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Corticotropic Axis Drive of Overnight Cortisol Secretion is Suppressed in Adolescents and Young Adults with Type 1 Diabetes Mellitus

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Abstract

Context—Type 1 diabetes mellitus (T1DM) is a pro-inflammatory stress state, which, with its attendant hyperglycemia, likely disrupts hypothalamo-pituitary-adrenal (HPA) control, further dysregulating glucose homeostasis.

Objective—To test the hypothesis that endogenous ACTH-cortisol dose-responsive drive, estimated analytically, is significantly accentuated in adolescents and young adults with T1DM compared with healthy individuals.

Design, Setting, Patients, Interventions—This was a pilot study of 11 volunteers with T1DM and 10 controls, ages 16–30 yr, at a medical center. Subjects underwent overnight frequent blood sampling (every 10 min for ACTH and cortisol and every 60 min for blood glucose) from 10 pm to 8 am. T1DM volunteers maintained their home insulin regimen.

Main Outcomes—Deconvolution analysis and dose-response estimates were the key outcomes.

Results—Mean free cortisol, but not ACTH, concentrations were lower in the T1DM group compared with controls ($P=0.012$). Noninvasive ACTH-cortisol dose-response estimates revealed that T1DM patients had reduced ACTH efficacy (maximal cortisol secretion, $P=0.009$), reduced ACTH potency as quantified by greater EC_{50} (ACTH concentration driving half-maximal cortisol secretion, $P=0.04$), and increased ACTH sensitivity (more positive ACTH-cortisol slope, $P=0.03$). *Post-hoc* gender comparisons indicated that these differences were limited to females. Linear regression in women showed a strong correlation of both ACTH efficacy and EC_{50} with C-peptide levels (both $P<0.01$).

Conclusion—Compared with healthy individuals, T1DM patients manifest decreased overnight adrenal responsiveness to endogenous ACTH leading to lower free cortisol concentrations. These findings suggest impaired stress-related adaptations of the HPA axis in T1DM.

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Keywords

ACTH; Cortisol; Adolescents; HPA axis; Diabetes

Introduction

Ultradian ACTH-cortisol rhythms reflect a complex interplay of hormone signals. While early pulsatility analyses were restricted to concentration studies, more recent methods allow quantification of burst-like (pulsatile) hormone secretion via deconvolution along with non-invasive quantification of ACTH-cortisol secretion and clearance in various pathophysiologic states (1,2). Regulation of the ACTH-cortisol axis in type 1 diabetes mellitus (T1DM), however, remains largely unexplored.

In animal studies, poorly controlled diabetes mellitus leads to an activated basal HPA axis. Responses include increased hypothalamic corticotropin-releasing hormone (CRH) mRNA and hippocampal mineralocorticoid receptor (MR) mRNA concentrations (3), as well as elevated baseline plasma ACTH and total cortisol levels (50% and 100% higher, respectively) (3). Excessive CRH, ACTH and cortisol output from an excitable HPA axis may worsen glycemic control via multiple channels (4–7). Elevated CRH expression stimulates the sympathoadrenal pathway, which promotes the release of epinephrine and norepinephrine, as well as glucagon. All three signals promote glycogenolysis. Moreover, high cortisol concentrations amplify hyperglycemia by stimulating gluconeogenesis via the rate-limiting enzymes phosphoenolpyruvate carboxykinase (PEP-CK) and glucose-6-phosphatase (G6Pase), and by inhibiting glucose utilization by muscles (8). In some studies, insulin replacement in animals leads to partial resolution of ACTH and glucocorticoid hypersecretion, but does not normalize elevated CRH and MR expression (3,9,10). Other investigations have shown diminished cortisol feedback at the level of hippocampal/hypothalamic glucocorticoid receptor (GR) and MR in streptozotocin (STZ)-induced diabetes in rats (11,12). Limited human studies have reported varying results, ranging from HPA axis overdrive (as seen in animal models) to HPA suppression, including blunted counterregulatory HPA responses to hypoglycemia (7,13–15).

