

Hypothalamic-Pituitary-Adrenal Axis Dysregulation and Memory Impairments in Type 2 Diabetes

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Context: There is evidence of both hypothalamic-pituitary-adrenocortical (HPA) axis and cognitive dysfunction in type 2 diabetes mellitus (T2DM). However, the exact nature and the associations between these abnormalities remain unclear.

Objectives: The aim of the study was to characterize the nature of the HPA dysregulation in T2DM and ascertain whether impaired cognition in T2DM could be attributed to these abnormalities.

Design: A cross-sectional study was performed, contrasting matched groups on HPA axis function and cognition by using the combined dexamethasone (DEX)/CRH test and a neuropsychological battery assessing declarative and working memory, attention, and executive function.

Setting: The study was conducted in a research clinic in an academic medical center.

Participants: Participants were volunteers functioning in the cognitively normal range. We studied 30 middle-aged individuals with T2DM, on average 7.5 yr since diabetes diagnosis, and 30 age-, gender-, and education-matched controls.

Main Outcome Measures: Basal cortisol levels, cortisol levels during the DEX/CRH test, and performance on neuropsychological tests were measured.

Results: Individuals with T2DM had elevated basal plasma cortisol levels, higher levels after DEX suppression, and a larger response to CRH (all $P \leq 0.005$). Among individuals with T2DM, cortisol levels during the DEX/CRH test were positively associated with glycosylated hemoglobin ($P = 0.05$), independent of age, body mass index, hypertension, and dyslipidemia. Diabetic subjects showed cognitive impairments restricted to declarative memory. Across all subjects, declarative memory was inversely associated with cortisol levels; however, these associations were subsumed by glycemic control (glycosylated hemoglobin).

Conclusions: HPA hyperactivity and declarative memory deficits are present in T2DM. Both alterations may reflect the negative impact of poor glycemic control on the hippocampal formation. (*J Clin Endocrinol Metab* 92: 2439–2445, 2007)

THE HYPOTHALAMIC-PITUITARY-adrenocortical (HPA) axis responds to stress. CRH from the hypothalamus leads to ACTH release from the pituitary. This in turn stimulates secretion of glucocorticoids (GCs) from the adrenal cortex. Once elevated, GCs exert a negative feedback via the pituitary, hypothalamus, and hippocampus (1). By acting on a wide array of target tissues, GCs are important for successful adaptation. Although acute cortisol elevations during stress are protective, chronically elevated levels have mostly negative effects (2).

GCs and glucose regulation are closely linked. For example, when cortisol levels increase, they increase blood glucose levels (3). It has been speculated that hypercortisolism can result in visceral obesity, dyslipidemia, and insulin resis-

tance (4). This order of cause and effect is clear in the case of Cushing's disease (5). However, in other conditions, the reverse order may also be operant, with disturbances in glucose regulation leading to hypercortisolemia.

Several studies have focused on the effect of type 2 diabetes mellitus (T2DM) on HPA axis functioning (6). Overall, these studies point toward increased HPA axis activity, however, results have been inconsistent, which can in part be attributed to differences in protocols and subject characteristics across studies.

Reports of elevations in basal cortisol levels in plasma are inconsistent, with one study showing elevated levels (6) and another reporting no alterations (7). A study using salivary cortisol measures observed elevated evening levels in T2DM (8). Cortisol levels among individuals with diabetes were shown to be associated with glycemic control (9), further suggesting that HPA axis dysregulation is linked to T2DM.

Neuroendocrine challenge studies demonstrate increased responsiveness of the HPA axis in T2DM. For example, a stronger elevation of cortisol occurs after CRH administration (10). Evidence for reduced feedback inhibition in the dexamethasone (DEX) suppression test has been found in some instances (11), but not all (7). Again, the varying populations studied as well as differing DEX doses used might explain these divergent results.

