A Nonlinear Relationship of Generalized and Central Obesity with Diurnal Cortisol Secretion in the Whitehall II Study

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Context: Evidence for an association of measures of generalized and central obesity with salivary cortisol secretion is equivocal.

Objective: The objective of this study was to assess the relationship between body mass index (BMI), waist circumference, and salivary cortisol.

Design: The design was a cross-sectional study of BMI, waist circumference, and salivary cortisol from phase 7 (2002–2004) of the Whitehall II study.

Setting: The occupational cohort was originally recruited in 1985–1988.

Participants: Participants included 2915 men and 1041 women aged 50–74 yr with complete information on height, weight and waist circumference, and cortisol secretion.

Outcome Measures: Saliva samples were taken on waking, waking plus 0.5, 2.5, 8, and 12 h, and bedtime for the assessment of cortisol. The cortisol awakening response and slope in diurnal secretion were calculated.

Results: After adjustment for age, sex, social position, waking time, and time since waking of sample collection, increasing central and generalized obesity was associated with lower waking cortisol ($P = 0.001$). U-shaped associations were apparent between diurnal slope in salivary cortisol and both BMI and waist circumference ($P < 0.0001$ for quadratic term). For example, the shallowest (most adverse) slopes in salivary cortisol were associated with highest (>31 kg/m^2) and lowest (<21 kg/m^2) levels of BMI, and the steepest slopes were apparent for those with BMI of 26 kg/m^2, independently of the 12 covariates examined. No associations were apparent for the cortisol awakening response ($P > 0.05$).

Conclusion: The associations of measures of generalized and central obesity with diurnal slope in salivary cortisol are not linear in older adults. These nonlinear associations may explain previously described mixed findings. **(*J Clin Endocrinol Metab* 95: 4415–4423, 2010)**

It is hypothesized that the hypothalamic-pituitary-adrenal (HPA) axis and obesity are linked (1). In particular, central obesity and syndromes of increased cortisol (a biomarker of the HPA axis) share several clinical metabolic and cardiovascular outcomes (2, 3). Diurnal cortisol release is typically characterized by high levels on waking, peaking at approximately 30 min [called the cortisol awakening response (CAR)], and a subsequent decline over the remainder of the day. The use of salivary cortisol allows measurement of this diurnal rhythm in a naturalistic setting, with minimal inconvenience for participants. Assessment of cortisol from saliva samples, to capture the

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-2105 Received October 1, 2009. Accepted May 18, 2010.
First Published Online June 30, 2010

Abbreviations: BMI, Body mass index; CAR, cortisol awakening response; HPA, hypothalamic-pituitary-adrenal.

CAR and levels over the day, is now routinely used in the epidemiological setting (4). Several studies suggest that a large or small CAR and shallow or flat slopes, *i.e.* small declines in cortisol secretion across the day, is associated with poor health and associated outcomes (5–8). However, the association of generalized obesity and central obesity with salivary cortisol is poorly understood.

Pathological activation of the HPA axis, such as found in Cushing's syndrome, is associated with increased central obesity. In nonclinical populations, however, the reported associations of salivary cortisol levels and measures of anthropometry are equivocal. For example, Wallerius *et al.* (9) described a positive association of morning cortisol levels with waist circumference in 28 healthy men, and Steptoe *et al.* (10) reported a positive association of the CAR with waist circumference in 58 healthy white men but no association in healthy white women or with cortisol measured in the rest of the day. Therrien *et al.* (11) similarly described a positive association of the CAR in 51 men but found no association in 31 women. In contrast, Rosmond *et al.* (12) described lower morning cortisol levels associated with increased waist/hip ratio in 50 middle aged men, and Ljund *et al.* (13) described an inverse association of body mass index (BMI) and waist circumference with an arithmetic mean of seven cortisol measures collected across the day in 284 men. Ranjit *et al.* (14) described diminished morning cortisol levels associated with obesity in 188 women, and Power *et al.* (15) described an inverse association of waist circumference and BMI with morning cortisol release in 6470 middle-aged men and women. Recently, a study of 491 middle-aged healthy men and women reported no association of BMI with morning, evening, or diurnal slope in cortisol secretion (16).

Reasons for mixed findings remain unknown. The different directions of association have been attributed to different mechanisms; positive associations between measures of adiposity and cortisol secretion were cited as evidence supportive of an etiological role of the HPA axis in obesity, whereas inverse associations are attributed to increased clearance of cortisol associated with increased adiposity (3, 12, 17, 18). It is also possible that there is a U-shaped association such that adverse cortisol profiles are linked with both obesity and underweight (a possible marker of disease), because both obesity and underweight are known correlates of morbidity and increased mortality risk (19, 20). Nonlinear associations with cortisol are not necessarily detected in previous analyses based on linear testing or crude BMI categories.

