Diurnal Salivary Cortisol Is Associated with Body Mass Index and Waist Circumference: The Multiethnic Study of Atherosclerosis

Shivam Champaneri[1], Xiaoqiang Xu[1], Mercedes R. Carnethon[2], Alain G. Bertoni[3], Teresa Seeman[4], Amy S. DeSantis[5], Ana Diez Roux[5], Sandi Shrager[6] and Sherita Hill Golden[1,7]

Objective: Neuroendocrine abnormalities, such as activation of the hypothalamic-pituitary-adrenal (HPA) axis, are associated with obesity; however, few large-scale population-based studies have examined HPA axis and markers of obesity. We examined the cross-sectional association of the cortisol awakening response (CAR) and diurnal salivary cortisol curve with obesity.

Design and Methods: The Multiethnic Study of Atherosclerosis Stress Study includes 1,002 White, Hispanic, and Black men and women (mean age 65 ± 9.8 years) who collected up to 18 salivary cortisol samples over 3 days. Cortisol profiles were modeled using regression spline models that incorporated random parameters for subject-specific effects. Cortisol curve measures included awakening cortisol, CAR (awakening to 30-min postawakening), early decline (30 min to 2-h postawakening), late decline (2-h postawakening to bedtime), and the corresponding areas under the curve (AUC). Body mass index (BMI) and waist circumference (WC) were used to estimate adiposity.

Results: For the entire cohort, both BMI and WC were negatively correlated with awakening cortisol ($P <$ 0.05), AUC during awakening rise, and early decline and positively correlated to the early decline slope ($P < 0.05$) after adjustments for age, race/ethnicity, gender, diabetes status, socioeconomic status, β-blockers, steroids, hormone replacement therapy, and smoking status. No heterogeneities of effects were observed by gender, age, and race/ethnicity.

Conclusions: Higher BMI and WC are associated with neuroendocrine dysregulation, which is present in a large population sample, and only partially explained by other covariates.

Introduction

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction is associated with obesity and the metabolic syndrome (1). Greater adrenocorticotropin hormone responses to stimulatory testing have been observed in subjects with higher waist-to-hip ratios (WHR) (2) or higher body mass index (BMI) (3). Other studies have suggested that higher WHR or abdominal sagittal diameter is associated with blunted dexamethasone suppression of cortisol (4), higher cortisol awakening response (CAR) (5), higher serum cortisol during stress (6), and lower awakening cortisol levels (7-9). Higher BMI has been found

to be associated with lower morning cortisol (10); however, higher urine-free cortisol (UFC) has been positively associated with WHR (2,11), subcutaneous adipose tissue (12), BMI (13), visceral fat area by magnetic resonance imaging (14), and the presence of metabolic syndrome (15). Purnell et al. (16) found increased cortisol production rate and free cortisol levels to be associated with higher intra-abdominal fat via computed tomography (CT).

In contrast, other studies have reported conflicting results. Some analyses of small studies have described no correlations between UFC and visceral fat as measured by CT (17) and WHR (7,17,18). Other studies

[1] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. Correspondence: Sherita Hill Golden (sahill@jhmi.edu)
[2] Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA [3] Division of Public Health Sciences, Wake Forest University Health Sciences, Winston-Salem, North Carolina, USA [4] Division of Geriatrics, David Geffen School of Medicine, University of California, Los Angeles, California, USA [5] Department of Epidemiology, University of Michigan, Ann Arbor, Michigan [6] Department of Biostatistics, University of Washington, Seattle, Washington, USA [7] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

have shown no association between WHR and dexamethasone-suppressed cortisol levels (7,11), and in one study, higher BMI actually associated with lower dexamethasone-suppressed cortisol levels (13).

