ORIGINAL ARTICLE

Altered Prefrontal Glutamate–Glutamine–γ-Aminobutyric Acid Levels and Relation to Low Cognitive Performance and Depressive Symptoms in Type 1 Diabetes Mellitus

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Context: Neural substrates for low cognitive performance and depression, common long-term central nervous system–related changes in patients with type 1 diabetes mellitus, have not yet been studied.

Objective: To investigate whether prefrontal glutamate levels are higher in patients with type 1 diabetes and whether an elevation is related to lower cognitive performance and depression.

Design: Cross-sectional study.

Setting: General clinical research center.

Participants: One hundred twenty-three patients with adult type 1 diabetes with varying degrees of lifetime glycemic control and 38 healthy participants.

Main Outcome Measures: With the use of proton magnetic resonance spectroscopy, prefrontal glutamate–glutamine–γ-aminobutyric acid (Glx) levels were compared between patients and control subjects. Relationships between prefrontal Glx levels and cognitive function and between Glx levels and mild depressive symptoms were assessed in patients with type 1 diabetes.

Results: Prefrontal Glx concentrations were 9.0% (0.742 mmol/L; $P=.005$) higher in adult patients with type 1 diabetes than in healthy control subjects. There were positive linear trends for the effects of lifetime glycemic control on prefrontal Glx levels (P for trend=.002). Cognitive performances in memory, executive function, and psychomotor speed were lower in patients ($P=.003$, .01, and $<.001$, respectively) than in control subjects. Higher prefrontal Glx concentrations in patients were associated with lower performance in assessment of global cognitive function (0.11 change in z score per 1-mmol/L increase in Glx) as well as with mild depression.

Conclusions: The high prefrontal glutamate levels documented in this study may play an important role in the genesis of the low cognitive performance and mild depression frequently observed in patients with type 1 diabetes. Therapeutic options that alter glutamatergic neurotransmission may be of benefit in treating central nervous system–related changes in patients with adult type 1 diabetes.

Arch Gen Psychiatry. 2009;66(8):878-887

Patients with type 1 diabetes mellitus (T1DM), particularly those with poor glycemic control, often undergo central nervous system (CNS)–related changes, manifested through low cognitive performance[1-3] and depression.[4-7]

Evidence from large-scale longitudinal studies of patients with T1DM suggests that prolonged hyperglycemia may cause low cognitive performance.[8] Microvascular or macrovascular complications in the peripheral organ systems, reflective of long-term exposure to hyperglycemia, have frequently co-occurred with low cognitive performance in patients with T1DM.[2,9,10] However, few studies have assessed the relationship between alterations in neural substrates and cognitive performance in patients with T1DM.[2,11]

A proton brain magnetic resonance spectroscopy (MRS) study with a clamp technique design documented a linear relationship between peripheral hyperglycemia and cerebral glucose levels in healthy volunteers.[12] Other proton MRS studies in T1DM cohorts of small sample sizes (range, 6-17),

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without a clamp design, showed elevated cerebral glucose levels.[11,13-15]

Given that cerebral glucose catabolism and the tricarboxylic acid cycle are closely coupled to the glutamine-glutamate cycle,[16,17] high cerebral glucose levels in patients with T1DM might be expected to lead to increased glutamate synthesis. Furthermore, antibodies to the glutamate decarboxylase (GAD) enzyme, which may also alter cerebral glutamate levels, have been found in the majority of patients with T1DM tested even during the early phase of disease.[18-20] Although glutamate, a major excitatory neurotransmitter, and its receptors have key roles in processes of learning and memory,[21] overstimulation potentially damages neuronal cells owing to calcium overload.[22] Maintaining the optimal balance of this major neurotransmitter in the CNS may thus be critically important.[23]

However, to our knowledge, the possibility of a sequential cascade starting from an increased cerebral glucose level, resulting in disturbed cerebral glutamate homeostasis and the eventual lowering of cognitive performance and depression, has not yet been studied in patients with diabetes. In an era when measurement of cerebral glucose and glutamate levels is increasingly available,[16,17] this knowledge in the target organ might provide more direct information for controlling CNS-related changes in diabetes, including suggestions for better treatment options.

We conducted proton MRS imaging to measure prefrontal glutamate–glutamine–γ-aminobutyric acid (Glx) and glucose levels and assessed cognitive function and current depressive symptoms in a large cohort of adult patients with T1DM with varying degrees of lifetime glycemic control and in healthy control subjects. This study provided a unique opportunity to examine the association between prefrontal metabolite levels and cognitive function in a naturalistic and clinical environment.

We hypothesized that prefrontal Glx levels would be higher in adult patients with T1DM than in healthy control subjects and that higher prefrontal Glx levels would be associated with lower cognitive function and depression in patients with T1DM. We also aimed to determine whether the relationship between prefrontal Glx levels and cognitive function in adult patients with T1DM would differ from that in healthy control subjects, thus indicating differential effects of prefrontal Glx on cognitive function in normal and pathological conditions.

Considering the beneficial effects of stringent glycemic control on cognitive function,[8,24] we additionally examined whether maintaining the glycemic control within target (lifetime glycated hemoglobin [HbA_{1c}] level $<7\%$) rather than beyond target ($\geq 7\%$) would be beneficial in keeping cerebral glucose and glutamate homeostasis within the normal range. (To convert HbA_{1c} level to a proportion of total hemoglobin, multiply by 0.01.)

METHODS

PARTICIPANTS

Study participants consisted of 123 adult patients with T1DM and 38 healthy control subjects. Patients with T1DM who consecutively visited the Joslin Diabetes Center, Boston, Massachusetts, and McLean Hospital's Brain Imaging Center, Belmont, Massachusetts, from January 2003 through June 2005, who were between the ages of 25 and 40 years, and who had a disease duration of 15 to 25 years were eligible for the initial inclusion.

Diabetic complications that served as exclusion criteria included proliferative diabetic retinopathy requiring laser treatment, clinically significant diabetic nephropathy (evidenced by urinary albumin to creatinine ratio >300 μg/mg or serum creatinine levels >1.5 mg/dL for men and >1.4 mg/dL for women [to convert to micromoles per liter, multiply by 88.4]), painful or symptomatic neuropathy, or gastroparesis. Potential participants with major medical and neurologic disorders and contraindications to magnetic resonance (MR) imaging were excluded, as were those with a history of psychosis, schizophrenia, bipolar disorder, attention-deficit/hyperactivity disorder, or cocaine, heroin, or alcohol dependence or with a current major depressive episode as assessed with the Structured Clinical Interview for *DSM-IV*.[25] Patients with hypoglycemic symptoms or diabetic ketoacidosis at the time of imaging were also excluded.