We hypothesize that the associations of cortisol secretion with measures of obesity involve multiple mechanisms and are therefore nonlinear. To test this hypothesis, we examine the association between BMI, waist circumference, and salivary free cortisol levels in a large cohort of late middle-aged men and women. We will examine waking cortisol, the CAR, and slope in cortisol measures separately because these are typically assessed parameters of diurnal cortisol secretion (4). Our study has the advantage of being large enough to account for correlates of cortisol levels in the analysis and also to examine any nonlinear associations that may be apparent in the data.

Subjects and Methods

Participants

Data reported here are from phase 7 (2002–2004) of the Whitehall II study. The Whitehall II cohort was initially recruited between 1985 and 1988 (phase 1) from 20 London-based civil service departments; 10,308 (74.0%) people participated, and details of the clinical assessment and cohort profile have been reported previously (21). The number participating at phase 7 was 6941, and 6484 (93.4%) had a clinical assessment. Collection of saliva for the assessment of cortisol was instigated partway through phase 7. Of those eligible for cortisol assessment, 4609 (90.1%) returned samples. In analyses reported here, fewer participants were in the lowest employment grades compared with phase 1 of the study, but this difference was small. Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research. Informed consent for involvement in the study was gained from every participant.

Cortisol collection and analysis

The collection of cortisol from phase 7 of the study has been described previously (22). Briefly, in a face-to-face interview, participants were requested to provide six saliva samples in salivettes over the course of a normal weekday at waking, at waking plus 30 min, 2.5 h, 8 h, and 12 h, and at bedtime. Participants were instructed to not brush teeth or eat or drink anything for 15 min before sample collection. An instruction booklet was used to record information on the day of sampling, including wake time, time each sample was taken, and stressful events. The salivettes and booklet were returned via post. Salivettes were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL-Hamburg, Hamburg, Germany). The lower concentration limit of this assay is 0.44 nmol/liter; intraassay and interassay coefficients of variance were $<8\%$. Any sample over 50 nmol/liter was repeated.

BMI and waist circumference

BMI was assessed by measurement of height and weight at the clinical assessment. Height was assessed using a stadiometer with the head in the Frankfort plane, and weight was assessed using a portable digital scale (Tanita, Yiewsley, Middlesex, UK). BMI was calculated as weight (in kilograms)/height (in meters) squared. For presentational purposes, BMI is categorized to underweight (<20 kg/m^2), desired/healthy weight (20–24.99 kg/m^2), overweight ($\geq$25–29.9 kg/m^2), and obese ($\geq$30 kg/m^2).

Waist circumference was measured in the standing position and unclothed, using a fiberglass tape measure at 600 g tension. The waist circumference was taken as the smallest circumference at or below the costal margin. Waist categories used were low waist (<90 cm in men and 80 cm in women), medium waist (≥90–102 cm in men and ≥80–88 cm in women), and high waist (>102 cm in men and 88 cm in women) (23).

Covariates

Age, sex, and current or previous employment grade were assessed by questionnaire. Smoking status (22) was defined as current smokers *vs.* the noncurrent smokers. Alcohol consumption was calculated using the units consumed in 1 wk, and high alcohol intake was assigned if greater than 21 U for men and 14 U for women. Waking time was assessed by participants' records on the day of the collection of saliva. Participants were also asked to record the time of falling asleep the night before sample collection. Sleep duration before cortisol measurement was calculated from these responses. Time difference between waking and taking first sample was categorized into 5-min intervals. Self-rated health was assessed by questionnaire asking the participants how they rated their health in the past 12 months with response items "very good," "good," "average," "poor," and "very poor." The Center for Epidemiological Studies Depression Scale (CES-D) was used to assess depressive symptoms using a cut point of 16 as reported previously (24). Fatigue was assessed using the vitality subscale of the Short Form-36 (25). Stress on the day of cortisol sampling was measured by questions on whether the participant had experienced a stressful event and, if yes, how stressful this was. Responses were grouped into the binary categories "no/not at all" and "somewhat/very stressful." Financial insecurity was assessed as described previously by the question "thinking of the next 10 years, how financially secure do you feel?" (26). Participants who responded that they felt fairly insecure and insecure were classified as financially insecure.

Statistical techniques

Sex has been shown to have an effect on adiposity and cortisol levels. Assessment of the interaction between sex, the two measures of anthropometry, and the three measures of cortisol secretion revealed no significant interactions (all $P > 0.05$), and therefore associations were examined in the total population with sex entered as covariate. The CAR is calculated as the difference in cortisol secretion at 30 min after waking and cortisol at waking. Because of non-normal distribution, cortisol values were log transformed for analysis of waking cortisol and slope.

Associations between participant characteristics and measures of adiposity were examined using χ^2 analysis for categorical variables and linear regression for continuous variables (Table 1). Linear regression models with waking cortisol and CAR as the outcome were used to analyze the association with measures of adiposity, adjusted for age, sex, time of waking on day of sampling, time since waking, and employment grade as a measure of social position. All analyses were then tested for nonlinear effects by using quadratic terms for BMI and waist circumference (Table 2). Data were also examined using standardized cut points for BMI and waist circumference, and estimated mean values for waking cortisol, CAR, and slope in cortisol release were calculated for each category (Table 2).

