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Longitudinal Assessment of Neuroanatomical and Cognitive Differences in Young Children With Type 1 Diabetes: Association With Hyperglycemia

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COMPLICATIONS

Significant regional differences in gray and white matter volume and subtle cognitive differences between young diabetic and nondiabetic children have been observed. Here, we assessed whether these differences change over time and the relation with dysglycemia. Children ages 4 to <10 years with (*n* = 144) and without (*n* = 72) type 1 diabetes (T1D) had high-resolution structural MRI and comprehensive neurocognitive tests at baseline and 18 months and continuous glucose monitoring and HbA_{1c} performed quarterly for 18 months. There were no differences in cognitive and executive function scores between groups at 18 months. However, children with diabetes had slower total gray and white matter growth than control subjects. Gray matter regions (left precuneus, right temporal, frontal, and parietal lobes and right medial-frontal cortex) showed lesser growth in diabetes, as did white matter areas (splenium of the corpus callosum, bilateral superior-parietal lobe, bilateral anterior forceps, and inferior-frontal fasciculus). These changes were associated with higher cumulative hyperglycemia and glucose variability but not with hypoglycemia. Young children with T1D have significant differences in total and regional gray and white matter growth in brain regions involved in complex sensorimotor processing and cognition compared with age-matched control subjects over 18 months, suggesting that chronic hyperglycemia may be detrimental to the developing brain.

Maintenance of near normoglycemia in young children with diabetes is limited by parental fears of the risks of hypoglycemia and impaired cognitive development. However, human and experimental animal data suggest that both hyper- and hypoglycemia, depending on age and severity, can lead to altered brain structure and

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*A complete list of the members of the DirecNet Study Group can be found in the APPENDIX.

neurocognitive function (1,2). Subjects with early-onset diabetes show altered brain morphology, suggesting that the young developing brain may be particularly vulnerable to neurodevelopmental insult associated with type 1 diabetes (T1D) (3,4). The hippocampus may be specially sensitive to damage from hypoglycemia (5,6), providing a potential mechanism for learning and memory dysfunction observed in some studies of children with diabetes (7–12). Ho et al. (13) reported their MRI findings in a group of 62 children (mean age 9.8 years) with early-onset diabetes (~3 years) showing a high prevalence of central nervous system structural abnormalities and mesial temporal sclerosis. This suggests that early-onset diabetes per se is associated with identifiable central nervous system abnormalities. In addition, greater hyperglycemia exposure correlated with reduction in white matter volume within the parietal cortex in children (14) and with smaller cortical cells with reduced myelin content in rodents (15). Cross-sectional and prospective cohort studies have examined cognitive functioning in youth with T1D versus control subjects, and similar to adults, youth with diabetes tend to show lower intelligence quotients (IQs) and deficits in executive functioning, particularly attention, episodic and spatial working memory, and processing speed, as compared with control subjects (1,3,4,8–11). However, longitudinal studies of cognition in youth with T1D have shown more mixed results (16,17).

We conducted a study in young children with (n = 144) and without (n = 72) T1D (ages 4 to <10 years) to prospectively examine the impact of dysglycemia on neuroanatomical growth and cognitive development. We used high-resolution structural MRI, a comprehensive battery of cognitive metrics, and serial glycemic measures from continuous glucose monitoring (CGM) (18–20). Baseline assessments showed that hyperglycemia was associated with lower scores for executive functions, intelligence, learning, and memory (19) as well as gray matter and white matter structural changes (18,20).

Although baseline cognitive differences were relatively subtle, neuroanatomical differences were striking. Using voxel-based morphometry, we observed significant differences in specific brain regions in children with diabetes versus control subjects (20)—areas involved in visual-spatial processing, executive functions, and working memory. Greater hyperglycemia was associated with smaller gray matter volume in medial-frontal and temporal-occipital regions and greater gray matter volume in lateral prefrontal regions (18,20). We also found significant differences in white matter microstructure, suggesting widespread aberrant fiber coherence in young children with diabetes; differences correlated with longer disease duration, increased glycated hemoglobin, and greater hyperglycemia (18). These cross-sectional observations raised important questions concerning longitudinal trajectories of neurodevelopment in childhood-onset diabetes (21).

