Original Article

Effects of Type 1 Diabetes on Gray Matter Density as Measured by Voxel-Based Morphometry

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The effects of type 1 diabetes and key metabolic variables on brain structure are not well understood. Sensitive methods of assessing brain structure, such as voxel-based morphometry (VBM), have not previously been used to investigate central nervous system changes in a diabetic population. Using VBM, we compared type 1 diabetic patients aged 25–40 years with disease duration of 15–25 years and minimal diabetes complications with an age-matched, nondiabetic control group. We investigated whether lower than expected gray matter densities were present, and if so, whether they were associated with glycemic control and history of severe hypoglycemic events. In comparison with control subjects, diabetic patients showed lower density of gray matter in several brain regions. Moreover, in the patient group, higher HbA_{1c} levels and severe hypoglycemic events were associated with lower density of gray matter in brain regions responsible for language processing and memory. Our study represents the first comprehensive study of gray matter density changes in type 1 diabetes and suggests that persistent hyperglycemia and acute severe hypoglycemia have an impact on brain structure. *Diabetes* 55:326–333, 2006

Type 1 diabetes can lead to peripheral and autonomic neuropathies (1). However, the extent to which diabetes impacts the integrity of the central nervous system (CNS) has been the subject of relatively few studies (2–4). Although persistent elevation in blood glucose level is a critical risk factor for other neuropathic complications of type 1 diabetes (1), there is little information about its long-term effect on the CNS (5). Moreover, although a single severe hypoglycemic event may rarely lead to signs of brain damage (6), investigations examining whether recurrent severe hypoglycemic events have a cumulative effect on the brain have yielded equivocal results (1,7). Many type 1 diabetic patients adhere to intensive diabetes management therapeutic approaches that greatly increase the likelihood of severe hypoglycemic events (8), and thus concern has been raised that these may result in damage to the CNS (9). Furthermore, the gradual progression of these CNS changes may make them difficult to detect until years after onset of type 1 diabetes (10). Thus, methods likely to detect subtle CNS changes are essential for evaluating the effects of type 1 diabetes and its metabolic perturbations on the CNS.

Past studies on the effect of diabetes on the brain suggest that it may lead to neurophysiological alterations (6), cognitive abnormalities (11), and changes in both brain function (12) and structure (2,13,14), such as white matter hyperintensities (3,4,15). Most of these studies have revealed that recurrent, severe hypoglycemic events, persistent hyperglycemia, and disease duration are related to brain structural changes. Earlier studies conducted in the 1960s showed that patients with severe diabetes complications showed signs of cerebral atrophy that were likely to be associated with persistent hyperglycemia (16).

Despite some indications that diabetes affects the brain, not all reports have been consistent. One study showed cortical atrophy to be associated with recurrent severe hypoglycemic events requiring external assistance (2), but this effect was not replicated in a later study (4). Several studies have reported that white matter hyperintensities occur commonly in type 1 diabetic patients despite their uncertain relationship to glycemic control (4,15). Importantly, none of these studies evaluated nondiabetic control subjects, thereby limiting the interpretation of the findings. More recently, we compared type 1 diabetic patients with control subjects, and preliminary data suggest a greater prevalence of white matter hyperintensities in the patient population and no association with either HbA_{1c} (A1C) or severe hypoglycemic events (17). Thus, diabetes appears to be a risk factor for white matter changes in the brain, but the underlying mechanism is unclear.

The present study examines how diabetes and its metabolic disturbances are correlated with changes in CNS gray matter as measured using voxel-based morphometry (VBM) analyses of magnetic resonance imaging (MRI) data. VBM analysis is an appropriate method for use in evaluating brain structure changes in diabetic patients, because it is sensitive to subtle brain alterations in gray matter (18) that may develop early in the course of the illness and that may initially be present without accompa-

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CNS, central nervous system; MRI, magnetic resonance imaging; STG, superior temporal gyrus; VBM, voxel-based morphometry; WASI, Wechsler Abbreviated Scale of Intelligence.

TABLE 1
Demographic and clinical characteristics of type 1 diabetic patients and control subjects

Clinical and demographic factors	Patients (n = 82)	Control subjects (n = 36)
Age (years)	32.6 ± 3.2	31.3 ± 5.1
Sex (M/F)	33/49	16/20
Education (years)	16.3 ± 2.4	17.3 ± 2.1
Handedness (right handed/ ambidextrous)	80 (98)/2 (2)	34 (94)/2 (6)
Lipid levels		
HDL (mg/dl)	57.5 ± 16.7	51.5 ± 13.9
LDL (mg/dl)	113.4 ± 34.6	113.5 ± 29.7
Triglycerides (mg/dl)	85.1 ± 72.6	110.1 ± 67.0
Cholesterol (mg/dl)	186.2 ± 43.7	185.1 ± 30.5
Systolic blood pressure (mmHg)	118 ± 14.7	115 ± 11.4
Diastolic blood pressure (mmHg)	75 ± 8.9	75 ± 7.9

Data are means ± SD or n (%).

nying disease-related cognitive dysfunction (19). In effect, VBM methods permit the detection of changes in gray matter before overt cortical atrophy is apparent (19). Earlier detection of brain structural changes may increase the likelihood that treatment interventions can slow the progression of these impairments. In this report, we focus primarily on gray matter loss. Severe hypoglycemia affects the hippocampus (20) and the superior temporal gyrus (21). Studies in rats suggest that persistent hyperglycemia affects synaptic plasticity in the hippocampus (22). Both persistent hyperglycemia and severe hypoglycemic events may result in gray matter density reduction. Secondarily, we examine whether increased gray matter density is also detectable. Little is known about how to interpret elevated gray matter density levels (23). They may result from heavy reliance on particular brain regions (24) and may develop in brain regions functionally connected to other areas experiencing gray matter density loss.

