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Diurnal Salivary Cortisol and Urinary Catecholamines Are Associated With Diabetes Mellitus: The Multi-Ethnic Study of Atherosclerosis

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Abstract

Objective—To examine the cross-sectional association of diurnal salivary cortisol curve components and urinary catecholamines with diabetes status.

Methods—Up to 18 salivary cortisol samples over 3 days and overnight urinary catecholamines were collected from 1,002 participants in the Multi-Ethnic Study of Atherosclerosis. Diabetes was defined as a fasting blood glucose ≥ 126 mg/dL or medication use. Cortisol curve measures included awakening cortisol, cortisol awakening response (CAR), early decline, late decline, and cortisol area under the curve (AUC). Urinary catecholamines included epinephrine, norepinephrine, and dopamine.

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AUTHOR CONTRIBUTIONS

S. Champaneri wrote the manuscript and interpreted the analyses. S. Golden designed the analyses, provided supervision to S. Champaneri, assisted in developing the first draft of the manuscript and interpreting the analyses, and provided critical revisions to the manuscript drafts. A. Diez Roux obtained funding, designed and wrote the protocol for the MESA Stress Study, and critically reviewed the manuscript. A. Diez Roux and T. Seeman were involved in data collection for MESA Stress. X. Xu performed statistical analyses. All authors contributed to critical manuscript revisions and have approved the final manuscript.

Results—Participants with diabetes had significantly lower CAR ($\beta=-0.19$; 95% CI: -0.34 to -0.04) than those without diabetes in multivariable models. While men with diabetes had a non-significant trend toward lower total AUC ($\beta=-1.56$; 95% CI: -3.93 to 0.80), women with diabetes had significantly higher total AUC ($\beta=2.62$; 95% CI: 0.72 to 4.51) ($p=0.02$ for interaction) compared to those without diabetes. Men but not women with diabetes had significantly lower urinary catecholamines, compared to those without diabetes ($p<0.05$).

Conclusions—Diabetes is associated with neuroendocrine dysregulation, which may differ by sex. Further studies are needed to determine the role of the neuroendocrine system in the pathophysiology of diabetes.

Keywords

diabetes; hypothalamic-pituitary-adrenal (HPA) axis; salivary cortisol; catecholamines; epidemiology

Hypercortisolism can induce insulin resistance and lead to type 2 diabetes by promoting development of visceral adiposity and activating lipolysis and free fatty acid release [1]. Higher fasting and mean overnight serum cortisol levels have been associated with higher homeostasis model assessment of insulin resistance and fasting glucose [1]. Individuals with the metabolic syndrome have also been shown to have higher circulating cortisol concentrations in both the basal setting and in response to dynamic hypothalamic-pituitary-adrenal (HPA) axis testing [2,3]. Subclinical hypercortisolism has been documented in individuals with type 2 diabetes, where they have been found to have higher 24-hour urine free cortisol [4], higher dexamethasone suppressed cortisol [4,5], higher basal plasma cortisol [4], and larger adrenal gland volume [6] than individuals without diabetes. In one study there was a positive association between dexamethasone-suppressed cortisol and glycated hemoglobin, suggesting that hypercortisolism may be related to glycemic control [5]. In a cohort of European males, decreased diurnal cortisol variation, indicating a dysregulated HPA axis, predicted incident type 2 diabetes [7]. Thus, the literature suggests HPA axis hyperactivity and dysregulation in the setting of type 2 diabetes; however, most of the studies have had small sample sizes or were conducted in a clinic-based and not population-based setting.

Catecholamines can also induce insulin resistance and glucose intolerance, as seen in patients with a pheochromocytoma [8]. Pheochromocytoma removal resulted in improvement in whole body glucose uptake and lowering of insulin levels in individuals with and without type 2 diabetes [8] supporting the contribution of catecholamines to insulin resistance. While some studies have shown higher fasting norepinephrine [9] and mean daily epinephrine [10] in individuals with diabetes compared to those without, one study found no difference in plasma norepinephrine levels [11]. The majority of studies have found lower 24-hour urine catecholamines [12-14] and plasma norepinephrine [15,16] in individuals with diabetes compared to those without diabetes. Thus, the role of catecholamines in the pathophysiology of type 2 diabetes remains unclear.