The slope of the decline in cortisol levels over the day was calculated by regressing (log) cortisol values (for samples 1, 3, 4, 5, and 6) on time after waking. The diurnal slope in cortisol secretion across the day was derived from a hierarchical linear (or multilevel) model to predict log cortisol in which measurement occasion was used as a level 1 identifier and person as a level 2 identifier with sample time (linear and quadratic terms), obesity measures, and potential confounders as the independent variables. A negative coefficient for linear sample time indicates a decline in log cortisol across the day, whereas a positive coefficient for quadratic sample time indicates that this linear decline tails off later in the day. The interaction terms between the obesity variables and time (hours) since awakening were included in models to test whether the cortisol slope differed between the obesity categories as the day proceeded (see Fig. 2) (Supplemental Fig. 2, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

The diurnal slope in log cortisol was estimated from the multilevel analysis for each person from the overall slope (which was negative) plus the level two slope residual; lower (more negative) slopes indicate more rapid the decline in cortisol levels, whereas slope values closer to zero reflect flatter diurnal rhythms. These slope estimates were then examined for associations with generalized and central obesity (see Fig. 1) (Supplemental Fig. 1). Confounders were added to the final models to see whether the effects of the obesity variables on slope in cortisol secretion were independent of confounding effects.

The data were analyzed using SAS version 14, and multilevel analysis was done in MLWin version 2.

Results

From the samples returned, 168 individual samples were not taken by participants, which equates to 0.6% of the total number of samples expected. During analysis, a total of 1002 individual samples (3.6%) were not assayed because of loss of sample in shipping to the laboratory or low saliva yield. Participants taking medications that affect cortisol levels were removed from the analysis (n = 236). Studies have shown that a delay in taking sample 1 results in a reduced CAR, because the morning cortisol peak is already substantially underway (27). In our study, participants who reported collecting their first sample more than 10 min after waking (sample 1 taken >10 min; n = 634) were marginally less likely to be obese compared with those who reported collecting their sample within 10 min of waking ($P = 0.052$). Analyses were therefore conducted with and without these participants and found not to vary. Data are presented with all these participants included in the analyses. When we retained these participants, the time difference between waking and time of sample collection was included within all models.

The final number of participants with reliable cortisol and anthropometric measurements is 3956. The mean BMI of the population is 26.6 kg/m^2, and the mean waist circumference is 94.0 cm in men and 83.6 cm in women; 109 (2.8%) were underweight, 1375 (34.8%) had desired/healthy weight, 1744 (44.1%) were overweight, and 728

TABLE 1. Participant characteristics for men and women who completed saliva sampling at phase 7 (2002–2004) of the Whitehall II study

	BMI (kg/m^2)					Waist circumference (sex-specific cut points)[a]			
	Underweight (<20)	Healthy weight (20–24.99)	Overweight (25–29.99)	Obese (≥30)		Lowest	Middle	Highest	
Percentage male	56.0	72.7	80.0	65.0	0.02	76.4	77.9	64.3	<0.001
Mean age (95% CI)		60.90 (60.77–61.03)	61.72 (61.58–61.86)	60.21 (59.93–60.49)	<0.001	60.90 (60.77–61.03)	61.72 (61.58–61.86)	60.21 (59.93–60.49)	<0.001
Lowest employment grades (%)	9.2	7.8	8.5	15.8	<0.001	7.6	8.4	15.0	<0.001
Health behaviors									
High weekly alcohol consumption (%)[b]	7.3	8.8	12.6	11.7	0.001	8.8	13.7	13.3	<0.001
Current smoker (%)	10.2	7.1	6.5	6.2	0.48	6.9	5.9	7.3	0.19
Short sleep (%)[c]	5.1	4.9	5.0	8.8	0.0002	4.7	4.6	8.2	0.0001
Health									
Poor or very poor self-rated health (%)		11.6	13.2	24.7	<0.001	10.9	12.6	22.6	<0.001
Fatigue (%)[d]	22.9	16.9	17.5	29.2	0.001	16.4	17.8	26.2	<0.001
Stress									
Stressful event on the day of sampling (%)	7.5	5.9	6.9	9.7	0.04	6.1	7.5	8.4	0.05
Financially insecure (%)	12.0	9.1	9.3	13.6	0.01	9.4	9.1	12.7	0.01
Depressive symptoms according to CES-D (%)[e]	19.2	12.4	13.1	18.3	0.02	12.7	13.0	16.3	0.03

Linear regression was used to assess association with age and χ^2 test for all other variables. CI, Confidence interval.

[a] Eighty and 102 cm in men and 72 and 88 cm in women.

