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Neuroanatomical Correlates of Dysglycemia in Young Children With Type 1 Diabetes



Studies of brain structure in type 1 diabetes (T1D) describe widespread neuroanatomical differences related to exposure to glycemic dysregulation in adults and adolescents. In this study, we investigate the neuroanatomical correlates of dysglycemia in very young children with early-onset T1D. Structural magnetic resonance images of the brain were acquired in 142 children with T1D and 68 age-matched control subjects (mean age 7.0 ± 1.7 years) on six identical scanners. Whole-brain volumetric analyses were conducted using voxel-based morphometry to detect regional differences between groups and to investigate correlations between regional brain volumes and measures of glycemic exposure (including data from continuous glucose monitoring). Relative to control subjects, the T1D group displayed decreased gray matter volume (GMV) in bilateral occipital and cerebellar regions ($P < 0.001$) and increased GMV in the left inferior prefrontal, insula, and temporal pole regions ($P = 0.002$). Within the T1D group, hyperglycemic exposure was associated with decreased GMV in medial frontal and temporal-occipital regions and increased GMV in lateral

prefrontal regions. Cognitive correlations of intelligence quotient to GMV were found in cerebellar-occipital regions and medial prefrontal cortex for control subjects, as expected, but not for the T1D group. Thus, early-onset T1D affects regions of the brain that are associated with typical cognitive development.

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Glucose dysregulation in type 1 diabetes (T1D) can result in physiological complications, such as neuropathies (1), and has also been linked to an increased risk for cognitive deficits and psychological dysfunction (2,3). Neuroanatomical insult from dysglycemia may be particularly consequential in early childhood, which is a period of dynamic brain development that includes rapid myelination of neurons as well as maturation, modification, and pruning of synapses (4). Although the effects of metabolic perturbations in T1D on central nervous system structure are not fully understood, neuronal damage is generally believed to be a consequence of both hyper- and hypoglycemic states. More specifically, nonenzymatic glycation of neural tissue is believed to lead to increased

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See accompanying commentary, p. 62.

oxidative stress during hyperglycemia (5), and ultimately neurodegradation, while cell death may be instigated through deficiencies in insulin-sensitive signaling pathways (6). Conversely, energy deprivation during hypoglycemia may abet defective apoptotic processes, spur reactive gliosis, or lead to cellular necrosis via excitotoxicity of glutamate receptors (7,8). Accordingly, an improved understanding of the relation between dysglycemia and brain development, especially in young children, is necessary to better inform medical treatment and ultimately improve clinical outcomes of individuals with T1D.

Previous investigations of T1D brain structure have shown increased rates of cerebral atrophy during the course of normal aging (9), with more pronounced effects observed in adults with early-onset diabetes (10). These findings suggest increased vulnerability of the younger brain to the disease. However, differences in total gray matter volume (GMV) and total white matter volume (WMV) relative to control subjects have only been observed in middle-age or older adults (mean age 44 years) (11). More fine-grain analyses using voxel-based morphometry (VBM) in adults with T1D relative to control subjects have detected smaller regional GMV in frontal, temporal, and parieto-occipital regions (12), areas that are responsible for language processing, executive function, and cognition. Neuroanatomical variations in adult T1D also have been linked to severe hypoglycemia exposure, lifetime hemoglobin A_{1c} (HbA_{1c}), disease duration, and severity of microangiopathy (12,13).

Using cross-sectional and longitudinal analyses in older children and adolescents with T1D (mean age ~12.6 years), our colleagues at Washington University in St. Louis (14–16) and Stanford University (17) observed significant correlations between neuroanatomical changes in occipital, temporal, frontal, and hippocampal regions and either greater exposure to hyperglycemia (as evidenced by lifetime weighted average HbA_{1c}) or severe hypoglycemic episodes. However, some of the brain-glycemia associations observed in these studies were contradictory to those reported in adults (12), a phenomenon that may be due to developmentally specific responses to glycemic insults or to the heterogeneity of glycemic exposure in the disease.

The current study was designed to determine if T1D in very young children (age range 4.0–10.0 years) is associated with significant changes in GMV or WMV relative to age- and sex-matched nondiabetic control subjects. We also sought to determine if such neuroanatomical variations within the T1D group were correlated with measures of glycemic exposure (e.g., HbA_{1c} or glucose levels measured with continuous glucose monitors [CGMs]) or with cognitive function. We hypothesized that rapidly developing brain regions are especially vulnerable to deviation from a euglycemic state and that this vulnerability would be reflected in

regional differences in GMV and WMV in young children with T1D.

