# Regional Brain Volume Differences Associated With Hyperglycemia and Severe Hypoglycemia in Youth With Type 1 Diabetes

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**OBJECTIVE** — Despite interest in the effects of type 1 diabetes on the developing brain, structural brain volumes in youth with this disease have not previously been examined. This study is the first to quantify regional brain volume differences in a large sample of youth with diabetes.

**RESEARCH DESIGN AND METHODS** — Magnetic resonance images (MRIs) were acquired from youth with diabetes ($n = 108$) and healthy sibling control subjects ($n = 51$) aged 7–17 years. History of severe hypoglycemia was assessed by parent interview and included seizure, loss of consciousness, or requiring assistance to treat. A1C values since diagnosis were obtained from medical records; median A1C was weighted by duration of disease. Voxel-based morphometry was used to determine the relationships of prior hypo- and hyperglycemia to regional grey and white matter volumes across the whole brain.

**RESULTS** — No significant differences were found between diabetic and healthy control groups in grey or white matter. However, within the diabetic group, a history of severe hypoglycemia was associated with smaller grey matter volume in the left superior temporal region. Greater exposure to hyperglycemia was associated with smaller grey matter volume in the right cuneus and precuneus, smaller white matter volume in a right posterior parietal region, and larger grey matter volume in a right prefrontal region.

**CONCLUSIONS** — Qualitatively different relationships were found between hypo- and hyperglycemia and regional brain volumes in youth with type 1 diabetes. Future studies should investigate whether these differences relate to cognitive function and how these regions are affected by further exposure.

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Type 1 diabetes is known to have cumulative deleterious effects on the body, most notably on the retina, kidney, nerves, and blood vessels (1,2). The effects of diabetes on central nervous system structure and function are less well understood. A number of studies associate exposure to hypo- and hyperglycemia during childhood with deficits in specific cognitive domains (3,4). These findings suggest that during development, exposure to glycemic extremes may alter the structure or function of specific pathways or regions in the brain. Recent brain imaging studies in diabetic adults have reported differences in grey or white matter integrity associated with prior hypo- or hyperglycemia (5,6). However, the effects of diabetes on the developing brain have not been assessed in any large-scale study to date (7). Assessing brain integrity earlier in the course of brain development and diabetes, followed by prospective monitoring, would be essential to determine when differences may emerge. Such knowledge could shed light on the neural basis of observed cognitive effects in children and adults with diabetes and determine whether there are developmental time periods during which the brain may be particularly vulnerable to the negative effects of hypoglycemia or hyperglycemia.

The present study is the first to examine the structural integrity of the brain in a large sample of children and adolescents with type 1 diabetes. We used high-resolution structural magnetic resonance imaging (MRI) and voxel-based morphometry (VBM), an objective method of quantitatively analyzing MRI data, to determine whether exposure to hypo- or hyperglycemia in youth with type 1 diabetes is associated with differences in grey or white matter volumes.

## RESEARCH DESIGN AND METHODS

— Children aged 7–17 years with type 1 diabetes and nondiabetic siblings (healthy control subjects) were recruited from the Diabetes Clinic at St. Louis Children's Hospital, which is affiliated with Washington University in St. Louis. Subjects were excluded for mental retardation, chronic disease other than type 1 diabetes (e.g., hypothyroidism), significant neurological history not due to diabetes, diagnosed psychiatric disorder, current use of psychoactive medications, prematurity at birth >4 weeks early with

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**Abbreviations:** MRI, magnetic resonance imaging; VBM, voxel-based morphometry.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

complications, and contraindications to MRI (e.g., metal implants). To reduce the likelihood of residual β-cell function, diabetic subjects were required to have been diagnosed and on insulin for at least 2 years. Handedness was assessed with a modified Edinburgh Handedness Inventory (8). Procedures were approved by the Washington University School of Medicine's Human Studies Committee, and all participants and their parents or guardians signed informed consents.