Studies of the association of the diurnal cortisol profile with anthropometry measures of adiposity have shown mixed results. Higher WHR has been associated with lower diurnal cortisol variability (19,20) and lower cortisol levels (21). Although CAR has been positively associated with WHR (5) and WC (22), others found an inverse association between awakening or morning cortisols and BMI (23,24). Kumari et al. (24) noted from a large population-based study that the most flat diurnal salivary cortisol slopes were observed among individuals with the highest and lowest BMI and WC, possibly explaining the mixed findings in other studies. With the exception of two large studies (23,24), studies to date have been limited by small sample size, single gender, and/or single race/ethnicity. The Multiethnic Study of Atherosclerosis (MESA) Stress Study collected diurnal salivary cortisol profiles on a subset of 1,002 ethnically diverse adult men and women and offered a unique opportunity to examine the association of the HPA axis profile with BMI (as a marker of overall adiposity) and WC (as a marker of central adiposity). MESA is unique from other studies in having detailed salivary cortisol collection (8 time points per day over 3 days) to permit examination of multiple components of the diurnal cortisol curve with body fat measures. We were also unique in being able to exploit additional data collected from the main MESA study to examine potential confounders/explanatory factors in these associations. Based on prior reported studies, we hypothesized that lower diurnal cortisol variability, higher CAR, and higher cortisol area under the curve (AUC) as a marker of cortisol production would be associated with higher BMI and WC.

Methods

Study population

MESA is a multicenter longitudinal cohort study of the prevalence and correlates of subclinical cardiovascular disease and the factors that influence its progression (25). Individuals were not eligible to participate in MESA if they had clinical cardiovascular disease at baseline (25). Between July 2000 and August 2002, 6,814 men and women who identified themselves as White, Black, Hispanic, or Chinese and aged 45-84 years were enrolled from six different US communities. Details on the sampling frames and the cohort examination procedures are published (25).

Between July 2004 and November 2006, in conjunction with the second and third follow-up examinations of the full MESA sample, a subsample of 1,002 White, Hispanic, and African-American participants from either Los Angeles County, California or Northern Manhattan and the Bronx, New York participated in a substudy of biological stress markers (the MESA Stress Study), which included repeat assessments of salivary cortisol (26). Enrollment continued until ~500 participants were enrolled at each site. Informed consent was obtained from each participant, and the study was approved by the Institutional Review Boards of each institution.

Hormonal measures

MESA Stress Study participants were instructed, by trained staff, to collect six salivary cortisol samples a day (directly on awakening,

30 min after waking, 10:00 a.m., 12:00 p.m. or before lunch whichever was earlier, 6:00 p.m. or before dinner whichever was earlier, and at bedtime). Salivary cortisol measure is free as opposed to bound cortisol; thus, it is less prone to variability due to changes in cortisol binding proteins (i.e., corticotrophin binding globulin and albumin) and is the preferred method of measurement for dynamic HPA axis studies (27). This daily collection protocol was repeated on each of three successive week days; thus, each participant provided up to 18 cortisol measures. Participants recorded collection time on special cards; in addition, a time-tracking device (Track Caps) automatically registered the time at which cotton swabs were extracted to collect each sample. The participants were informed of this time-tracking device.

Saliva samples were collected using cotton swabs and stored at −20°C until analysis. Before biochemical analysis, samples were thawed and centrifuged at 3000 rpm for 3 min to obtain clear saliva with low viscosity. Cortisol levels were determined using a commercially available chemiluminescence assay with a high sensitivity of 0.16 ng/ml (IBL-Hamburg; Germany). Intra-assay and interassay coefficients of variation are below 8%. For our analyses, all cortisol measures at each time over 3 days were treated as repeated measures and not averaged. Awakening cortisol was defined as the salivary cortisol obtained at time zero. CAR rise was the cortisol rise from time zero to 30-min postawakening. Early decline in cortisol (CAR decline) was defined as 30-min postawakening to 2-h postawakening. Late decline in cortisol was from 2-h postawakening to bedtime (26). A few unusually high salivary cortisol values were noted that did not seem physiologically plausible, and 20 such values were excluded based on being inappropriately high when compared with other values for that time of day within the same subject.