RESEARCH DESIGN AND METHODS

Recruitment and Exclusion Criteria

Children between 4.0 and 10.0 years of age were recruited for this study at five clinical centers in the Diabetes Research in Children Network (DirecNet) consortium (Nemours Children's Clinic, Stanford University, The University of Iowa, Washington University in St. Louis, Yale University, and the Jaeb Center for Health Research). The institutional review board at each participating center approved the study protocol. Informed written consent was obtained from the parent or legal guardian of all participants, and verbal assent was obtained from study participants as per local guidelines. Eligibility criteria for the T1D participants included age of onset of T1D ≥ 6 months, positive islet cell autoantibody testing (GAD65, ICA512, and mIAA) if onset was < 12 months of age, and use of insulin for at least 1 month. Requirements for nondiabetic control subjects included HbA_{1c} $< 6.0\%$ (42 mmol/mol), fasting glucose < 110 mg/dL, and no history of abnormal glycemic control. Sibling control subjects of T1D participants were considered for inclusion in the control group if they had negative antibody testing within the prior year. Exclusions for both groups included genetic or mental disorders that could impair neurological development, history of intellectual disability or significant learning disabilities, psychiatric treatment, premature birth (< 34 weeks gestation), low birth weight ($< 2,000$ g), and magnetic resonance imaging (MRI) contraindications (e.g., metal implants).

Participants

Of 214 participants for whom an MRI scan was obtained, four were excluded due to poor image quality. Imaging data for 142 T1D subjects (mean age 7.0 ± 1.7 years; 66 female and 76 male) and 68 control subjects (mean age 7.0 ± 1.8 years; 33 female and 35 male) were included in the analyses. Table 1 provides a further description of the cohort, which includes average T1D onset age of 4.1 ± 1.9 years with an average T1D duration of 2.9 ± 2.0 years.

Cognitive Testing

Trained examiners collected data on intelligence quotients (IQs) using the age-appropriate Wechsler Preschool and Primary Scale of Intelligence, the Wechsler Intelligence Scale for Children IV, or the Wechsler Adult Scale of Intelligence test for parents (18–20). Cognitive results for full-scale IQ (FSIQ) were converted to normalized *z* scores (Table 1).

Diabetic Exposure and CGM

Within the T1D group, a hyperglycemic index was determined based on all available HbA_{1c} values since diagnosis and up to the time of participation in this study

Table 1—Descriptive statistics of study cohort

	T1D	Control	<i>P</i>
<i>n</i> = 210	142 (66 female, 76 male)	68 (33 female, 35 male)	
Age (years)	7.0 ± 1.7	7.0 ± 1.8	0.94
GMV (mL)†	688 ± 44	691 ± 38	0.73
WMV (mL)†	446 ± 31	446 ± 28	0.89
GMV+WMV (mL)†	1,134 ± 72	1,137 ± 64	0.79
Clinical measures			
T1D onset age (years)	4.1 ± 1.9	N/A	N/A
T1D duration (years)	2.9 ± 2.0	N/A	N/A
HbA _{1c} at D _x (<i>n</i> = 116) % (mmol/mol)	10.4 ± 1.9 (90 ± 21)	N/A	N/A
HbA _{1c} at baseline % (mmol/mol)	7.9 ± 0.9 (63 ± 10)	5.2 ± 0.2 (33 ± 2)	N/A
Incremental HbA _{1c} >6.0%	5.8 ± 4.6	N/A	N/A
CGM data			
Hours of CGM data	268	N/A	N/A
Average mean BG	194 ± 37	N/A	N/A
Cognitive measures			
FSIQ (<i>z</i> score)	-0.09 ± 1.0	0.19 ± 0.9	0.02

†Adjusted for age and sex.

by computing the area under the curve >6.0% (HbA_{1c}AUC_{6%}) according to the trapezoid rule (additional glycemic measures are shown in Table 1). A CGM device was used to obtain a minimum of 72 h of glycemic data in a 6-day period (with at least 24 h of overnight data) and within 90 days prior to the MRI. Data within 28 days post-MRI were included if a subject had <30 h of pre-MRI CGM data. CGM measures included mean glucose value, coefficient of variation, SD, and mean amplitude of glycemic excursions (MAGEs) (21).

MRI Preparation

All study participants were introduced to a mock MRI environment to desensitize them to the sights and sounds they would encounter during the scan. Research staff was trained to identify and correct behaviors that might result in motion-related artifacts in the images.

Magnetic Resonance Data Acquisition

Unsedated MRI was performed on Siemens 3T Tim Trio whole-body magnetic resonance systems using a standard 12-channel head coil. All six imaging sites had the same scanner hardware, and an identical imaging protocol was uploaded to every scanner. Sagittal T1 images of the brain were acquired (right to left) using a magnetization-prepared rapid gradient echo (MP-RAGE) pulse sequence with the following parameters: repetition time (TR) = 2,300 ms, echo time (TE) = 2.98 ms, inversion time (TI) = 900 ms, flip angle = 9°, slice thickness = 1 mm, field of view (FOV) = 25.6 cm × 24 cm, 160 slices, matrix = 256 × 256, voxel size = 1.0 × 1.0 × 1.0 mm, and duration = 4:54 min. By default, two MP-RAGE acquisitions were obtained

for all participants to increase the probability that at least one scan would be collected with minimal head motion (22). A second MRI session was performed on a separate day if the initial scan could not be successfully completed or if image quality was deemed unacceptable after the first attempt. Additionally, for diabetic subjects, a finger stick blood glucose (BG) level was required to be between 70 and 300 mg/dL within 60 min prior to all scan sessions.