### Clinical variables

Detailed information about each diabetic youth's history of severe hypoglycemia, hyperglycemia, and other diabetes complications was collected by parental and child interview. Severe hypoglycemia was defined as events with neurological dysfunction, including seizure, loss of consciousness, or inability to arouse from sleep, or those requiring assistance of someone other than the patient for treatment (9). Hyperglycemic history was estimated from all available A1C test results, collected from participants' medical records at St. Louis Children's Hospital. A1C tests approximate blood glucose control over the previous 2–3 months. The amount of time represented by the A1C tests was calculated by multiplying the number of tests by 3 months and dividing by duration of diabetes in months. Participants with A1C coverage for <30% of their duration of diabetes ($n = 10$) were excluded from hyperglycemia analyses. Less-than-complete coverage was due to clinical appointments >3 months apart, transfers from other clinics, or use of total glycated hemoglobin tests. To account for duration of exposure to hyperglycemia, a "hyperglycemia exposure score" was calculated. Because a child with an average A1C of 8% and duration of diabetes for 10 years has had more exposure to hyperglycemia than has a child with the same average A1C and duration of diabetes for 2 years, a score that weighted duration and A1C equally was calculated by adding each patient's $z$ score of median A1C to the $z$ score of duration of diabetes. This method of calculation results in a near-normal distribution of scores, with higher scores indicating more exposure to hyperglycemia. Each child's hyperglycemia exposure score can be interpreted relative to this sample only.

### Image acquisition

Structural images were acquired for each subject on a Siemens Sonata 1.5 Tesla imaging system with a standard Siemens 30-cm circularly polarized RF head coil. For each subject, three to five images consisting of 128 contiguous 1.25-mm sagittal slices were acquired using magnetization-prepared rapid gradient echo. Subjects with movement or other artifact were excluded ($n = 10$). Images with suspected anatomical abnormalities were referred to a neuroradiologist for review; three subjects were excluded for confirmed brain abnormalities. For each subject, three high-quality images were averaged after being coregistered by an automated, validated technique (10).

### Image analysis

VBM was performed with statistical parametric mapping software (SPM5; Wellcome Department of Cognitive Neurology [available at www.fil.ion.ucl.ac.uk]). Images were simultaneously normalized to Montreal Neurological Institute space, corrected for intensity inhomogeneity, and tissue segmented as grey matter, white matter, and cerebrospinal fluid based on a priori probability maps (11). Grey and white segments were modulated to produce images representing grey and white matter volume (11). After this processing, voxel dimensions were $2 \times 2 \times 2$ mm. Modulated segments were smoothed with a 12-mm full-width at half-maximum Gaussian kernel to promote normality of residuals (12).

Voxels with segmented intensities <0.1 were masked out with an absolute threshold to reduce voxels possibly belonging to other tissue classes and because these voxels are less likely to adhere to assumptions of normality (13). Images were analyzed by SPM5, performing standard parametric tests (e.g., regressions) at each voxel, which results in statistical parametric maps on which every voxel's intensity corresponds to a $t$ value. The statistical parametric maps were then thresholded to show only voxels with $t$ values corresponding to uncorrected $P < 0.001$. The probability of resulting clusters was corrected for multiple comparisons using the stat_threshold script from Worsley's fmristat package (14). This cluster level method of multiple comparisons correction takes into account nonuniformity due to intrinsically inhomogeneous smoothness (15).

Total volume of each tissue class (grey matter or white matter) was calculated by summing modulated voxel intensities for that class. Covarying total grey/white matter volume ensures that differences are not attributable to global differences in volume, such as those expected between sexes and with age (16). Age, sex, and total volume of the relevant brain tissue (grey/white) were removed as covariates from all models. In addition, in models using only diabetic subjects, age of onset was also covaried. Independent sample $t$ tests were performed for comparisons between groups, defining contrasts in each direction (e.g., any hypoglycemia > no hypoglycemia; any hypoglycemia < no hypoglycemia). For analyses of hyperglycemia exposure, multiple regressions were performed with contrasts in each direction (negative and positive correlations). Cluster level multiple comparisons–corrected $P$ values <0.05 were considered significant.