Assessment of BMI, waist circumference, and obesity

Weight and height were measured using a balanced beam scale and a vertical ruler, respectively, with participants wearing light clothing and no shoes. Height was recorded to the nearest 0.5 cm and weight to the nearest 0.5 lb. BMI was calculated as weight (kg) divided by height squared (m^2). BMI categories are as defined by the World Health Organization (1995) as normal (<25 kg/m^2), grade 1 overweight (25-30 kg/m^2), grade 2 overweight (30-40 kg/m^2), and grade 3 overweight (>40 kg/m^2) (28). Waist circumference (WC) was measured at the minimum abdominal girth. All anthropometric measures were taken in duplicate and averaged.

Covariates

Data on age, race/ethnicity, sex, years of education, cigarette smoking, highest level of education achieved, and annual income by self-report using standard protocols were previously described (25). The analysis of socioeconomic status was simplified by the use of a single wealth-income index variable described by Hajat et al. (29). This wealth-income index is a composite socioeconomic status measure that incorporates annual income and information about assets. Prescription and over-the-counter medications were determined by transcription of medications brought into clinic during each exam (25). Smoking was assessed by self-report and categorized according to current, ever, and never smokers. Because Badrick et al. (30) showed higher salivary cortisol levels in current smokers only and no differences among ex-smokers and never-smokers, we accounted

for smoking by current smoking or noncurrent smoking status. Diabetes status was defined according to the 2003 American Diabetes Association criteria as fasting glucose $\geq$ 7.0 mmol/l (126 mg/dl) or use of hypoglycemic medication (oral agents and/or insulin). Impaired fasting glucose was defined as a fasting glucose of 5.5-6.9 mmol/l (100 to 125 mg/dl) (31).

Statistical analysis

Analysis overview. In each of our analyses, we determined the association of cortisol curve parameters with BMI and WC. For the purpose of our analyses, we have defined our exposure variable as the cortisol measurements and our outcome variables as BMI and WC. Cortisol distribution was log-transformed due to skewed distributions. BMI was maintained as a continuous variable in some analyses and a categorical variable in others. Because only 49 participants met criteria for the grade 3 overweight category, our analyses combined the grade 2 and grade 3 overweight groups. Specific details of our analyses are summarized below; however, in general, the regression coefficients derived from our linear regression models represent the change in BMI (kg/m^2) or WC (cm) per one unit increase in the log of the cortisol variable. In the case of categorical BMI, the regression coefficients represent the mean difference in the log of the cortisol variable in individuals in the overweight categories when compared with normal weight individuals (reference category). Because of significant differences seen in cortisol according to diabetes status in prior MESA analyses (32), secondary analyses were performed excluding individuals with diabetes or impaired fasting glucose.

In the base model for these analyses, adjustments were made for age, race/ethnicity, gender, and diabetes status. To explore potential confounding or explanatory factors in our associations, we performed the following additional multivariable adjustments: wealth-income index (socioeconomic status marker), current smoking status, and medications that have the potential to affect cortisol, namely β-blockers, steroids, and hormone replacement therapy (33). Adjustments for wake up time were made.

Derivation of salivary cortisol curve variables. Cortisol secretion has a well-documented circadian pattern typified by a rapid early morning peak within 30 to 45 min after waking followed by a decline throughout the remainder of the day (26). To capture all the relevant inflections of the circadian rhythm, we modeled our data using the approach proposed by Ranjit et al. (26). Daily cortisol values were modeled as a function of time since wake up, using linear regression mixed model splines with "knots" located to capture the ascending and descending phases of the CAR and the slower decline over the course of the remainder of the day. Knots were located at 30 and 120 min after wake up based on prior MESA analyses (26). The choice of the two knots was confirmed by the locally weighted scatterplot smoothing (LOESS) curve on the diurnal cortisol level. The presence of the knots allowed the relationship between time after wake up and cortisol levels (i.e., slope associated with time) to vary over the day.