To date, no population-based studies have tested whether HPA axis and sympathetic nervous system (SNS) activity are altered in type 2 diabetes. The Multi-Ethnic Study of Atherosclerosis (MESA) Stress Study collected diurnal salivary cortisol profiles and overnight urine catecholamines on a subset of 1,000 ethnically diverse adult men and women and offered a unique opportunity to examine the association of neuroendocrine hormones with diabetes status. We examined the cross-sectional association of the cortisol awakening response (CAR), diurnal salivary cortisol curve, and urine catecholamines with diabetes status in this population. Additionally, we used data collected from the main MESA Study to examine potential confounders/explanatory factors in these associations. Because

prior studies have suggested sex and racial differences in HPA response to psychological stressors [17,18], we also looked for interactions by sex and race/ethnicity.

METHODS

Study Population

MESA is a multi-center, longitudinal cohort study of the prevalence and correlates of subclinical cardiovascular disease and the factors that influence its progression [19]. Between July 2000 and August 2002, 6814 men and women without clinical cardiovascular disease who identified themselves as white, black, Hispanic, or Chinese, and were 45 to 84 years of age were recruited from six US communities: Baltimore City and Baltimore County, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; Northern Manhattan and the Bronx, New York; and St. Paul, Minnesota. Details on the sampling frames and the cohort examination procedures have been published elsewhere [19].

Between July 2004 and November 2006, in conjunction with the second and third follow-up examinations of the full MESA sample, a subsample of 1002 white, Hispanic, and African-American participants at the New York and Los Angeles sites of MESA were recruited for a sub-study of biological stress markers (the MESA Stress Study), which included repeat assessments of salivary cortisol and collection of overnight urinary catecholamines [20]. Enrollment continued until approximately 500 participants were enrolled at each site [20]. 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 upon awakening, 30 minutes after waking, 10:00 am, 12:00 pm or before lunch whichever was earlier, 6:00 pm or before dinner whichever was earlier, and at bedtime). 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. Participants were told 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 minutes to obtain clear saliva with low viscosity. Cortisol levels were determined employing a commercially available chemiluminescence assay with a high sensitivity of 0.16 ng/ml (IBL-Hamburg; Germany). Intra- and interassay coefficients of variation were below 8%. Awakening cortisol was defined as the salivary cortisol obtained at time zero. Cortisol awakening response (CAR) rise was the cortisol rise from time zero to 30 minutes post-awakening. Early decline in cortisol was defined as 30 minutes post-awakening to 2 hours post-awakening. Late decline in cortisol was from 2 hours post-awakening to bedtime [20]. A few unusually high salivary cortisol values were noted which did not seem physiologically plausible, and 20 such values were excluded based on being inappropriately high compared to other values for that time of day within the same subject.

MESA Stress participants also collected 12 hour overnight urine samples to measure catecholamines in a specially prepared urine bottle with sodium metabisulfate (NA2S2O5, crystal form; reagent ACS grade, Fisher Scientific No. S244) as a preservative. Participants were instructed to void at the start time and to collect the last urine at the stop time. Aliquots of urine for catecholamines assays were acidified to a pH of 3. If the pH was greater than 3

by pH paper testing, the urine was acidified with (~6N) HCl. High performance liquid chromatography was employed for measurement of urinary epinephrine, norepinephrine, and dopamine [21]. Intra and interassay coefficients of variation were 2.3% and 2.69% for norepinephrine, 3.2% and 6.7% for epinephrine, and 2.8% and 3.2% for dopamine, respectively. Results of the three outcomes (epinephrine, norepinephrine, and dopamine) are reported as ng/ml volume.

Diabetes Status

Participants fasted for 12 hours and avoided smoking and heavy physical activity for 2 hours before each examination. Fasting blood samples were drawn between 7:30 and 10:30 am. Serum was frozen and stored at -70°C as previously described [19]. Impaired fasting glucose (IFG; 100 to 125 mg/dL) and type 2 diabetes (fasting glucose ≥ 126 mg/dL; or use of oral hypoglycemia medication, insulin or both) were defined according to the 2003 American Diabetes Association criteria [22].

Covariates

Data on age, race/ethnicity, sex, years of education, cigarette smoking, highest level of education achieved, and annual income were self-reported using standard protocols previously described [19]. Analysis of socioeconomic status was simplified by use of a single wealth-income index variable described by Hajat A et al [23] and 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 [19]. Because Badrick E et al [24] showed higher salivary cortisol levels in current smokers only and no differences among ex-smokers and never-smokers, we categorized smoking as current smoking or non-current smoking status.