## RESULTS

— A total of 108 youth with type 1 diabetes and 51 healthy control subjects were included in these analyses. See Table 1 for demographic and clinical information and Table 2 for a summary of imaging results.

### Type 1 diabetic versus healthy control subjects

Diabetic and healthy control groups did not differ significantly in sex distribution ($\chi^2 = 0.58$, $P = 0.45$), mean age ($t = -0.59$, $P = 0.56$), or mean parental education ($t = 0.42$, $P = 0.68$) (Table 1). The diabetic group had proportionally more left-handed or ambidextrous subjects than the healthy control subjects ($\chi^2 = 3.86$, $P = 0.05$) (Table 1). In VBM analyses comparing diabetic versus healthy control groups, there were no significant grey or white matter volume differences. Covarying handedness did not change these results.

### Hypoglycemia

Because the distribution of severe hypoglycemic episodes was skewed, with most subjects having few or no episodes (median 1 episode [range 0–50]; lower quartile = 0, median quartile = 1, upper quartile = 2), subjects were categorized as having no ($n = 42$) or any ($n = 66$) severe hypoglycemic episodes. Age, handedness, sex, parental education, estimated IQ, and median A1C did not differ between the no ($n = 42$) and any ($n = 66$) hypoglycemia groups, but the any hypoglycemia group had longer duration of diabetes ($t = -5.03$, $P < 0.001$), earlier age of onset ($t = 3.49$, $P = 0.001$), and higher hyperglycemia exposure scores ($t = -4.73$, $P < 0.001$) (Table 1).

**Table 1—*Demographic and clinical variables***

| | Control subjects | Diabetic subjects | No hypoglycemia | Any hypoglycemia |
|---|---|---|---|---|
| *n* | 51 | 108 | 42 | 66 |
| Age | 12.3 ± 2.7 | 12.6 ± 2.7 | 12.3 ± 2.4 | 12.8 ± 2.9 |
| Sex [male/female (% male)] | 26/25 (52) | 62/46 (57) | 27/15 (64) | 35/31 (53) |
| Race [white/minority (% minority)]* | 43/7 (14) | 95/12 (11) | 37/7 (16) | 60/5 (8) |
| Handedness [right-handed/other (% right-handed)] | 50/1 (98) | 96/12 (89)† | 37/5 (88) | 59/7 (89) |
| Parental education‡ | 15.2 ± 2.2 | 15.0 ± 2.2 | 14.8 ± 2.5 | 15.2 ± 2.0 |
| Duration of diabetes | | 5.7 ± 2.9 | 4.1 ± 1.9 | 6.7 ± 3.0§ |
| Age of onset | | 6.9 ± 3.3 | 8.2 ± 2.9 | 6.1 ± 3.2§ |
| Median A1C¶ | | 8.4 ± 1.0 | 8.2 ± 1.1 | 8.4 ± 0.8 |
| Hyperglycemia exposure score¶ | | 0.0 ± 1.4 | −0.8 ± 1.2 | 0.5 ± 1.3§ |

Data are means ± SD unless otherwise indicated. *Not reported for two participants. †Significantly different from healthy control subjects; $P < 0.05$. ‡Not reported for five participants; §Any hypoglycemia significantly different from no hypoglycemia; $P < 0.001$. ¶Ten subjects with <30% coverage excluded.

In VBM analyses, the any hypoglycemia group had less grey matter volume than that in the no hypoglycemia group in the left superior temporal/occipital cortex ($P = 0.001$) and left inferior occipital cortex ($P = 0.0002$) (Fig. 1*A*). Because the any hypoglycemia group also had higher hyperglycemia exposure scores, we additionally covaried hyperglycemia exposure scores. Notably, the volume of the left temporal/occipital region was still smaller ($P = 0.008$), but the left inferior occipital cortex region was not ($P = 0.13$). There were no differences in grey matter in the other direction (any hypoglycemia > no hypoglycemia) and no differences in white matter volume in either direction. No differences were found comparing the healthy control group to the any or the no hypoglycemia groups.