No clinical study of T1DM has employed contemporary analytical methods to evaluate ACTH-cortisol pulsatility and dose-response behavior (1,2,16). The present investigation proposes that T1DM significantly alters HPA axis regulation in adolescents and young adults compared with healthy individuals, and that the degree of HPA disruption is proportionate to endogenous C-peptide depletion and/or glucose dysregulation.

Materials and Methods

Human subjects

Participants were 11 volunteers with T1DM (6 males and 5 females) and 10 healthy controls (5 males and 5 females), ages 16–30 years. The sample size estimate was based on the log-transformed EC₅₀ parameter, which is a potency measure defining the endogenous ACTH concentration stimulating pulsatile cortisol secretion one-half maximally (2). In particular,

analysis of 22 participants (11 T1DM and 11 controls) was predicted to have 90% power to detect a 30% difference in this endpoint, assuming a pooled standard deviation of the log EC₅₀ of 0.6, the value observed in a large study on 111 individuals (17), and a two-sided type I error rate of 0.05 ($\alpha=0.05$).

Inclusion criteria for T1DM patients included a diagnosis of T1DM for at least 12 months treated with multiple daily insulin injections or bolus/continuous subcutaneous insulin infusions. Controls had normal fasting plasma glucose (<100 mg/dL). Women were studied in the early and mid-follicular phase of the normal menstrual cycle (within 12 days of menses onset), after a negative pregnancy test. Participants maintained conventional work and sleeping patterns and reported no recent (within 10 days) trans-meridian travel, significant weight loss or gain, intercurrent psychosocial stress, substance abuse, neuropsychiatric illness, or systemic disease. A complete medical history, physical examination, and screening tests of hematological, renal, hepatic, metabolic and endocrine function were normal.

Study Design

Volunteers were admitted to the Mayo Clinical Translational Research Unit (CRU) in the evening at or before 2000. In diabetic individuals, the evening dose of short-acting insulin was administered at home according to their prescribed insulin regimen. The evening dose of long-acting insulin was administered either at home or in the CRU. No hypoglycemic events were documented at least 24 h prior to the study visit.

A single forearm intravenous catheter was placed to permit repetitive overnight blood sampling commencing at 2200 h, continuing every 10 min, and ending at 0800 h (total duration, 10 h) for subsequent assays of ACTH and cortisol. Plasma was obtained every 60 min for glucose measurements by the hexokinase reaction. Patients with T1DM also had bedside reflectance-meter glucose (RMG) estimates at the beginning of blood sampling and next morning. Morning glucose values were used by patients to adjust their morning dose of short-acting insulin, administered in the CRU after study completion followed by breakfast and subsequent dismissal.

Hormone Assays

Estradiol and testosterone (E₂ and T) were quantified by mass spectrometry in the Mayo Medical Laboratories (18). Plasma ACTH was assayed via sensitive and specific, solid-phase sequential immunochemiluminescent assay (*Siemens Healthcare Diagnostics, Deerfield, IL 60015*). The detection threshold was 5 ng/L. The intra-assay coefficients of variations (CoV) were 6.4%, 2.7%, and 3.2% at 5.7 ng/L, 28 ng/L, and 402 ng/L respectively. Plasma cortisol was assayed via competitive binding immunoassay (*Beckman Coulter, Inc., Fullerton CA 92834*). The detection limit was 0.4 µg/dL and intra-assay CoVs were 13 %, 9.4%, and 6.6% at 1.6 µg/dL, 2.8 µg/dL, and 30 µg/dL respectively. Glucose samples were collected in a sodium fluoride tube and measured photometrically utilizing a hexokinase reagent. Intra-assay CoVs are 0.5% at 42 mg/dL, 0.6% at 83 mg/dL and 0.5% at 124 mg/dL. C-peptide was measured via Cobas e411 electrochemiluminescence assay (Roche Diagnostics, Indianapolis, IN), as described (19). Transcortin (CBG) was

assayed by ELISA (BioVendor Laboratory Inc., Ashville, NC, 28806) (20). Estimated free cortisol concentrations were calculated by Coolen's equation, as modified by Mazer thereafter (21,22).