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. Body-mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at the minimum abdominal girth and the hip circumference was measured at the level of the symphysis pubis and maximum protrusion of the buttocks. All anthropometric measures were taken in duplicate and averaged.

Statistical Analysis

Analysis Overview—We began by comparing the distribution of baseline characteristics by diabetes status using t-tests for continuous variables and Chi-square tests for categorical variables. In each of our analyses, we determined the association of our exposure variable, type 2 diabetes status with two outcome variables: 1) cortisol curve parameters and 2) urinary catecholamines. Both cortisol and catecholamine concentrations were log-transformed due to right skewed distributions. Initial analyses showed that the association of cortisol curve parameters and urinary catecholamines were similar in those with normal fasting glucose and impaired glucose tolerance, so those two groups were combined for all analyses. The regression coefficients derived from our linear regression models represent the mean difference in the log of each cortisol or catecholamine variable in individuals with type 2 diabetes compared to those without type 2 diabetes as the reference category.

Our preliminary analysis showed a strong association of sex with outcomes and significant interaction between sex and diabetes status. We therefore included an interaction term for sex and diabetes in the pooled models, and this term was found to be statistically significant ($p < 0.03$) in all models. We also performed sex-stratified analyses to further assess the association of different hormonal variables with diabetes status. In the base model, we

adjusted for age and race/ethnicity. To explore potential confounding or explanatory factors in our associations, we performed the following additional multivariable adjustments: waist circumference, wealth-income index, current smoking status, and medications that have the potential to affect cortisol and catecholamine levels [25]. In the cortisol analyses, we adjusted for use of beta-blockers, steroids, and hormone replacement therapy [25]. In the catecholamine analysis, we adjusted for use of beta-blockers and aspirin. Only a small number of participants were taking tricyclic antidepressants (n=14) and alpha blockers (n=34) so a sensitivity analysis was performed to assess for their effects. No participants were taking monoamine oxidase inhibitors. Data on other medications that could affect catecholamines such as acetaminophen, caffeine, decongestants, or antibiotics were not available.

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 minutes after waking followed by a decline throughout the remainder of the day [24]. To capture all the relevant inflections of the circadian rhythm, we modeled our data utilizing the approach of Ranjit N et al [20]. 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 minutes after wake up based on prior MESA analyses [20]. The choice of the two knots was confirmed by the 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. Adjustments for wake up time were made.

Similar to Ranjit N et al., we obtained the 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 minutes post-awakening (the rapidly changing ascending CAR slope, called CAR), the slope in cortisol from 30 minutes to 120 minutes post-awakening (the rapidly descending CAR slope, called “early decline”), and the slope in cortisol from 120 minutes post-awakening to bedtime, called “late decline” [20]. 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 diabetes status, after adjusting for other risk factors and/or confounders [20].

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 [20]. 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 area under curve (AUC) to estimate the total amount of cortisol exposure. For the purpose of this analysis, bedtime was defined as 16 hours post-awakening [23].

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 by diabetes status are summarized in Table 1. Compared to those without diabetes, participants with diabetes were less likely to be

Caucasian, less likely to have obtained a college education, and had higher BMI and waist circumference. Individuals with diabetes had lower annual income than those without diabetes. There were no differences in smoking by diabetes status, and individuals without diabetes were younger. Twenty-four of the 177 diabetic participants were on insulin therapy. Another twenty-four were untreated, and the remainder were treated with oral hypoglycemic medications. Medications that potentially could confound the association between hormonal measures and diabetes status are shown in Table 1. Beta-blocker and aspirin use was significantly greater among individuals with diabetes compared to those without diabetes.

Association of awakening cortisol, CAR, early decline, and late decline with diabetes status

Figure 2a shows the cortisol curve from awakening to bedtime, and figure 2b shows the cortisol curve from awakening to 3-hours post-awakening (which also includes the CAR). It shows that individuals with diabetes appeared to have a lower CAR than individuals with impaired and normal fasting glucose. Linear spline modeling was used to quantify this difference with knots at 30 minutes after awakening and 2 hours after awakening. In the minimally adjusted base model (age, sex, and race), there was no significant difference in the awakening cortisol at time 0 by diabetes status, although there was a trend for lower levels in participants with diabetes (Table 2). Findings were similar following adjustment for waist circumference, wealth-income index, smoking, and medications (beta blockers, steroids, and hormone replacement therapy). The CAR (awakening to 30 minutes post-awakening) was significantly lower in individuals with compared to those without diabetes (Table 2). This persisted following multivariable adjustment. In addition, the early cortisol decline (30 minutes post-awakening to 2 hours post-awakening) was significantly slower in individuals with diabetes compared to those without diabetes following multivariable adjustment (Table 2). There was no difference in late cortisol decline (2 hours post-awakening to bedtime) by diabetes status. In analyses examining the cortisol AUC, there was no significant difference between the three groups in all the models. Substitution of BMI for waist circumference in our models yielded similar results (data not shown). No interaction by race was found, likely due to lack of power.

