Association of diurnal patterns in salivary cortisol with type 2 diabetes in the Whitehall II study

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Context: The hypothalamic pituitary-adrenal (HPA) axis is thought to play a role in Type 2 Diabetes (T2D). However, the evidence for an association between diurnal cortisol patterns and T2D is equivocal.

Objective: The aim was to examine the association of cortisol patterns throughout the day with T2D status in a community-dwelling population.

Design: This was a cross-sectional study of T2D status and salivary cortisol from phase 7 (2002–2004) of the Whitehall II study, United Kingdom.

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

Participants: 3508 white men and women including 238 participants with T2D aged 50–74 years with complete information on cortisol secretion.

Outcome Measures: We measured diurnal cortisol (nmol/l) patterns from six saliva samples obtained over the course of a normal day: at waking, $+30$ min, $+2.5$ h, $+8$ h, $+12$ h, and bedtime. The cortisol awakening response and slope in diurnal secretion were calculated.

Results: T2D status was associated with a flatter slope in cortisol decline across the day ($B = 0.004$, C.I. $= 0.001$ to 0.007, $p = 0.014$) and greater bedtime cortisol ($B = 0.063$, C.I. $= 0.010$ to 0.117, $p = 0.020$) independent of a wide range of covariates measured at the time of cortisol assessment. There was no association between morning cortisol, the cortisol awakening response and T2D ($p > 0.05$).

Conclusions: In this non-clinical population, T2D was associated with a flatter slope in cortisol levels across the day and raised bedtime cortisol values.

Type 2 diabetes (T2D) is a chronic metabolic and endocrine disorder characterized by impaired insulin resistance and pancreatic β-cell dysfunction (1). The hypothalamic pituitary adrenal (HPA) axis is thought to play a role in T2D (2). Pathological (3) and experimental (4) exposure to excessive cortisol (a product of the HPA system) is related to metabolic disturbances such as hypertension, hyperlipidemia and central obesity, all of which are risk factors for T2D. Elevated cortisol concentrations, assessed from single plasma samples (5) and 24 hour urinary free samples (6) have been associated with raised

plasma glucose (5) and insulin resistance (5, 6) in healthy participants. T2D is also a recognized complication of long-term cortisol excess as seen in Cushing's syndrome (7) and in glucocorticoid-treated patients (8).

Increasingly, the marked diurnal patterning in the release of cortisol has been the focus of large-scale HPA axis research (9). The diurnal cortisol pattern is typically characterized by high cortisol levels on waking, followed by a rise that reaches a peak 30 minutes after waking (termed the cortisol awakening response (CAR)) and subsequent decline across day (9). Flatter slope in cortisol over the day

Abbreviations:

has been associated with diabetes-related outcomes such as central adiposity (10) and an increased risk of cardiovascular disease (11). However, the reported associations between diurnal cortisol patterns and T2D are equivocal.

In the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, participants with T2D were found to have a significantly lower CAR than those without T2D (12). Bruehl et al (13), similarly observed a blunted CAR in participants with T2D, but found no association of T2D with slope in cortisol across the day. In contrast, Lederbogen et al (14), observed a flattened slope in diurnal cortisol secretion among those with diabetes. Whereas, Vreeburg et al (15), observed no association between diabetes status and the CAR or diurnal cortisol slope.

The reasons for these divergent findings are unknown. It is possible that differences in participant characteristics or in the number and timing of cortisol samples between studies may be involved. We therefore sought to examine the association of diurnal cortisol secretion with diabetes status in sample of 3508 community-dwelling men and women of the Whitehall II study. We predicted that individuals with T2D would have a greater CAR and flatter slope in cortisol across the day.

Methods and Measures

Participants

We used data from phase 7 (2002–2004) of the British Whitehall II study. The cohort of 10,308 participants was initially recruited between 1985 and 1988 from 20 London-based civil service departments. The total number of participants at phase 7 was 6967, and of these, 6484 had a clinical assessment. Saliva collection for the assessment of cortisol was instigated partway through phase 7 and of those participants that were asked to collect saliva samples, 90.1% (n = 4608) returned samples. This group had fewer participants in the lowest civil service employment grades compared with phase 1 of the study: however, this difference was small. We restricted our analysis to those with complete information on time of waking, cortisol measures and diabetes status giving a final sample of 3508 participants (see Figure 1). 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 obtained from all participants.