Neuroimaging was repeated after 18 months in these same children with and without diabetes to investigate whether brain differences would lessen, persist, or worsen over time and to correlate these neuroanatomical changes with longer (18 months) exposure to hypo- and hyperglycemia and with targeted measures of neurocognitive function longitudinally.

RESEARCH DESIGN AND METHODS

Studies were conducted after institutional review board approval at all centers, and informed written consent was obtained.

Study Subjects

Children with T1D (n = 144) and healthy, nondiabetic control subjects (n = 72) between 4 and <10 years of age at study entry participated (18–20). Inclusion criteria included ≥34 weeks' gestation; diabetes onset after 6 months of age; birth weight ≥2,000 g; no genetic, neurologic, or psychiatric disorders or intellectual, language, or learning disability; no enrollment in special education programs; no visual or auditory deficits; and no MRI contraindications. Healthy control subjects had similar inclusion criteria except that their glycated hemoglobin was <6.0% and fasting blood glucose <110 mg/dL.

Study Procedures

At enrollment and 18 months, subjects underwent brain imaging and neurocognitive testing. Glycemic measures were assessed every 3 months in the diabetic group.

Glycemic Measures—T1D

Glycated hemoglobin was measured quarterly (DCA 2000), and severe hypoglycemia and ketoacidosis were recorded. CGM was performed to collect glycemic data for at least 72 h (24 overnight) every 3 months for 18 months using either the patient's clinically prescribed devices, an iPro2 (Medtronic MiniMed, Northridge, CA), or Dexcom SEVEN Plus (Dexcom, San Diego, CA).

Neurocognitive Testing

Trained examiners obtained cognitive data and IQs using age-appropriate scales. Parents also completed abbreviated intelligence testing (see Supplementary Data).

MRI

Subjects had unsedated brain MRIs using previously described desensitization protocols (22) in six Siemens 3T Tim Trio instruments with identical pulse sequences and 12-channel head coils for optimal reproducibility. Scanners were tested for geometric distortions and noise levels using human phantoms who visited each site three times in 18 months. Reproducibility across sites was excellent (coefficient of variation <0.4%) (see Supplementary Data). Blood glucose was between 70 and 300 mg/dL during both the imaging and cognitive assessments.

Statistical Methods

Glucose Data

All glycated hemoglobin levels since diagnosis, and quarterly for 18 months, were used to compute a lifelong cumulative

index of hyperglycemia exposure based on average amount >6% ($HbA_{1c}AUC_{6\%}$) using the trapezoidal rule. A normal $HbA_{1c}AUC_{6\%}$ is considered 0. The incremental lifetime score across time points was taken as the 18-month exposure for the analyses. In addition, HbA_{1c} was measured near the time of each scan, and we used the average ($avgHbA_{1c}$) of these values across longitudinal time points as a covariate of interest.

Glycemic variables from CGM data were computed from the average of all CGM wears (usually seven per participant) during the 18-month interval. These included the following: mean glucose (gluMean), area under the curve glucose >180 mg/dL, area under the curve glucose >250 mg/dL, area over the curve glucose <70 mg/dL, SD, and mean amplitude of glycemic excursions (MAGE) (23). Except for MAGE (which is based on individual excursions), each glycemic index was calculated giving equal weight to each of the 24 h of the day. Hypoglycemic area above the curve was defined as the mean value of max (0, 70 glucose). That is, glucose values outside the hypoglycemic range (>70 mg/dL) were counted as 0, and values within the hypoglycemic range were counted as to how far below 70 mg/dL they fell. Similarly, hyperglycemic area under the curve was defined as the mean value of max (0, 180 glucose).