Sex-stratified Analysis—In sex-stratified analyses, men with diabetes had significantly lower CAR than men with NFG despite multivariable adjustment (Table 3). Similar trends were seen in women; however, they were not statistically significant (Table 3). Both men and women with diabetes had a slower early cortisol decline than those without diabetes; however, no statistical significance was achieved following multivariable adjustment (Table 3). In contrast to male participants with diabetes, females with diabetes had significantly higher overall AUC compared to those without diabetes, and this persisted following multivariable adjustment.

Association of urinary catecholamines with diabetes status

In the analysis of overnight urine catecholamines, individuals with diabetes had lower dopamine, epinephrine, and norepinephrine levels than those without diabetes following multivariable adjustment (Table 4). Only 14 participants were on tricyclic antidepressants, and 34 individuals were on alpha blockers (none were on both). Sensitivity analyses demonstrated no differences in our findings when these participants were excluded. Analyses performed with adjustment for creatinine instead of volume yielded similar findings with some attenuation in statistical significance in the model that adjusted for waist circumference (data not shown).

Sex-stratified Analysis—In sex-stratified analyses, men with diabetes had significantly lower urinary norepinephrine, epinephrine, and dopamine than those without diabetes

following multivariable adjustment; however, urinary catecholamines were not related to diabetes status among women (data not shown). Sensitivity analyses excluding participants on tricyclic antidepressants or alpha blockers showed no difference in our findings other than mild attenuation in the association of urinary epinephrine with diabetes status in men. Analyses performed with adjustment for creatinine instead of volume yielded similar findings except the association of urinary epinephrine with diabetes status in men was no longer statistically significant (data not shown).

DISCUSSION

In this study we found a cross-sectional association between components of the diurnal cortisol profile and diabetes status, independent of demographics, socioeconomic status, waist circumference, medications and smoking, and some associations differed by sex. The CAR was lower in those with compared to those without diabetes; however, this association was primarily driven by men. Those with diabetes had a slower early cortisol decline, that was no longer significant in the full model, than individuals without diabetes; however, there was no difference in the late cortisol decline by diabetes status in either sex. When assessing cortisol exposure via AUC measures, there was a significant difference in the associations with diabetes by sex. While men with diabetes tended to have lower total cortisol AUC, women with diabetes had higher total cortisol AUC. The latter associations in women persisted following multivariable adjustment. We also found that men, but not women, with diabetes had significantly lower urinary catecholamines compared to those without diabetes following multivariable adjustment. None of our neuroendocrine parameters were associated with IFG status in either sex (data not shown).

Two prior studies have yielded conflicting results regarding the association of the CAR with diabetes status. The largest study of 491 individuals did not find an association between the CAR and diabetes status [26]; however, a smaller study by Bruehl et al found a blunted CAR in individuals with type 2 diabetes not on insulin compared to matched controls [27]. Although the literature has been mixed, prior studies suggest that the CAR response may vary by sex, even among non-diabetic individuals. Some studies have reported higher CAR in women compared to men [26, 28-31] while others have shown no difference by sex [32, 33]. Prior studies examining the association between the CAR and diabetes status have had small sample sizes and so lacked power to examine sex differences [27].

In our study, women with diabetes had a higher total diurnal cortisol exposure than women without diabetes. Because our study is cross-sectional, we cannot determine whether higher cortisol exposure led to diabetes or whether the hypercortisolism among the women was a consequence of having diabetes. Higher cortisol levels can induce visceral fat accumulation and increased free fatty acid release, leading to insulin resistance and type 2 diabetes [1]. It is also possible that diabetes and its associated treatments and/or co-morbidities induce hypercortisolism. We did not find higher diurnal cortisol AUC among women with IFG, compared to normal women; however, longitudinal studies are needed to further explore our hypotheses.