First Published Online April 10, 2007

Abbreviations: ANCOVA, Analysis of covariance; AUC, area under the curve; BMI, body mass index; CVLT, California Verbal Learning Test; DEX, dexamethasone; GC, glucocorticoid; HbA1c, glycosylated hemoglobin; HPA, hypothalamic-pituitary-adrenocortical; IQ, intelligence quotient; MANOVA, multivariate analysis of variance; MMSE, Mini Mental State Examination; QUICKI, quantitative insulin sensitivity check index; T2DM, type 2 diabetes mellitus; WAIS-R, Wechsler Adult Intelligence Scale, Revised; WMS-R, Wechsler Memory Scale-Revised.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

It is well established that both T2DM (12) and elevated levels of GCs (13) can negatively affect cognition. T2DM is associated with problems in memory, attention, executive function, and psychomotor speed (14–16), and there is some evidence that these deficits are related to duration and severity of illness (17). The hippocampus, which is essential for declarative memory, has been shown to be smaller among elderly individuals with T2DM (18). Our group has demonstrated that well-controlled middle-aged individuals with T2DM have specific hippocampal volume reductions and declarative memory deficits (19). Furthermore, we have demonstrated hippocampal volume reductions among non-diabetic individuals with insulin resistance (20).

Chronic elevations of GC levels can have deleterious effects on the hippocampus (2). It is important to note that this region, which is affected by both elevated GC levels and T2DM, plays a central role in HPA axis feedback inhibition (21). In addition, the hippocampus has the highest colocalization of insulin and GC receptors in the brain (21), adding to the possible links between impaired HPA axis regulation and T2DM. We have recently postulated a model by which insulin resistance, particularly when coupled with cortisol dysregulation, may lead to hippocampal damage (22).

Although several studies have either reported associations between T2DM and HPA axis dysfunction or impaired cognition, to our knowledge, no study to date has evaluated all three domains in the same sample. The objective of this study was 2-fold. First, we sought to ascertain how basal cortisol levels as well as feedback control of the HPA axis (by the DEX/CRH test) differed between patients with T2DM and controls. Based on prior literature, we hypothesized that individuals with T2DM would show dysregulation of the HPA axis and impairments in cognition. The second goal was to investigate whether the expected cognitive impairments in T2DM could be attributed to the HPA axis dysregulation.

Subjects and Methods

Subjects

A total of 60 volunteers, 30 with T2DM and 30 healthy controls, participated in the study. All were community residing individuals, living independently. Subjects responded to advertisements, were referred by collaborating endocrinologists, or were participating in our studies of normal aging. Participants were between 43 and 74 yr of age, and had a minimum of a high school education. All subjects were in the cognitively normal range. Evidence of neurological, medical (other than diabetes, dyslipidemia, or hypertension), or psychiatric (including depression and alcohol or other substance abuse) signs and symptoms that allowed for a diagnosis to be made, excluded individuals from participation in the study. Participants gave informed written consent and were compensated for their participation. A subset of these subjects also participated in a related study (19). The study protocol was approved by the New York University School of Medicine Institutional Board of Research Associates.

Participants with T2DM

The diabetic group was composed of individuals evaluated as part of a National Institutes of Health sponsored project. Diabetics met one or more of the following criteria: 1) had a fasting glucose value greater than 125 mg/dl on two separate occasions; 2) had a 2-h glucose value greater than 200 mg/dl during a 75-g oral glucose tolerance test; or 3) had a prior diagnosis of T2DM, and were being treated with hypoglycemic agents and/or diet and exercise. None of the patients were being treated with insulin or insulin secretagogues. Although 22 (73%) of the individuals with diabetes were being treated pharmacologically, eight subjects were being treated only with a lifestyle intervention. A total of 25 (83%) subjects met criteria for hypertension, and 25 also met criteria for dyslipidemia (Table 1).

Control participants

Control subjects were part of a larger study of normal aging, and were selected to be age-, gender-, and education-matched to the diabetic group. The maximum difference between matched pairs was 4 yr for age and 2 yr for education. Control subjects were selected not to have evidence of overt insulin resistance, as reflected by the quantitative insulin sensitivity check index (QUICKI) (23); only individuals with QUICKI values above 0.35 were included. Given the associations between insulin resistance and obesity, no attempt was made to match groups on body mass index (BMI). Within the control group, 15 subjects