Deconvolution analysis

Overnight ACTH and cortisol concentration-time series were analyzed by way of a recently developed variable-waveform deconvolution method (2). The model estimates basal secretion, a slow-phase half-life, secretory-burst mass, random effects on burst mass, and a 3-parameter flexible Gamma probability distribution to embody secretory-burst waveform (23). The rapid phase half-life was assumed to be 3.5 min for ACTH and 2.4 min for cortisol, representing 37% of total decay (16,24). Units of parameters are burst frequency (number per 10-h session), slow half-life (min), basal and pulsatile secretion rates (concentration units/session), mass secreted per burst (concentration units), and waveform mode (time delay to maximal secretion after burst onset, min).

Dose-response analysis

The dose-response model is a 3-parameter logistic function relating ACTH concentrations to delayed pulsatile cortisol secretion (24–26). Specific dose-response properties are defined by the following parameters: 1) efficacy (asymptotically projected maximal ACTH-stimulated cortisol secretion rate, $\mu\text{g/dL/min}$); 2) ACTH concentration mediating one half-maximal cortisol secretion (EC_{50} , ng/L); and 3) sensitivity (maximal positive slope of the ACTH-cortisol dose-response relationship).

Statistical analysis

Baseline subject characteristics in the 2 subject groups were compared via an unpaired two-tailed t-test. Ten-hour mean plasma ACTH and serum cortisol concentrations derived from 61 serial measurements, and glucose derived from 10 serial measurements served as the primary endpoints. The (natural logarithm of) mean ACTH, cortisol and glucose concentrations in the T1DM and control group were compared using Student's two-tailed unpaired t-test and confirmed with a Mann-Whitney U test when appropriate. Power was >85% to detect a 30% contrast in the primary endpoint (17).

Mechanistic outcome measures were deconvolution-based secretory parameters and ACTH-cortisol dose-response estimates. Protected experiment-wise $P < 0.05$ was construed as significant. Data are presented as the mean $\pm$ SEM. Systat version 13.1 (Systat Software Inc. Chicago, IL, www.systat.com) was used for statistical analysis, and SigmaPlot version 12.3 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com) for illustrations.

Results

Baseline (screening 0800 h) subject characteristics in the T1DM and control group were first compared by unpaired 2-tailed Student's t-testing of T1DM vs controls independently of gender. Mean glucose ($P = 0.002$, 11.8 ± 1.8 vs 4.6 ± 0.09 mmol/L), HgA1c ($P < 0.001$, 8.6 ± 0.58 vs $4.9 \pm 0.05\%$), and C-peptide concentrations ($P < 0.001$, 0.21 ± 0.12 vs 1.7 ± 0.28 ng/mL) were predictably different between the T1DM and control groups, respectively. The

11 T1DM patients averaged 4 yr younger than the 10 controls ($P=0.03$, 21 ± 1.1 vs 24 ± 1.3 yr). However, BMI (26 ± 1.9 vs 24 ± 1.2 kg/m², $P=0.38$), estradiol (79 ± 31 vs 49 ± 8.1 pg/mL, $P=0.70$), testosterone (339 ± 95 vs 353 ± 111 ng/dL, $P=0.81$), transcortin (CBG 502 ± 12 vs 473 ± 11 nmol/L, $P=0.18$), total cortisol (14 ± 1.6 vs 15 ± 1.2 µg/dL, $P=0.22$) and ACTH (25 ± 5.5 vs 24 ± 3.2 ng/L, $P=0.67$) did not differ by group. When gender was included, Table 1, ANOVA of the 4 subsets of subjects revealed expected sex differences in serum testosterone (T) and estradiol (E₂) measurements, and expected group differences between T1DM and controls in glucose, glycated hemoglobin, and C-peptide. There were trends toward lower free cortisol ($P=0.08$) and ACTH ($P=0.07$) concentrations in diabetic women than healthy adults of either sex.