[b] More than 21 U/wk for men and >14 U/wk for women.

[c] Less than 5 h.

[d] Score of <50 on the Short Form-36 vitality scale.

[e] CES-D: score of 16.

TABLE 2. BMI and waist circumference and cortisol awakening response at phase 7 (2002–2004) of the Whitehall II study

	n (%)	Waking cortisol (nmol/liter), 95% CI	Cortisol awakening response (nmol/liter), 95% CI	Slope in cortisol secretion (nmol/liter/h), 95% CI
BMI				
Underweight	109 (2.8%)	15.3 (13.7, 17.1)	8.7 (6.6, 10.8)	−0.124 (−0.128, −0.125)
Desired BMI	1375 (34.7%)	14.6 (14.1, 15.0)	7.2 (6.6, 7.8)	−0.129 (−0.130, −0.127)
Overweight	1744 (44.0%)	13.7 (13.3, 14.1)	7.5 (7.0, 8.1)	−0.130 (−0.131, −0.129)
Obese	728 (18.4%)	13.7 (13.1, 14.3)	7.0 (6.2, 7.6)	−0.126 (−0.128, −0.125)
Test for linear trend		$P = 0.001$	$P = 0.42$	$P = 0.69$
Test for quadratic trend		$P = 0.19$	$P = 0.26$	$P = 0.005$
Waist circumference				
Low	1521 (38.5%)	14.5 (14.0, 14.9)	7.4 (6.9, 7.9)	−0.129 (−0.130, −0.128)
Medium	1482 (37.5%)	13.8 (13.4, 14.2)	7.3 (6.6, 8.0)	−0.129 (−0.130, −0.127)
High	953 (24.1%)	13.5 (13.0, 14.1)	7.1 (6.3, 7.8)	−0.128 (−0.129, −0.126)
Test for linear trend		$P = 0.04$	$P = 0.13$	$P = 0.46$
Test for quadratic trend		$P = 0.87$	$P = 0.33$	$P = 0.0002$

P values from linear regression with BMI and waist entered as linear and quadratic terms. Means adjusted for age, sex, employment grade, waking time, and time since waking are from linear regression with generalized or central obesity entered as categorical variables in the models. Cut points for waist are 80 and 102 cm in men and 72 and 88 cm in women. CI, Confidence interval.

(18.4%) were obese. For the total population that participated in the clinic at phase 7 (n = 6450), 186 (2.9%) were underweight, 2148 participants (33.3%) had a desired/healthy weight, 2891 (44.8%) were overweight, and 1225 (19.0%) were obese and thus are not different from the participants with reliable cortisol measures.

The participant characteristics by BMI and waist circumference are shown in Table 1. There were significant differences observed between the groups. Obese participants were younger, more likely to be women, and more likely to be in the lower employment grades. Obese participants had a higher weekly alcohol consumption, they reported poorer self-rated health, more fatigue, less sleep, and more depressive symptoms, and they felt more financially insecure. Corresponding differences were apparent between waist circumference groups.

Table 2 shows that increasing BMI and waist circumference was associated with decreasing waking cortisol. Both linear terms (test of linear association) and quadratic terms (test of curvilinear association) were nonsignificant for the CAR in general linear models. In contrast, quadratic terms were highly significant for slope in cortisol secretion.

Figure 1 presents the association of slope in cortisol secretion by BMI. The equivalent data for waist circumference can be found in Supplemental Fig. 1. This clearly shows that the normal and overweight BMI categories had the steepest diurnal slope. Thus, compared with BMI of less than 22 kg/m^2 or greater than 32 kg/m^2, significantly flatter slopes were apparent than for those between 23 and 31 kg/m^2. Similarly shaped associations were apparent for waist circumference. For both BMI and waist circumference, adjustment for health behaviors (smoking, heavy alcohol intake, and short sleep), health (fatigue, self-rated health, and depressive symptoms), or "stress" (financial insecurity and stressful events on the day of sampling) failed to alter the shape of association with diurnal slope in cortisol secretion.

Table 3 displays the results of the multilevel model of diurnal log cortisol regressed on time since awakening (linear and quadratic terms) and obesity measures. The average log cortisol on awakening was approximately 3.01 nmol/liter. After 1 h, log cortisol decreased by approximately 0.17 nmol/liter, although this decrease tails off later in the day (indicated by the positive coefficient for the "time squared" term). On awakening, the under-