TABLE 1. Description of the control and T2DM groups

	Control group	Diabetic group
Age (yr)	59.12 ± 8.40	59.16 ± 8.58
No. of females/males	14/16	14/16
Education (yr)	16.17 ± 1.86	15.45 ± 2.44
Time from diagnosis of T2DM (yr)	NA	7.43 ± 7.26
No. on antidiabetic medication	NA	22
Height (m)	1.71 ± 0.11	1.69 ± 0.10
Weight (kg)	75.65 ± 15.79	94.14 ± 19.67
BMI (kg/m ²) ^a	25.71 ± 4.15	33.11 ± 6.60
HbA1c (%) ^a	5.19 ± 0.37	7.5 ± 1.45
Glucose (mg/dl) ^a	79.83 ± 9.21	132.10 ± 49.37
Insulin (μIU/ml) ^a	5.56 ± 1.83	14.54 ± 10.11
No. with hypertension (medications or blood pressure elevation) ^a	15	25
No. on antihypertensive medications	4	23
No. with dyslipidemia ^a	6	25
No. on statin treatment	4	19
High-density lipoprotein (mg/dl) ^a	59.37 ± 14.08	41.79 ± 9.39
Triglycerides (mg/dl) ^a	89.73 ± 38.32	175.59 ± 117.50
Hamilton total score	2.94 ± 3.65	3.00 ± 2.65

Unless noted, values are expressed as mean ± SD. NA, Not applicable.
^a Significant group differences (*P* < 0.05).

(50%) met criteria for hypertension, and six (20%) met criteria for dyslipidemia.

Evaluations

All subjects underwent an assessment that included a physical examination, and endocrine, neuropsychological, and psychiatric evaluations.

Medical examination

Blood pressure and definition of hypertension. Blood pressure was measured during one of the visits. The readings were performed at 0830 h, 30 min after the participants arrived. Hypertension was defined according to the National Cholesterol Education Program guidelines. The following criteria were used: 1) systolic value ≥ 130 mm Hg, 2) diastolic value ≥ 85 mm Hg, or 3) use of antihypertensive medication.

Dyslipidemia. Dyslipidemia was also defined following National Cholesterol Education Program guidelines. The following criteria were used: 1) statin treatment, 2) triglyceride levels ≥ 150 mg/dl, or 3) high-density lipoprotein levels ≤ 40 mg/dl for men and 50 mg/dl for women.

Glycosylated hemoglobin (HbA1c), glucose, and insulin. We assessed plasma levels of glucose, insulin, and HbA1c after an overnight fast. Glucose was measured using a glucose oxidase method (VITROS 950 AT; Ortho-Clinical Diagnostics, Inc., Amersham, UK), insulin by chemiluminescence (Advia Centaur; Bayer Corp., Leverkusen, Germany), and HbA1c using an automated HPLC method (Tosoh Corp., Kanagawa, Japan) certified by the National Glycohemoglobin Standardization Program.

HPA axis endocrine measures

Basal cortisol secretion. Two independent blood samples were collected within 2 min of each other just before the glucose ingestion at 0930 h during a standardized glucose tolerance test. They were averaged and used as an estimate of basal cortisol secretion.

DEX/CRH challenge test. HPA axis feedback was evaluated using the short version of the DEX/CRH test (24). Subjects took 1.5 mg DEX at 2300 h. On the next day, subjects arrived at 1300 h and received a standardized lunch. Afterward, an iv catheter was placed in the forearm and kept patent with a heparin lock. Subjects were asked to sit quietly (but not allowed to sleep) in an easy chair and were allowed to read. No blood sample was drawn for at least 1 h after catheter insertion to allow sufficient time for cortisol to return to baseline. To avoid a response to the unexpected effects of the CRH administration, all subjects were carefully prepared for the possibility that they may experience a sense of breathlessness for a few seconds after injection, or flushing in the face for a few minutes. No subject reported feeling anxious after the CRH injection. Two samples were drawn at 1452 and 1455 h to measure DEX, cortisol, and ACTH levels. At 1500 h, 100 μ g ovine CRH was injected iv. Blood samples were subsequently drawn at 1530, 1545, 1600, and 1615 h. Bloods were kept on ice and spun down in a refrigerated centrifuge (4 C). Plasma was separated into aliquots and frozen (-80 C). The two samples drawn before CRH administration were averaged to obtain a measure of the suppressed HPA axis. The samples drawn after CRH injection, together with those two samples were used to compute an area under the curve (AUC).