Impact of T1DM on HPA axis

Mean ACTH concentrations, Figure 1 (*left*), as well as deconvolved ACTH secretory and kinetic parameters (Table 2) were determined by 10-min sampling overnight for 10 h. The values were comparable between the T1DM and the control group. Mean total cortisol concentrations overnight in the T1DM arm showed a trend towards lower levels (5.0 ± 0.40 µg/dL) compared with controls (6.1 ± 0.4 µg/dL, $P=0.057$): Figure 1 (*middle*). In conjunction with a numerical trend toward higher CBG noted in T1DM vs controls, T1DM patients had significantly lower free cortisol concentrations (calculated from baseline albumin, cortisol and transcortin levels) than controls: 0.34 ± 0.03 µg/dL vs. 0.48 ± 0.04 µg/dL, respectively less ($P=0.012$): Table 2..

Deconvolution analysis of the 10-h data revealed that the T1DM group also had lower total (basal plus pulsatile) 10-h cortisol secretion, $P=0.019$, and nearly significantly lower 10-h pulsatile cortisol secretion, $P=0.06$. Mean hourly glucose concentrations were significantly higher in T1DM than controls (158 ± 20 mg/dL vs. 93 ± 1.7 mg/dL; $P=0.001$): Figure 1 (*right*). Linear regression analysis did not reveal a correlation between mean 10-h glucose and either mean total cortisol concentrations ($R=-0.11$, $P=0.65$) or pulsatile cortisol secretion ($R=-0.02$, $P=0.94$) in the combined cohorts ($N=21$).

ACTH concentration-cortisol secretion dose-response estimates disclosed that the T1DM group had greater ACTH EC₅₀ (14.2 ± 3.6 ng/L vs 4.9 ± 1.9 ng/L, $P=0.04$), lower estimated endogenous ACTH efficacy than controls (0.32 ± 0.03 µg/dL/min vs 0.45 ± 0.04 µg/dL/min, respectively, $P=0.009$), and higher ACTH sensitivity (1.5 ± 0.04 vs 0.45 ± 0.2 slope units, $P=0.03$): Figure 2. In the combined groups, T1DM's effects on ACTH-cortisol dose-response parameters were supported by the positive correlation of ACTH efficacy and negative correlation of ACTH-cortisol sensitivity with C-peptide levels ($R=0.60$, $P=0.004$ and $R=-0.43$, $P=0.04$, respectively).

Impact of gender on HPA axis

Analysis of a possible gender impact on mean 10-h overnight ACTH concentrations in the combined cohorts revealed significant differences in mean ACTH levels between men ($N=11$) 15 ± 1.2 ng/L and women ($N=10$) 10 ± 1.1 ng/L, $P=0.014$. This was due to significantly lower mean ACTH concentrations in T1DM women than T1DM men (9.1 ± 0.9 ng/L vs 15 ± 1.8 ng/L, $P=0.018$): Figure 3. No gender differences were seen in the

control arm. Deconvolution analysis indicated that decreased mean ACTH concentrations in T1DM women compared with T1DM men were a result of lower mean pulsatile ACTH secretion (137 ± 29.2 ng/L/10 h vs 270 ± 34.2 ng/L/10 h, $P=0.03$) and lower total ACTH secretion (312 ± 31.2 ng/L/10 h vs 501 ± 54.3 ng/L/10 h, $P=0.017$). Gender differences in diabetics were accompanied by a positive correlation between mean T concentrations and mean ACTH concentrations in the ($N=11$) T1DM group ($R=0.60$, $P=0.05$) with similar trends between T levels and pulsatile and total ACTH secretory rates ($R=0.55$, $P=0.079$, and $R=0.58$, $P=0.064$, respectively).