VBM

All images were visually inspected to identify the highest quality MP-RAGE scan for each participant. Participants without at least one high-quality scan (two T1D and two control) were eliminated from further analysis. Voxel-wise volumetric analysis of MP-RAGE data were performed on the remaining 210 scans by well-established methods using Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, University College London, London, U.K., <http://www.fil.ion.ucl.ac.uk/spm>) in MATLAB (MathWorks, Natick, MA). Each image volume was manually aligned onto the axis of anterior-posterior commissures (23). Data were corrected for magnetic field inhomogeneity and subsequently segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid volumes based on a priori adult tissue probability maps and spatially constrained tissue classification based on neighboring voxels (24,25). High-dimensional, intersubject registration was then performed by generating a cohort-specific template using the DARTEL toolbox in SPM (26). Images were warped and modulated into Montreal Neurological

Institute (MNI) space for reporting of results, down-sampled to $1.5 \times 1.5 \times 1.5$ mm voxels, and spatially smoothed using a 6-mm full-width at half-maximum Gaussian smoothing kernel.

Whole-Brain Between-Group Analysis

Regional differences in brain morphology between T1D and control subjects were analyzed based on the general linear model using voxel-wise two-sample Student *t* tests to create a *t* statistic parametric map of the whole brain. Analyses of GMV and WMV were independently restricted to voxels with a mean tissue class probability >0.15 . Statistical inference was evaluated using the VBM toolbox (C. Gaser, University of Jena, Jena, Germany, <http://dbm.neuro.uni-jena.de/vbm/>) to threshold the voxel-level *t* statistics at a height corresponding to $P < 0.05$ (uncorrected) and at a cluster extent threshold of $P < 0.05$ (corrected for family-wise error [FWE]) while accounting for nonstationary smoothness of the data (27).

Whole-Brain Regression Analysis

Within each group (T1D and control subjects analyzed separately), voxel-wise multiple linear regression was used to examine whole-brain correlations between imaging data and glycemic or cognitive variables after first accounting for the effects of total GMV (or WMV), age, and sex. For age-related clinical variables (e.g., age of onset, disease duration, and $HbA_{1c}AUC_{6\%}$), an alternative two-step analysis was used to separate the effects of age and exposure. First, VBM was applied using only GMV (or WMV) as a covariate, and the mean value of each significant cluster was extracted for each subject for a region-of-interest (ROI) analysis. Then, a hierarchical linear regression analysis (IBM SPSS Statistics v19) removed the effect of total GMV from the ROI and analyzed the independent contributions of age, sex, and the clinical variable to the residual volumes. To investigate differences in cognitive correlations between groups, an ANOVA of mean cluster volume by GMV, age, sex, group, FSIQ, and group \times FSIQ interaction was performed in SPSS to test for significant interaction effects. In defining the analyses performed in this study, pre-existing hypotheses of critical factors in diabetes were used to identify clinical variables of interest, and child FSIQ was adjusted for parent IQ. Accordingly, the analyses are parallel tests of hypotheses, rather than exploratory analyses, and so further corrections for multiple comparisons (beyond the used FWE cluster extent correction for imaging data) were not applied.

Multisite Effects

Identical scanners were used at every site, and continued calibrations were performed to confirm the repeatability of measurements across sites. The same two adult human phantoms were scanned on every machine prior to study start and again annually for 2 years. A standard plastic phantom (American College of Radiology [ACR]; available

at each site) was also scanned at each site quarterly to monitor for possible temporal variations in scan quality. Additional human and/or ACR data were acquired both before and after hardware or software upgrades. We observed $<0.5\%$ variation in geometric size across sites, which was not significantly larger than the observed intrasite variation of 0.4%. The possibility of multisite effects affecting the VBM statistical results were investigated using a full factorial design with fixed factors for diagnosis (two levels: T1D and control) and scanner (six levels: scanners 1–6), while covarying for the effects of total GMV (or WMV), age, and sex (28). *F* tests showed a significant main effect of scanner in the GM in left anterolateral inferior temporal gyrus and fusiform gyrus ($-22, 10, -40$ [*x, y, z*]; $F = 6.62, P = 0.01, k = 4,826$), and a significant diagnosis \times scanner interaction bilaterally in central and posterior WM ($-36, -45, 33$ [*x, y, z*]; $F = 5.47, P = 0.002, k = 5,947$). Consequently, all potential diabetes results in these regions were analyzed using scanner as a covariate in the analyses. To further examine the effect of multiple scanners, between-group analyses of T1D versus control subjects were performed using a two-sample Student *t* test (also covaried for total GMV [or WMV], age, and sex) both with and without the inclusion of scanner as a nuisance variable (by coding *n* scanners into $n - 1$ dichotomous variables). It was determined that the inclusion of scanner as a covariate had a negligible impact on the results.

RESULTS

Total Brain Volumes

No significant differences were detected between T1D and control subjects in total GMV, WMV, or GMV+WMV, adjusted for age and sex (Table 1).