It is unclear whether gray matter changes and related CNS changes are a direct result of glycemic factors or are due to secondary effects associated with micro- and macrovascular complications attributable to the glycemia-related metabolic aberrations (25). Therefore, in this study, we restricted age and disease duration to reduce any contributions that microvascular and macrovascular complications might have on brain structure. Specifically, we examined three primary research questions related to gray matter densities: *1*) Do patients with type 1 diabetes, in comparison with nondiabetic control subjects, exhibit lower density of gray matter? *2*) Is a history of severe hypoglycemia events leading to unconsciousness, coma, and/or seizures associated with lower density of gray matter? *3*) Is a history of persistently elevated blood glucose levels, as measured by A1C, associated with lower density of gray matter?

RESEARCH DESIGN AND METHODS

The study sample consisted of 82 type 1 diabetic patients and 36 healthy, age-matched control subjects. All subjects were between the ages of 25–40 (average 32.6 ± 3.2), and all type 1 diabetic patients had disease duration between 15–25 years (average 20.3 ± 3.6 years) (Table 1). We intentionally restricted the age range and disease duration to decrease the contributions of vascular changes to any brain structural abnormalities examined in the study and to isolate the effects of metabolic factors on brain structure. We excluded patients who had painful neuropathy and clinically significant nephropathy indicated through chart review of medical records or self-report. As shown in Table 1, we collected information on retinopathy and psychiatric status and excluded participants with a history of psychosis; schizophrenia; bipolar disorder; attention deficit hyperactivity disorder; cocaine, heroin, or alcohol dependence; or current depression as assessed by the Structured Clinical Interview for DSM-IV. Any contraindications to MRI, such as gunshot wound, pacemaker, pregnancy, and claustrophobia, were also exclusionary factors. Hand preference was assessed using the Edinburgh Handedness Inventory (26), and only right-handed and ambidextrous subjects were included.

After approval from the Joslin Diabetes Center Institutional Committee on Human Subjects, each patient and healthy volunteers provided the following information during screening: date of birth, psychiatric history, medical history, and current medications. Diabetic patients additionally provided date of diagnosis and self-report of lifetime experience of severe hypoglycemic events leading to unconsciousness (1).

Medical history data. We obtained all available medical records to determine lifetime average A1C history. Glycemic control "scores" were calculated by grouping results in 4-year increments, beginning with the 1st year of diagnosis and working to the present. This allowed us to obtain an estimate of glycemic control over time. To assess the strength of associations between severe hypoglycemic events on brain structure, we divided patients into two groups: *1*) no severe hypoglycemic events, and *2*) one or more hypoglycemic event according to the Diabetes Control and Complications Trial strict criteria in which the event leads to coma or unconsciousness (27).

MRI image acquisition. All patients underwent MRI at McLean Brain Imaging Center in Belmont, Massachusetts, using a 1.5-Tesla GE whole-body imaging system (Horizon Echo-Speed, General Electric Medical Systems, Milwaukee, WI) and a custom-made linear birdcage coil with ~40% improvement in signal-to-noise ratio and improved homogeneity over standard quadrature head coil (28). A three-dimensional spoiled gradient echo pulse sequence was used to produce 124 1.5-mm-thick contiguous coronal images (echo time = 5 ms, repetition time = 35 ms, 256 × 192 matrix; field of view = 24 cm, flip angle = 45°, number of excitations = 1). Axial proton density and T-2 weighted images (echo time = 30/80 ms, repetition time = 3,000 ms, 256 × 192 matrix; field of view = 24 cm, flip angle = 45°, number of excitations = 0.5, 3-mm-thick slices, no skip) were obtained to screen for brain structural abnormalities.

VBM. The 124 contiguous coronal images were transferred to a Dell 530 Workstation and interpolated to 0.938 mm^3 voxels, using Analyze 3.1 (BIR 2001) (29). After inspecting individual MRI volumes, we used spatial filtering to correct for image nonuniformity.

Spatial normalization. Each MR volume was transferred and coregistered into a standardized stereotaxic proportional space using the Montreal Neurological Institute brain-averaged space based on 152 brains using SPM99 (30). Spatial normalization requires two steps: *1*) 12-parameter affine transformations (31) to correct for head size, and *2*) nonlinear warping using basis functions (7 × 8 × 7) (30), which reduces variability in nonlinear dimensions. Resulting images had isotropic voxels of 1.5 mm.

Segmentation. After normalization, gray matter, white matter, and cerebrospinal fluid were segmented using a modified mixture model cluster analysis technique (31).

Smoothing. Images were smoothed by convolving with a 8-mm full-width at half-maximal isotropic Gaussian kernel.

Cognitive assessment. We administered the Wechsler Abbreviated Scale of Intelligence (WASI) (32), four subtests in the Delis-Kaplan Executive Function System (33), two working memory tasks, the verbal paired associate task from the Wechsler Memory Scale (34), the Digit Symbol Substitution task from the Wechsler Adult Intelligence Scale (35), and the Grooved Pegboard (36). This battery allowed us to assess general intelligence, executive function, memory, and psychomotor speed, respectively.

Data analyses. Linear regression modeling methods were used to compare age, education, HDL, LDL, triglyceride, total cholesterol, and blood pressure distributions between diabetic patients and control subjects. χ^2 analyses were used to assess for significant between-group differences in categorical measures, including sex and alcohol and drug use. Between-group t tests were used to compare cognitive results. The α-level was set to $P < 0.05$ for these two-tailed statistical comparisons.