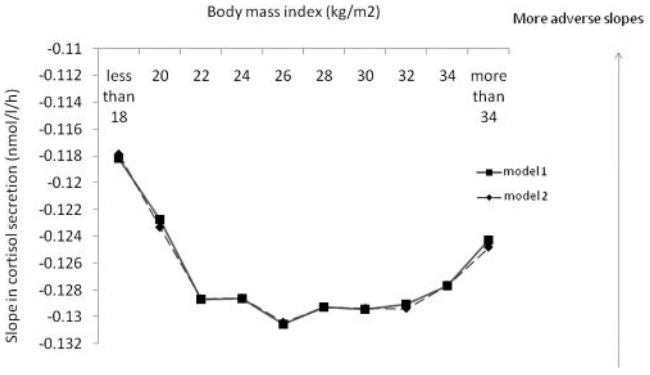

FIG. 1. Diurnal slope in cortisol secretion (from a 2-class multilevel model with cortisol sampled at waking, at waking plus 2.5, 8, and 12 h, and at bedtime as level 1 variables and individual as a level 2 variable) by BMI. The figure represents mean slope in cortisol secretion adjusted for age, sex, last known employment grade, waking time, and time since waking (*squares and continuous line*), plus additional adjustments for alcohol consumption, smoking, poor self-rated health, short sleep, fatigue, depressive symptoms, financial insecurity, and stress on day of sampling (*dots and dashed line*). $P < 0.0001$ for quadratic term.

TABLE 3. Diurnal slope in cortisol secretion by BMI categories, adjusted for confounders and mediators

	Diurnal slope in cortisol (samples 1–6, excluding sample 2)		
	Model A: adjusted for age, grade, gender, waking time [Regr. Coeff. (SE)]	**Model B: model A + health and health behaviors[a] [Regr. Coeff. (SE)]**	**Model C: model B + psychosocial factors[b] [Regr. Coeff. (SE)]**
Average cortisol (log)	3.01 (0.10)	−2.78 (0.09)	−2.787 (0.12)
Time (since awakening)	−0.17 (0.02)	−0.17 (0.02)	−0.16 (0.02)
Time squared	0.002 (0.001)	0.003 (0001)	0.003 (0001)
Obesity categories			
BMI			
Underweight BMI	Ref.	Ref.	
Desired BMI	−0.11 (0.07)	−0.12 (0.07)	−0.10 (0.08)
Overweight	−0.17 (0.07)	−0.17 (0.07)	−0.15 (0.08)
Obese	−0.16 (0.07)	−0.17 (0.07)	−0.16 (0.08)
P for BMI categories	<0.01	<0.01	<0.01
Interaction of time (linear term) × BMI			
Underweight BMI	Ref.	Ref.	Ref.
Desired BMI	0.01 (0.02)	0.02 (0.02)	0.02 (0.02)
Overweight	0.01 (0.02)	0.02 (0.02)	0.01 (0.02)
Obese	<0.001 (0.02)	0.006 (0.02)	0.003 (0.02)
P for interaction term	<0.01	<0.01	<0.01
Interaction of time (quadratic term) × BMI			
Underweight BMI	Ref.	Ref.	Ref.
Desired BMI	−0.001 (0.001)	−0.001 (0.001)	−0.002 (0.001)
Overweight	−0.001 (0.001)	−0.001 (0.001)	−0.001 (0.001)
Obese	<0.001 (0.001)	<−0.001 (0.001)	<−0.001 (0.001)
	0.05	0.07	0.01

Regr. Coeff., Regression coefficient.

[a] Alcohol consumption, smoking, poor self-rated health, short sleep, and fatigue.

[b] Depression symptoms, financial insecurity, and stress on the day of sampling.

weight BMI category had the highest cortisol levels, whereas the overweight category had the lowest cortisol levels. There were significant interactions between the BMI categories and time since awakening (linear and quadratic terms). The patterns of these interaction effects are displayed in Fig. 2. The multilevel analyses for diurnal slope were repeated using waist as the measure of adiposity (Supplemental Table 1).

We show that, when categorizing BMI (Fig. 2) and waist circumference (Supplemental Fig. 2), flatter slopes in cortisol secretion were caused by a combination of lower waking cortisol combined with higher evening cortisol levels in those with increasing obesity. The highest evening values of cortisol were found in the obese and underweight groups rather than the desired/healthy weight.

All analyses were repeated after removal of those that collected their first sample greater than 10 min after waking and also separately for men and women, and the findings were unaffected.

Discussion

We have shown in a large population of middle-aged people that adiposity is associated with cortisol in a nonlinear manner. The most adverse (flattest) slopes in cortisol secretion are found at the extremes of BMI and waist circumference, and these effects were apparent after adjustment for a wide variety of covariates. Flat slopes occur as a result of a combination of lower waking cortisol and higher evening cortisol in those with generalized or central obesity. No associations are seen for the CAR.

Previous reports of the association of adiposity and cortisol secretion are mixed (8–15). Our findings may help to explain these mixed findings as they suggest that associations are dependent on whether anthropometry measures are used continuously or categorized (28) associations vary with respect to different aspects of cortisol release and in some cases are nonlinear.