Between-Group Differences

Voxel-wise whole-brain volumetric analysis revealed significantly decreased GMV in T1D subjects relative to control subjects bilaterally in cerebellum, precuneus, cuneus, calcarine, lingual gyrus, and fusiform gyrus (Table 2 and Fig. 1). Conversely, increased GMV was observed in T1D subjects relative to control subjects in left lateral prefrontal cortices (middle frontal gyrus [MFG], inferior frontal gyrus [IFG], and superior frontal gyrus [SFG]), extending into the superior temporal gyrus (STG), middle temporal gyrus (MTG), and insula. GMV was 3.6% less for T1D relative to control subjects averaged over the posterior cluster, and 4.2% greater in the left lateral frontal cluster. These effect sizes are much larger than the calibrated differences across scanners (0.5%), and both clusters remained significant after accounting for the effect of multiple scanners. No significant regional WMV differences were observed between T1D and control subjects, both with and without accounting for the effects of multiple scanners.

Table 2—VBM results table

Model	Covariates	Regions contained in cluster	Peak voxel (x, y, z)	t score	Volume (voxels)	P value*
Between groups						
Control > T1D	GMV, age, sex	Bilateral cerebellum, precuneus, cuneus, calcarine, lingual, fusiform	14, -53, 0	4.09	16,788	<0.001
T1D > control	GMV, age, sex	Left MFG, IFG, SFG, temporal pole (STG, MTG), insula	-28, 40, 33	3.91	9,853	0.002
Negative glycemic correlations with brain volume for T1D						
HbA _{1c} AUC _{6%} (-)	GMV, age, sex	Bilateral lingual, fusiform, right parahippocampal, bilateral cerebellum	26, -20, -27	3.88	7,532	<0.001**
T1D duration (-)	GMV, age, sex WMV, age	Bilateral medial orbital frontal, rectal gyri, and anterior cingulate	-4, 22, -3	3.98	7,464	0.001**
T1D duration (-)	Sex	Left anterior frontal lobe	-21, 34, -6	4.24	5,158	<0.001**
CGM mean glucose (-)	GMV, age, sex	Right cerebellum, vermis, fusiform, lingual, inferior temporal gyrus	30, -81, -27	3.8	8,064	0.007
Positive glycemic correlations with brain volume for T1D						
HbA _{1c} Dx (+)	GMV, age, sex	Left insula, IFG, temporal pole (STG, MTG)	-52, -5, 0	3.67	7,884	0.01
CGM mean glucose (+)	GMV, age, sex	Right insula, IFG, temporal (STG, MTG), precentral Left IFG, insula, MFG, temporal pole (STG)	52, 10, -19 -50, 43, 9	3.43 4.53	5,434 6,083	0.054 0.03
Positive IQ correlations with brain volume for control subjects	GMV, age, sex, parent IQ	Bilateral medial SFG, supplementary motor area, anterior cingulate, medial orbitofrontal gyrus, rectal gyri	8, 46, -10	4.46	21,520	<0.001
Control subjects, posterior regions	GMV, age, sex, parent IQ	Bilateral superior cerebellum, lingual, calcarine, cuneus, fusiform, left parahippocampal gyrus	30, -41, -21	3.72	9,174	0.007

*All P values are cluster extent corrected for FWE and nonstationary smoothness. **P value is from ROI analysis.

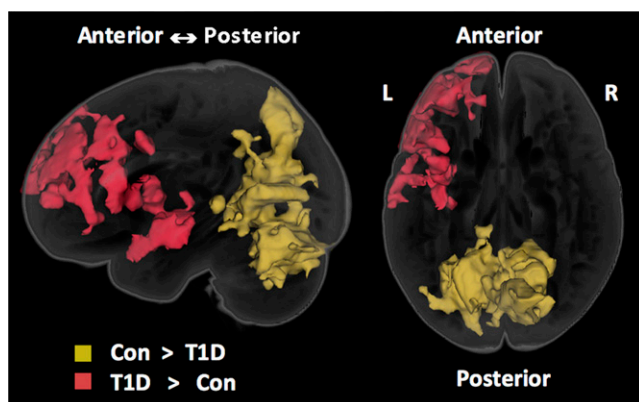


Figure 1—Sagittal (*left*) and axial (*right*) three-dimensional renderings of clusters of significant differences between T1D and control subjects. Increased GMV in lateral temporo-frontal regions in T1D relative to control subjects (red cluster, $P < 0.01$) was also found to be associated with higher $HbA_{1c}D_X$. Decreased GMV in occipital regions and cerebellum in T1D relative to control subjects (yellow cluster, $P < 0.001$) was found to be associated with higher $HbA_{1c}AUC_{6\%}$ and higher mean glucose.