Smoothed brain images were compared between diabetic patients and control subjects using the analytic program SPM99. T maps from the statistical comparisons were transformed into Z values, and the statistical significance was estimated using random Gaussian fields methods. We used two-sample regression models for the comparisons between type 1 diabetes and control subjects for these well-defined hypotheses. For the effects of the covariates (hypoglycemic events, average A1C, age of onset, and disease duration), we used generalized linear regression modeling methods, controlling for potentially confounding variables. Control variables included age, sex, education, recreational drug use, alcohol use, and handedness.

TABLE 2
Diabetes-specific clinical data

Disease duration (years)	20.3 ± 3.6
Current A1C (%)	7.8 ± 1.3 (5.5–11.3)
Lifetime average A1C (%)	8 ± 1.2 (5.5–12.1)
Age of onset (years)	
<7	11 (13.4)
7–11	25 (30.5)
12–16	32 (39)
17–21	9 (11)
22–25	5 (6.1)
Number of severe hypoglycemic episodes*	
0	31 (37.8)
1	14 (17.1)
2	12 (14.6)
3	3 (3.7)
4	3 (3.7)
5	6 (7.3)
8	3 (3.7)
10	5 (6.1)
11	5 (6.1)
Retinopathy level†	
No retinopathy	28 (35.4)
Mild nonproliferative retinopathy	27 (34.2)
Moderate nonproliferative retinopathy	13 (16.4)
Proliferative retinopathy without high-risk characteristics‡	5 (6.3)
Proliferative retinopathy with high-risk characteristics	6 (7.7)

Data are means ± SD (range) or *n* (%). *Percentages do not total 100% because of a rounding error. †Data for three patients were unavailable. ‡High-risk proliferative diabetic retinopathy refers to neovascularization of the optic disc one-fourth to one-third of a disc diameter in size and neovascularization elsewhere with any vitreous hemorrhage.

We used a whole-brain analysis for all of our statistical comparisons evaluating gray matter density. Parameters to define regions of significant difference include P value <0.001 (uncorrected), height threshold $t = 3.16$, and extent threshold = 100 voxels. This α-level is consistent with published standards for applying VBM when there are clearly defined a priori hypotheses for regional differences (37). All statistical tests were two tailed.

First, we examined whether there were gray matter density differences between type 1 diabetic patients and healthy control subjects. Next, we conducted a multiple regression analysis of data from a single region (the left superior temporal gyrus [STG]) using several covariates that, a priori, were considered likely to be associated with gray matter densities; these covariates included age, sex, handedness, years of education, drug use, depression history status, and alcohol abuse history. The objective of this multivariate regression analysis was to determine whether the lower gray matter density pattern observed in diabetic patients was maintained after adjustment for important covariates. Finally, for the diabetes-specific analyses, we evaluated whether gray density differences existed in the patient population as a function of lifetime A1C values and number of severe hypoglycemic events. For lifetime A1C values, we calculated Pearson correlation coefficients (and their 95% CIs) between gray matter densities and lifetime A1C values using SPM99 within group whole-brain analyses. For the number of severe hypoglycemic events, we dichotomized the diabetes study sample according to whether they had experienced a severe hypoglycemic event. We then examined gray matter density difference between these two diabetes subgroups in whole-brain analyses using SPM99.

RESULTS

Diabetes versus healthy control subjects. Clinical and demographic characteristics of the 82 type 1 diabetic patients and 36 age-matched healthy control subjects are summarized in Table 1. There were no important differences between diabetes and comparison subjects on any of the demographic measures outlined in Table 1. Table 2 shows diabetes-specific demographics.

When gray matter densities of 82 type 1 diabetic patients and 36 age-matched healthy control subjects were contrasted, we found that the left and right superior temporal gyri (STG), left angular gyrus, left middle temporal and middle frontal gyri, and left thalamus were less dense in type 1 diabetic patients relative to control subjects. The differences in gray matter densities between diabetes and