### Hyperglycemia

The mean ± SD number of A1C values per subject was 14.3 ± 6.6; the percentage of the duration of diabetes represented by A1C tests was 68 ± 14%. Hyperglycemia exposure scores were normally distributed (0.00 ± 1.42 [range −2.45 to 3.69]) and treated as a continuous variable. In VBM analyses, higher hyperglycemia exposure scores correlated with less grey matter volume in the right cuneus and precuneus ($P = 0.02$) (Fig. 1*B*). Hyperglycemia exposure scores also correlated with larger grey matter volume in the right frontal middle gyrus ($P = 0.008$) (Fig. 1*C*) and with smaller white matter volume in right superior parietal white matter ($P = 0.01$) (Fig. 1*D*). A similar cluster appeared in the left superior parietal white matter but did not survive multiple comparisons correction ($P = 0.13$). Higher hyperglycemia exposure scores were not associated with greater white matter volume in any region.

### Age of onset

Given the possibility that age of onset might confound our results (despite having covaried it from those analyses), we performed an exploratory analysis correlating age of onset to grey and white matter volume. These analyses found that earlier age of onset was related to larger white matter volume in the left precuneus region (voxel extent = 177; $P = 0.02$). There were no significant differences for white matter in the other direction or for grey matter in either direction.

## CONCLUSIONS

— This is the first study to examine the effects of type 1 diabetes on brain structure in a large sample of children and adolescents. We found that youth with type 1 diabetes did not significantly differ from nondiabetic siblings in regional grey or white matter volumes. However, within the diabetic group, we found qualitatively different relationships between exposure to severe hypoglycemia and chronic hyperglycemia and regional grey and white matter volumes. These differences were statistically significant despite the relatively short duration of diabetes in our sample.

### Hypoglycemia

Compared with their hypoglycemia-naive diabetic peers, diabetic youth with one or more prior severe hypoglycemic episodes had smaller grey matter volume at the left temporal-occipital junction. Interestingly, smaller grey matter in a similar region (left superior temporal and angular gyri) has been reported in adults with type 1 diabetes (5). In addition, human neuropathological case studies of profound hypoglycemia in adults have reported defects in temporal and/or occipital regions (17,18); however, in one report, these regions were relatively preserved (19). This region has been associated with the episodic memory system (20); severe hypoglycemia has previously been found to affect episodic memory in children (3,21), but the relation of such cognitive changes to the current brain findings remains to be examined. This area is also part of the "default system," a set of interconnected brain regions with high resting state neuronal activity that decreases in response to cognitive challenges (22). It is possible that this high baseline rate of blood flow creates a heightened vulnerability of the neurons to significantly reduced blood glucose during hypoglycemia.

While no direct comparisons were made between the left and right hemispheres, our data may suggest a stronger effect of severe hypoglycemia on the left side of the brain than that on the right side. At least three neuropathological case studies also described more extensive damage in the left than in the right hemisphere with severe hypoglycemia (18,23,24). Additionally, in a single photon emission computed tomography (SPECT) study, children with diabetes had abnormal left-right blood flow ratios, suggestive of left hemisphere dysfunction (25). Interestingly, in the default system, this same region has a greater response to cognitive challenge on the left than on the right side (22).