Glycemic-Structural Correlations

Within the T1D group, the ROI analysis of clinical measures of glycemic exposure (Fig. 2) showed that GMV was negatively correlated with $HbA_{1c}AUC_{6\%}$ in bilateral lingual gyrus, fusiform gyrus, right parahippocampal gyrus, and cerebellum ($P < 0.001$). Similarly, GMV was negatively correlated with duration of diabetes in the bilateral medial orbitofrontal and rectal gyri and the anterior cingulate ($P = 0.001$), and WMV was negatively correlated with disease duration in the left anterior frontal lobe ($P < 0.001$). Additionally, HbA_{1c} at diagnosis ($HbA_{1c}D_X$, $n = 116$) was positively correlated with GMV in a lateral frontal cluster, including the left middle and inferior prefrontal cortex (MFG and IFG), lateral orbitofrontal gyrus, and insula and extending into the anterior STG and MTG ($P = 0.01$). A similar trend ($P = 0.05$) was observed for a cluster in the right prefrontal cortex (Table 2).

Voxel-wise regression for the CGM measures showed that mean glucose was negatively correlated with GMV in a posterior region, including right cerebellum, lingual, fusiform, and inferior temporal regions ($P = 0.007$) (Fig. 2). Conversely, mean glucose was positively correlated with GMV in the left inferior frontal, insula, and anterior STG regions ($P = 0.03$). Measures of glucose variability, including coefficient of variation, SD, and MAGE, did not show significant GMV correlations (SD and MAGE were corrected for mean BG).

Spatial overlaps of between-group differences and within-group glycemic correlations for T1D were observed in several brain regions (Table 3). In the right cerebellum, GMV was smaller in T1D than control subjects, and reduced GMV was associated with higher $HbA_{1c}AUC_{6\%}$ and higher mean BG. Conversely, in the left

insula, GMV was larger in T1D than control subjects, and increased GMV was associated with higher mean BG and $HbA_{1c}D_X$. Thus, correlations of regional brain volumes with measures of glycemic exposure in the T1D group were consistent with observed volumetric differences between T1D and control subjects in these conjunction regions.

Cognitive-Structural Correlations

Within the control group, whole-brain voxel-wise regression analysis revealed that IQ was significantly positively correlated with GMV in a large medial frontal cluster including bilateral anterior cingulate, superior medial frontal gyrus, and medial orbitofrontal gyrus, and in a posterior cluster including bilateral superior cerebellum and inferior occipital regions (Table 2 and Fig. 3). A similar whole-brain analysis for the T1D group did not detect any significant correlations of regional GM with IQ. An ROI analysis within each cluster derived from the control group revealed a significant interaction of group \times FSIQ (frontal cluster, $P < 0.001$; posterior cluster, $P < 0.006$), indicating that children with T1D had atypical trajectories of GM development in these regions.

Regions With Both Glycemic-Structural and Cognitive-Structural Effects

Conjunction analyses were performed on the brain regions associated with both glycemic exposure in T1D and FSIQ in control subjects. Regions were defined by the spatial overlaps of glycemic-structural clusters and cognitive-structural clusters (Table 4 and Fig. 3). In the medial orbitofrontal and anterior cingulate conjunction, GMV was positively correlated with FSIQ for both control subjects ($P = 0.007$) and T1D ($P = 0.014$), whereas the group \times FSIQ interaction was not significant ($P = 0.13$). In the right fusiform, lingual gyrus, and cerebellum, FSIQ was positively correlated with GMV in control subjects ($P < 0.001$) but not T1D, and the group \times FSIQ interaction was significant ($P < 0.001$). Similarly, in the bilateral cerebellum, right lingual and right cuneus conjunction region, FSIQ was positively correlated with GMV only in control subjects ($P < 0.005$), and the group \times FSIQ interaction was significant ($P < 0.025$). Thus, T1D is associated with decreased GMV, and a decrease in GMV-IQ correlations, in the same cerebellar-occipital regions where increased GMV is associated with higher IQ in control subjects. No significant IQ correlations were found in regions that showed hyperglycemic-associated increases in GMV in T1D.

Glycemic Measures at Time of MRI

Blood glucose measurements for T1D subjects immediately prior to the scan session were not significantly correlated with regional brain volumes (mean glucose 165 ± 64 mg/dL), nor were mean glucose measurements across the scan session (mean glucose: 183 ± 64 mg/dL) and absolute changes in BG at the end of the MRI (mean change 56 ± 44 mg/dL).