Cortisol collection and analysis

The collection of cortisol from phase 7 of the study has been described previously (16). Participants were provided with a set of Salivettes and asked to take six samples over the course of a normal weekday (Monday-Friday) at waking, after 30 minutes, 2.5 hours, 8 hours, and 12

hours, and at bedtime. They were instructed not brush teeth or consume any food or beverages for 15 minutes prior to 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 by post. Salivettes were centrifuged at 3000g for 5 minutes resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial chemiluminescence immunoassay (CLIA; IBL Hamburg, Germany). The lower concentration limit of the assay was 0.44 nmol/liter and the intra- and interassay coefficients of variance were less than 8%. Any sample over 50 nmol/liter was reanalyzed.
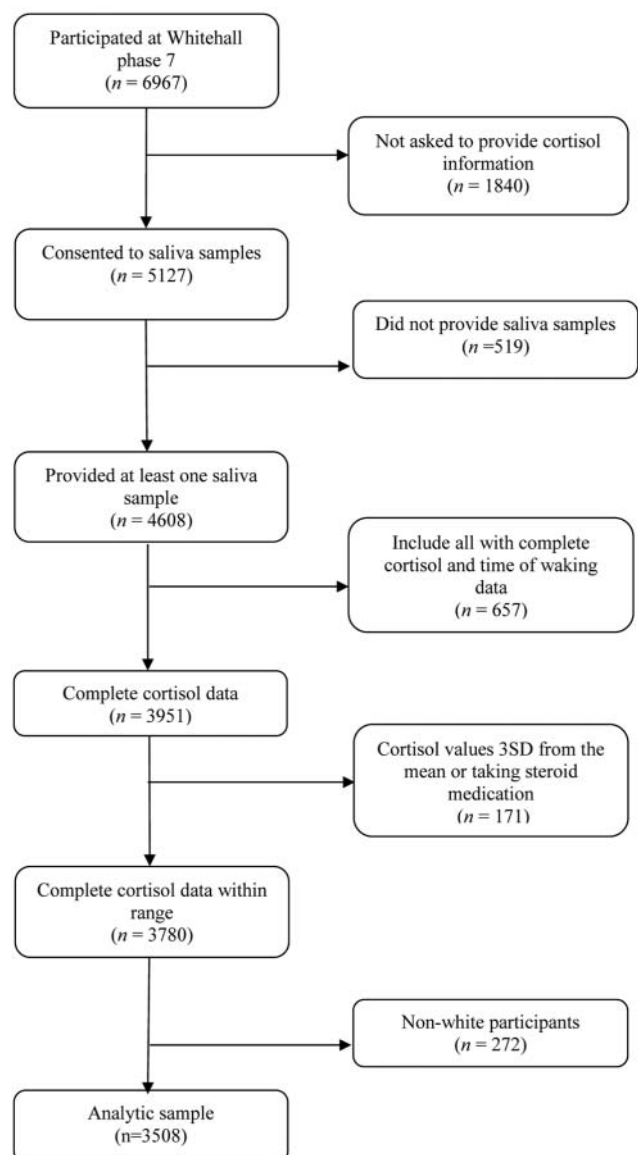
Figure 1. Flow diagram of participant included and excluded from the analyses

type 2 Diabetes

Type 2 diabetes was defined as a fasting glucose ≥ 7.0 mmol/l or a 2-hour post load glucose ≥ 11.1 mmol/l during the oral glucose tolerance test (OGTT) performed at the Whitehall clinical assessment or by reported doctor diagnosed diabetes, or the use of diabetes medication (17). For the purposes of the OGTT, participants provided a venous blood sample 8 hours after fasting and at 2 hours post administration of a 75g glucose solution. Blood glucose was measured using the glucose oxidase method (18) on a YSI MODEL 2300 STAT PLUS Analyzer (YSI Corporation, Yellow Springs, OH; mean coefficient of variation 1.4–3.1%) (19). Of the 238 participants with prevalent diabetes in our sample, 126 had known diabetes (confirmed by report of doctor diagnosis and diabetic medication) at the beginning of phase 7. A further 112 participants with diabetes were identified by the OGGT carried out at the Whitehall clinical assessment.