### Hyperglycemia

The extent of exposure to hyperglycemia was associated with differences in both grey and white matter volumes. Smaller grey matter volume was found in posterior cortical areas (right cuneus and precuneus). This region is associated with higher-order visuospatial function and episodic memory (26); the relation of findings in this region to diminished per-

**Table 2—*Summary of models, contrasts, and results***

| Tissue type | Model | Covariates | *n* | Contrast | Region | Brodmann's area(s) | Cluster size (voxels) | Corrected cluster *P* | Peak voxel MNI coordinates (x, y, z) | Peak voxel *t* |
|---|---|---|---|---|---|---|---|---|---|---|
| Grey | Diabetic vs. HC | Age, sex, GM | 108 vs. 51 | Diabetic < HC | L calcarine, precuneus | 18, 17 | 287 | 0.14 | −22, −66, 14 | 3.95 |
| | | | | Diabetic > HC | None | | | | | |
| Grey | Any vs. NH | Age, sex, GM, onset | 66 vs. 42 | **AH < NH** | **L temporal-occipital** | **39, 37, 19** | **901** | **0.001** | **−52, −72, 20** | **4.88** |
| | | | | | **L inferior occipital** | **19, 18** | **508** | **0.002** | **−42, −84, −12** | **4.65** |
| | | | | AH > NH | None | | | | | |
| Grey | Hyperglycemia exposure score | Age, sex, GM, onset | 98 | **Negative correlation** | **R cuneus, precuneus** | **18, 19, 7** | **464** | **0.02** | **12, −74, 22** | **4.68** |
| | | | | **Positive correlation** | **R prefrontal** | **45, 46, 9, 8** | **473** | **0.008** | **46, 34, 36** | **3.87** |
| | | | | | L ventral prefrontal | 11 | 163 | 0.18 | −10, 58, −24 | 4.39 |
| White | Diabetic vs. HC subjects | Age, sex, WM | 108 vs. 51 | Diabetic < HC | None | | | | | |
| | | | | Diabetic > HC | None | | | | | |
| White | AH vs. NH | Age, sex, WM, onset | 66 vs. 42 | AH < NH | None | | | | | |
| | | | | AH > NH | None | | | | | |
| White | Hyperglycemia exposure score | Age, sex, WM, onset | 98 | **Negative correlation** | **R superior parietal** | **Near 5, 7** | **297** | **0.01** | **22, −54, 52** | **4.29** |
| | | | | | L superior parietal | Near 5, 7 | 178 | 0.13 | −22, −54, 40 | 4.22 |
| | | | | Positive correlation | None | | | | | |

Regions with multiple-comparison corrected cluster level $P < 0.20$ are shown; significant results are in bold. Voxel dimensions are 2 × 2 x 2 mm. Peak voxel refers to the voxel with the greatest *t* value. AH, any hypoglycemia; GM, total grey matter volume; HC, healthy control; L, left; MNI, Montreal Neurological Institute; NH, no hypoglycemia; R, right; WM, total white matter volume.

formance in these cognitive domains in diabetic children (3,21) remains to be investigated. A study in adults with type 1 diabetes also found less grey matter in the right cuneus in those with higher lifetime A1C averages (5). In addition, another study reported lower grey matter density in the right occipital lobe in diabetic adults with retinopathy compared with that in healthy control subjects (27). The mechanism for this regional effect is unknown, but Wessels et al. (27) speculated that the occipital lobe is at risk for hypoperfusion because of its location in a "watershed area" of circulation. One might expect these effects to correlate to vascular changes. Although vascular disease and retinopathy were not assessed in this sample, some degree of early vascular changes might be present in diabetic children within a few years of onset (1). Therefore, a vascular mechanism cannot be ruled out as accounting for this finding even in this age range. This region, also in the "default system," has been noted for having the highest baseline metabolism of the whole brain (22) and may be preferentially vulnerable to insults (28).

Our analyses also revealed greater grey matter volume in a right prefrontal region with greater exposure to hyperglycemia. Overall, grey matter decreases with development across this age range (29), so this finding may reflect an abnormal developmental trajectory. Alternatively, this finding may indicate a compensatory reaction to the lower grey matter volume that we found in the right cuneus and precuneus. Musen et al. (5) also reported greater grey matter density associated with higher lifetime A1C in adults with diabetes, but the affected region was in a different location (parietal lobe) than the ones that we report here in children.