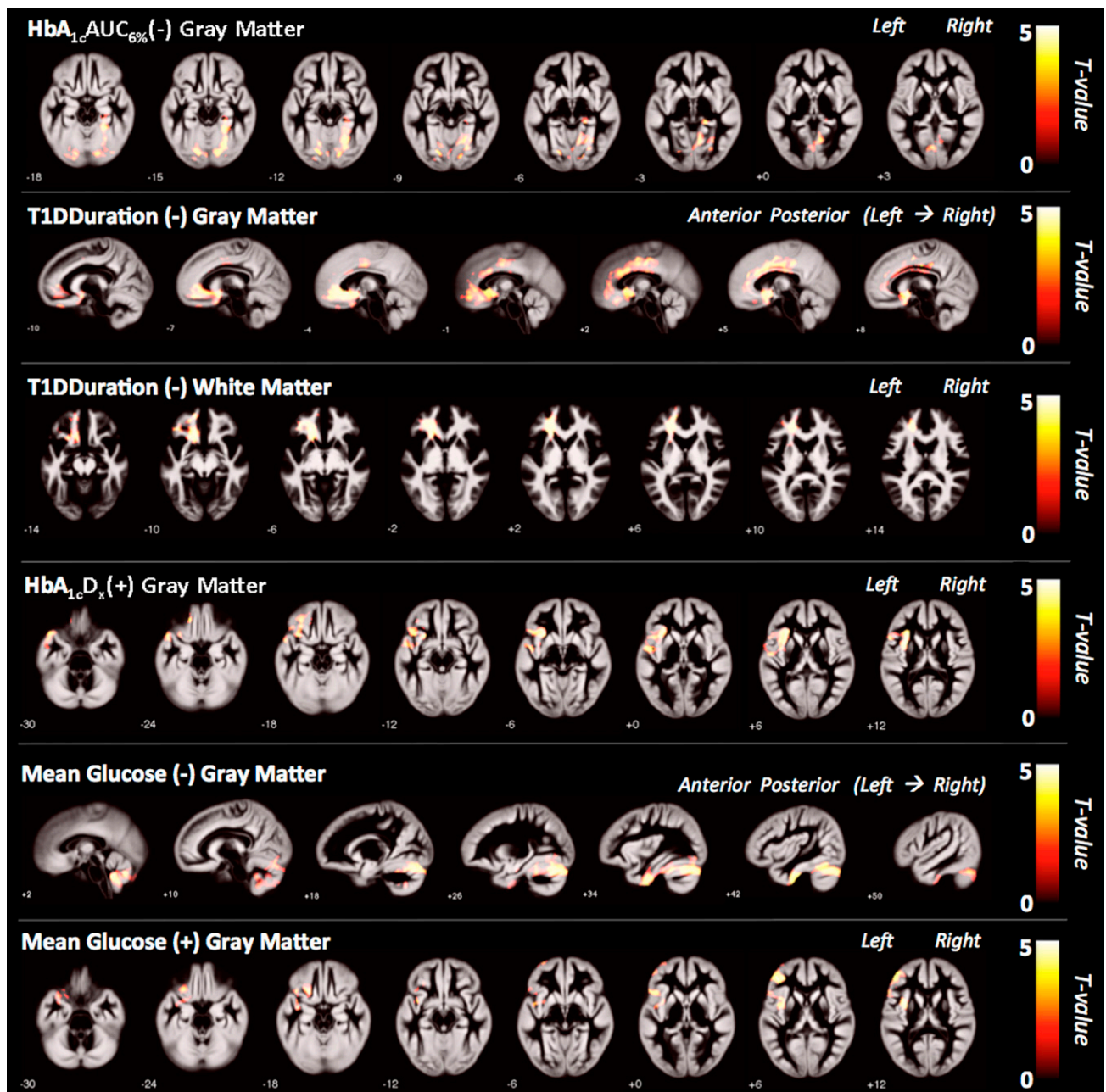


Figure 2—Significant GMV clusters from whole-brain regression analysis of glycemic measures in the T1D group (height threshold of $T = 1.66$, cluster extent threshold of $P < 0.05$, and corrected for FWE and nonstationary smoothness). Clusters are overlaid on the average GM template. $HbA_{1c}AUC_{6\%}(-)$ ($P < 0.001$), T1D duration (-, GMV) ($P = 0.001$), T1D duration (-, WMV) ($P < 0.001$), $HbA_{1c}D_x(+)$ ($P = 0.01$), mean BG (-) ($P = 0.007$), and mean BG (+) ($P = 0.03$).

DISCUSSION

The current study is the first to investigate the neuro-anatomical effects of glycemic dysregulation in a large cohort of very young children with T1D (mean age 7.0 years; mean duration 2.9 years). We report differences in regional brain volumes in young children with T1D relative to control subjects and in association with glycemic exposure. The results were concentrated in three different brain regions that are discussed below.

Occipital Regions and Cerebellum

Hyperglycemia was found to be associated with smaller GMV in bilateral temporal-occipital regions and cerebellum using multiple methods of analysis. We observed significantly smaller GMV for T1D in a between-group analysis for these regions (Fig. 1), as well as a negative correlation of GMV with increased $HbA_{1c}AUC_{6\%}$ exposure and higher mean glucose levels (Fig. 2). The occipital lobe findings in our study are consistent with reductions

Table 3—Regions with between-group differences and glycemic correlations

Combined clusters	Regions contained in conjunction	Centroid	Volume (voxels)	Overlap (%)*
T1D >control and mean glucose (+)	L insula, L anterior temporal gyrus, L dorsolateral prefrontal cortex	-36, 2, 3	1,561	10.8
T1D >control and HbA _{1c} D _x (+)	L insula, L inferior frontal pars operculum and pars triangularis	-54, 16, 12	734	4.3
Control >T1D and mean glucose (-)	R cerebellar lobes VI and crus I, vermis	20, -80, -24	2,061	9.0
Control >T1D and HbA _{1c} AUC _{6%} (-)	Bilateral lingual gyri, R fusiform, bilateral cerebellum lobes VI, L cuneus	18, -80, -16	1,203	5.2