Similar to Ranjit et al., we obtained the following key parameters of the spline models: the intercept (mean cortisol value at time 0 or at wake time, called "awakening cortisol"), the slope in cortisol from wake time to 30-min postawakening (the rapidly changing ascending CAR slope, called CAR), the slope in cortisol from 30 min to 120-min postawakening (the rapidly descending CAR slope, called
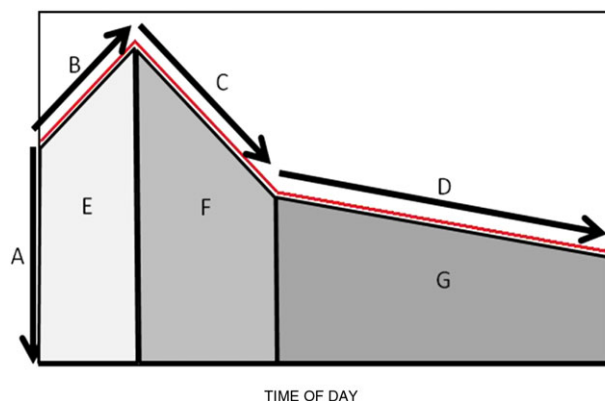
FIGURE 1 Summary of diurnal cortisol curve parameters derived from mixed model linear regression. Key: A = awakening cortisol at time zero; B = cortisol awakening response (CAR) denoting rise from awakening to 30-min postawakening; C = early decline from 30-min postawakening to 2-h postawakening; D = late decline from 2-h postawakening to bedtime; E = area under the curve (AUC) for CAR; F = AUC for early decline; G = AUC for late decline; the full AUC is the sum E + F + G. Reprinted from Metabolism, 61, Champaneri S et al. Diurnal salivary cortisol and urinary catecholamines are associated with diabetes mellitus: the Multi-Ethnic Study of Atherosclerosis, pp. 986–995, Copyright (2012), with permission from Elsevier (32).

"early decline"), and the slope in cortisol from 120-min postawakening to bedtime, called "late decline" (26). This is depicted in an exaggerated version of a diurnal cortisol curve in Figure 1. By including covariates in the model as main effects and in interaction with the different slope parameters, we were able to estimate how these parameters varied as a function of BMI, after adjusting for other risk factors and/or confounders (26).

We used mixed model linear regression to account for within-subject correlation between repeated measures as well as a variable number of repeated measures within a person and variations in the times of sample collection, as done previously (26). To account for correlations, the intercept and the time slope parameters for each person were treated as random, and an unstructured covariance matrix was used to obtain robust standard errors. All covariates were treated as fixed effects.

We also calculated AUC summary measures to estimate the total amount of cortisol exposure during the portions of the diurnal cortisol cycle outlined above. Based on the constructed spline model, we calculated several adjusted AUC summary measures by BMI and WC, including the full AUC (awakening to bedtime), CAR AUC (awakening to 30 min), early decline AUC (30 min to 2 h), and late decline AUC (2 h to bedtime) (Figure 1). For the purpose of this analysis, bedtime is defined as 16-h postawakening (29).

In all of our analyses, a two-sided *P*-value of <0.05 was considered statistically significant. All the analyses were carried out using SAS version 9.2.

Results
Baseline characteristics
Baseline characteristics of the study participants are summarized in Table 1 stratified by BMI category. Age and education were