The study was approved by respective institutional committees on human subjects, and all subjects gave informed consent prior to participation.

NEUROPSYCHOLOGICAL AND CLINICAL ASSESSMENTS

A battery of neuropsychological tests was administered to assess 3 cognitive domains: memory function, executive function, and psychomotor speed. After adjusting for age, sex composition, and educational level, scores of each neuropsychological test were converted to *z* scores with the use of group means and standard deviations of healthy control subjects. If necessary, test scores were reversed to indicate better performances with positive *z* scores.

The composite score for memory function was made by averaging *z* scores of the immediate memory and the delayed recall from the Wechsler Memory Scale III.[26] The composite score for executive function was constructed by averaging *z* scores of the trail-making number-letter switching, verbal fluency, design fluency, card-word interference, and card sorting from the Delis-Kaplan Executive Function System[27]; the digit symbol substitution from the Wechsler Adult Intelligence Scale[28]; and the letter-number sequencing and spatial span from the Wechsler Memory Scale III.[26] The composite score for psychomotor speed was calculated by averaging *z* scores of the dominant and nondominant hand on the grooved pegboard test.[29] The compound score for the global cognitive function was calculated by averaging *z* scores of composite scores of the 3 cognitive domains.

Internal consistency of the neuropsychological tests within each cognitive domain was assessed by means of Cronbach α coefficient analysis. Cronbach α coefficients for memory function, executive function, and psychomotor speed were 0.89, 0.82, and 0.71, respectively.

The 17-item Hamilton Depression Rating Scale[30] was used to assess current depressive symptoms.

Lifetime average HbA_{1c} level, which reflects the level of lifetime glycemic control, was defined as the average value of HbA_{1c} levels, grouped and time-weighted every 4 years for the entire duration of the disease.[31] Patients with T1DM were divided into 2 subgroups on the basis of whether the level of lifetime glycemic control was good (ie, within target [lifetime average HbA_{1c} level $<7\%$]) or poor ($\geq 7\%$).[24] A severe hypoglycemic episode was defined as an event that leads to a coma or to unconsciousness, on the basis of the Diabetes Control and Complications Trial Research Group criteria.[31-33] Date of diagnosis was obtained from

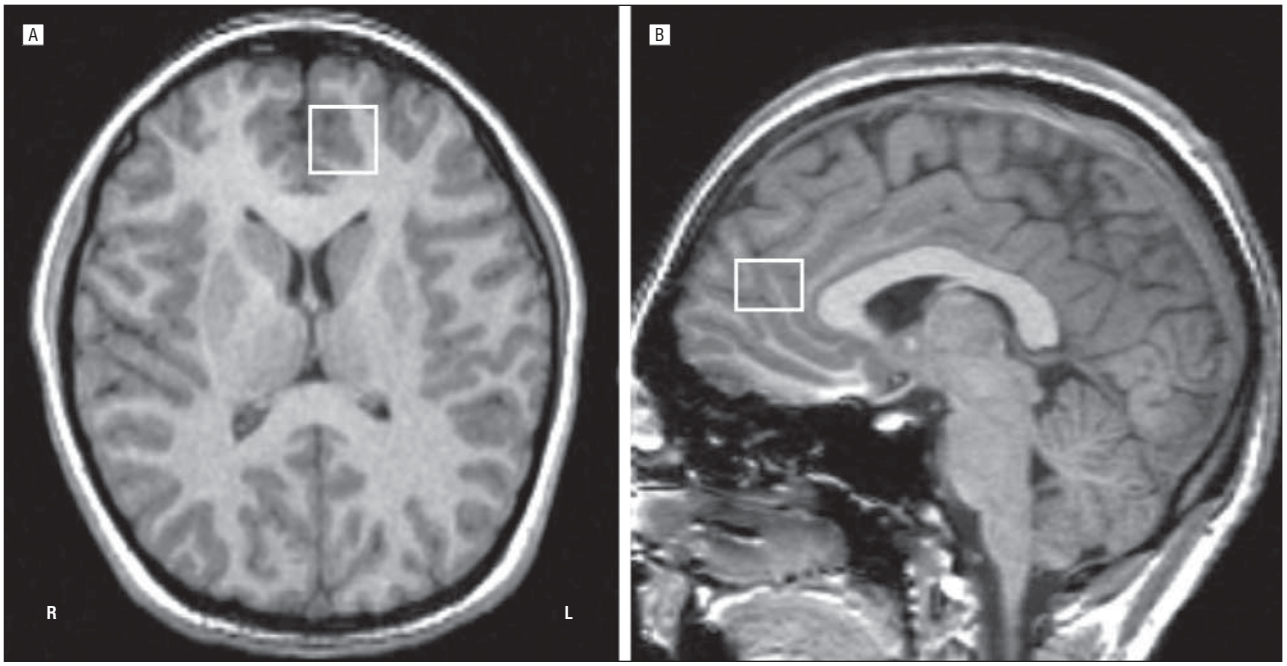

Figure 1. Axial (A) and midsagittal (B) planes of T1-weighted image showing the typical location (white box) of the voxel positioned on the left anterior cingulate cortex. The left anterior cingulate cortex voxel of interest was selected in an axial section at the level of the genu of the corpus callosum. The right boundary of the voxel was placed at the midline, and the posterior boundary was placed approximately 5 mm anterior to the genu of the corpus callosum. A 15-mm-thick voxel was built on this bottom voxel section. The upper left corner was placed approximately 10 mm from the inner cranial boundary.

medical records or patient self-report. Hand preference was evaluated by the Edinburgh Handedness Inventory.[34]

Current HbA_{1c} levels, measured approximately 30 minutes before the start of MR imaging, were used as a measure of recent ambient blood glucose levels in the present study. Detailed methods and results for the acquisition of data pertaining to the relationship between recent peripheral glycemia and prefrontal glucose levels are described in eSupplement 1 (http://www.archgenpsychiatry.com).