Covariates

We used standard protocols to assess characteristics of the participants at the time diurnal cortisol patterns were measured (phase 7, 2002–2004). Age, sex, and current or most recent civil service employment grade, a measure of social position, were assessed by questionnaire. Smoking status was defined as current smokers vs. noncurrent smokers (16). Waking up time was assessed by participants' records on the day of the collection of saliva. Time difference between waking and taking first sample was categorised into 5-minute intervals. Body mass index (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 categorised as obese (≥ 30 kg/m^2) or nonobese (< 29.9 kg/m^2). We have previously reported an association between fatigue and alterations in diurnal cortisol secretion (20), and fatigue is common in individuals with T2D (21). Therefore, we included fatigue as a covariate in the study. Fatigue was assessed using the vitality subscale of the Short Form-36(22). At the clinical assessment it was recorded whether participants had a history of coronary heart disease (CHD). Participants also provided details of current medication use and these were subsequently coded using the British National Formulary (23). Cardiovascular medication usage was defined as the use of β-blockers, antihypertensives, lipid lowering drugs, nitrates or antiplatelet medications.

Statistical analysis

Participants with cortisol values outside 3 SD from the mean and those taking steroid medications were removed from the analyses (n = 171). Despite this, cortisol data were skewed and were therefore logged for analysis. The CAR was calculated by subtracting cortisol measured at time 1 (waking) from cortisol measured at time 2 (+30 minutes). Conventionally, analyses are restricted to samples that are collected within 10 minutes of waking (sample 1 taken > 10 minutes, n = 646) because of a reduced CAR in those with longer delays (24). We did not see a difference in sample delays by diabetes status so all participants were retained. Most participants (n = 3395, 96.8%) took cortisol sample 2 on time. We not see a difference in late sample 2 collection by diabetes status so this was not included as a covariate in the analyses. The method used to calculate the diurnal slope in cortisol secretion has been previously described (10, 11, 20). In brief, the slope of the decline in cortisol levels over the day was calculated by regressing cortisol values on time after waking for samples 1 (waking), (+2.5 hours), 4 (+8 hours), 5 (+12 hours), and 6 (bedtime). Because it is suggested that the CAR and slope in cortisol secretion are under different neurobiological control systems (9), sample 2 was not included to ensure that the CAR does not obscure the slope calculation. Lower (more negative) slopes indicate a more rapid decline in cortisol levels, whereas slope values closer to zero reflect flatter diurnal rhythms. Descriptive and clinical characteristics of the sample were compared using t-tests for continuous variables and χ^2 tests for categorical variables. Associations between prevalent diabetes and the cortisol measures were analyzed using linear regression. Multivariable linear regressions using waking cortisol, CAR, slope and bedtime cortisol as outcome variables were performed to analyze associations with prevalent diabetes. Age, sex, grade of employment, smoking, waking time, late saliva collection, fatigue, BMI, cardiovascular medication and history of CHD were included as covariates in all analyses. Participants with missing covariate information were excluded from the analyses. Previous research has shown sex differences in the relationship between cortisol and diabetes status (12). Therefore, we investigated whether diabetes status interacted with sex, but found no significant associations with cortisol measures, so interaction terms were not included in the final models. We have previously shown a nonlinear relationship between BMI and slope (10). We investigated whether the pattern of results changed including BMI as a quadratic term. As the results were robust to controlling for the nonlinear effects of BMI, only BMI as a continuous variable was included in the final models. Of the Whitehall II participants who provided cortisol data 92.8% (n = 3508)

were of white ethnicity. We investigated whether prevalent diabetes interacted with ethnicity in the current sample. This interaction term was significant for slope ($P <$ .001) and bedtime cortisol ($P =$ .015). Therefore, we limited the present analysis to individuals of white ethnicity. Results are presented as unstandardised regression coefficients (B) with 95% confidence intervals (C.I.). The slope estimates were generated using 1 MLWin version 2.10 beta 6, all other analyses were conducted using SPSS version 21 (SPSS, Chicago, IL, USA).

Results

We restricted our analysis to those with complete information on time of waking, cortisol measures and diabetes status. This resulted in 3508 participants. The characteristics of participants included and excluded from the analysis are displayed in Table 1. The groups significantly differed in diabetic prevalence. However, this effect did not remain when nonwhite participants were removed from the excluded group ($P =$ .380, data not shown). The group with complete cortisol data were younger, more likely to be male and had fewer participants in the lowest civil service employment grades. They were less likely to take cardiovascular medication and have a history of CHD than the phase 7 group who did not provide saliva samples.