TABLE 1 Baseline characteristics of study population

		BMI category			
Variable	Category	Normal (*n* = 233)	Grade 1 overweight (*n* = 413)	Grades 2 and 3 overweight (*n* = 355)	*P*-value
Age (mean ± SD)		66.4 (10.4)	66.0 (10.0)	63.8 (8.9)	0.0014[a]
Adjusted income wealth index (mean ± SD)		4.13 (2.29)	3.73 (2.33)	3.59 (2.27)	0.020[a]
Mean waist circumference (cm) (mean ± SD)		85.4 (7.6)	96.9 (7.2)	111.3 (9.2)	<0.0001[b]
Gender	Male	48.5%	54.7%	38.3%	<0.0001[b]
	Female	51.5%	45.3%	61.7%	
Race/ethnicity	Caucasian	32.2%	17.9%	11.0%	<0.0001[b]
	African-American	25.8%	24.7%	34.9%	
	Hispanic	42.0%	57.4%	54.1%	
Diabetes status	Normal	78.1%	62.7%	49.0%	<0.0001[b]
	Impaired fasting glucose	14.6%	21.3%	24.8%	
	Diabetes	7.3%	16.0%	26.2%	
Elevated depressive symptom status	CES-D < 16	81.9%	82.1%	79.4%	0.595
	CES-D < 16	18.1%	17.9%	20.6%	
Education	<High school	18.9%	30.8%	27.6%	0.013[a]
	High school	20.2%	18.6%	22.0%	
	≥College	60.9%	50.6%	50.4%	
Income ($)	<16,000	21.4%	24.8%	25.6%	0.552
	16,000–24,999	14.3%	15.6%	15.2%	
	25,000–34,999	15.6%	17.6%	18.7%	
	35,000–49,999	15.6%	15.6%	15.2%	
	50,000–74,999	13.8%	11.2%	13.8%	
	≥75,000	19.2%	15.1%	11.5%	
β-Blocker use		32.3%	37.8%	34.5%	0.354
Smoking	Current smoker	8.2%	11.7%	7.7%	0.131
Inhaled steroid use		2.2%	2.7%	3.2%	0.793
Oral steroid use		0.9%	0.7%	1.4%	0.622
Hormone replacement therapy since last visit (Female only)		9.9%	5.1%	7.6%	0.067

Note: BMI categories are defined as follows: normal (<25 kg/m^2), grade 1 overweight (25–30 kg/m^2), grade 2 overweight (30–40 kg/m^2), and grade 3 overweight (>40 kg/m^2).
Abbreviation: CES-D, Center for Epidemiological Studies Depression Scale.
[a]$P < 0.05$.
[b]$P < 0.0001$.

inversely associated with weight status, whereas African-American or Hispanic race, female sex, WC, and prevalence of glucose disorders were positively associated with weight status. Income, depressive symptoms, and smoking status were not related to obesity. There were some individuals taking medications that might confound the association between hormonal measures and BMI or WC (33), including 10 on oral steroids, 27 on inhaled steroids, 71 on hormone replacement therapy, and 346 on *β*-blockers. Among those with normal BMI, there were only four individuals with BMI < 18 kg/m^2.

Association of awakening cortisol, CAR, early decline, late decline, and AUC with BMI and WC

Figure 2 depicts the variation in diurnal cortisol curve from awakening to bedtime based on BMI category with notably lower cortisol

values seen in those with higher BMI. This is more visibly apparent in the awakening hours as depicted in Figure 3. Table 2 demonstrates the individual cortisol variables relative to WC and BMI modeled as continuous variables and adjusted for age, sex, race/ethnicity, and diabetes status. Both WC and BMI showed a significant negative association with awakening cortisol, AUC awakening rising, and AUC early decline. No statistically significant differences were noted in the awakening rise or late decline; however, the early decline portion of the salivary curve was flattened with a significantly positive association in the slope for both BMI and WC. Following additional adjustments for socioeconomic status, *β*-blockers, steroid use, HRT use, and current smoking status, these associations persisted, although some associations with total AUC were attenuated. Findings were similar when BMI was modeled as a categorical variable, although BMI category was not significantly associated with the early decline and total AUC (data not shown). Because a
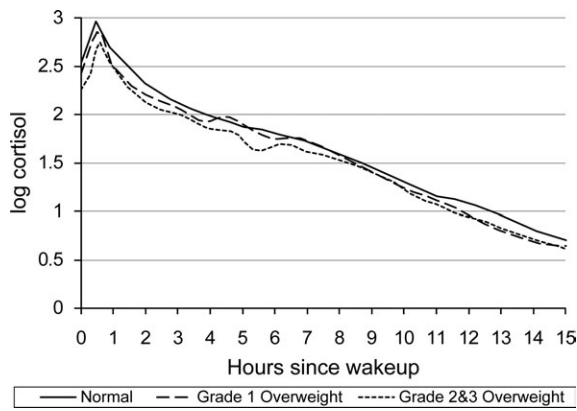
FIGURE 2 Diurnal curve for entire sample set.

prior study suggested a nonlinear association between the diurnal cortisol slope and BMI (29), we performed subsidiary analyses in which a quadratic term was included for BMI and WC. The results of these analyses were similar to those from the linear model, suggesting that there was no nonlinear association between cortisol and BMI and WC in our population.