MR IMAGE ACQUISITION AND PROTON MRS

The MR imaging was conducted at the McLean Brain Imaging Center with the use of a 1.5-T whole-body imaging system (Horizon LX; GE Medical Systems, Milwaukee, Wisconsin). All images were obtained with the use of a custom-made linear birdcage coil with approximately 40% improvement in the signal to noise ratio and improved homogeneity over a standard quadrature head coil.[35] A 3-dimensional spoiled gradient echo pulse sequence was used to produce 124 contiguous coronal images, 1.5 mm thick (echo time [TE], 5 milliseconds; repetition time [TR], 35 milliseconds; matrix, 256×192; field of view [FOV], 24 cm; flip angle [FA], 45°; and number of excitations [NEX], 1). Axial T2-weighted images (TE, 80 milliseconds; TR, 3000 milliseconds; matrix, 256×192; FOV, 20 cm; FA, 90°; NEX, 0.5; and section thickness/gap, 3/0 mm) and fluid attenuated inversion recovery axial images (TE, 133 milliseconds; TR, 9000 milliseconds; inversion time, 2200 milliseconds; matrix, 256×192; FOV, 20 cm; FA, 90°; and NEX, 1; section thickness/gap, 5/2 mm) were obtained to screen for gross brain structural abnormalities.

Although studies on T1DM-related structural and functional brain changes are rather scarce,[11] impaired prefrontal cortical function, including diminished psychomotor speed and mental flexibility, has consistently been documented in patients with T1DM.[1] On the basis of these neuropsychological studies, the anterior cingulate cortex was selected as our voxel of interest (VOI). Single-voxel proton MR spectra (20×20×15 mm, 6 cm^3) were acquired from the left anterior cingulate cortex VOI while it was viewed on orthogonal T1-weighted images (**Figure 1**).

After an automated shimming routine was used for each voxel, a point-resolved spectroscopy pulse sequence[36] was used with the following acquisition variables: TR, 1500 milliseconds; TE, 45 milliseconds; 2500-Hz spectral width; 8-step phase cycling; and 256 transients. Spectra were analyzed by means of the Linear Combination of Model Spectra (LCModel), a fully automated program for quantitating metabolite concentrations from spectroscopic data.[37]

With the use of a linear combination model of the basis set, absolute metabolite concentrations (in millimoles per liter) were estimated by using the unsuppressed water signal as an internal concentration reference[38] for *N*-acetyl aspartate–*N*-acetyl aspartyl glutamate, Glx, creatine-phosphocreatine (Cr), choline, *myo*-inositol, and glucose. The macromolecular and lipid basis spectra were also included into the LCModel fitting to control for their contribution.[39,40] Short-echo localized proton MRS has been used in detecting high cerebral glucose resonances, which are reflected by peaks at 3.43 ppm, in patients with T1DM.[13,15]

The relative MRS metabolite levels were expressed as the ratio of each metabolite peak to the Cr peak at 3.0 ppm. Spectral quality and reliability of fit for all metabolites were adequate, and the detailed descriptions are presented in eSupplement 2. The mode, median, and mean values of relative error estimates (Cramer-Rao lower bound values) for each metabolite, calculated by the LCModel, are presented in eTable 1.

To control for the effects of tissue composition on prefrontal metabolite levels, gray matter (GM), white matter, and cerebrospinal fluid volumes in the VOI were measured. There were no differences in the proportions of GM, white matter, or cerebrospinal fluid in the VOI between groups (eTable 2). Metabolite concentrations were corrected for cerebrospinal fluid volumes in the VOI.[41] Furthermore, to control for variations of metabolite concentrations between GM and white matter, final analyses were conducted with the GM proportion of each VOI used as a covariate.

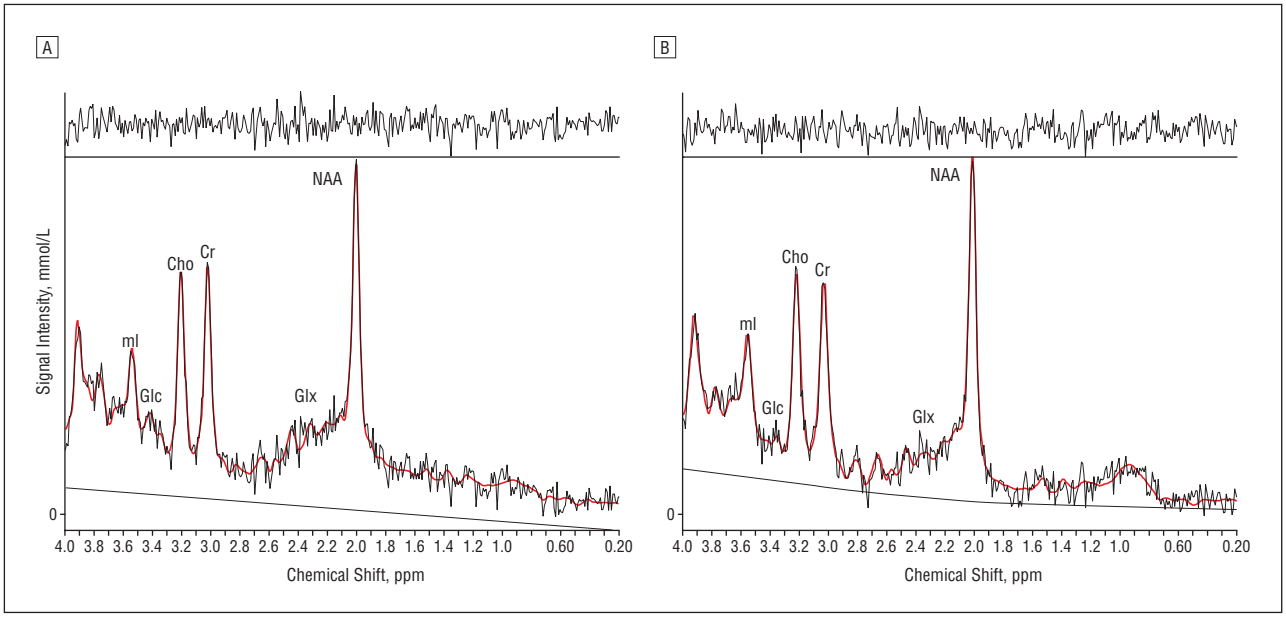

Figure 2. Sample magnetic resonance spectroscopy spectra extracted from the left anterior cingulate cortex voxel of interest from a patient with type 1 diabetes mellitus (A) and from a healthy control subject (B). Cr indicates creatine-phosphocreatine; Cho, choline; Glc, glucose; Glx, glutamate–glutamine–γ-aminobutyric acid; mI, *myo*-inositol; and NAA, *N*-acetyl aspartate–*N*-acetyl aspartyl glutamate. The Linear Combination of Model Spectra (LCModel) estimated baselines are shown by the smooth gray line. The LCModel fit to metabolite signals is shown by the red heavy line. The raw data are in the thin gray trace. At the top of each plot, the residual signal following fitting is displayed.