L, left; R, right. *Ratio of volume of intersection to volume of union.

in occipital GMV and WMV that have been linked to hyperglycemia exposure in older children and adolescents with T1D, thinner occipital cortex in adults with T1D, and the presence of microangiopathy (12,13,16,29). Results indicating a negative correlation of GMV with HbA_{1c}AUC_{6%} exposure in the fusiform and lingual gyri are located inferior to similar correlations in the cuneus and posterior cingulate reported by Musen et al. (12); however, their study was in adults and included patients with long durations of diabetes and widely varying ages of diabetes onset.

The between-group and hyperglycemic results located in the cerebellum have not been previously reported. The cerebellum is a region of active development during childhood, both in absolute volume and volume relative to the cerebrum (30). The superior-posterior cerebellum is known to be involved in many tasks including working memory, executive function, and motor control (31,32),

and the volume of the superior cerebellum has been found to be positively correlated with IQ (33). For control subjects, we found that IQ was positively correlated with regional GMV in superior cerebellum. However, T1D subjects did not show this correlation, and there was a significant group by region interaction for this region, indicating that cerebellar development underlying general cognition is disturbed in children with T1D relative to control subjects.

Medial Frontal Regions

The duration of diabetes was negatively correlated with GMV in medial-frontal regions (Fig. 2), consistent with the previously reported loss of GMV in this region for adults (12). Studies of normal brain development have shown that the frontal lobe, and particularly the medial frontal lobe, has rapid increases in GMV throughout early childhood, with frontal GMV cresting around 11 years of

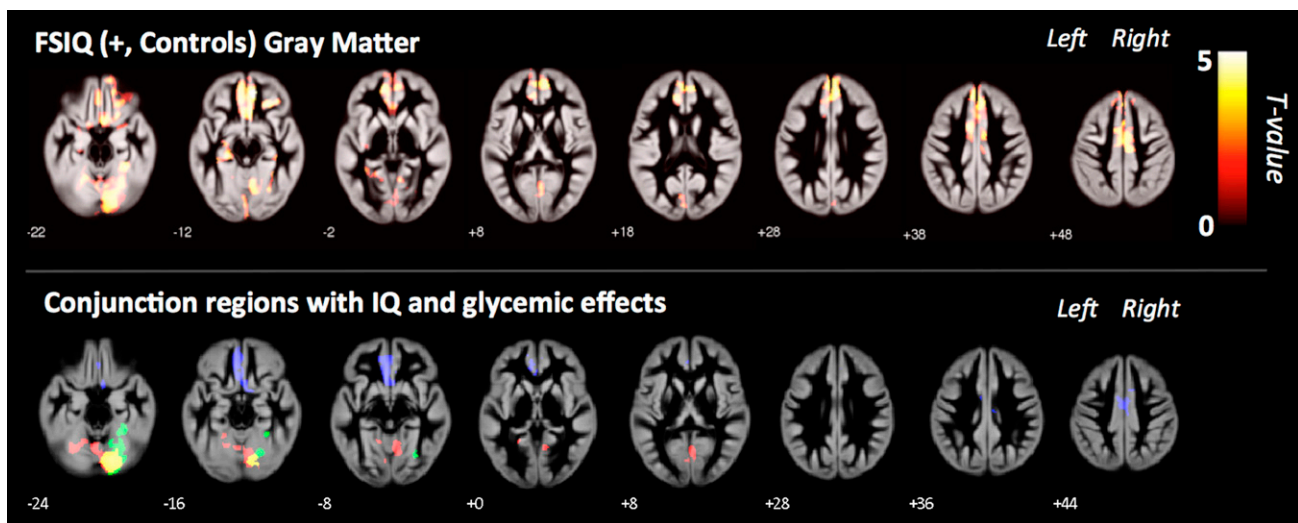


Figure 3—Regions with significant correlations between IQ and GMV. *Top*: Clusters from whole-brain regression analysis of IQ in the control group (height threshold of $T = 1.66$, cluster extent threshold of $P < 0.05$, and corrected for FWE and nonstationary smoothness). *Bottom*: Conjunction regions with IQ-GMV correlations in control subjects and glycemic-GMV correlations in T1D. Color denotes glycemic variable/contrast: T1D duration (-) (blue), mean glucose (-) (green), control >T1D (red), overlap (yellow). Clusters are overlaid on the average GM template.

age (34). In addition, increased IQ has been associated with more rapid thickening along the bilateral superior/medial prefrontal cortex (35). For control subjects, we found this well-established result, that IQ was positively correlated with GMV along the medial SFG and the medial orbitofrontal gyrus. However, children with T1D did not show this correlation and, in fact, had significantly different IQ-GMV correlations than control subjects for this cluster. Hyperglycemia may affect GM growth since it has been associated with reduced insulin-like growth factors, leading to deficiencies in neurotrophic growth factors and thus reduced dendritic arborization (36). Recent studies have also suggested that brain metabolites important for osmotic regulation are significantly altered at acute hyperglycemic onset and that defective cell volume regulation due to increased oxidative stress may lead to neuronal loss (37,38). Thus, these results indicate that early-onset diabetes may interfere with the normal growth trajectory of GM in this frontal region.