Because cortisol profiles may vary by sex (34), we determined whether there was effect modification by sex on the association between cortisol curve parameters and BMI and WC. In stratified analyses, the results suggested that the association between awakening cortisol and AUC parameters and BMI was slightly stronger in men than women (awakening cortisol: $\beta = -0.021$, 95% CI: -0.031 to -0.011 in men and $\beta = -0.011$, 95% CI: -0.019 to -0.002 in women; total AUC: $\beta = -0.16$, 95% CI: -0.28 to -0.035 in men and $\beta = -0.044$, 95% CI: -0.16 to 0.067 in women). Sex-stratified associations were similar for WC (data not shown). However, there was no evidence of statistical interaction, and the direction of association was similar in men and women (P-value $= 0.094$ and 0.079 for sex interaction of cortisol curve measures with BMI and WC, respectively). Measures of cortisol
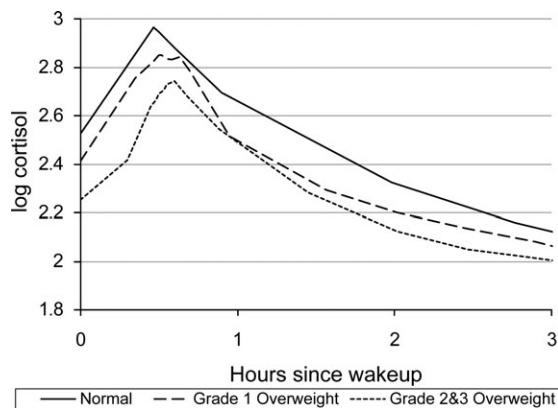

FIGURE 3 Diurnal curve during awakening hours.

TABLE 2 Mean differences in BMI and log WC for each one log-unit change in cortisol parameters for entire cohort by age, race/ethnicity, gender, and diabetes status

	β-Coefficient (95% CI)			
	BMI[a]	Waist circumference[a]	BMI[b]	Waist circumference[b]
Awakening (time zero)	−0.0152[c] (−0.0217 to −0.0087)	−0.0046[c] (−0.0071 to −0.0021)	−0.0141[c] (−0.0205 to −0.0076)	−0.0042[c] (−0.0067 to −0.0018)
Awakening rise (awakening 0 h to peak 30 min) (slope)	0.0008 (−0.0113 to 0.0129)	−0.0003 (−0.0049 to 0.0043)	−0.0008 (−0.0131 to 0.0116)	−0.0009 (−0.0055 to 0.0038)
Early decline (peak 30 min to 2 h) (slope)	0.0063[c] (0.0012–0.0114)	0.0022[c] (0.0001–0.0042)	0.0064[c] (0.0012–0.0115)	0.0023[c] (0.0002–0.0043)
Late decline (2 h to bedtime 16 h) (slope)	0.0001 (−0.0006 to 0.0008)	0.0000 (−0.0003 to 0.0002)	0.0001 (−0.0006 to 0.0008)	0.0000 (−0.0003 to 0.0002)
AUC full range (Waking 0 h to bedtime 16 h) (area)	−0.0857[c] (−0.1696 to −0.0018)	−0.0284 (−0.0596 to 0.0028)	−0.0782 (−0.1624 to 0.006)	−0.0253 (−0.0565 to 0.006)
AUC awakening rising (awakening 0 h to peak 30 min) (area)	−0.0075[c] (−0.0106 to −0.0044)	−0.0023[c] (−0.0035 to −0.0012)	−0.0071[c] (−0.0102 to −0.0041)	−0.0022[c] (−0.0034 to −0.0011)
AUC early decline (peak 30 min to 2 h) (area)	−0.0151[c] (−0.0233 to −0.0069)	−0.0047[c] (−0.0076 to −0.0017)	−0.0145[c] (−0.0227 to −0.0064)	−0.0044[c] (−0.0074 to −0.0015)
AUC late decline (2 h to bedtime 16 h) (area)	−0.0631 (−0.1399 to 0.0136)	−0.0214 (−0.05 to 0.0072)	−0.0565 (−0.1336 to 0.0206)	−0.0186 (−0.0474 to 0.0101)