Validation for quantifying metabolites in the phantom was conducted as a measure of the reproducibility (eTable 3). Coefficients of variation for each metabolite suggest that LCModel-fitted metabolite concentrations from the phantom were highly reproducible over the study period.

Figure 2 shows the sample spectra, which were measured in a patient with T1DM and a corresponding healthy control subject.

STATISTICAL ANALYSIS

Independent *t* test/analysis of variance and χ^2 test were used for comparing continuous and categorical variables, respectively. Prefrontal metabolite concentrations were compared between adult T1DM and healthy control groups by means of an analysis of covariance with age, sex composition, and GM proportion as covariates.

To evaluate how the level of long-term glycemic control is associated with prefrontal metabolite concentrations, values were compared between T1DM subgroups, as divided by degree of lifetime glycemic control (good vs poor) relative to control subjects, using the analysis of covariance with a test for linear trend.

Z-transformed neuropsychological test scores after adjusting for age, sex composition, and educational level were used in the analyses. Values were compared between adult patients with T1DM and healthy control subjects *and* between T1DM subgroups by means of independent *t* tests.

Multiple linear regression analyses were performed to assess the relationship between prefrontal metabolite (glucose and Glx) levels and behavioral measures (scores of cognitive domains and the Hamilton Depression Rating Scale) in healthy control subjects and in adult patients with T1DM. These models were controlled for age, sex composition, and educational level in healthy control subjects and additionally controlled for the duration of illness and presence of lifetime hypoglycemic episodes in adult patients with T1DM. Regression lines were compared, with an interaction term as an indicator, between healthy control subjects and adult patients with T1DM to evaluate whether the pattern of associations between prefrontal metabolite concentrations and cognitive function differ between groups.

Statistical significance, 2-tailed, was defined at $\alpha < .05$. Stata 5.0 (StataCorp, College Station, Texas) was used for all computations.

RESULTS

There was no significant difference in age, sex composition, educational level, handedness, or proportion of smokers between adult T1DM and healthy control groups or between the 2 T1DM subgroups (**Table 1**). However, the healthy control group included more nonwhites than did the adult T1DM group ($P < .001$). The 2 T1DM subgroups did not differ in most diabetes-specific clinical characteristics of duration of illness, age at onset, or the number of hypoglycemic episodes, but there was a difference in the lipid profile (Table 1).

Prefrontal metabolite concentrations and ratios to Cr are shown in **Table 2**. Prefrontal glucose concentrations were higher, by 88.9% (0.872 mmol/L), in adult patients with T1DM relative to healthy control subjects ($F_{1,142} = 26.10$, $P < .001$). There was a positive linear trend (a dose-responsive pattern, healthy control group vs good vs poor glycemic control T1DM subgroups) for the relationship between lifetime glycemic control and prefrontal glucose concentrations (P for trend $< .001$; **Figure 3**).

Prefrontal Glx concentrations were 9.0% higher (0.742 mmol/L) in adult patients with T1DM than in healthy control subjects ($F_{1,151} = 8.15$, $P = .005$). A linear trend for the relationship between lifetime glycemic control and prefrontal Glx concentrations was observed (P for trend = .002; Figure 3). This linear trend remained unchanged when the smoking status was included as an additional covariate (P values of the Glx difference between groups = .01; P for trend = .004).

Table 1. Demographic and Clinical Characteristics of 123 Patients With Type 1 Diabetes Mellitus and 38 Healthy Control Subjects

Characteristic	Control Subjects (n = 38)	All Diabetic Patients (n = 123)	*P* Value, Control Subjects vs All Patients[a]	Type 1 Diabetic Patients[b]		*P* Value, Good vs Poor Glycemic Control Subgroups[a]
				Good Glycemic Control (n = 22)	Poor Glycemic Control (n = 101)	
Demographics						
Age, mean (SD), y	30.8 (5.1)	32.3 (4.4)	.09	32.9 (4.6)	32.1 (4.4)	.46
Sex, No. (%) F	18 (47)	71 (58)	.26	11 (50)	60 (59)	.29
Educational level, mean (SD), y	17.4 (1.9)	16.4 (3.2)	.06	17.0 (2.2)	16.3 (3.3)	.31
Right-handed, No. (%)	35 (92)	119 (97)	.22	21 (95)	98 (97)	.55
Current smoker,[c] No. (%)	1 (3)	5 (4)	.60	0	5 (5)	.39
Race, No. (%)						
White	29 (76)	116 (94)	.001	20 (91)	96 (95)	.37
Nonwhite	9 (24)	7 (6)		2 (9)	5 (5)	
Medical						
Lipids, mean (SD), mg/dL						
HDL-C	51.4 (14.7)	57.5 (16.2)	.04	56.0 (14.3)	57.9 (17.0)	.66
LDL-C	106.6 (32.8)	112.0 (34.0)	.39	106.9 (20.1)	113.1 (36.3)	.43
Total cholesterol	179.2 (32.4)	185.3 (40.9)	.39	174.9 (24.3)	188.0 (43.6)	.17
Triglycerides	101.8 (56.6)	84.1 (62.8)	.12	58.4 (28.3)	89.4 (67.3)	.04
Blood glucose,[d] mean (SD), mg/dL	NA	164.0 (62.2)	NA	149.7 (56.4)	166.1 (63.4)	.55
Diabetes-specific clinical characteristics, mean (SD)						
Duration of illness, y	NA	19.9 (3.5)	NA	20.1 (3.4)	19.9 (3.5)	.77
Age at onset, y	NA	12.5 (5.2)	NA	12.8 (4.6)	12.4 (5.4)	.74
Glycated hemoglobin, %	5.08 (0.33)	7.83 (1.35)	<.001	6.70 (0.79)	8.08 (1.32)	<.001
Lifetime average glycated hemoglobin, %	NA	8.12 (1.16)	NA	6.59 (0.38)	8.46 (0.99)	<.001
No. of hypoglycemic episodes	NA	4.46 (11.0)	NA	4.23 (10.5)	4.51 (11.1)	.92

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NA, not available or not applicable.