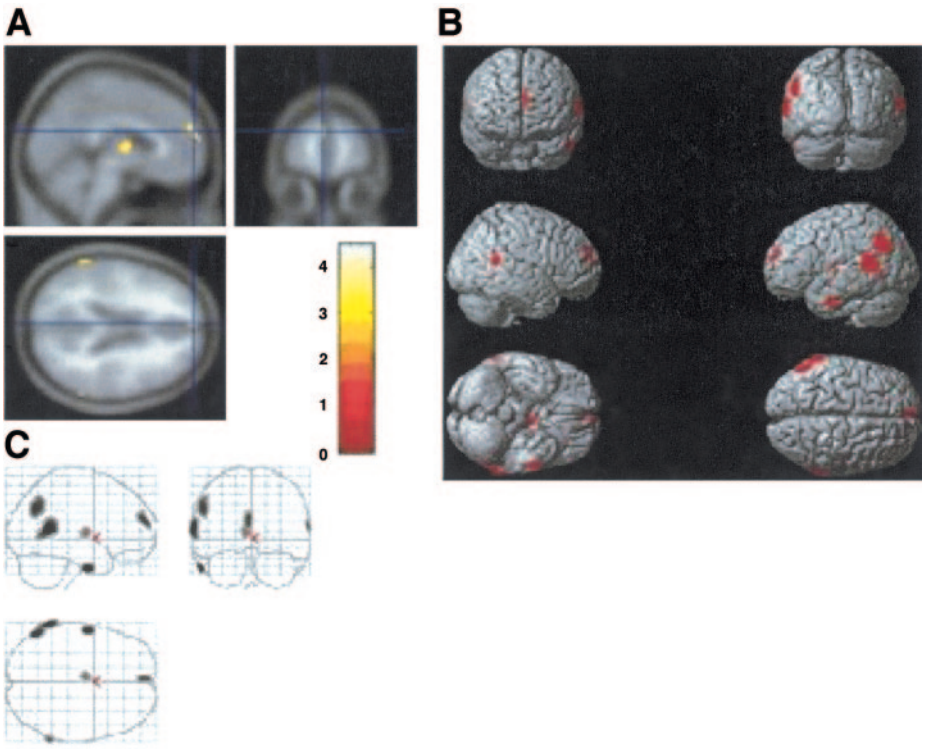

FIG. 1. Gray matter density loss in type 1 diabetic patients compared with healthy control subjects. Results shown on sectioned places of average smoothed brains (*A*), rendered brains (*B*), and glass brains (*C*). Colored areas refer to regions of reduced gray matter density.

TABLE 3
VBM-estimated brain region volume estimates in type 1 diabetic patients (n = 82) versus control subjects (n = 36)

Region (cluster level)	Stereotaxic coordinates (mm) X	Y	Z	T	Z*	P values	Gray matter density loss (patients versus control subjects) (%)
Left superior temporal gyrus (397)	−68	−50	11	4.39	4.21	<0.001	4.9†
Left middle temporal gyrus	−65	−59	3	3.69	3.58	<0.001	4.9†
Left inferior temporal gyrus (129)	−59	−5	−33	4.35	4.18	<0.001	5.0
Left angular gyrus (414)	−56	−65	36	4.12	3.97	<0.001	4.6
Left inferior parietal lobule	−54	−62	45	3.63	3.53	<0.001	4.6
Left medial frontal gyrus (179)	−5	59	20	3.92	3.79	<0.001	4.3
Right superior temporal gyrus (105)	66	−50	15	3.73	3.62	<0.001	4.6
Left thalamus	−6	−8	8	3.51	3.42	<0.001	5.2

*SPM99 T maps from the statistical comparisons were transformed into Z values, and the statistical significance was estimated using random Gaussian fields methods. †These areas represent connected regions on the SPM map and therefore have the same percentage of gray matter density loss.

control subjects in these regions are clearly indicated in Fig. 1 and are summarized in Table 2 expressed in terms of percentage of gray matter density. When we systematically controlled for age, sex, handedness, education, premorbid intelligence (measured using vocabulary scores derived from the WASI), and drug and alcohol use in each of the analyses summarized in Table 3, we found the same pattern of results (data not shown).

Additionally, as noted in RESEARCH DESIGN AND METHODS, we selected a single region (the STG) in which to examine the type 1 diabetes versus healthy control gray matter density differences in a multivariate model controlling for important covariates. This region was selected for more intensive analysis because type 1 diabetes versus control subject gray matter density differences were observed in multiple analyses within this region, and there are some research findings suggesting that the STG is affected by metabolic variables such as persistent hyperglycemia or severe hypoglycemic events (21). Diabetes status, age, sex, handedness, education, depression, and drug and alcohol use were entered as covariates. Importantly, type 1 diabetes remains a significant predictor of gray matter STG density loss after controlling for all other variables in the model.

Within–diabetes group analyses

Effect of A1C. Lifetime average A1C was analyzed as a continuous variable for all 82 type 1 diabetic patients. The results indicated that the higher the A1C level, the lower the gray matter density in several brain regions important for memory, language processing, and attention, including the left posterior cingulate, right parahippocampal gyrus, left hippocampus, and left superior temporal gyrus. Elevated A1C levels, suggesting compromised glycemic control, were associated with lower gray matter density in all of these brain regions. Additional regions in which there were strong correlations between lifetime average A1C levels and lower gray matter density levels were the right occipital cuneus and the left thalamus. These results are summarized in Table 4 and Fig. 2.

Effect of severe hypoglycemic events on gray and matter density. As noted in RESEARCH DESIGN AND METHODS, the number of severe hypoglycemic events was treated as a dichotomous variable. We divided patients into two groups: group 1 experienced no hypoglycemic events (n = 31) and group 2 experienced one or more severe hypoglycemic event (n = 51). We observed less gray matter density in the left cerebellar posterior lobe in patients who experienced at least one severe hypoglycemic event. These results are summarized in Table 5 and Fig. 3.

Effect of A1C and severe hypoglycemia. We were also interested in what brain areas had reduced gray matter density that was associated with both lifetime A1C levels and number of severe hypoglycemic events. We found that the limbic unci were associated with less gray matter density at high A1C and number of severe hypoglycemic events.

Age of onset. Because age of onset is strongly related to disease duration, we conducted the analysis holding dura-

TABLE 4
VBM-estimated brain region volume estimates in 82 type 1 diabetic patients: correlations (Pearson) between lifetime A1C levels and percentage gray matter density

Region (cluster level)	Stereotaxic coordinates (mm) X	Y	Z	SPM99 T map T	Z*	P values	Pearson [ρ (95% CI)]
Left posterior cingulate (634)	−20	−63	9	4.47	4.20	<0.001	−0.49 (−0.31 to −0.64)†
Left parahippocampal gyrus	−12	−36	6	4.26	4.02	<0.001	−0.49 (−0.31 to −0.64)†
Left sub-gyral	−23	−41	2	3.71	3.55	<0.001	−0.49 (−0.31 to −0.64)†
Right parahippocampal gyrus (331)	12	−38	2	4.08	3.87	<0.001	−0.44 (−0.24 to −0.60)
Right cuneus (545)	6	−90	8	4.06	3.86	<0.001	−0.45 (−0.25 to −0.61)†
Right posterior cingulate	15	−62	12	3.67	3.51	<0.001	−0.45 (−0.25 to −0.61)†
Right posterior cingulate	9	−68	12	3.36	3.23	<0.001	−0.45 (−0.25 to −0.61)†
Left superior temporal gyrus (102)	−59	9	3	3.87	3.69	<0.001	−0.39 (−0.18 to −0.56)

*SPM99 T maps from the statistical comparisons were transformed into Z values, and the statistical significance was estimated using random Gaussian fields methods. †These areas represent connected regions on the SPM map and therefore have the same percentage of gray matter density loss.