Mean total cortisol concentrations did not differ significantly between genders in either the T1DM arm or control arm, despite lower ACTH levels in women than men in both groups (above). This could be explained by a compensatory increase in ACTH-cortisol dose-response sensitivity in T1DM women, when compared with their male counterparts (2.5 ± 0.57 vs 0.71 ± 0.30 slope units; $P=0.03$). Moreover, there was a strong negative correlation between ACTH-cortisol sensitivity and T concentrations in the combined T1DM group ($N=11$); $R=-0.98$, $P=0.001$.

Gender comparison between cortisol secretion in the T1DM and control groups suggested that, compared with their healthy female counterparts, T1DM women had significantly lower pulsatile (52 ± 3.9 $\mu\text{g/dL}/10$ h vs 72 $\mu\text{g/dL}/10$ h, $P=0.028$) and total cortisol secretory rates (63 ± 4.1 $\mu\text{g/dL}/10$ h vs 88 ± 6.4 $\mu\text{g/dL}/10$ h, $P=0.028$). Lower cortisol output in T1DM women was likely secondary to their (1) lower ACTH potency defined by higher ACTH EC_{50} (20 ± 5.4 ng/L vs 4.1 ± 1.8 ng/L, $P=0.04$) and (2) lower ACTH efficacy (0.28 ± 0.04 $\mu\text{g/dL}/\text{min}$ vs 0.50 ± 0.05 $\mu\text{g/dL}/\text{min}$, $P=0.015$). Male subjects did not show significant differences for the same set of parameters: Figure 2. A linear regression model revealed that in the combined group of women ($N=10$), C-peptide levels correlated negatively with ACTH EC_{50} ($R=-0.80$, slope= -1.04 , $P=0.003$), positively with ACTH efficacy ($R=0.86$, slope= 1.14 , $P<0.001$) and negatively with ACTH-cortisol sensitivity ($R=-0.71$, slope= -1.11 , $P<0.01$): Figure 4. Higher E_2 levels in the same 10 women correlated with lower ACTH EC_{50} ($R=-0.70$, $P=0.02$), greater ACTH efficacy ($R=0.68$, $P=0.012$) and lower ACTH-cortisol sensitivity ($R=-0.72$, $P=0.005$).

Discussion

The purpose of this study was to explore HPA axis regulation in T1DM individuals using frequent overnight blood sampling and contemporary analytical tools. ACTH-cortisol dynamics are important clinically in this context, because current understanding of diabetes-related modulation of the HPA axis is derived mainly from animal models. For example, Chan *et al.* have shown that streptozotocin-induced diabetes in rats leads to HPA axis activation at the level of CRH gene expression in the paraventricular nucleus. This results in a strong feedforward cascade, comprising increased CRH, ACTH and corticosterone output, decreased hypothalamic-pituitary feedback sensitivity (3,12), and increased adrenal sensitivity to ACTH along with adrenal hypertrophy (27,28). In contrast, clinical studies are conflicting. Several authors have reported elevated baseline ACTH and total cortisol concentrations (5,29), increased adrenal sensitivity to ACTH (5) and impaired HPA axis feedback (4,30). At the same time, other studies identified no differences in HPA axis

regulation between diabetic and non-diabetic individuals (31–33). Two others noted an obtunded adrenal response to ACTH stimulation (14,15). No earlier study has estimated ACTH dose-responsiveness, plasma free cortisol concentrations or cortisol secretion rates.