Total cortisol was measured with an enzyme immunoassay (EIA; IBL, Hamburg, Germany) with a sensitivity of 0.1 μ g/dl. Intact ACTH was measured with a RIA (Nichols Institute, Bad Nauheim, Germany) with a sensitivity of 2 pg/ml. Assays had interassay and intraassay coefficients of variance less than 12%.

Neuropsychological and psychiatric assessment

All cognitive assessments used were standardized neuropsychological tests described in detail elsewhere (25). Briefly, declarative memory was assessed with the California Verbal Learning Test (CVLT), the Guild immediate and delayed paragraph recall, and the index scores from the Wechsler Memory Scale-Revised (WMS-R). Working memory was evaluated using the Digit Span Backwards from the WMS-R. Executive function was measured with the Controlled Word Association Test

(letters F, A, and S) and the interference score of the Stroop task. Attention was assessed with the perceptual speed test (a cancellation task) and the Digit Symbol Substitution Test from the Wechsler Adult Intelligence Scale-Revised (WAIS-R). General intellectual functioning was assessed using the Shipley Institute of Living Scale. Scores were used to estimate WAIS-R full-scale intelligence quotient (IQ) scores. Depressive symptoms were assessed with the Hamilton Depression Scale (26).

Statistical analyses

Group differences in demographic variables and other group descriptors were tested using independent samples *t* tests. χ^2 or Fisher's exact tests were used for nominal variables. Group differences in endocrine variables were tested using univariate ANOVAs.

For the analysis of the cognitive variables, we first placed variables into four cognitive domains (declarative memory, working memory, attention, and executive function). To control for multiple comparisons, we compared the groups by running multivariate analyses of variance (MANOVAs) for each of the cognitive domains. We then ran follow-up univariate analyses of covariance (ANCOVAs) for the individual variables within the domains that had significant MANOVAs.

Performance on cognitive tests may be influenced by intelligence and/or education (27). Even though our groups were tightly matched on education, they did differ significantly on the IQ. Thus, we used the IQ as a covariate to assess differences in test performance independent of overall intellectual ability.

Associations between cortisol and diabetes-related variables within each group were determined using linear regression analysis, controlling for age and gender.

To determine how much of the variance in the cognitive variables could be explained by HPA axis measures and level of glucose control (HbA1c), respectively, linear regressions including all subjects were used with cognition as the dependent variable. Using all subjects allows coverage of a broader spectrum of long-term glycemic control and its impact on cognition. Because cognition declines with age (28) and may be influenced by gender (29), we controlled for both variables (entered as the first step). As the marker of HPA axis function, we used the variable that was most closely associated with the cognitive variables (cortisol after DEX), and this was entered as the second step. To ascertain whether long-term glycemic control would add to the variance explained by the cortisol after DEX, we added HbA1c as the third step. We then inverted steps two and three to determine the amount of variance explained by the HPA marker after taking glycemic control into account.

Results

Demographic variables and group descriptors

The demographic variables and group descriptors are summarized in Table 1. The groups were closely matched on age, gender, and education. All subjects were functioning within the normal cognitive range, as can be seen by their high Mini Mental State Examination (MMSE) scores; all subjects scored above 26 out of 30. Neither diabetic nor control subjects exhibited signs of depression and did not differ on the Hamilton Depression Scale ($P = 0.936$). As expected, individuals with T2DM had higher BMIs, higher fasting glucose levels, higher HbA1c, as well as higher rates of dyslipidemia and hypertension than control subjects. The 27% of the diabetic subjects treated exclusively with lifestyle intervention did not differ on HbA1c from those that were on oral hypoglycemic agents (7.5% vs. 7.5%, respectively; $P = 0.959$).

HPA axis findings

Group differences in HPA measures. Because the endocrine data were skewed, they were logarithmically transformed. Table 2 shows mean group differences (raw values) and *P* values (derived from the log-transformed data) for these variables.