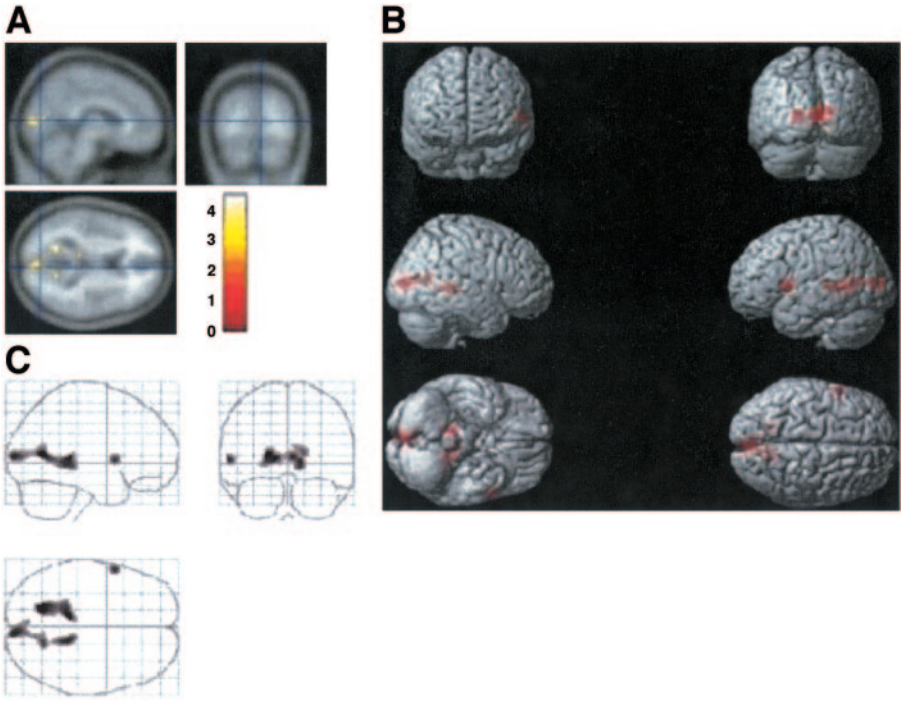

FIG. 2. Negatively correlated gray matter density with lifetime average A1C for type 1 diabetic patients. Results shown on sectioned planes of averaged smoothed brains (*A*), rendered brains (*B*), and glass brains (*C*). Colored areas refer to regions of reduced gray matter density.

tion of illness constant. Results from this analysis were similar to those observed when comparing type 1 diabetic patients with control subjects, except that an additional region, the cerebellum, showed gray matter density loss.

Duration of diabetes. Similarly, disease duration analyses were conducted holding age of onset constant. The results were also similar to those observed in the type 1 diabetes and control subject comparison with the addition that as disease duration increased, there was reduced gray matter density in the lentiform nuclei. For all of the diabetes-specific comparisons, the pattern of results remained the same after controlling for age, sex, handedness, education, and drug and alcohol use.

Additional exploratory analyses

Association between retinopathy and gray matter density. Retinopathy is a common complication in diabetes and has been shown to be associated with brain changes (4). No research has been conducted to determine whether gray matter changes are associated with cerebral microangiopathy. Our results revealed a statistically significant relationship between severity of retinopathy and gray matter density loss in the medial and superior frontal gyri bilaterally, the right middle temporal and parahippocampal gyri, and the left insula.

Elevated gray matter density levels. Secondarily, we examined the associations of explanatory variables with higher density of gray matter. Regions of elevated gray matter density were observed in the cerebellum and in the occipital gyrus in comparisons between type 1 diabetic patients and healthy control subjects. When comparing patients with higher lifetime A1C with those with lower A1C levels, we observed that patients with poorer glycemic control had a greater gray matter density volume in the parietal lobe ($P < 0.001$, uncorrected).

Cognitive results. A detailed discussion of these results is beyond the scope of this article. In summary, our test battery yielded 20 test scores. Diabetic patients showed normal performance on most of the tests (16 of 20), including three of four IQ subtests from the WASI. Using standardized scaled scores, patients scored lower than control subjects at a level of $P < 0.05$ on vocabulary (57.1 vs. 63.2), verbal paired associates (9.7 vs. 11.4), inhibition in the color-word task (10.8 vs. 11.8), and card sorting (9.6 vs. 11). The vocabulary score was used as our measure of premorbid intelligence and was entered as a covariate in our VBM analyses.

DISCUSSION

This is the first report of reduced gray matter densities in type 1 diabetic patients. We used carefully characterized patients who were identical to control subjects with respect to age, education, and sex ratios and who had minimal complications. We found lower levels of gray matter density in diabetic patients compared with control subjects. Among diabetic patients, we also found that

TABLE 5
Volume estimates in the left cerebellar posterior in type 1 diabetic patients who experienced no severe hypoglycemic events compared with patients with one or more event

Region (cluster level)	Stereotaxic coordinates (mm)			T	*Z**	*P* values	Gray matter density loss (0 vs. ≥1 hypoglycemic event) (%)
	X	Y	Z				
Left cerebellar posterior lobe (192)	−5	−60	−38	4.04	3.85	<0.001	3.62

*SPM99 T maps from the statistical comparisons were transformed into *Z* values, and the statistical significance was estimated using random Gaussian fields methods.