Our findings point toward decreased overnight free cortisol concentrations and deconvolved cortisol secretion rates in the T1DM group, particularly in women. Noninvasive ACTH-cortisol dose-response analyses indicated that hyposecretion of cortisol in T1DM was secondary to increased ACTH EC₅₀ (signifying reduced ACTH potency), and decreased ACTH efficacy (lower projected maximal ACTH action). Contrary to expectations, neither elevated glucose concentrations nor HgA1c predicted cortisol output, implying that neither short nor long-term glycemic control per se specifies this measure of adrenal steroidogenesis. However, C-peptide did predict ACTH-cortisol dose-response estimates and cortisol secretion. In particular, serum C-peptide levels correlated negatively with ACTH EC₅₀ and positively with ACTH efficacy when healthy and diabetic individuals were analyzed together. These outcomes are consistent with earlier studies showing that decreased insulin availability is associated with diminished ACTH efficacy (14,34), increased cortisol metabolism via 5- α reductase, and attenuated cortisol synthesis via 11- β hydroxysteroid dehydrogenase type I, culminating in lower total cortisol concentrations (32,35). Diminished free cortisol concentrations in the present T1DM group might also be explained via hyperglycemia-related glucose toxicity of zona fasciculata cells of the adrenal cortex, possibly analogous to pancreatic beta-cell glucose toxicity (36). Additionally, reactive oxygen species generated by the reduced form of nicotinamide adenine dinucleotide phosphate oxidase may cause adrenocortical cell injury and downregulate adrenocortical transcription factors in T1DM (37). Lastly, T1DM-related hyperglycemia might impair adrenocortical cell function by binding of advanced glycation end products to ACTH (MC2) receptors (38) or by altering glycosylation patterns of the physiologically essential MC2 receptor accessory protein (MRAP), thereby restricting ACTH binding and efficacy (39).

A plausible suggestion for our findings of lower ACTH efficacy and potency in T1DM is downregulation of ACTH signaling by intermittent hyperstimulation of the adrenal gland in diabetes mellitus. In a diabetic mouse model, blunted HPA counterregulatory stress responses are also noted to hypoglycemia (14). In this regard, cytokines, like IL-1, IL-6 and TNF- α , which are also implicated in the pathogenesis of T1DM, could be toxic to adrenocortical cells, while additionally impairing cortisol-ACTH/CRH feedback (40). We do not know the basis for increased adrenal sensitivity to ACTH inferred here in T1DM patients, although it may be a compensatory adaptation to lower ACTH potency.

Caveats include the small combined group size of 21 subjects evaluated in this pilot HPA study. Reduced ACTH drive in females compared with males with type 1 diabetes mellitus is a new finding, which would need to be confirmed in further studies in a larger number of men and women. Factors related to the dawn phenomenon, such as GH, epinephrine and glucagon, were not measured in this particular study. Finally, dose-varying injections of ACTH would be required to verify the analytical outcomes reported here, and to test their reversibility with good diabetes control.

In conclusion, in this study of 11 diabetic and 10 control subjects, T1DM conferred apparent resistance to endogenous ACTH despite adequate ACTH secretion. In particular, T1DM patients (specifically women) manifested lower ACTH efficacy and lower potency (higher ACTH EC₅₀) than healthy controls. The reversibility and gender selectivity of these outcomes will need to be confirmed in larger cohorts.

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Figure 1.

Time-varying overnight concentrations of plasma ACTH and matching serum cortisol (both sampled every 10 min) and plasma glucose (every hour). The T1DM group (N=11) is represented by closed circles, and the control group (N=10) by open circles. Glucose concentrations show expected differences in the two cohorts. Data are the mean $\pm$ SEM.

Figure 2.

Dose-response estimates of ACTH EC₅₀, ACTH efficacy and adrenal sensitivity (*slope*). Comparisons are made by two-way ANOVA between the T1DM and control arm (*uppercase letters*) and between genders (*lowercase letters*). Different (unshared) uppercase alphabetic letters represent significant differences between T1DM and control arms, while different lowercase letters designate significant differences between genders, at $P < 0.05$. Data are the mean $\pm$ SEM.

Figure 3.

ACTH concentrations sampled every 10 min overnight in 6 men with T1DM and 5 women with T1DM. Men are represented by closed circles and women by open circles. Data are the mean $\pm$ SEM.

Figure 4.