TABLE 2. HPA axis group comparisons

	Control group	Diabetic group	<i>P</i> value
Basal cortisol ($\mu\text{g/dl}$)	22.77 \pm 24.80	67.85 \pm 60.63	0.002
Cortisol after DEX ($\mu\text{g/dl}$)	3.19 \pm 3.67	11.91 \pm 13.77	0.001
Cortisol AUC (arbitrary unit)	70.95 \pm 81.30	254.29 \pm 269.50	0.002
ACTH after DEX (pg/ml)	5.68 \pm 5.46	8.36 \pm 14.46	0.102
ACTH AUC (arbitrary unit)	134.39 \pm 103.90	210.77 \pm 416.32	0.766

Reported values are raw values (mean \pm SD). *P* values are derived from analyses using logarithmically transformed data.

Overall, there was considerable overlap between the diabetic and control subjects for all three cortisol measures; only some 30% of diabetics showed higher levels than controls. At the group level, however, these differences were significant. As can be seen in Fig. 1 (*left bars*), the diabetic group exhibited elevated basal cortisol levels ($P = 0.002$). The two right bars in Fig. 1 show that post-DEX cortisol values were also significantly higher in the diabetic group ($P = 0.001$). Fig. 2 shows that after CRH administration, cortisol levels were more elevated for all time points in the diabetic group (AUC $P = 0.002$). Although basal ACTH and the ACTH AUC during the DEX/CRH test were also higher in the diabetic group, these differences were not statistically significant (Table 2). Excluding those diabetic subjects that were not taking antidiabetic medication did not change the results. Because there is evidence associating cortisol and ACTH levels with age (30) and gender (31), we controlled for those associations in these analyses. As expected, given that our groups were tightly matched on age and education, these adjustments did not change the results. All subjects had high DEX levels, indicating that they had taken the drug the night before. Controlling for DEX levels also did not change the results.

Within-group findings. After controlling for age and gender, we found significant positive associations between HbA1c and cortisol levels after DEX (R^2 change = 0.201 of total $R^2 = 0.241$ at $P = 0.016$, $\beta = 0.485$, $t = 2.573$, SE of estimate for entire model: 1.28) and cortisol AUC (R^2 change = 0.156 of total $R^2 = 0.353$ at $P = 0.027$, $\beta = 0.424$, $t = 2.356$, SE of estimate for entire model: 1.25) during the DEX/CRH test in the diabetic group. All of these associations remained significant after accounting for BMI, hypertension, and dyslip-

idemia. In contrast, basal cortisol levels were not significantly associated with HbA1c ($P = 0.143$). Within the control group, none of these associations were statistically significant.

Cognitive findings

Between group differences. Significant group differences were found for the IQ, with diabetic individuals having a normal but lower IQ than control subjects (individuals with T2DM: 106 ± 12 , controls: 116 ± 8 ; $P < 0.001$). The only cognitive domain that separated the groups was declarative memory [MANOVA P (Wilk's λ) = 0.004]. In follow-up ANCOVAs of this significant cognitive domain, most of the individual measures of declarative memory remained significant after controlling for the IQ. Individuals with diabetes had lower performance than control subjects on the Guild paragraph immediate ($P = 0.073$) and delayed recall ($P < 0.01$), and the CVLT short ($P < 0.05$) and long delay free recall ($P = 0.065$). There were no statistically significant differences between diabetic and control individuals for the other cognitive domains. However, to allow the reader to compare the results across all cognitive domains, Table 3 shows results from univariate ANCOVAs for all variables. Controlling for hypertension, which could potentially have a negative impact on cognition (32), did not change the results (Table 3).