Similar to what prior studies have reported [9, 12-16], we found that urinary catecholamines were significantly lower among diabetic participants compared to those without diabetes; however, this association was noted predominantly in men. This sex difference has not been previously reported in the literature. One explanation for lower catecholamine levels in individuals with diabetes is that it may be a manifestation of neuropathy and/or diabetes severity [16, 34-40]. Plasma norepinephrine levels have been shown to be low among individuals with long duration of diabetes and clinically significant neuropathy [16, 34]. Urinary and plasma catecholamine responses to insulin-induced hypoglycemia and mental

stress were reduced in diabetic individuals with autonomic neuropathy compared to those without this complication [35,39]. Finally, lower plasma dopamine levels in diabetic individuals with other microvascular complications suggest that changes in the sympathoadrenal system may be related to late diabetic vascular complications [41]. The majority of these studies have had small sample sizes and were derived from clinical settings. Unfortunately, our study did not have information on diabetic complications documented at the time of catecholamine measurement. Because autonomic neuropathy is a risk factor for cardiovascular disease in diabetes [42], lower catecholamine levels in diabetes may identify those at increased risk for cardiovascular disease.

Some data suggest that dysfunction and burnout of the HPA axis, resulting in low total and morning values of cortisol, as we saw in men with diabetes, may be accompanied by compensatory increased sympathetic nervous system activity to maintain allostatic functions [43, 44]. In our study, however, diabetic men with a blunted cortisol profile also had lower urinary catecholamines, suggesting downregulation of sympathetic nervous system activity. Since the HPA axis and sympathetic nervous system co-activate each other [45], downregulation of the HPA axis in men with diabetes may therefore result in understimulation of the sympathetic nervous system as well. In our study, individuals with IFG had hormonal measures that more closely reflected those of normal individuals rather than those with diabetes. This suggests that the hormonal differences seen in diabetes may become more pronounced during the disease course rather than necessarily precede it. Because our study is cross-sectional, we cannot determine the temporality of this association.

Another consideration in the cause versus effect issue is whether hypoglycemia, which may result from diabetes treatment, plays a role in HPA axis alteration. Some cross-sectional studies have suggested that exposure to recurrent episodes of hypoglycemia may predispose to damage of cerebral neurons and cognitive decline [46]. HPA axis dysregulation in subjects with diabetes is also thought to be associated with cognitive decline [46]. Whether hypoglycemia in subjects with diabetes predisposes to HPA axis dysregulation is not clear and our study did not have data on hypoglycemic events or frequency to examine this hypothesis. Finally, HPA axis dysfunction is associated with depression [47], which is more prevalent among individuals with diabetes [48]; however, in the MESA Stress population, depression was not associated with diabetes status (data not shown).

Our study has several strengths. First, to our knowledge, this is the largest population-based study to date reporting the association of awakening and diurnal salivary cortisol and urinary catecholamines with diabetes status. Because of the large sample size, we were able to preliminarily explore whether these associations differed by sex. Second, we used well-established criteria to determine diabetes status and did not rely on self-report. Third, salivary cortisol sample collection included the use of track caps for recording sample times to increase compliance in sample collection. Fourth, salivary cortisol sample collection occurred over 3 days, 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.

Our findings should be interpreted in light of some limitations. First, this is a cross-sectional study so we are unable to determine whether hormonal dysfunction preceded or occurred after diabetes onset. Second, the urinary catecholamine samples were twelve hour overnight samples rather than conventional twenty-four hour collections used in clinical endocrinology. Third, many medications can theoretically affect salivary cortisol [25] and catecholamine levels, and our study only had data on a subset of these. Fourth, we did not know diabetes duration, which may be related to hormonal function in individuals with

diabetes. We also lacked data on diabetes-specific complications, which have previously been shown to be related to catecholamine levels, and on glycemic control at the time hormonal measures were assessed. The only glycemic marker was a hemoglobin A1c value from visit 2, which would have been 2-4 years before hormonal data were collected. Although most women, given the age of inclusion, were postmenopausal, it was not clear whether hormonal data in premenopausal women were collected in the follicular or luteal phase. Finally, although our population had multiple races, we did not have enough power to examine racial differences in the associations.