SI conversion factors: To convert HDL-C, LDL-C, and total cholesterol to millimoles per liter, multiply by 0.0259; glucose to millimoles per liter, multiply by 0.0555; glycated hemoglobin to a proportion of total hemoglobin, multiply by 0.01; and triglycerides to millimoles per liter, multiply by 0.0113.

[a] Group differences were tested by independent *t* tests for age, educational level, and lipid level and by χ^2 tests for sex composition, handedness, and race.

[b] Patients with type 1 diabetes were divided into good (within the treatment target range) and poor glycemic control subgroups according to the level of lifetime average glycated hemoglobin. Patients with good glycemic control were defined as those with a lifetime average glycated hemoglobin level less than 7%. Patients with poor glycemic control were defined as those with a lifetime average glycated hemoglobin level of 7% or greater.

[c] Data were available in 119 patients with type 1 diabetes and 34 healthy comparison subjects.

[d] Blood glucose levels were measured at the time of imaging in a subsample (n = 45; 6 patients with good and 39 with poor glycemic control) of patients with type 1 diabetes.

Table 2. Prefrontal Metabolite Concentrations and Ratios in Patients With Type 1 Diabetes Mellitus and Healthy Control Subjects[a]

	Degree of Metabolic Control			*P* Value for Trend[d]	All Diabetic Patients	*P* Value[e]
	Normal	Good[b]	Poor[c]			
Metabolite concentrations, mmol/L[f]						
Glucose	0.99 (0.35)	1.63 (0.77)	1.92 (0.96)	<.001	1.87 (0.93)	<.001
Glx	8.32 (1.76)	8.67 (1.93)	9.15 (1.63)	.002	9.07 (1.69)	.005
Cr	6.71 (0.76)	6.52 (0.76)	6.69 (0.80)	.82	6.66 (0.80)	.84
NAA	7.04 (0.63)	6.92 (0.60)	7.05 (0.78)	.53	7.03 (0.75)	.75
Choline	1.59 (0.24)	1.57 (0.19)	1.60 (0.28)	.66	1.59 (0.26)	.93
Myo-inositol	3.66 (0.64)	3.78 (0.52)	3.82 (0.71)	.20	3.81 (0.68)	.22
Metabolite ratios						
Glucose:Cr	0.15 (0.05)	0.25 (0.12)	0.29 (0.15)	<.001	0.28 (0.14)	<.001
Glx:Cr	1.23 (0.21)	1.34 (0.28)	1.38 (0.32)	.004	1.37 (0.31)	.004
NAA:Cr	1.06 (0.11)	1.07 (0.10)	1.06 (0.10)	.82	1.06 (0.10)	.65
Choline:Cr	0.24 (0.03)	0.24 (0.02)	0.24 (0.03)	.72	0.24 (0.03)	.72
Myo-inositol:Cr	0.55 (0.08)	0.58 (0.09)	0.57 (0.09)	.20	0.57 (0.09)	.11

Abbreviations: Cr, creatine-phosphocreatine; Glx, glutamate–glutamine–γ-aminobutyric acid; NAA, *N*-acetyl aspartate–*N*-acetyl aspartyl glutamate.

[a] Data are mean (SD) values. Glucose measurements in 14 cases and Glx measurements in 5 cases were excluded due to poor spectral fit.

[b] Lifetime average glycated hemoglobin level less than 7% (to convert to a proportion of total hemoglobin, multiply by 0.01).

[c] Lifetime average glycated hemoglobin level 7% or greater.

[d] Values were compared among the groups by means of analysis of covariance with a test for linear trend controlling for age, sex composition, and gray matter proportion.

[e] Values were compared between healthy control subjects and patients with type 1 diabetes mellitus by means of analysis of covariance with age, sex composition, and gray matter proportion as covariates.

[f] Data are values corrected for cerebrospinal fluid.

There were no significant differences in concentrations of other prefrontal metabolites, including Cr, choline, *myo*-inositol, or *N*-acetyl aspartate–*N*-acetyl aspartyl glutamate between adult T1DM and healthy control groups.

In addition to analyses using absolute metabolite concentrations, the same sets of analyses using ratios of each metabolite to Cr were conducted. Prefrontal glucose:Cr ($F_{1,142}$=26.42, $P<.001$) and Glx:Cr ($F_{1,151}$=8.55, $P=.004$) ratios were also higher in patients with T1DM than in healthy control subjects (Table 2). Positive linear trends between the level of lifetime glycemic control and of prefrontal glucose:Cr and Glx:Cr ratios were observed (*P* for trend $<.001$ and $=.004$, respectively).

Adult patients with T1DM showed substantially lower performances in 3 domains of cognitive function (memory function: effect size [ES] as defined by the adjusted mean difference in *z* scores, –0.65; $P=.003$; executive function: ES, –0.29; $P=.01$; and psychomotor speed: ES, –0.55; $P<.001$) relative to healthy control subjects. There was no difference in cognitive performance between the 2 T1DM subgroups. Detailed adjusted *z* scores of each neuropsychological test are summarized in eTable 4.

Multiple regression analysis showed that higher prefrontal Glx concentrations were associated with lower performance in global cognitive function (β [SE]=–0.11 [0.04], $P=.002$), and specifically in memory function (β [SE]=–0.23 [0.07], $P=.001$) and executive function (β [SE]=–0.07 [0.03], $P=.045$) among adult patients with T1DM. Each increase of 1 mmol/L in prefrontal Glx concentrations was associated with 0.23 ES lower performance in memory function and 0.07 ES lower performance in executive function in adult patients with T1DM (**Figure 4**). Prefrontal Glx concentrations in adult patients with T1DM were also positively correlated with Hamilton Depression Rating Scale scores (β [SE]=0.51 [0.22], $P=.02$). The similar negative association between prefrontal Glx:Cr ratio and global cognitive function was observed in patients with T1DM (β [SE]=–0.53 [0.19], $P=.007$). Glx:Cr ratios were also associated with lower performance in memory and executive function (β [SE]=–1.04 [0.36], $P=.005$, and β [SE]=–0.43 [0.17], $P=.02$, respectively). A similar set of analyses was conducted with a subgroup of patients with T1DM who received the rapid-acting insulin analogues while covarying for insulin dose and time between insulin injections and MR imaging. Results from this subgroup analysis were similar to analyses with all patients with T1DM, although there were slight decreases in significance levels.