Associations between HPA axis function and cognition. Across all subjects, after accounting for age and gender (8.9% of the variance), the HPA marker (cortisol levels after DEX suppression) explained an additional 8.3% ($\beta = -0.044$) of variance in the Guild delayed paragraph recall. HbA1c, when added next to this prediction model, accounted for an additional 15.6% ($\beta = -0.472$) of the variance explained, for a total of 32.8%. The last two steps were statistically significant

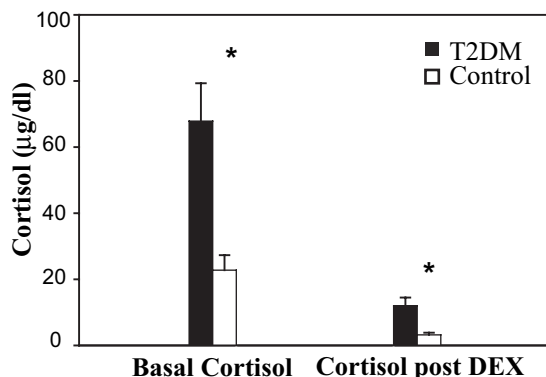


FIG. 1. Basal cortisol levels (mean \pm SEM) for the diabetic and control groups (*left bars*) and the cortisol values post-DEX suppression (before CRH administration, *right bars*). Both basal and suppressed cortisol are significantly higher in the diabetic group, as indicated by an asterisk ($P < 0.05$).

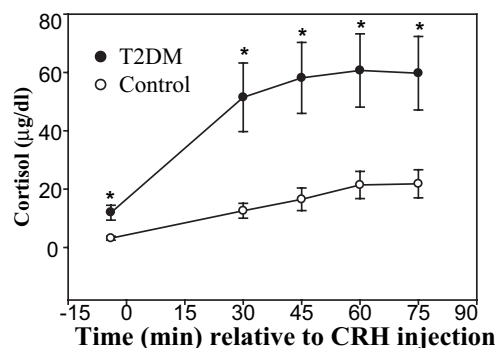


FIG. 2. Cortisol secretion (mean \pm SEM) throughout the DEX/CRH test. Solid black circles represent the diabetic group and open circles the control group. CRH injection occurred at 0 min. Cortisol at all time points, as well as the AUC, was significantly different between groups ($P < 0.05$).

TABLE 3. Cognitive group comparisons derived from ANOVA with the IQ as covariate

	Control group	Diabetic group	P value
Declarative memory immediate recall			
Guild immediate paragraph	8.37 ± 2.18	6.08 ± 2.75	0.073
General index WMS-R	133.31 ± 4.34	125.72 ± 12.59	0.261
Declarative memory delayed recall			
Guild delayed paragraph	9.82 ± 3.02	6.67 ± 2.89	0.007
CVLT short delay	13.07 ± 2.36	10.43 ± 3.79	0.040
CVLT long delay	13.27 ± 2.15	11.20 ± 3.52	0.065
Delayed index WMS-R	125.82 ± 15.18	114.18 ± 15.53	0.221
Working memory			
Digit Span Backwards WMS-R	8.48 ± 2.38	7.04 ± 2.62	0.834
Executive function			
Stroop Interference Score	4.41 ± 7.22	1.17 ± 7.67	0.231
Verbal fluency	16.99 ± 4.20	15.13 ± 4.41	0.963
Attention			
Digit Symbol Substitution Test	55.17 ± 10.21	49.25 ± 10.77	0.629
Perceptual speed correct	77.41 ± 13.46	70.86 ± 11.32	0.256
Attention index WMS-R	112.83 ± 15.07	101.55 ± 16.91	0.228

Values are expressed as mean ± SD.

($P < 0.05$). For the other measures of declarative memory (Guild immediate paragraph recall, CVLT short delay recall, CVLT long delay recall), we found equivalent results (Table 4). When we inverted the order of the second and third steps, HbA1c explained a large amount of variance (23.8%, $\beta = -0.472$; $P < 0.001$) in the Guild delayed paragraph recall score, but the HPA marker when entered as the last step did not add significantly to the explained variance (1%, $\beta = -0.044$; $P = 0.743$). Similar associations were found for the other measures of declarative memory (Table 4). In addition, similar results were found for the other HPA markers (data not shown).

Discussion

Relative to control subjects, our group with T2DM had higher basal cortisol secretion as well as impairments in feedback inhibition of the HPA axis. However, it should be noted that cortisol levels in the diabetic and control groups partly overlapped; only about 30% of the diabetic subjects had levels above those of control subjects. In addition, cortisol elevations were specifically associated with declarative memory impairments across all study subjects, but these associations were mediated by glycemic control (HbA1c).