In summary, compared with individuals without diabetes, our study found lower CAR, total cortisol AUC, and urinary catecholamines in men with diabetes and higher total cortisol AUC in women with diabetes. The findings in men may represent neuroendocrine burnout in the setting of diabetes or they may be a manifestation of early autonomic neuropathy among men with diabetes. The findings in women suggest the presence of subclinical hypercortisolism and HPA axis hyperactivity in the women with diabetes in our study. It is possible that neuroendocrine burnout and/or hyperactivity are accelerated in the setting of diabetes or that it may be related to the pathophysiology of diabetes, but our hypothesis-generating cross-sectional study cannot determine this. A major implication of our findings is that longitudinal studies are needed to (1) determine neuroendocrine tone prior to as well as after the onset of diabetes and (2) to determine if there are long-term metabolic and cardiovascular consequences for diabetic individuals with these neuroendocrine profiles. The long-term effects 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 [49]. Whether the neuroendocrine system might be a potential therapeutic target for preventing and/or treating these disorders is dependent on future longitudinal studies to determine whether it contributes to their pathophysiology.

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Abbreviations

HPA	hypothalamic-pituitary-adrenal
MESA	Multi-Ethnic Study of Atherosclerosis
CAR	cortisol awakening response
IFG	impaired fasting glucose
BMI	body mass index
NFG	normal fasting glucose
AUC	area under curve

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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 minutes post-awakening; C=early decline from 30 minutes post-awakening to 2 hours post-awakening; D=late decline from 2 hours post-awakening to bedtime; E=area under curve (AUC) for CAR; F=AUC for early decline; G=AUC for late decline; the full AUC is the sum E+F+G

Figure 2.

Overall diurnal cortisol curve from awakening to bedtime (2a) and awakening to 2-hour post awakening cortisol curve (2b)

Abbreviations: IFG=impaired fasting glucose

TABLE 1

Baseline Characteristics of Study Population

Variable		Diabetes status		P-value [†]
		No diabetes (Normal & IFG [*]) N = 825	Diabetes n=177	
Age (Mean (std))		65.0 (9.9)	66.9 (9.0)	0.018
Gender	Female	435 (52.7%)	91 (51.4%)	0.751
	Male	390 (47.3)	86 (48.6%)	
Race	Caucasian	179 (21.7%)	9 (5.08%)	<0.0001
	African-American	227 (27.5%)	59 (33.33%)	
	Hispanic	419 (50.8%)	109 (61.58%)	
BMI (Mean (std))		28.5 (5.5%)	31.5 (5.6%)	<0.0001
Mean waist circumference (cm) (Mean (std))		98.8 (14.3)	107.6 (13.6)	<0.0001
Education	< High school	211 (25.6%)	59 (33.3%)	0.029
	High school	162 (19.6%)	40 (22.6%)	
	College	452 (54.8%)	78 (44.1%)	
Income	<\$16,000	173 (21.5%)	65 (37.6%)	<0.0001
	\$16,000 ~ \$24,999	119 (14.8%)	29 (16.8%)	
	\$25,000 ~ \$34,999	142 (17.7%)	29 (16.8%)	
	\$35,000 ~ \$49,999	130 (16.2%)	21 (12.1%)	
	\$50,000 ~ \$74,999	106 (13.2%)	18 (10.4%)	
	>\$74,999	133 (16.6%)	11 (6.4%)	
Insulin		0 (0.0%)	24 (13.87%)	<0.0001
Inhaled steroid use		21 (2.6%)	6 (3.39%)	0.528
Oral steroid use		8 (1.0%)	2 (1.13%)	0.845
HRT use since last visit		64 (7.8%)	7 (4.0%)	0.074
Aspirin		236 (28.6%)	85 (48.0%)	<0.0001
Beta blockers		255 (31.6%)	91 (52.6%)	<0.0001
Smoking	Current smoker	78 (9.5%)	16 (9.1%)	0.239
	Non-current smoker	743 (90.5%)	160 (90.9%)	

* IFG=Impaired fasting glucose

[†] For continuous covariates, the P-value was generated from the *t*-test. For categorical covariates, the Pvalue was generated from the Chi-square test.