The characteristics of the participants who provided cortisol samples are displayed in Table 2. Two hundred and thirty eight (6.78%) participants had prevalent diabetes at the time of saliva collection. The group with diabetes were older on average and were more likely to be in the lowest civil service employment grades. They were more likely to be obese, have a history of CHD and take cardiovascular medicine than those without diabetes. Cortisol collection measures, such as waking time on day of sampling did not differ by diabetes status.

The average CAR in the sample was 7.33 (SD = 11.575). As shown in Table 3 the CAR did not differ by

diabetes status ($B = 0.002$, C.I. $= -0.036$ to 0.039, $p=$ 0.923). The average diurnal slope estimated from the hierarchical linear model was -0.1290 nmol/l per h (SD = 0.023). Participants with diabetes had a flatter slope in cortisol across the day than those without diabetes ($B =$ 0.004, C.I. $= 0.001$ to 0.007, $P =$ .014). This association was robust to adjustment for age, sex, grade of employment, smoking, waking time, late saliva collection, fatigue, BMI, cardiovascular medication and history of CHD. A flatter slope in cortisol patterns across the day can be due to low waking values or high evening values of cortisol. We examined the association of these cortisol measures with diabetes status. While participants with diabetes had higher waking levels on average compared to those without diabetes, this difference was not significantly different ($B = 0.014$, C.I. $= -0.018$ to 0.046, $P =$ .383). In contrast, cortisol measures at bedtime differed significantly between the groups. Participants with diabetes had significantly greater bedtime cortisol values than those without controlling for covariates ($B = 0.063$, C.I. $= 0.010$ to 0.117, $P =$ .020). This suggests that raised evening cortisol levels accounted for the difference in slope between the two groups.

Discussion

This study investigated the cross-sectional association between components of the diurnal cortisol profile and diabetes status in a large population of community dwelling adults. We found that the slope in cortisol across the day was flatter in those with compared to those without T2D. Our data suggest that the flat slope in cortisol in individuals with T2D is due to raised late evening cortisol levels rather than depressed morning levels. These findings were robust to adjustment for a range of covariates. No association emerged for the CAR.

Previous reports of the association between cortisol secretion and diabetes status are mixed (12–15). In the pres-

Table 1. Characteristics of participants at phase 7 (2002–2004) of Whitehall II Study Means ± standard deviations and N (%)

	Participants included in the cortisol analyses (n = 3508)	Participants excluded from the cortisol analyses (n = 3459)	P value
Sex (% men)	2636 (75.1%)	2257 (65.3%)	<0.001
Mean age (SD)	61.04 (5.94)	61.44 (6.06)	= 0.005
Current Smoker (% yes)	230 (6.6%)	274 (8.0%)	= 0.056
Employment grade (% lowest)	271 (7.7%)	489 (14.6%)	<0.001
Mean BMI (SD)	26.68 (4.29)	26.84 (4.49)	= 0.131
Fatigued (% yes)	682 (19.5%)	700 (21.3%)	= 0.068
Cardiovascular medication (% yes)	1005 (28.6%)	1135 (33.1%)	<0.001
History of CHD (% yes)	467 (13.7%)	570 (17.2%)	<0.001
Type 2 Diabetes (% yes)	238 (6.78%)	309 (8.9%)	<0.001

Table 2. Characteristics of participants at time of cortisol assessment by diabetes status Means ± standard deviations and N (%)

	N of participants	No diabetes (n = 3270)	Prevalent Diabetes (n = 238)	P value
Mean age (SD)	3508	60.85 (5.89)	63.64 (5.99)	<0.001
Sex (% men)	3508	2455 (75.1%)	181 (76.1%)	= 0.816
Current Smoker (% yes)	3506	212 (6.5%)	18 (7.6%)	= 0.522
Employment grade (% lowest)	3498	237 (7.3%)	34 (14.3%)	<0.001
Obese (% yes)	3494	567 (17.4%)	80 (33.8%)	<0.001
Fatigued (% yes)	3491	625 (19.2%)	57 (24.1%)	= 0.075
Late saliva collection (% yes)	3508	597 (18.3%)	49 (20.6%)	0.386
Cardiovascular medication (% yes)	3508	861 (26.3%)	144 (60.5%)	<0.001
History of CHD (% yes)	3398	407 (12.8%)	60 (26.2%)	<0.001