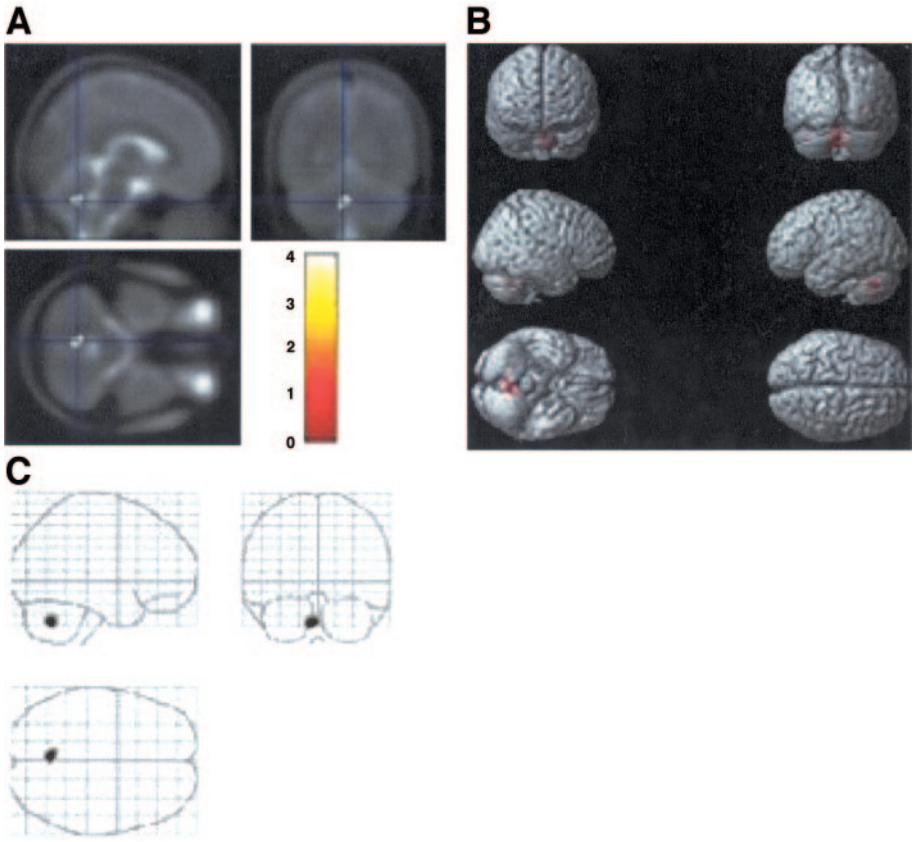

FIG. 3. Negatively correlated gray matter density with one or more severe hypoglycemic events in type 1 diabetic patients. Results shown on sectioned planes of averaged smoothed brains (*A*), rendered brains (*B*), and glass brains (*C*). Colored areas refer to regions of reduced gray matter density.

lower levels of gray matter density was associated with worse glycemic control and higher frequency of recurrent severe, hypoglycemic events. These structural changes are consistent with other findings in the literature that suggest that the integrity of brain tissue is affected by diabetes and its metabolic perturbations (2–4,13,38).

We found that a greater degree of retinopathy was associated with more gray matter density loss in brain regions used for cognition (frontal and temporal regions), and Ferguson et al. (4) reported white matter lesions in the basal ganglia in the absence of changes in cerebral atrophy using volumetric measurements. The methods used in these studies are quite different, thus yielding two separate perspectives on the possible ways in which retinopathy may be mirrored in different types of changes in the CNS.

We found that the posterior, temporal, and cerebellar regions of the brain were the areas that were primarily impacted, at least in terms of gray matter densities, by type 1 diabetes and its associated metabolic perturbations. In particular, our data suggest that the STG may be particularly vulnerable to the effects of diabetes, because gray matter density variation within this region was associated not only with diabetes but also with elevated A1C levels.

Our study also suggested that diabetes, per se, and persistent hyperglycemia may be associated with lower levels of gray matter density in areas of the brain that contribute to memory such as the hippocampus and parahippocampal gyrus. Diabetes, per se, was also associated with gray matter density loss in areas necessary for language processing (STG and angular gyrus). Although there is no evidence that type 1 diabetic patients suffer from language disturbances, these structural changes detected by VBM are subtle. Furthermore, the relationship between cognitive dysfunction and behavior is not always observable (39). On most tests of cognition, we did not observe any differences between diabetic patients and control subjects. On the WASI vocabulary test, which was our measure of premorbid intelligence, the patients scored more poorly. Vocabulary is thought to be resilient to brain changes (40) and, as such, is not considered to be a consequence of diabetes. In subsequent research, we plan to further examine the relationship of cognition to these types of subtle structural changes.

In addition to the effects of persistent hyperglycemia, our data show that severe hypoglycemic events may be associated with lower gray matter density in the cerebellum. It is generally assumed that the cerebellum is efficient in using glucose to help protect it from the effects of hypoglycemia. One small study contradicts this assumption (41). The cerebellum is involved in cognition, in particular with executive function (42). Working memory has been shown to be impaired in type 1 diabetic patients with early age of onset (43). The influence of hypoglycemic events on cognition is unresolved with conflicting results from the few available studies (7,44,45).