White matter volume was lower in the right superior parietal region in subjects with greater hyperglycemia exposure, with a similar finding in the homologous area on the left side. The significant region on the right was adjacent to the hyperglycemia-associated grey matter area (precuneus), and these two findings may be related. By reconstructing white matter fiber bundles and their neuroanatomical connectivity to grey matter regions, diffusion tensor tractography could further address whether these two regions may be connected. A magnetic resonance spectroscopy study in children with a history of significant hyperglycemia reported low metabolite ratios in posterior parietal

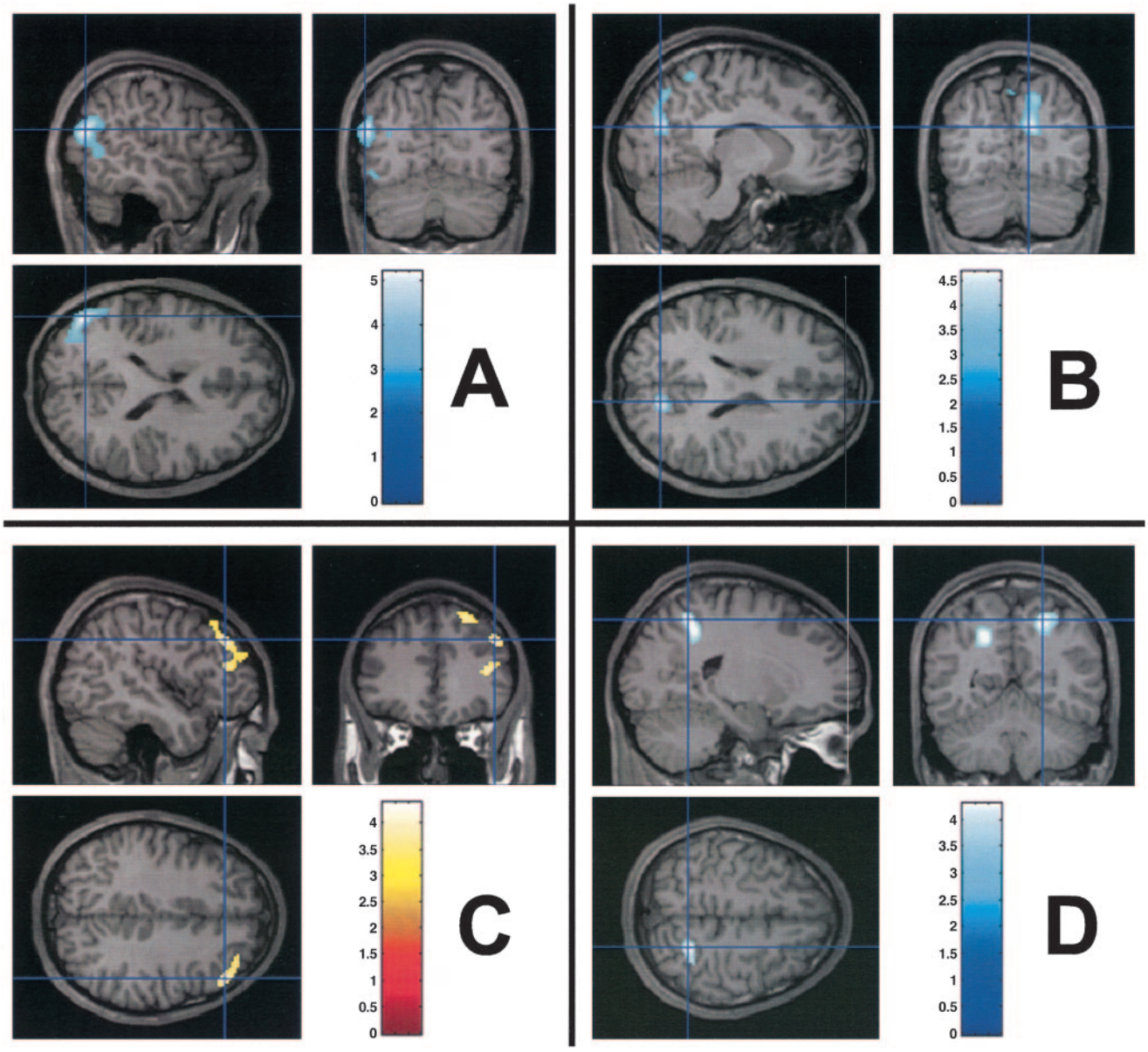


**Figure 1**—*Results overlaid on individual subject's brain in Montreal Neurological Institute space.* A: *Regions with smaller grey matter volume in diabetic youth with histories of severe hypoglycemia compared with those in diabetic youth without histories of severe hypoglycemia. Regions of less grey matter* (B)*, more grey matter* (C)*, and less white matter* (D) *associated with greater hyperglycemia exposure. Crosshairs indicate location of peak voxel of the significant region. The color bar indicates* t *values. In the coronal view, the left side of the images depicts the left side of the brain. A supplementary figure is available in the online appendix at http://dx.doi.org/10.2337/dc07-0351.*

white matter, indicating possible dysfunction or reduced axonal density in this region (30). In normal children, anisotropy in the parietal white matter has been found to increase with age (31) and to correlate with IQ (32). Thus, it is possible that altered white matter volume in this region could be reflected in aspects of cognitive performance.

### Age of onset

Age of onset of diabetes was examined as a potential confounder to the observed effects of hypoglycemia and hyperglycemia. The finding that age of onset was not associated with differences in grey matter is consistent with a previous report, which found no effect of diabetes-onset age on traced temporal lobe or amygdalohippocampal volumes in young adults with type 1 diabetes (33). The association of earlier age of onset with larger white matter volume near the left precuneus was unexpected, but there have been no previous studies examining the effects of age of onset on white matter volume. Our finding was in the opposite direction (larger) and on the opposite side (left) from the volume difference in the precuneus region found to be associated with hyperglycemia exposure (see above). Thus, the anatomical location and the direction of the relationship suggest that age of onset does not explain the results from our analyses of the effects of hypoglycemia and hyperglycemia.