Our findings fail to accord with reports suggesting that morning cortisol levels are positively correlated with adiposity (8–10). Neither BMI nor waist circumference were positively associated with the CAR. Our findings are difficult to compare with those of Power *et al.* (15) in which they described a negative correlation between measures of anthropometry and cortisol at 45 min and 3.45 h after waking, but we do find that cortisol secretion at waking is negatively associated with general and central obesity in linear analyses. In this regard, our data are somewhat sup-

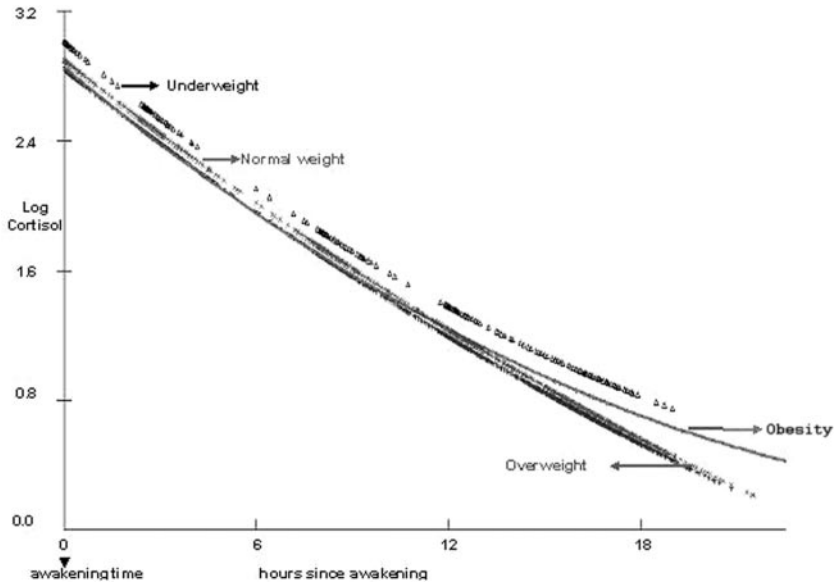

FIG. 2. Diurnal slope in cortisol secretion (from a 2-class multilevel model with cortisol sampled at waking, at waking plus 2.5, 8, and 12 h, and at bedtime as level 1 variables and individual as a level 2 variable) by obesity status. Figure represents mean cortisol secretion adjusted for age, last known employment grade, waking time, and time since waking estimated from Table 3. $P < 0.0001$ for quadratic term.

portive of Power *et al.* (15) and Ranjit *et al.* (14) who also described diminished morning cortisol associated with obesity and may explain the failure by Vreeberg *et al.* (16) to find a linear association between BMI and diurnal cortisol secretion in a healthy community-based cohort. Our findings of a nonlinear association of slope in cortisol release are difficult to compare with previous reports. Steptoe *et al.* (10) failed to find an association of cortisol during the day with adiposity. In our analyses, when obesity was conventionally categorized, the flatter slope in cortisol was composed of a generally lower mean morning cortisol and raised cortisol at bedtime in those classified as obese compared with the rest of the population. It is possible that averaging data from samples collected in the afternoon and evening may serve to obscure this diurnal effect.

The potential pathways by which adiposity is related to the HPA axis are numerous, and the literature is unclear about the mechanisms that could be mediating the effect (29). Our findings of U-shaped curves suggest that bidirectional and or multiple feedback and control mechanisms may mediate the associations between HPA and obesity. These may include both central and peripheral mechanisms. Our findings of a nonlinear association between anthropometry and cortisol secretion were robust to adjustment for a number of potential mediators, including health behaviors and psychosocial or psychological factors. It has been suggested that adipocytes themselves may be a source of cortisol through local conversion of cortisone (30, 31). Our findings suggest that this is unlikely to make a substantial contribution to the pattern of salivary cortisol found here. Additional research is needed to examine whether "stress" may underlie the association of flat curves at the extremes of adiposity. Our findings remained independent of acute stress assessed by stressful events on the day of sampling. This measurement of stress was undertaken on the day of sample collection only, but literature suggests that "stressful daily problems" cause some people to eat more and others to eat less (32). Accordingly, data from Whitehall II suggest that BMI response to work stress varied by initial BMI status such that those that experienced work stress and had low BMI at baseline declined in BMI by year 5 of follow up, whereas those that experienced work stress and had high BMI at baseline experienced a rise in BMI (33). Thus, groups that experience work stress either gain or lose weight depending on initial status. These patterns have not been described for waist circumference. It is problematic to investigate the role of work stress in the population under investigation here because 42% of men and 49% of women were retired at the time of cortisol sampling, 19 yr after the baseline for the Whitehall II study. Central adiposity is associated with "stress eating" in women (34), and Vicenetti *et al.* (35) also describe increased cortisol in 14 women that gained weight in response to stress compared with 21 controls.

