, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) Study. Radiology 2005;234:35–43.
18. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–32.
19. Warnick GR. Enzymatic methods for quantification of lipoprotein lipids. In: Albers JJ, Segrest JP, eds. Methods in Enzymology, vol 129. New York: Academic Press, 1986:101–22.
20. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 1994;19:313–33.
21. Reid JD, Intrieri RC, Susman EJ, Beard JL. The relationship of serum and salivary cortisol in a sample of healthy elderly. J Gerontol Psychol Sci 1992;47:176–9.
22. Kirschbaum C, Read GF, Hellhammer DH. Assessment of Hormones and Drugs in Saliva in Biobehavioral Research. Gottingen, Germany: Hogrefe & Huber, 1992.
23. Dressendorfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ. Synthesis of a cortisol–biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J Steroid Biochem Mol Biol 1992;43:683–92.
24. Bjorntorp P. Body fat distribution, insulin resistance, and metabolic diseases. Nutrition 1997;9:795–803.
25. Epel ES, McEwan B, Seeman T, Matthews K, Castellazzo G, Brownell KD, Bell J, Ickovics JR. Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. Psychosom Med 2000;62:623–32.
26. Broadley AJM, Korszun A, Abdelaal E, Moskvina V, Jones CJH, Nash GB, Ray C, Deanfield J, Frenneaux MP. Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. J Am Coll Cardiol 2005;46:344–50.
27. Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid resistance model. Health Psychol 2002;21:531–41.
28. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease. Is interleukin-6 the link? Atherosclerosis 2000;148:209–14.
29. Bray PJ, Du B, Mejia VM, Hao SC, Deutsch E, Fu C, Wilson RC, Hanauske-Abel H, McCaffrey A. Glucocorticoid resistance caused by reduced expression of the glucocorticoid receptor in cells from human vascular lesions. Arterioscler Thromb Vasc Biol 1999;19:1180–9.
30. Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Pyschoneuroendocrinology 2000;25:1–35.