As with complications in other organs and systems in youth with type 1 diabetes (e.g., neuropathy, retinopathy, and nephropathy) (1,2), the effects of blood glucose extremes on the structural integrity of the brain, although significant, may be

subclinical at this early stage. These subtle differences are observable at a group level and are not likely to be apparent in individual patients at this age. However, these differences were generated with conservative statistical methods designed to minimize the probability of false positives (34), so their potential consequences should be examined. Currently, it is unknown whether these differences are associated with cognitive consequences. It is possible that, as with other complications, these brain differences and their consequences could be compounded by further cumulative exposure to glycemic extremes with advancing age and increasing duration of diabetes.

We propose that the regional volume differences detected in this study reflect the impact of hyper- and hypoglycemia on neural integrity and/or development. In animal models, hypoglycemia has been shown to induce neuronal death and dysfunction (35), and hyperglycemia is reported to cause injury to myelin and neurons (36); these data support our supposition that glycemic extremes precede measurable differences in the brain. However, because of the retrospective and correlative nature of this study, we cannot rule out that the differences reported here were present before exposure to glycemic extremes or diabetes. Retrospective report of severe glycemic experiences has been found to be fairly reliable in adults (37), but prospective measures are likely to be more accurate, especially over long time periods (38). Prospective follow-up of our sample is ongoing and should be able to determine if further exposure to hyper- and hypoglycemia accentuates the pattern of regional volume differences reported here. It should also be noted that glycemic extremes could affect the function of the brain with or without altering regional volumes. Future studies using methods such as functional MRI would be needed to determine whether functional abnormalities exist in this population.

We conclude that hypo- and hyperglycemia are associated with differences in regional grey and white matter volumes in the brain in youth with type 1 diabetes. Longitudinal follow-up of well-characterized samples such as ours is necessary to determine the course of brain changes with age and further exposure to glycemic extremes. Ultimately, an understanding of the implications of these findings for optimal cognitive and academic function must be obtained to place these observations in proper clinical context.

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