Table 3. Mean scores of measures of cortisol by diabetes status at phase 7 Means ± standard deviations

	Prevalent Diabetes (n = 238)	No diabetes (n = 3270)	P value	Adjusted P value
Waking cortisol (nmol/liter)	16.32 (7.74)	15.82 (7.18)	0.383	
CAR (nmol/)	7.35 (10.64)	7.54 (10.96)	0.923	
Slope across the day (nmol/liter per h)	−0.125 (0.022)	−0.129 (0.023)	0.002	0.014
Bedtime cortisol (nmol/)	2.59 (2.57)	2.34 (2.95)	0.002	0.020

*Adjusted for age, sex, smoking, grade of employment, waking time, fatigue, late saliva collection, BMI, CVD medication, and history of CHD.

ent study, we observed a flattened diurnal cortisol slope in participants with T2D. This corroborates the results of Lederbogen et al (14), who found an association between diabetes status and flatter daily cortisol profiles in 979 individuals from a community cohort. Similar to our analysis, individuals with T2D were observed to have raised evening cortisol concentrations compared to nondiabetic controls. Elevated late-night cortisol levels have been suggested as a diagnostic criterion for Cushing's syndrome (25). We removed participants with very high cortisol concentrations from our analysis, which would serve to exclude individuals with Cushing's syndrome. Our findings were also independent of obesity, which is strongly associated with the disorder. Indeed, raised late-night salivary cortisol levels have been previously been described in individuals with diabetes but without Cushing's syndrome (26).

In contrast to our findings, Vreeburg et al, (15) found no association between T2D and diurnal cortisol slope in 491 individuals without psychopathology from the Netherlands Study of Depression and Anxiety (NESDA) cohort. Participants in the study provided four saliva samples within an hour of waking and two late-evening samples. The additional samples collected in the late morning and afternoon in the present investigation may account for the diverging findings, as we were better able to define the shape of the diurnal cortisol curve. It is possible that the lack of information on late morning and afternoon cortisol levels reduced the ability of Vreeburg et

al to examine the curvilinear nature of the decline in cortisol across the day (9).

We failed to find an association between diabetes status and the CAR. This result is in contrast to the findings of Bruehl et al (13) and Champaneri et al (12) and who observed a blunted CAR in T2D individuals relative to controls. The reasons for the inconsistent results are unclear. However, our study is considerably larger than previous studies and consisted of a well-defined group of white community dwelling individuals. In contrast, the study by Bruehl et al (13), was limited by low participant numbers and a lack of adjustment for potential confounding factors. Champaneri et al (12), investigated the association between diabetes status and cortisol secretion in a cohort of over a 1000 individuals. However, the sample used was ethnically diverse and over 60% of the participants were of Hispanic origin. The present study was unpowered to detect the potential ethnic differences in the association between cortisol secretion and T2D and this may account for the differing findings between the studies.

Waking cortisol was not related to diabetes status in the current analysis. We have previously reported a relationship between fatigue and lower cortisol on waking (20) and fatigue is a common complaint among individuals with diabetes (21). Fatigue was independently associated with the diurnal cortisol slope and bedtime cortisol in the present study (data not shown). However, the association between T2D and these cortisol measures was robust to adjustment for this factor.

The causes of a flattened slope in diurnal cortisol are unknown and the mechanisms by which T2D is related to the HPA axis also remain to be elucidated. Cortisol plays a pivotal role in many physiological processes relevant to diabetes. It directly reduces insulin sensitivity and decreases insulin secretion by acting through glucocorticoid receptors, which are expressed on pancreatic β-cells. It triggers hepatic gluconeogenesis, promotes lipolysis and the release of fatty free acids into the circulation and the accumulation of triglycerides in adipose tissue (2). Obesity is common in T2D and visceral adipose tissue expresses high levels of glucocorticoid receptors (27). It has been hypothesized that adipocytes are a source of cortisol. Research has shown that transgenic mice overexpressing 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme activating the inactive form of glucocorticoids, have increased adipose levels of corticosterone (28). Increased 11β-HSD1 activity in human visceral adipose tissue has been associated with symptoms of the metabolic syndrome (29). Thus, obesity offers one possible mechanism through which T2D might be associated with alterations in cortisol secretion.