The negative associations between prefrontal Glx concentrations and cognitive function, however, were not observed in analyses of healthy control subjects. Multiple linear regression analysis using interaction terms confirmed that the effects of prefrontal Glx concentrations on cognitive function in adult patients with T1DM differed from those in healthy control subjects (*P* for interaction=.003, .046, and .001 for memory function, executive function, and global cognitive function, respectively; Figure 4). The pattern of associations between prefrontal Glx:Cr ratio and cognitive function also differed between groups (*P* for interaction=.005, .04, and .004 for memory function, executive function, and global cognitive function, respectively).

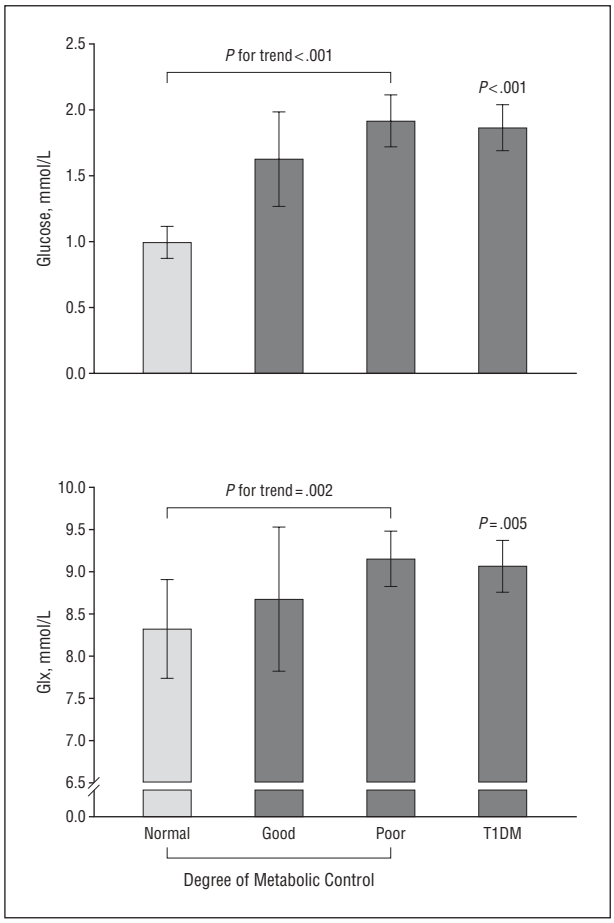

Figure 3. Cerebral metabolite concentrations according to degree of metabolic control. T1DM indicates type 1 diabetes mellitus; Glx, glutamate–glutamine–γ-aminobutyric acid. Error bars represent 95% confidence intervals. Mean glucose and Glx concentrations are cerebrospinal fluid–corrected values. Prefrontal glucose ($P<.001$) and Glx ($P=.005$) concentrations were higher in patients with T1DM than in healthy control subjects. There were linear trends for the effect of metabolic control on prefrontal glucose ($P<.001$) and Glx ($P=.002$) concentrations.

There were no significant associations between prefrontal glucose concentrations and cognitive function in adult patients with T1DM or healthy control subjects. However, an exploratory subgroup analysis including patients with T1DM (n=58) with lifetime HbA_{1c} levels of greater than 8% showed a negative association between prefrontal glucose concentrations and psychomotor speed (β [SE]=–0.27 [0.11], $P=.02$).

COMMENT

The present report documents that adult patients with T1DM have high prefrontal Glx levels and that higher prefrontal Glx levels are associated with lower performance in cognitive function and mild depression.

Although low cognitive performance has been reported in some patients with T1DM, particularly those with long-term exposure to hyperglycemia,[1-3,8] there is a less clear understanding regarding potential mediators for this CNS-related change. The present study may provide a new insight into its underlying pathophysiologic processes.