Elevations in basal cortisol levels in T2DM are in line with

previous studies (6). After DEX administration, cortisol levels were higher among individuals with T2DM, which again is in line with findings reported by others (33). In addition, CRH administration after DEX suppression resulted in a much larger cortisol response in the diabetic group. This increased responsiveness to CRH, coupled with diminished suppression after DEX, indicates an abnormality in HPA feedback sensitivity in T2DM. Previous studies have observed an exaggerated HPA response to this challenge test among depressed patients as well as among the elderly (24, 34). This has been interpreted as a reduction in feedback regulation due to a hippocampal GC receptor deficit (35), a conclusion in line with the model we propose later.

While we found clear cortisol elevations in the T2DM group, ACTH levels, although also higher among diabetics, did not significantly separate the groups. This is likely due to the large variability in ACTH, particularly among individuals with T2DM, although cortisol hypersecretion in the absence of a clear ACTH elevation could also imply that the abnormality in T2DM is more peripheral than central. However, our other findings of decreased declarative memory performance among diabetics, possibly reflecting hippocampal dysfunction, advocate more for a central origin of the HPA axis dysregulation.

TABLE 4. Associations between cognition, HPA marker (DEX-suppressed cortisol), and HbA1c for all subjects, derived from linear regression analyses

	Step 1 (age/gender)			Step 2 (HPA marker)			Step 3 (HbA1c)			
	ΔR^2	β	P value	ΔR^2	β	P value	ΔR^2	β	P value	SE
Guild immediate	<u>0.031</u>	-0.151/-0.043	0.414	<u>0.016</u>	0.111	0.341	<u>0.147</u>	-0.457	0.003	2.54
Guild delayed	<u>0.089</u>	-0.270/-0.066	0.074	<u>0.083</u>	-0.044	0.022	<u>0.156</u>	-0.472	0.001	2.84
CVLT short delay	<u>0.171</u>	-0.240/-0.274	0.005	<u>0.122</u>	-0.191	0.003	<u>0.065</u>	-0.305	0.023	2.81
CVLT long delay	<u>0.156</u>	-0.198/-0.294	0.009	<u>0.098</u>	-0.216	0.010	<u>0.025</u>	-0.190	0.173	2.71

	Step 1 (age/gender)			Step 2 (HbA1c)			Step 3 (HPA marker)			
	ΔR^2	β	P value	ΔR^2	β	P value	ΔR^2	β	P value	SE
Guild immediate	<u>0.031</u>	-0.151/-0.043	0.414	<u>0.154</u>	-0.457	0.002	<u>0.009</u>	-0.111	0.445	2.54
Guild delayed	<u>0.089</u>	-0.270/-0.066	0.074	<u>0.238</u>	-0.472	0.000	<u>0.001</u>	-0.044	0.743	2.84
CVLT short delay	<u>0.171</u>	-0.240/-0.274	0.005	<u>0.161</u>	-0.305	0.001	<u>0.026</u>	-0.192	0.143	2.81
CVLT long delay	<u>0.156</u>	-0.198/-0.294	0.009	<u>0.090</u>	-0.190	0.013	<u>0.033</u>	-0.216	0.121	2.71

The columns represent the order of steps, and the numbers that are shown for the total models are the change in R^2 (ΔR^2 , *underlined*), β value, P value for the ΔR^2 , and the SE of the estimate for the final model (in column 3 only).

As also described previously by others (9), HbA1c, a marker of long-term glycemic control, was associated, independent of age, with HPA axis dysregulation in the diabetic group. Interestingly, these associations remained after controlling for BMI, hypertension, and dyslipidemia, suggesting that they result from a direct impact of T2DM on the HPA axis. Dysregulation of the HPA axis in T2DM appears to become more prominent when the disease is more poorly controlled. Although the design of our study does not permit us to draw conclusions as to the order of events, we suggest that T2DM leads to hippocampal damage, which in turn leads to disruptions in HPA axis regulation. However, other investigators have proposed that these events occur in the reverse order (36).