Participants with diabetes in our sample were significantly more likely to be obese and obesity has been associated with a flattened slope in diurnal cortisol secretion (10). As previously reported (10), BMI as a continuous measure was not associated with the slope in diurnal cortisol secretion. However, obesity was independently associated with the diurnal cortisol slope and bedtime cortisol (data not shown). Despite this the relationship between diabetes status and these cortisol measures was robust to adjustment for obesity.

Inflammation is another pathway through which T2D might be related to alterations in HPA axis function. Inflammatory cytokines are involved in the pathogenesis of T2D. Circulating cytokine levels are elevated in diabetic individuals (30) and heightened concentrations are predictive of T2D development in initially healthy samples (31). Cortisol is involved in the regulation of inflammation (2) and circadian rhythms are regulated at the hypothalamic level by the suprachiasmatic nuclei. It has been suggested that circadian control is an important aspect of hypothalamic-immune communication, and that glucocorticoids may dysregulate the immune response via circadian-immune communication (32). It is also possible that disturbances in circadian rhythms may act on T2D through the alteration of glucose metabolism. Recent experimental work indicates that circadian disruption increases both fasting and postprandial plasma glucose concentrations through inadequate pancreatic insulin secretion (33). Additional research is needed to examine whether changes in inflammation and alterations in glu-

cose metabolism may underlie the association between flatter slopes in diurnal cortisol section and T2D.

Another mechanism that may explain the relationship between diurnal cortisol slope and T2D is psychosocial stress. Results from meta-analyses and longitudinal studies indicate that psychosocial factors increase the risk of developing T2D (34) and contribute to disease progression in diabetic individuals (35). Cortisol levels are elevated by exposure to stress (36) and the flattening of the diurnal cortisol slope has been associated with both acute and chronic stress factors (37). Acute stress assessed by stressful events on the day of saliva sampling was not associated with the slope in cortisol or evening cortisol in the current analysis and our findings remained independent of acute stress (data not shown). However, it is possible that the findings could be attributed to long-term changes in circadian regulation as a result of chronic stress in people with T2D. Additional research is needed to examine whether chronic stress factors may underlie the association of flattened diurnal cortisol slopes with T2D.

In the present study we assessed cortisol across the day in a large community-based sample. The participants with T2D were well-characterized and we were able to use data from the larger cohort study to adjust for a number of potentially confounding factors in our analysis. However, our findings should be interpreted in light of some limitations. The Whitehall II study is an occupational cohort of civil servants and as such, our sample is not representative of the general population. The larger cohort study is predominately of white ethnicity, and due to ethnic differences in the pathogenesis of T2D we restricted the current analysis to white individuals. Therefore, the present results may not generalize to other populations. T2D was assessed by self-report of doctor diagnosis, use of diabetic medications or OGGT rather than clinical diagnosis. We also lacked data on the duration of T2D, which may be related to neuroendocrine function. The associations observed in the analyses were small. However, these patterns are thought to be representative of chronic differences that are present on an everyday basis. Under these circumstances, even modest effects may contribute to substantial accumulated differences in cortisol output over time. Studies specifically designed to test the association between T2D and neuroendocrine function would provide richer data. The cross-sectional design makes it impossible to draw causal conclusions about the temporal relationship between aberrant cortisol output and diabetes. Cortisol was assessed over a single day and it has been suggested that this may obscure the CAR to situational rather than chronic correlates (38). Furthermore, the night release of cortisol was not assessed and therefore it was not possible to evaluate total 24 hour circadian cortisol exposure. We

only measured free cortisol levels in the study and did not assess glucocorticoid receptor function. It is possible that the findings reflect reduced episodic cortisol release which has been reported to modify the regulation of glucocorticoid sensitive genes (39). We relied on self-report for the timing of sample collection. Our prevalence of 'late' reporting was similar to previously reported rates (24) and evidence suggests that participants are generally accurate in their recording of this information (40) .

Despite these considerations, our findings indicate that flat slopes in salivary cortisol, particularly raised evening levels of cortisol are associated with T2D in nonclinical population of middle-aged men and women. The mechanisms by which these associations occur remain to be determined. It is possible that neuroendocrine dysfunction may be related to the pathophysiology of T2D. However, longitudinal studies are required to assess the prognostic properties of cortisol secretion for T2D.

Acknowledgments

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