The effect of hyperglycemia on cognition is less consistent. Although cognitive dysfunction directly related to chronic hyperglycemia as indexed by measures of metabolic control in type 1 diabetic patients has not previously been reported in the literature, the effects of acute hyperglycemia on cognition are more equivocal (46,47). However, nonproliferative retinopathy (4,11), proliferative retinopathy, autonomic neuropathy, and elevated blood pressure (11) may be linked to psychomotor slowing in type 1 diabetic adults. It is also possible that the observed structural changes predict future cognitive problems that are subclinical and may reflect early macrovascular damage.

Elevated levels of gray matter density were also obtained. High gray matter densities were observed in the left cerebellum and occipital gyrus in type 1 diabetic patients compared with control subjects. Additionally, high A1C levels were associated with elevated gray matter density in the parietal lobe. The clinical significance of elevated gray matter density is not well studied (24). One interpretation is that use-dependent brain expansion may occur to support heavy reliance on particular brain regions (24). Accordingly, we speculate that higher levels of gray matter in the occipital gyrus may serve to compensate for early retinal changes that occur in type 1 diabetic patients (even in patients without clinically evident diabetic retinopathy) (48). We know that retinal blood flow is reduced in type 1 diabetic patients with early stages of diabetic retinopathy (49) and elevated gray matter density in the occipital gyrus may be another early sign of retinopathy.

Our study represents the first comprehensive study of gray matter density changes in type 1 diabetes but is limited in the following ways: *1*) it is cross-sectional, which rules out the possibility of considering the timing of important events such as patient ages during severe hypoglycemic events, time between events, and duration of time with high A1C values; *2*) we used a self-report measure for severe hypoglycemic events; and *3*) we had varying amounts of A1C data within the diabetic patients subgroup with a range of 2–78 A1C readings. Incomplete records of glycemic control and self-report measures of severe hypoglycemic events may have affected data reliability, and this can complicate interpretations of the data and can reduce our ability to evaluate any synergistic effect these two metabolic conditions can have on brain structure.

The study also has important strengths. Of these, one of the most important is the use of VBM neuroimaging methods, which permit evaluation of structural changes in the brain that may appear before clinically relevant changes are observed. If such alterations could be detected at an early stage through use of VBM methods or other means, it might be possible to implement treatment regimens that minimize risks to the patient in terms of hypo- and hyperglycemia and its effects on the CNS.

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REFERENCES

1. DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. Perros P, Deary IJ, Sellar RJ, Best JJK, Frier BM: Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care* 20:1013–1018, 1997
3. Dejgaard A, Gade A, Larsson H, Balle V, Parving A, Parving H: Evidence for diabetic encephalopathy. *Diabet Med* 8:162–167, 1991
4. Ferguson SC, Blane A, Perros P, McCrimmon RJ, Best JJ, Wardlaw J, Deary IJ, Frier BM: Cognitive ability and brain structure in type 1 diabetes: relation to microangiopathy and preceding severe hypoglycemia. *Diabetes* 52:149–156, 2003
5. Brands AM, Kessels RP, de Haan EH, Kappelle LJ, Biessels GJ: Cerebral dysfunction in type 1 diabetes: effects of insulin, vascular risk factors and blood-glucose levels. *Eur J Pharmacol* 490:159–168, 2004
6. Soltész G, Acsádi G: Association between diabetes, severe hypoglycemia, and electroencephalographic abnormalities. *Arch Dis Child* 64:992–996, 1989
7. Deary I, Crawford J, Hepburn DA, Langan SJ, Blackmore LM, Frier BM: Severe hypoglycemia and intelligence in adult patients with insulin-treated diabetes. *Diabetes* 341–344, 1993
8. Bolli GB: How to ameliorate the problem of hypoglycemia in intensive as well as nonintensive treatment of type 1 diabetes. *Diabetes Care* 22 (Suppl. 2):B43–B52, 1999
9. Deary IJ, Frier BM: Severe hypoglycaemia and cognitive impairment in diabetes: link not proven. *Br Med J* 313:767–768, 1996
10. Biessels GJ, van der Heide LP, Kamal A, Bleys RL, Gispen WH: Ageing and diabetes: implications for brain function. *Eur J Pharmacol* 441:1–14, 2002
11. Ryan C, Geckle M, Orchard T: Cognitive efficiency declines over time in adults with type 1 diabetes: effects of micro- and macrovascular complications. *Diabetologia* 46:940–948, 2003
12. MacLeod KM, Hepburn DA, Deary IJ, Goodwin GM, Dougall N, Ebmeier KP, Frier BM: Regional cerebral blood flow in IDDM patients: effects of diabetes and of recurrent severe hypoglycaemia. *Diabetologia* 37:257–263, 1994
13. Lunetta M, Damanti AR, Fabbri G, Lombardo M, Di Mauro M, Mughini L: Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients. *J Endocrinol Invest* 17:241–245, 1994
14. Makimattila S, Malmberg-Ceder K, Hakkinen AM, Vuori K, Salonen O, Summanen P, Yki-Jarvinen H, Kaste M, Heikkinen S, Lundbom N, Roine RO: Brain metabolic alterations in patients with type 1 diabetes-hyperglycemia-induced injury. *J Cereb Blood Flow Metab* 24:1393–1399, 2004
15. Jacobson AM, Weinger K, Hill TC, Parker JA, Suojanen JN, Jimerson DC, Soroko DJ: Brain functioning, cognition and psychiatric disorders in patients with type 1 diabetes (Abstract). *Diabetes* 49 (Suppl.):537, 2000
16. Reske-Nielson E, Lundbaek K, Rafaelsen OJ: Pathological changes in the central and peripheral nervous system of young long-term diabetics. *Diabetologia* 1:232–241, 1965
17. Musen G, Lyoo I, Sparks C, Burwood A, Lee HK, Weinger K, Driscoll A, Ryan C, Renshaw P, Jacobson A: White matter lesions in type 1 diabetes patients with minimal diabetes complications (Abstract). *Diabetes* 54: A219, 2005
18. May A, Ashburner J, Buchel C, McGonigle DJ, Friston KJ, Frackowiak RS, Goadsby PJ: Correlation between structural and functional changes in brain in an idiopathic headache syndrome. *Nat Med* 5:836–838, 1999
19. Baron JC, Chetelat G, Desgranges B, Perchey G, Landeau B, de la Sayette V, Eustache F: In vivo mapping of gray matter loss with voxel-based morphometry in mild Alzheimer's disease. *Neuroimage* 14:298–309, 2001
20. Mohseni S: Hypoglycemic neuropathy. *Acta Neuropathol (Berl)* 102:413–421, 2001
21. Akyol A, Kiylioglu N, Bolukbasi O, Guney E, Yurekli Y: Repeated hypoglycemia and cognitive decline: a case report. *Neuro Endocrinol Lett* 24:54–56, 2003
22. Biessels GJ, Kamal A, Ramakers GM, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH: Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. *Diabetes* 45:1259–1266, 1996
23. Bothwell S, Meredith GE, Phillips J, Staunton H, Doherty C, Grigorenko E, Glazier S, Deadwyler SA, O'Donovan CA, Farrell M: Neuronal hypertrophy in the neocortex of patients with temporal lobe epilepsy. *J Neurosci* 21:4789–4800, 2001
24. Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RS, Frith CD: Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci U S A* 97:4398–4403, 2000
25. Sima AA, Kamiya H, Li ZG: Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. *Eur J Pharmacol* 490:187–197, 2004
26. Oldfield RC: The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9:97–113, 1971
27. Diabetes Control and Complications Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 46:271–286, 1997
28. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF: Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry* 61:450–458, 2004
29. Biomedial Imaging Resource at Mayo Clinic: *Analyze (3.1)*. Rochester, MN, Mayo Clinic, 2001
30. Ashburner J, Friston KJ: Nonlinear spatial normalization using basis functions. *Hum Brain Mapp* 7:254–266, 1999
31. Ashburner J, Friston K: Multimodal image coregistration and partitioning: a unified framework. *Neuroimage* 6:209–217, 1997
32. Wechsler D: *WASI Manual*. San Antonio, TX, Psychological Corporation, 1999
33. Delis DC, Kaplan E, Kramer JH: *The Delis-Kaplan Executive Function*