Neurocognitive Scores

A *z* score was calculated for each measure pooling all participants (n = 216) at both baseline and 18 months (19,24) and measures averaged within each prespecified domain. Repeated-measures least squares regression models were used to account for correlations between siblings, adjusting for age, sex, parent IQ, and parent-reported child depression scores (25). Primary outcome domains included learning and memory, executive functions, processing speed, and IQ. Secondary outcomes included parent ratings of executive functioning, externalizing behavior, and internalizing mood symptoms. Spearman partial correlations were conducted between disease-specific variables and each cognitive domain, adjusting for age, sex, and parent intelligence.

MRI Analyses

Whole Brain and Regional. Regional differences in brain morphology between groups were analyzed based on general linear models using voxel-wise two-sample Student *t* tests, creating a whole-brain parametric map. Each scan was segmented into gray and white matter images to generate volumes, restricting analyses to voxels with mean tissue class probability >0.15. Statistical inference was evaluated using the voxel-based morphometry toolbox (http://dbm.neuro.uni-jena.de/vbm/) to threshold the voxel-level *t* statistics and a cluster extent of $P < 0.05$ accounting for nonstationary data smoothness (26). Statistical Parametric Mapping software (SPM8 [http://www.fil.ion.ucl.ac.uk/spm]) was used. Regional differences in gray and white matter images at baseline and 18 months were calculated as indices of brain growth, covarying for age, sex, gray or white matter volume, and time between studies (see Supplementary Data).

Whole-Brain Regression Analysis. Voxel-wise multiple linear regression was used to examine whole-brain correlations between imaging data and glycemic or cognitive variables after accounting for effects of total gray and white matter volumes, age, and sex (see Supplementary Data).

Multiple Comparisons. The Hochberg step-up approach (27) was used to investigate between-group differences for the four primary neurocognitive domains. Each individual brain MRI analysis reports the *P* value for family-wise error after using random field theory to correct for the multiple voxels and regions in the image while accounting for the nonstationary smoothness of the data (26) (see Supplementary Data).

RESULTS

Table 1 shows clinical characteristics of the study subjects. Groups were well matched for age, BMI, and socioeconomic status; 25% of the control subjects were siblings of children with diabetes. At 18 months, 144 children with diabetes completed cognitive testing and 143 MRI; 70 control subjects had MRI and 69 cognitive testing (Supplementary Fig. 1*A*).

Glycemic Data

Glycated hemoglobin levels at baseline and 18 months were similar in children with diabetes (Table 1). Median device use over 18 months was 943 h (interquartile range 789, 1,554). Cumulative median time spent in hyperglycemia (glucose >180 mg/dL) over 18 months was 50% and >250 mg/dL 25% (18,33) and MAGE 159 mg/dL. $HbA_{1c}AUC_{6\%}$, a cumulative index of hyperglycemia since diagnosis, was high both at baseline and 18 months.

Neurocognitive Data

Cross-sectional analysis at 18 months showed children with diabetes had worse scores than control subjects for internalizing behavior (P = 0.002); no other cognitive domains were significantly different between groups over time (Table 2). Within the diabetic group, CGM measures did not correlate with change in cognitive domains over 18 months (data not shown).

Total Brain Volume

There were no significant between-group differences in total brain volumes and no group-by-sex interaction at 18 months (Supplementary Table 2*A*). However, longitudinal brain growth over 18 months was greater in the control than diabetic group both for total gray ($P < 0.001$) and white matter volumes (P = 0.046) (Fig. 1*A*). Within the diabetic group, change in total gray matter volume was significantly negatively correlated with change in glycated hemoglobin across baseline and 18-month time points ($P < 0.002$).