Interestingly, the pattern of association between BMI and diurnal slope in cortisol mirrors the recently described associations of BMI and mortality (8) and waist circumference and mortality (36). It is unclear whether a flatter slope in cortisol is attributable to stress-related elevations, resulting from a stressful day, or long-term changes in circadian regulation as a result of chronic stress. A flattened slope may reflect reduced feedback in the HPA axis, and our findings accord with this (37) and evidence of altered HPA function in overeating and undereating syndromes (38). However, given the nature and age of the cohort, we speculate that the prevalence of these syndromes is likely to be low in our population. A flattened slope in cortisol secretion is a risk factor because it has been related to earlier mortality in cancer patients (8). The prognostic properties of cortisol secretion for disease and mortality remain to be determined in our cohort.

The strengths and weaknesses of our study need to be considered. This is a large study with an excellent response

rate that allows us to examine potential nonlinear associations. Indicators suggest that the participants understood the instructions and took samples in the correct manner, confirming the reliability of the dataset. A large number of possible confounders are measured and adjusted for, and the results are still significant. The Whitehall II study is an occupational cohort of civil servants, and, although not representative of the general United Kingdom population, it is likely that the observed association between adiposity and cortisol rhythm is free of substantive bias. Sample collection times were obtained by self-report, but evidence suggests that people are generally accurate in their data collection (39). It has been reported that cortisol secretion assessed on a single day only obscures the CAR to situational rather than chronic correlates (40), but speculatively, this should have greater relevance to psychological or psychosocial correlates than objective biomeasures. Our findings are from cross-sectional analyses, and so we cannot ascertain whether the low or high levels of obesity have caused changes in cortisol secretion or vice versa.

In conclusion, these results indicate that the association of salivary cortisol and adiposity is nonlinear in a cohort of middle-aged men and women. The mechanisms by which these associations occur remain to be determined but appear not to involve confounding by social position or health.

Acknowledgments

We thank Ellena Badrick for data collection and Clemens Kirschbaum for conducting the cortisol measurements. We thank the Whitehall II study team (the team comprises research scientists, statisticians, study coordinators, nurses, data managers, administrative assistants, and data entry staff) who make the study possible.

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The Whitehall II study has been supported by grants from the Medical Research Council, the Economic and Social Research Council, the British Heart Foundation, the Health and Safety Executive, the Department of Health, the National Heart Lung and Blood Institute/National Institutes of Health (NIH), the National Institute on Aging/NIH, and the John D. and Catherine T. MacArthur Foundation Research Networks on Successful Midlife Development and Socio-Economic Status and Health and Agency for Health Care Policy Research Grant HS06516. M.Ki is supported by Academy of Finland and the Bupa Foundation. T.C. is supported by the Medical Research Council (MRC) and Economic and Social Research Council Grant RES 596-28-0001. M.Ku was partially supported by MRC, Economic and Social Research Council Grant RES 596-28-0001, and National Heart Lung and Blood Institute Grant HL36310.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Bjorntorp P** 2001 Do stress reactions cause abdominal obesity and comorbidites? Obes Rev 2:73–86
2. **Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, Seckl JR, Papadopoulos A, Checkley S, Rumley A, Lowe GD, Stansfeld SA, Marmot MG** 2002 Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. Circulation 106:2659–2665
3. **Rosmond R, Dallman MF, Bjorntorp P** 1998 Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab 83:1853–1859
4. **Adam EK, Kumari M** 2009 Assessing salivary cortisol in large scale epidemiological research. Psychoneuroendocrinology 34:1423–1436
5. **Chida Y, Steptoe A** 2009 Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol Psychol 80:265–278
6. **Kumari M, Badrick E, Chandola T, Adam EK, Stafford M, Marmot MG, Kirschbaum C, Kivimaki M** 2009 Cortisol secretion and fatigue: associations in a community based cohort. Psychoneuroendocrinology 34:1476–1485
7. **Kumari M, Badrick E, Ferrie J, Perski A, Marmot M, Chandola T** 2009 Independent associations of sleep duration and sleep disturbance with cortisol secretion in an ageing cohort: findings from the Whitehall II study. J Clin Endocrinol Metab 94:4801–4809
8. **Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D** 2000 Diurnal cortisol rhythm as a predictor of breast cancer survival. J Natl Cancer Inst 92:994–1000.
9. **Wallerius S, Rosmond R, Ljung T, Holm G, Bjornorp. P** 2003 Rise in morning saliva cortisol is associated with abdominal obesity in men: a preliminary report. J Endocrinol Invest 26:616–619
10. **Steptoe A, Kunz-Ebrecht SR, Brydon L, Wardle J** 2004 Central adiposity and cortisol response to waking in middle aged men. Int J Obes Relat Metab Disord 28:1168–1173
11. **Therrien F, Drapeau V, Lalonde J, Lupien SJ, Beaulieu S, Tremblay A, Richard D** 2007 Awakening cortisol response in lean, obese, and reduced obese individuals: effect of gender and fat distribution. Obesity 15:377–385
12. **Rosmond R, Holm G, Björntorp P** 2000 Food-induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men. Int J Obes Relat Metab Disord 24:416–422
13. **Ljung T, Holm G, Friberg P, Andersson B, Bengtsson BA, Svensson J, Dallman M, McEwen B, Björntorp P** 2000 The activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. Obes Res 8:487–495
14. **Ranjit N, Young EA, Raghunathan TE, Kaplan GA** 2005 Modeling cortisol rhythms in a population-based study. Psychoneuroendocrinology 30:615–624
15. **Power C, Li L, Hertzman C** 2006 Associations of early growth and adult adiposity with patterns of salivary cortisol in adulthood. J Clin Endocrinol Metab 91:4264–4270
16. **Vreeburg SA, Kruijtzer BP, van Pelt J, van Dyck R, DeRijk RH, Hoogendijk WJ, Smit JH, Zitman FG, Penninx BW** 2009 Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. Psychoneuroendocrinology 34:1109–1120
17. **Vicennati V, Pasquali R** 2000 Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obe-