NOTE: β-coefficients reflect relative increase in slope or AUC per unit increase in BMI or WC.
[a]Adjusted values for age, race/ethnicity, sex, and diabetes status.
[b]Additional adjustments for socioeconomic status, β-blockers, steroids, HRT, and current smoking.
[c]$P < 0.05$.

TABLE 3 Mean differences in BMI and log WC for each one log-unit change in cortisol parameters for individuals with normal fasting glucose, adjusted for age, race/ethnicity, gender, socioeconomic status, β-blockers, steroids, HRT, and current smoking

	β-Coefficient (95% CI)	
	BMI	Waist circumference
Awakening (time zero)	−0.0205[a] (−0.029 to −0.012)	−0.0065[a] (−0.0099 to −0.0032)
Awakening rise (awakening 0 h to peak 30 min) (slope)	0.0016 (−0.016 to 0.019)	−0.0001 (−0.0065 to 0.0062)
Early decline (peak 30 min to 2 h) (slope)	0.0063 (−0.001 to 0.013)	0.0026 (−0.0002 to 0.0054)
Late decline (2 h to bedtime 16 h) (slope)	−0.0002 (−0.001 to 0.001)	−0.0001 (−0.0005 to 0.0002)
AUC full range (Waking 0 h to bedtime 16 h) (area)	−0.1963[a] (−0.297 to −0.096)	−0.0627[a] (−0.1007 to −0.0247)
AUC awakening rising (awakening 0 h to peak 30 min) (area)	−0.0101[a] (−0.014 to −0.006)	−0.0033[a] (−0.0049 to −0.0017)
AUC early decline (peak 30 min to 2 h) (area)	−0.0225[a] (−0.033 to −0.012)	−0.0070[a] (−0.011 to −0.0031)
AUC late decline (2 h to bedtime 16 h) (area)	−0.1638[a] (−0.256 to −0.072)	−0.0524[a] (−0.0869 to −0.0179)

Note: β-coefficients reflect relative increase in slope or AUC per unit increase in BMI or WC.
[a]$P < 0.05$.

slope (awakening rise, early decline, and late decline) were not associated with BMI or WC in either sex (data not shown).

Secondary analysis with exclusion of impaired fasting glucose and diabetes subjects

The association between WC and BMI and cortisol variables among individuals with normal fasting glucose is summarized in Table 3, as the association of diabetes with WC and BMI and HPA measures might confound the observed association. Following multivariable adjustment, higher BMI and WC showed a significant inverse association with awakening cortisol, total AUC, AUC awakening rise, AUC early decline, and AUC late decline. BMI and WC were not associated with awakening rise, early decline, or late decline.

Discussion

In this study, we found a cross-sectional association of the diurnal cortisol profile with markers of overall and abdominal obesity. These findings were independent of demographics, socioeconomic status, medications, and smoking. We noted a significant negative association of BMI and WC with awakening cortisol and early decline, as well as the respective AUC.

Studies to date analyzing cortisol levels in relation to obesity markers have yielded mixed results. Our findings of an inverse association of BMI and WC with morning cortisol levels are consistent with those of three other studies (8,20,35), all of which were fairly large population-based samples; however, these studies were more limited in age range and/or race/ethnicity. A few studies have noted a positive correlation between urinary cortisols and WHR (2,11), subcutaneous adipose tissue (12), and BMI (13). As suggested by Walker et al. (8), this paradox may be due to enhanced peripheral metabolic clearance of cortisol in obesity or altered central HPA control. Bjorntorp et al. (20) have also noted that urinary collections have their own inherent weaknesses, including lack of information on the kinetics of HPA axis regulation.