WM was negatively correlated with diabetes duration in the nearby left anterior frontal lobe. A recent study of changes in WM pathology in a rat model of diabetes found that abnormalities such as myelin loss were not present in early disease stages but instead developed over time (39), suggesting that large-scale WMV variations might not yet be fully discernable in our young cohort. Previous diffusion tensor imaging studies in young children and adolescents (40,41), as well as a similar companion study of our cohort (unpublished data), showed reduced diffusivity in multiple brain regions in subjects with T1D relative to control subjects, which may precede detectable WMV loss.

Left Lateral Frontal and Insula Regions

Using multiple analysis methods, hyperglycemia was associated with increased GMV in the left lateral prefrontal cortex, insular cortex, and anterior temporal lobe. These regions showed a significant between-group difference in GMV for T1D greater than control subjects, and within the T1D cohort, these regions showed positive correlations of GMV with HbA_{1c} at diagnosis (HbA_{1c}D_X) and higher mean BG levels. Similar to the medial prefrontal region, the insula is also one of the fastest growing regions during childhood (34). Although these regions are associated with executive function (42), interoceptive awareness (43), and auditory and language processing (44), we did not find cognitive correlations with FSIQ for these clusters. It is possible that the increase in GMV in these regions may be due to a neuroinflammatory response to increased oxidative stress from the formation of advanced glycation end products during hyperglycemia exposure (45) or, more speculatively, may compensate for loss of GMV in the cerebellum and occipital regions.

Limitations

The current study is limited by several factors. First, the cross-sectional nature of this study limits conclusions about developmental changes that occur in brain growth

Table 4—Correlations of GMV with IQ in regions with both glycemic and cognitive effects

Combined clusters	Regions contained in conjunction	Centroid	Volume (voxels)	P value	
				control subjects	T1D
IQ and duration	Bilateral medial orbitofrontal gyrus and dorsal anterior cingulate	-6, 38, -8	2,919	0.007 (<i>r</i> = 0.28)	0.014 (<i>r</i> = 0.10)
IQ and mean glucose (-)	Bilateral superior cerebellum, vermis, R fusiform, R lingual	18, -80, -24	2,838	0.015 (<i>r</i> = 0.33)	NS (<i>r</i> = 0.09)
IQ and HbA _{1c} AUC _{6%}	R fusiform, lingual, cerebellar lobes VI and crus I	26, -20, -27	1,255	<0.001 (<i>r</i> = 0.30)	NS (<i>r</i> = -0.01)
IQ and Control > T1D	Bilateral cerebellar lobes VI, vermis, R crus I, R lingual, R cuneus	12, -76, -21	4,326	0.005 (<i>r</i> = 0.33)	NS (<i>r</i> = 0.03)

R, right; *r*, standardized regression coefficient of GMV with IQ. All interaction results covaried by GMV, group, age, and sex. NS, not significant.

due to T1D. Results from an ongoing longitudinal analysis may help to further clarify the neurodevelopmental growth of young children with T1D. Second, this study reports large, nonlocalized clusters because the algorithm parameters were set under the assumption that diabetes may cause weak, but widespread, effects. Future studies should try to better localize these brain regions. Finally, this study did not consider factors such as exposure to diabetic ketoacidosis and severe hypoglycemic events, or performance on measures assessing more specific cognitive and behavioral domains.

Summary

Neuroanatomical differences in the lateral frontal, medial frontal, occipital, and cerebellar brain regions were found to be associated with dysglycemia in young children with early-onset T1D relative to a well-matched healthy control group. Overlapping regional results were obtained for both between-group studies and regressions within the T1D group for hyperglycemic variables. The medial prefrontal regions, insula, and cerebellum are known to be regions of rapid development in childhood and may be particularly vulnerable to glycemic effects from early onset of the disease. Control subjects had a significant positive correlation of IQ with GMV in the medial prefrontal lobe and cerebellum-occipital regions; these correlations were not present in the diabetic group. Thus, these regions may be associated with the effect of early-onset diabetes on cognitive function. Conversely, the only WMV effect was in the left anterior frontal lobe, perhaps due to the limited disease duration in this young cohort. A longitudinal analysis of this and other young pediatric cohorts will help better define the neuroanatomical profile and developmental trajectory of the T1D brain.

APPENDIX

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Author Contributions. M.J.M., P.K.M., and A.L.R. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. N.B.-G., T.H., E.T., W.T., N.M., N.H.W., B.B., R.W.B., K.J.R., C.K., and P.C. researched data, contributed to discussion, and reviewed and edited the manuscript. R.W.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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