TABLE 2

Beta coefficients depicting log difference in salivary cortisol curve for participants with diabetes compared to those without diabetes (normal fasting glucose + impaired fasting glucose; n=1002)

Awakening (time zero)			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	−0.061 (−0.171, 0.049)	−0.029 (−0.14, 0.082)	−0.016 (−0.127, 0.096)
Awakening rise (awakening – 0 hr to peak – 30 minutes) (slope)			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	−0.203 (−0.353, −0.054) *	−0.191 (−0.342, −0.039) *	−0.19 (−0.342, −0.039)*
Early decline (peak – 30 minutes to 2 hours) (slope)			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	0.113 (0.036, 0.191) *	0.096 (0.017, 0.176) *	0.091 (0.011, 0.172) *
Late decline (2 hours to bedtime – 16hour) (slope)			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	0.004 (−0.006, 0.014)	0.004 (−0.006, 0.014)	0.003 (−0.007, 0.013)

Full model = Base model + waist circumference, socioeconomic status by wealth-income index, beta blockers, steroid, hormone replacement therapy, wake up time, and current smoking

* p<0.05 for comparison of diabetes versus no diabetes

TABLE 3

Beta coefficients depicting log difference in salivary cortisol curve for participants with diabetes compared to those without diabetes (normal fasting glucose + impaired fasting glucose) stratified by sex (476 males and 526 females)

Awakening (time zero)						
Diabetes status	Male			Female		
	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-0.156 (-0.331, 0.019)	-0.127 (-0.303, 0.048)	-0.113 (-0.29, 0.064)	0.03 (-0.104, 0.164)	0.06 (-0.077, 0.196)	0.07 (-0.068, 0.208)
Awakening rise (awakening – 0 hr to peak – 30 minutes) (slope)						
Diabetes status	Male			Female		
	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-0.289 (-0.502, -0.075) *	-0.28 (-0.497, -0.064) *	-0.281 (-0.497, -0.065) *	-0.127 (-0.334, 0.079)	-0.105 (-0.315, 0.105)	-0.105 (-0.316, 0.106)
Early decline (peak – 30 minutes to 2 hours) (slope)						
Diabetes status	Male			Female		
	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	0.119 (0, 0.237) *	0.108 (-0.013, 0.23)	0.106 (-0.016, 0.228)	0.112 (0.01, 0.213) *	0.088 (-0.018, 0.193)	0.079 (-0.029, 0.187)
Late decline (2 hours to bedtime – 16 hour) (slope)						
Diabetes status	Male			Female		
	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	0.001 (-0.014, 0.015)	0.002 (-0.013, 0.016)	0.001 (-0.013, 0.016)	0.007 (-0.006, 0.02)	0.006 (-0.007, 0.02)	0.006 (-0.008, 0.02)
AUC full range (Waking 0 hr to bedtime – 16 hr) (area)						
Diabetes status	Male			Female		
	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	0.001 (-0.014, 0.015)	0.002 (-0.013, 0.016)	0.001 (-0.013, 0.016)	0.007 (-0.006, 0.02)	0.006 (-0.007, 0.02)	0.006 (-0.008, 0.02)

Awakening (time zero)						
Diabetes status	Male			Female		
	Base model (age, race)	Base model + Waist circumference	Full model	Base model (age, race)	Base model + Waist circumference	Full model
Diabetes status	Base model (age, race, sex)	Base model + Waist circumference	Full model	Base model (age, race, sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-2.065 (-4.283, 0.154)	-1.673 (-3.919, 0.573)	-1.565 (-3.929, 0.798)	2.61 (0.73, 4.49) *	2.669 (0.824, 4.515)	2.616 (0.725, 4.507) *

P-value for interaction by sex 0.02

Full model = Base model + waist circumference, socioeconomic status by wealth-income index, beta blockers, steroid, hormone replacement therapy, wake up time, and current smoking

* p<0.05 for comparison of diabetes versus no diabetes

TABLE 4

Beta coefficients depicting log difference in urinary catecholamines for men and women with compared to those without diabetes (normal + impaired fasting glucose; n=1002)

Norepinephrine			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-0.174 (-0.279, -0.068) *	-0.194 (-0.302, -0.086) *	-0.173 (-0.284, -0.062) *
Epinephrine			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-0.226 (-0.37, -0.082) *	-0.179 (-0.326, -0.033) *	-0.174 (-0.325, -0.023) *
Dopamine			
Diabetes status	Base model (age, race,sex)	Base model + Waist circumference	Full model
No Diabetes	0 (Ref)	0 (Ref)	0 (Ref)
Diabetes	-0.259 (-0.367, -0.15)	-0.261 (-0.372, -0.149) *	-0.213 (-0.327, -0.099) *

Full model = Base model + waist circumference, Socioeconomic status by wealth-income index, beta blockers, aspirin, and current smoking

*
p<0.05 for comparison of diabetes versus no diabetes