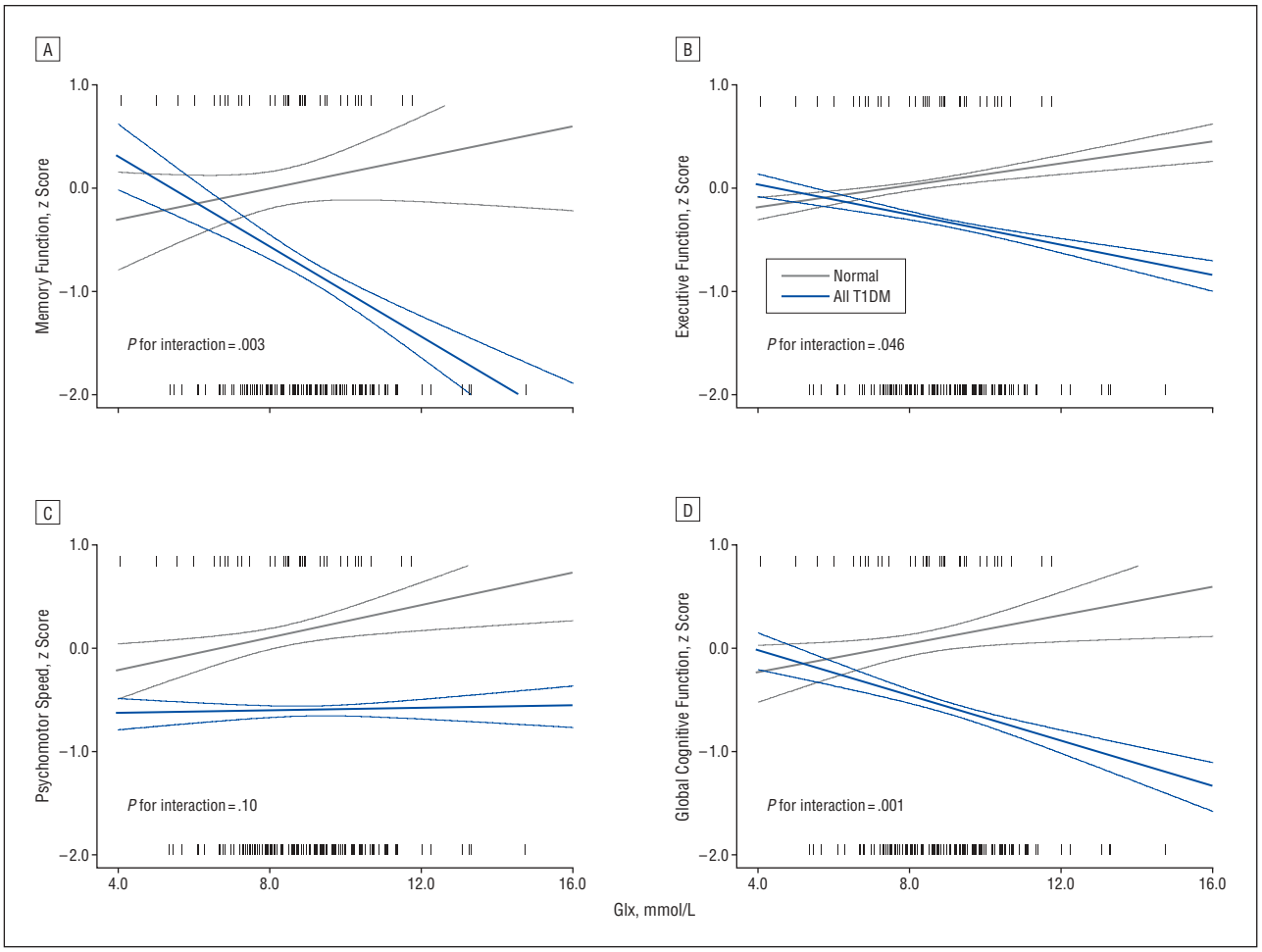

Figure 4. Regression lines of prefrontal glutamate–glutamine–γ-aminobutyric acid (Glx) concentrations and estimated cognitive function in patients with type 1 diabetes mellitus (T1DM) (blue lines) and healthy control subjects (gray lines). Heavy blue and gray lines represent the fit of linear regression modeling in all patients with T1DM and healthy control subjects, respectively; thin lines denote their fitted 95% confidence intervals. Tick marks above the fitted lines represent cerebrospinal fluid–corrected prefrontal Glx concentrations of healthy control subjects, whereas tick marks below the fitted lines represent those of patients with T1DM. Estimates for function of each cognitive domain were made after controlling for age, sex composition, and educational level. Higher prefrontal Glx concentration was associated with lower performance in memory function ($P<.001$) (A), executive function ($P=.045$) (B), and global cognitive function ($P=.001$) (D) in patients with T1DM. This pattern was not observed in healthy control subjects. Regression lines were compared between healthy control subjects and patients with T1DM by means of interaction terms. Regression lines for memory ($P=.003$), executive function ($P=.046$), and global cognitive function ($P=.001$) differed between groups.

Glutamate, a primary excitatory neurotransmitter of the brain and a major component of the Glx spectrum, is a key molecule in the processes of learning and memory.[21,42] When present beyond the capacity of glial uptake, however, glutamate accumulates extracellularly and has an excitotoxic effect on neurons.[22]

Differences between the effects of prefrontal glutamate on the cognitive function of healthy control subjects and on the cognitive function of our patients with T1DM, as we have noted, suggested that high prefrontal glutamate levels above the normal range in patients with T1DM may cause neurotoxic effects[22] instead of serving as a substrate for neuronal plasticity.[42] High prefrontal glutamate levels may in part explain reductions in cortical gray matter density, alterations we have recently seen in patients with T1DM.[31]

In animal models of diabetes, alterations in the composition and function of glutamate receptor subtypes and subunits frequently occur.[43] This diabetes-induced perturbation of the glutamate receptor system may, in part, explain the differential effects of prefrontal glutamate on the cognitive function of adult patients with T1DM and on the cognitive function of healthy control subjects.

Preclinical and clinical reports suggest that alterations in the synthesis and function of a family of neurotrophins may play an important role in several T1DM-related CNS changes.[44-46] Considering the neurotrophins' neuroprotective effects against glutamate-induced excitotoxicity,[47,48] the altered function of neurotrophins in patients with T1DM may partly be responsible for the association between high prefrontal Glx levels and low cognitive performance in our T1DM cohort.

Although preventive optimal glycemic control, recommended for the reduction of T1DM-related complications, is likely to be beneficial in preventing decreases in cognitive performance, specific interventional treatments have not yet been suggested.[2] Our finding regarding the relationship between high prefrontal glutamate levels and low cognitive performance may provide an insight into expanding treatment options. Drugs acting on

homeostasis of the glutamatergic system may be of benefit in preventing low cognitive performance in patients with diabetes.[49,50] Interestingly, *N*-methyl-D-aspartic acid receptor antagonists have recently been used in slowing cognitive decline in Alzheimer disease.[23,51]

Prefrontal glucose levels, which were also higher in patients with T1DM, were not related to cognitive function in analyses of patients with T1DM and T1DM subgroups. Patients with more poorly controlled diabetes who have a lifetime average HbA_{1c} level greater than 8%, however, showed a negative association between prefrontal glucose levels and cognitive function. This exploratory analysis indirectly suggests the presence of a threshold for glycemic control or a range of cerebral glucose levels at which cerebral glucose may directly affect cognitive function in patients with T1DM.

Depression affects up to 25% of patients with T1DM,[6,7] increasing diabetic complications and mortality as well as decreasing overall quality of life.[5-7] Although poor glycemic control is regarded as a risk factor for depression,[6] its mechanism remains to be further clarified.[7] Associations between dysregulated glutamatergic system and mild depressive symptoms, observed in the present study, may provide clues to uncovering a neurochemical mechanism of depression in T1DM. Because depression in patients with medical disorders is often resistant to standard treatments,[52] alternative treatment options may be suggested in view of the current findings. Considering that the glutamatergic system has recently emerged as an important target for drug development, particularly in mood disorders with an atypical nature,[53-55] drugs acting on the glutamatergic system, such as ketamine hydrochloride, lamotrigine, and riluzole,[56] may have the potential to help improve depressive symptoms in patients with T1DM.

Our findings in patients with T1DM are different from those of a recent study on depressed patients with type 2 diabetes, which reported decreased Glx resonances in subcortical brain regions.[57] This discrepancy may stem from several factors, including differences in sample characteristics, such as age, type of diabetes, comorbid medical conditions, and presence of current depression; in MRS methods used; and in brain regions examined.

In the present study, prefrontal Glx levels were 9.0% higher in patients with T1DM than in healthy control subjects, and they increased uniformly as the level of glycemic control worsened from normal through good to poor (4.2% and 10.0% of Glx level increment compared with healthy control subjects, respectively). A similar pattern of uniform increases in prefrontal glucose levels was observed.