sity and relations with insulin resistance: evidence for a central and a peripheral alteration. J Clin Endocrinol Metab 85:4093–4098
18. **Strain G, Zumoff B, Strain J** 1980 Cortisol production in obesity. Metab Clin Exper 29:980–985
19. **Zaninotto P, de Oliveira C, Kumari M** 2008 Anthropometric measures and health. In: Living in the 21st century: older people in England. London: Institute for Fiscal Studies
20. **Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R** 2009 Body-mass index and cause-specific mortality in 900,000 adults: collaborative analyses of 57 prospective studies. Lancet 28:3731083–3731096
21. **Marmot M, Brunner E** 2005 Cohort profile: the Whitehall II study. Int J Epidemiol 34:251–256
22. **Badrick E, Kirschbaum C, Kumari M** 2007 The relationship between smoking status and cortisol secretion in the Whitehall II study. J Clin Endocrinol Metab 92:819–824
23. **Flegal KM** 2007 Waist circumference of healthy men and women in the United States. Int J Obes Relat Metab Disord 31:1134–1139
24. **Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ** 1977 Assessing depressive symptoms in five psychiatric populations: a validation study. Am J Epidemiol 106:203–214
25. **Ware Jr JE, Sherbourne CD** 1992 The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 30:73–83
26. **Ferrie JE, Shipley MJ, Stansfeld SA, Smith GD, Marmot M** 2003 Future uncertainty and socioeconomic inequalities in health: the Whitehall II study. Soc Sci Med 57:637–646
27. **Kudielka BM, Broderick JE, Kirschbaum C** 2003 Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. Psychosom Med 65:313–319
28. **Royston P, Altman DG, Sauerbrei W** 2006 Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med 25:127–141
29. **Rask E, Olsson T, Söderberg S, Andrew R, Livingstone DE, Johnson O, Walker BR** 2001 Tissue specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab 86:1418–1421
30. **Seckl JR, Walker BR** 2001 11β-Hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376
31. **Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M, Stewart PM** 2004 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr Rev 25:831–866
32. **Stone AA, Brownell K** 1994 The stress-eating paradox: multiple daily measurements in adult males and females. Psychol Health 9:425–436
33. **Kivimäki M, Head J, Ferrie JE, Shipley MJ, Brunner E, Vahtera J, Marmot MG** 2006 Work stress, weight gain and weight loss: evidence for bidirectional effects of job strain on body mass index in the Whitehall II study. Int J Obes Relat Metab Disord 30:982–987
34. **Epel E, Jimenez S, Brownell K, Stroud L, Stoney C, Niaura R** 2004 Are stress eaters at risk for metabolic syndrome? Ann NY Acad Sci 1032:208–210
35. **Vicennati V, Pasqui F, Cavazza C, Pagotto U, Pasquali R** 2009 Stress-related development of obesity and cortisol in women. Obesity (Silver Spring) 17:1678–1683
36. **Koster A, Leitzmann MF, Schatzkin A, Mouw T, Adams KF, van Eijk JT, Hollenbeck AR, Harris TB** 2008 Waist circumference and mortality. Am J Epidemiol 167:1465–1475
37. **Mattsson C, Reynolds RM, Simonyte K, Olsson T, Walker BR** 2009 Combined receptor antagonist stimulation of the hypothalamic-pituitary-adrenal axis test identifies impaired negative feedback sensitivity to cortisol in obese men. J Clin Endocrinol Metab 94:1347–1352
38. **Dallman MF, Pecoraro N, Akana SF, LaFleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD, Manolo S** 2003 Chronic stress and obesity: a new view of "comfort food." Proc Natl Acad Sci USA 30:11696–11701
39. **Dockray S, Bhattacharyya MR, Molloy GJ, Steptoe A** 2008 The cortisol awakening response in relation to objective and subjective measures of waking in the morning. Psychoneuroendocrinology 33:77–82
40. **Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D** 2007 Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state- and trait components. Psychoneuroendocrinology 32:80–86