Linear regressions of ACTH-cortisol dose-response estimates on C-peptide levels in 10 women. The female T1DM group (N=5) is represented by closed circles and female control group (N=5) by open circles. The slopes and P values are given.

Table 1

Baseline characteristics by diagnosis and gender

Characteristics	T1DM (N=11)		Controls (N=10)		P-Value ANOVA
	Males	Females	Males	Females	
N	6	5	5	5	
Age (years)	20 ± 1.5	20 ± 1.7	23 ± 1.8	25 ± 1.8	0.17
BMI (kg/m ²)	25 ± 2.3	26 ± 3.5	24 ± 0.9	23 ± 2.4	0.81
Glucose (mmol/L)	14 ± 3.2 ^A	9.6 ± 1.2 ^A	4.7 ± 0.2 ^B	4.6 ± 0.1 ^B	0.008
HgA1c (%)	8.4 ± 0.7 ^A	8.8 ± 1.0 ^A	5.0 ± 0.1 ^B	4.9 ± 0.1 ^B	<0.001
C-peptide (ng/mL) *	0.1 ± 0.1 ^A	0.4 ± 0.3 ^A	1.5 ± 0.2 ^B	1.9 ± 0.2 ^B	<0.001
Estradiol (pg/mL) *	31 ± 4.5 ^A	136 ± 62 ^B	40 ± 3.6 ^A	59 ± 16 ^B	0.02
Testosterone (ng/dL) *	594 ± 66 ^A	32 ± 6.4 ^B	679 ± 53 ^A	27 ± 2.5 ^B	<0.001
Cortisol total (µg/dL) *	15 ± 2.3	12 ± 2.1	14 ± 2.0	15 ± 1.8	0.70
Cortisol, free (µg/dL) *	0.37 ± 0.06	0.30 ± 0.03	0.49 ± 0.04	0.48 ± 0.08	0.08
ACTH (ng/L) *	33 ± 8.6	15 ± 3.5	29 ± 3.9	19 ± 4.5	0.07
Transcortin, CBG (nmol/L) *	487 ± 15	516 ± 18	463 ± 8	483 ± 21	0.18

Data are presented as mean ± SEM.

* To convert to SI units, multiply C-peptide by 331, estradiol by 3.68, testosterone by 0.0347, cortisol by 27.6 and ACTH by 0.220.

Different alphabetic superscripts across any given row designate P<0.05 by *post hoc* Tukey's honestly significantly different test.

Table 2

Overnight 10-h ACTH and cortisol parameters between groups

Parameters	Type 1 DM (N=11)	Control (N=10)	P-Value
A. ACTH			
Mean ACTH concentration (ng/L)	12.3 ± 1.4	13.2 ± 1.2	0.55
ACTH burst mass (ng/L)	37.1 ± 6.9	37.2 ± 4.4	0.59
ACTH basal secretory rate (ng/L/10h)	205 ± 19	197.3 ± 18	0.76
ACTH pulsatile secretory rate (ng/L/10h)	210 ± 30	251 ± 34	0.29
ACTH total secretion (ng/L/10h)	415 ± 43	448 ± 42	0.51
B. Cortisol			
Mean Cortisol concentration (µg/dL)	5.0 ± 0.40	6.1 ± 0.4	0.057
Mean Free Cortisol concentration (µg/dL)	0.34 ± 0.03	0.48 ± 0.04	0.012
Cortisol burst mass (µg/dL)	12.9 ± 2.2	13.0 ± 0.76	0.46
Cortisol basal secretory rate (µg/dL/10h)	11.1 ± 1.6	15.5 ± 2.8	0.44
Cortisol pulsatile secretory rate (µg/dL/10h)	59.2 ± 4.4	69.7 ± 3.0	0.06
Cortisol total secretory rate (µg/dL/10h)	70.3 ± 4.8	85.2 ± 3.5	0.019

Boldface denotes P<0.05.

Data are the mean ± SEM of 10-h overnight sampling data.