The physiology of cortisol metabolism may explain these findings. 11-β-Hydroxysteroid dehydrogenase-1 (11β-HSD1) is an enzyme that converts hormonally active glucocorticoids like cortisol into inactive metabolites like cortisone; its expression is thought to cause higher intra-adipose tissue cortisol levels in obese subjects, perhaps through its reverse reductase action (1). Rask et al. (13) found that higher BMI was associated with higher 11β-HSD1 by adipose tissue biopsy, which would be difficult to detect in serum and salivary samples. They also suggest that perhaps the reductase activity is impaired at the level of the liver, yielding lower serum levels. This may imply that in obesity, there may be tissue-specific alterations in cortisol metabolism (13). Travison et al. (9) found among 999 subjects followed up over two separate visits that there was a negative correlation between changes in serum cortisol levels collected within 4 h of waking and increases in BMI over time. They also suggest that perhaps overexpression of 11β-HSD1 in adipose tissue may cause local regeneration of cortisol, but with minimal effect on circulating levels.

Long-term activation of HPA axis is associated with decreased diurnal variability of cortisol (36). It has been noted in men with ''burned-out'' diurnal cortisol secretion (including lower awakening cortisol and flatter slope) that there is a negative correlation with WHR (37). Total cortisol secretion was lower in this group with a flatter diurnal cortisol curve when compared with men with a more normal high-variability diurnal curve (37). Thus, low daily cortisol variability may reflect a prior overstimulated HPA axis, and previously high cortisol levels may have stimulated visceral fat accumulation (3). Because our study was cross-sectional, we cannot confirm a temporal sequence.

Our study has several strengths. First, this is one of the largest population-based studies to date reporting the association of awakening and diurnal salivary cortisol with markers of overall and central obesity. Second, salivary cortisol sample collection included the use of track caps for recording sample times to increase compliance in sample collection. Third, salivary cortisol sample collection occurred over 3 days, which has not been done in prior studies, allowing a more accurate determination of each participant's diurnal cortisol pattern. Finally, we were able to adjust for smoking, many medications, and other potentially confounding variables using data collected in MESA.

Several limitations should be kept in mind, however, in interpreting our data. First, this is a cross-sectional study, and thus, we were unable to determine whether hormonal dysfunction preceded or occurred after developing higher levels of obesity. Second, many medications can theoretically affect salivary cortisol (33), and our study only had data on a subset of these. Third, we did not have data to more objectively assess visceral fat content via abdominal imaging. Associations of metabolic risk factors have been noted to be less pronounced in peripheral obesity rather than central obesity (37); however, we found similar patterns of association using our estimate of overall obesity (BMI) and our estimate of central adiposity (WC). Fourth, we did not have data on specific subgroups of obese individuals who have been shown to have flattened diurnal cortisol profiles, including those with atypical depression (38) and sleep apnea (39); therefore, we could not examine these factors as potential mediators and/or effect modifiers of our observed associations. Fifth, although we found significant associations between cortisol curve parameters and BMI and WC, they were small and their clinical significance uncertain; however, our associations are of similar magnitude to that observed in other studies. For example, the unadjusted correlation coefficient between waking cortisol and BMI and WC were -0.15 ($P < 0.0001$) and -0.11 ($P < 0.0001$), respectively, in our population. Other studies have found correlations of -0.09 to -0.26 between morning cortisol and BMI (12, 14, 24). Finally, this study lacked data concerning sex steroid and growth hormone levels, which may have been potential confounders in the association as they may affect cortisol and body fat levels (20).

Our population-based study comprehensively examined components of the diurnal cortisol curve in relation to obesity markers. The long-term effect of dysregulation of the neuroendocrine stress system (i.e., allostatic load) is thought to be one potential mechanism through which individuals are predisposed to metabolic disorders (40). Longitudinal studies to assess neuroendocrine tone over time as adiposity develops are necessary to further delineate the role of the HPA axis in the development of obesity.O

Acknowledgments

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