System: Examiner's Manual. San Antonio, TX, Psychological Corporation, 2001
34. Wechsler D: *Wechsler Memory Scale*. 3rd ed. San Antonio, TX, Psychological Corporation, 1997
35. Wechsler D: *WAIS-III Wechsler Adult Intelligence Scale*. 3rd ed. San Antonio, TX, Psychological Corporation, Harcourt Brace & Company, 1997
36. Matthews CG, Klove H: *Instruction Manual for the Adult Neuropsychology Test Battery*. Madison, WI, University of Madison Medical School, 1964
37. Ashburner J, Csernansky JG, Davatzikos C, Fox NC, Frisoni GB, Thompson PM: Computer-assisted imaging to assess brain structure in healthy and diseased brains. *Lancet Neurol* 2:79–88, 2003
38. McCall AL: The impact of diabetes on the CNS. *Diabetes* 41:557–570, 1992
39. Salthouse TA: What and when of cognitive aging. *Curr Dir Psychol Sci* 13:140–144, 2004
40. Lezak MD: *Neuropsychological Assessment.* New York, Oxford University Press, 1995
41. Kim DE, Park SH, Kim SK, Nam HW, Lee YS, Chung JK, Roh JK: Hypoglycemia-induced cerebellar dysfunction and quantitative positron emission tomography study. *Neurology* 55:418–422, 2000
42. Schmahmann JD, Sherman JC: The cerebellar cognitive affective syndrome. *Brain* 121:561–579, 1998
43. Northam EA, Anderson PJ, Jacobs R, Hughes M, Warne GL, Werther GA: Neuropsychological profiles of children with type 1 diabetes 6 years after disease onset. *Diabetes Care* 24:1541–1546, 2001
44. Wredling R, Levander S, Adamson U, Lins PE: Permanent neuropsychological impairment after recurrent episodes of severe hypoglycaemia in man. *Diabetologia* 33:152–157, 1990
45. Diabetes Control and Complications Trial Research Group: Effects of intensive diabetes therapy on neuropsychological function in adults in the Diabetes Control and Complications Trial. *Ann Intern Med* 124:379–388, 1996
46. Draelos MT, Jacobson AM, Weinger K, Widom B, Ryan CM, Finkelstein DM, Simonson DC: Cognitive function in patients with insulin-dependent diabetes mellitus during hyperglycemia and hypoglycemia. *Am J Med* 98:135–144, 1995
47. Cox DJ, Kovatchev BP, Gonder-Frederick LA, Summers KH, McCall A, Grimm KJ, Clarke WL: Relationships between hyperglycemia and cognitive performance among adults with type 1 and type 2 diabetes. *Diabetes Care* 28:71–77, 2005
48. Bursell SE, Clermont AC, Kinsley BT, Simonson DC, Aiello LM, Wolpert HA: Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Invest Ophthalmol Vis Sci* 37:886–897, 1996
49. Feng D, Bursell SE, Clermont AC, Lipinska I, Aiello LP, Laffel L, King GL, Tofler GH: von Willebrand factor and retinal circulation in early-stage retinopathy of type 1 diabetes. *Diabetes Care* 23:1694–1698, 2000