As anticipated, subjects with T2DM showed specificity for cognitive impairments in declarative memory. Our groups did not differ on tasks assessing working memory, attention, and executive functioning. This specificity is likely due to the relative short time from diagnosis (7.43 ± 7.26 yr), the majority of the patients being relatively young (<60 yr of age in average), and the high vulnerability of the hippocampus to any type of damage (37). The notion that in T2DM hippocampal-based functions are particularly affected early on is supported by neuropsychological and structural imaging studies by our group as well as others (18–20). As the disease progresses and other less vulnerable brain areas become affected, the cognitive impairments may spread to other cognitive domains.

Although we found negative associations between cortisol levels and declarative memory across all subjects, those associations were mediated by the level of glycemic control; when adjusting for HbA1c, those associations disappeared. Based on the data presented here, in conjunction with another study conducted by our group, which showed associations between HbA1c and hippocampal volumes (19), we hypothesize that T2DM may cause primary damage to the hippocampus (with its associated declarative memory dysfunction), which then leads to a disruption of the HPA axis. The hippocampus is not only a target structure for GCs but also plays a fundamental role in HPA axis feedback regulation (21). Dysregulation of the HPA axis caused by hippocampal damage results in elevated cortisol levels, which further impacts on the hippocampus (37), thus creating a vicious cycle.

We recently proposed a model suggesting that this hippocampal damage may be, at least in part, a result of the endothelial dysfunction that accompanies insulin resistance (22). Briefly, endothelial dysfunction may result in functional hypoglycemia during periods of increased glucose demand, *i.e.* during brain activation. This relative hypoglycemia, when coupled with cortisol elevations, may result in damage to vulnerable regions like the hippocampus (22). Alternative mechanisms, suggesting damage from toxic species (38), advanced glycation end products (39), and nutrient-excess alterations of the activity of mammalian target of rapamycin (40) have also been postulated.

Some limitations of this study should be noted. First, although our sample was sufficiently large to detect significant differences in HPA axis as well as memory, it is relatively small (30 subjects per group). Second, despite our groups

being tightly age-matched and also controlling for age in our regression analyses, the sample included a relatively wide age range. Third, our measure of basal cortisol secretion was a single time point, which, although standardized at 0930 h across subjects, may not be representative of basal secretion. Fourth, we did not assess corticosteroid-binding globulin levels and cannot directly evaluate the role of free cortisol levels in our findings. Fifth, we included participants who were receiving blood pressure or lipid-lowering therapies, which could have influenced our results. However, because high blood pressure and dyslipidemia are very common in T2DM, we believe that our approach increases the generalizability of our findings. Finally, the study design does not permit us to draw conclusions about the order of events, and future research is needed to address this issue.

In summary, we demonstrated that individuals with T2DM have elevated basal cortisol levels and disturbances in HPA axis feedback regulation. HPA axis hyperactivity was associated with the degree of glycemic control as represented by HbA1c. In addition, we showed that subjects with T2DM showed specificity for deficits in declarative memory. Finally, reductions in declarative memory performance appear to be primarily related to the level of glycemic control rather than resulting from HPA axis dysregulation because the associations between declarative memory and cortisol were subsumed by HbA1c. We propose that early on in the course of T2DM, the hippocampus is damaged, which in turn leads to impaired HPA axis feedback regulation, and, thus, to the abnormalities in HPA axis reported here. This might create a vicious cycle of chronic elevations in cortisol, leading to more hippocampal dysfunction, resulting in even further cortisol elevations.

Future longitudinal studies should focus on subjects in the preclinical stages of the illness and follow them during their transition into diabetes. This will provide data on the order of events leading to HPA axis dysregulation and hippocampal dysfunction. In addition, improvements in glycemic control might lead to improvements in cognition (41). Therefore, treatment studies may provide valuable information on the role of the HPA axis on cognition in T2DM. Future research should include more comprehensive longitudinal evaluation of endocrine status, cognition, and brain imaging.

Acknowledgments

We thank Professor Clemens Kirschbaum at the University of Dresden, Germany, for kindly having conducted the cortisol and ACTH assays for this project.

Received November 20, 2006. Accepted April 2, 2007.

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This work was supported by the National Institutes of Health DK064087 and P30-AG-08051 and support from the New York University General Clinical Research Center (NCRR M01 RR00096).

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Disclosure Information: The authors have no conflict and nothing to report.

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