We reasoned that sustained exposure to hyperglycemia would alter cerebral energy metabolism and change cerebral metabolite levels. Considering that high intracellular glucose concentrations lead to increased oxidative phosphorylation and, possibly, an accelerated glutamate-glutamine cycle,[16] high prefrontal glutamate levels in patients with T1DM are likely to stem from increased de novo synthesis, ie, cellular adaptations to hyperglycemia. Preclinical evidence indicates that elevated glucose levels increase cerebral glutamate levels and that this link, ultimately, contributes to neuronal damage in diabetic as well as ischemic conditions.[58-60] Data from the current study suggest that keeping glycemic control in the near-normal range is likely to help diabetic patients maintain optimal prefrontal glutamate levels, which may then reduce the risk of having lower cognitive performance.

High prefrontal Glx concentrations in patients with T1DM may be attributed in part to T1DM-specific conditions. Patients with T1DM frequently have antibodies to GAD,[18,61] the rate-limiting enzyme that catalyzes the decarboxylation of glutamate to γ-aminobutyric acid. These autoimmune abnormalities may lead to excessive glutamate accumulations.[62] Consequently, patients with T1DM, especially those with high levels of GAD antibodies, may be vulnerable to glutamate-induced neuronal damage.

Patients with T1DM have been reported to have high cerebral *myo*-inositol levels.[13,14] Considering *myo*-inositol's function as an osmotic agent and a storage form for glucose,[17,63-65] an increase in *myo*-inositol levels may reflect osmotic changes in the brain or inadequate glycemic control in patients with T1DM. Although there was no difference in prefrontal *myo*-inositol levels between subjects in our T1DM and control groups, patients with T1DM with a lifetime HbA_{1c} level higher than 8% showed a higher *myo*-inositol:Cr ratio than healthy control subjects ($F_{1,93}=4.41$, $P=.04$). This is partly in accord with a previous report that suggested that *myo*-inositol may work as a cerebral osmolyte, particularly in diabetic patients with poor glycemic control.[66]

Several potential confounding factors should be considered in interpreting our results. Although hyperglycemia-induced microvascular and macrovascular complications are relatively uncommon in patients before the age of 40 years,[2] vascular injury in the brain may have contributed to the occurrence of low cognitive performance and depression. In our recent study, conducted with a similar but smaller T1DM cohort,[32] however, we did not find an increased prevalence of brain T2 white matter hyperintensities, which are indirect markers of vascular injury. In addition, the relatively young ages (mean, 32.3 years) and short disease durations (mean, 19.9 years) of our patients with T1DM lessen the likelihood of the confounding by aging and cerebral vascular changes.

Although cognitive function in patients with T1DM may be influenced by previous severe hypoglycemic episodes,[2] this association was not confirmed in a recent large-scale longitudinal trial.[8,67] In the present study, the number or pattern of hypoglycemic episodes was not associated with cognitive function.

Mild ketosis, which frequently occurs in patients with T1DM,[68-70] can influence cerebral glutamate metabolism. In cerebral ketone body metabolism, glutamate is transformed to γ-aminobutyric acid through the GAD pathway.[71,72] Reduced GAD activity in patients with T1DM[62] may further contribute to increases in cerebral glutamate levels.[71,72]

Depressive symptoms in our patients with T1DM might be psychological responses to chronic medical disorders.[73] Also, because our patients with T1DM had relatively mild depression and were not diagnosed as having clinical depression on the basis of the structured interview, our findings should not be generalized to diabetic patients with depression.

Our interpretation of high cerebral Glx levels as greater cerebral glutamate levels should be considered with cau-

tion given the potential overlapping of the chemical spectra of glutamate, glutamine, and γ-aminobutyric acid as Glx resonance at 1.5-T imaging.[16,63,74]

Because plasma glucose levels were measured in a subset of patients with T1DM, the immediate effects of peripheral glucose levels cannot be effectively controlled in the present study assessing long-term effects of hyperglycemia on cerebral glucose metabolism (see eSupplement 1 for details). More controlled conditions, such as those using the experimental clamping technique during MR examination, would be necessary to accurately control for the confounding influence of the peripheral glucose levels—especially extreme hypoglycemia or hyperglycemia—on evaluating long-term cerebral metabolic changes in patients with T1DM.

The present MRS study, conducted with a large T1DM cohort, has shown that prefrontal Glx level is increased in adult patients with T1DM compared with healthy control subjects. Higher prefrontal Glx levels are associated with lower cognitive function and depressive symptoms in adult patients with T1DM. Randomized controlled studies are necessary to confirm the causal relationship between the level of glycemic control and cerebral glutamate changes.

Future studies are also recommended to evaluate the effectiveness of therapeutic options acting on glutamatergic neurotransmission in the treatment or prevention of CNS-related changes in patients with T1DM.

In addition, our findings show that patients with T1DM who maintain glycemic control within the recommended treatment target of HbA_{1c} less than 7% would have the benefit of keeping optimal prefrontal glutamate levels, thereby potentially reducing the risk of CNS-related changes. A randomized clinical trial assessing the causal relationship between glycemic control and cerebral glutamate levels could confirm our interpretation.

Submitted for Publication: June 19, 2008; final revision received February 9, 2009; accepted February 11, 2009.
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Author Contributions: Dr Lyoo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: Dr Lyoo has received research support from Eli Lilly and Company and GlaxoSmithKline. Dr Renshaw has been a consultant to Novartis, Hoffman-La Roche Pharmaceuticals, and Kyowa Hakko and has received research support from GlaxoSmithKline. Dr Jacobson has received speaking honoraria from Novo Nordisk and travel support from Eli Lilly and Company.

Funding/Support: This study was supported by grant DK-060754 from the National Institutes of Health (Dr Jacobson), Independent Investigator Awards (Drs Lyoo and Renshaw) from the National Alliance for Schizophrenia and Depression, grant KRF-2008-220-E00021 from the Korea Research Foundation (Dr Lyoo), and grant 2009K001272 from the Brain Research Center of the 21st Century Frontier Research Program funded by the Korean Ministry of Education, Science, and Technology (Dr Lyoo).

Role of the Sponsor: The sponsors of the study had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Previous Presentation: An abstract of the preliminary data from this study was presented at the Poster Session of the 68th Scientific Session of the American Diabetes Association; June 7, 2008; San Francisco, California.

Additional Information: The eSupplements and eTables are available at http://www.archgenpsychiatry.com.

Additional Contributions: We thank all the study participants as well as the whole international collaborative team, including Rosemond Villafuerte, MA (Brain Imaging Center, McLean Hospital), and Hengjun J. Kim, MD, MS, and Sujin Bae, MS (Interdisciplinary Program in Brain Science, Seoul National University), for technical assistance.

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