Table 1—Clinical characteristics of study subjects

	T1D (*n* = 144)	Control (*n* = 72)
Female, *n* (%)	66 (46)	34 (47)
Race/ethnicity*	81% W, 7% H, 4% AA, 1% A, 6% O	86% W, 6% H, 6% AA, 0% A, 2% O
Parent with college degree, *n* (%)	123 (85)	67 (93)
Age at diabetes onset (years), mean ± SD	4.1 ± 1.9	NA
Sibling of T1D subject, *n* (%)	NA	18 (25)
DKA at diagnosis, *n* (%)†	46 (32)	NA
Baseline characteristics		
Age (years), mean ± SD	7.0 ± 1.7	6.9 ± 1.8
BMI percentile, median (25th, 75th percentile)	72 (58, 87)	61 (35, 82)
Diabetes duration (years), median (25th, 75th percentile)	2.5 (1.2, 4.4)	NA
DKA history, *n* (%)†	51 (36)	NA
Severe hypoglycemia history, *n* (%)‡	23 (16)	NA
Glycated hemoglobin, % (mmol/mol), mean ± SD	7.9 ± 0.9 (63 ± 10)	5.2 ± 0.2 (33 ± 2)
Averaged $HbA_{1c}AUC_{6\%}$, mean ± SD‖	2.2 ± 0.9	NA
18-month characteristics		
Age (years)	8.5 ± 1.7	8.5 ± 1.8
BMI percentile, median (25th, 75th percentile)	69 (52, 85)	66 (33, 81)
Interval DKA history, *n* (%)	4 (2.8)	NA
Interval severe hypoglycemia history, *n* (%)§	6 (4.2)	NA
Glycated hemoglobin, % (mmol/mol), mean ± SD	7.9 ± 0.9 (63 ± 10)	5.2 ± 0.3 (33 ± 3)
Average $HbA_{1c}AUC_{6\%}$, mean ± SD‖	2.0 ± 0.7	NA
Average CGM indices over 18 months, median (IQR)¶		
% Glucose within target (71–180 mg/dL)	45 (38, 52)	NA
% Glucose >180 mg/dL	50 (41, 57)	NA
% Glucose >250 mg/dL	25 (18, 33)	NA
% Glucose <70 mg/dL	4.6 (3.2, 7.1)	NA
Mean glucose (mg/dL)	191 (175, 209)	NA
Glucose SD (mg/dL)	82 (74, 90)	NA
MAGE (mg/dL)	159 (141, 171)	NA

*AA, African American; A, Asian; H, hispanic; IQR, interquartile range; O, other/more than one race; W, white. †Excluded two subjects with unknown DKA history at enrollment. ‡Includes 18 participants with one episode, 3 with two episodes, 1 with three episodes, and 1 with five episodes. §Includes 4 participants with one episode and 2 participants with two episodes. ‖Area under the curve for glycated hemoglobin >6% since diagnosis divided by diabetes duration. ¶Excluded nine subjects who used CGM less than five of seven visits.

Regional Gray and White Matter Changes

Children with T1D had less cortical gray matter growth than control subjects in widespread connected regions reported as a single cluster with >90,000 voxels, $P < 0.001$ (Fig. 1*B*). Within this cluster, the most significant localized differences were in the left precuneus extending to the left parietal and posterior-occipital regions ($P < 0.001$); the right temporal, frontal, and parietal lobes ($P < 0.001$); and the right medial-frontal cortex ($P = 0.008$) (see Supplementary Data). Similarly, there were widespread clusters (>12,000 voxels) in each hemisphere where white matter grew much slower ($P < 0.001$) in the diabetic group than control subjects, including bilateral anterior forceps, inferior-frontal fasciculus, superior-parietal lobule white matter, and splenium (Fig. 1*C*). The strongest localized effects were in the right anterior-frontal lobe near the corpus callosum ($P < 0.001$).

Structural-Glycemic Correlations

Using an index of cumulative hyperglycemia measured as lifelong $HbA_{1c}AUC_{6\%}$ and MAGE, a metric of glucose variability, we found negative correlations with gray and white matter growth in multiple brain areas (Table 3). Significant correlations with $HbA_{1c}AUC_{6\%}$ and gray matter growth included one cluster in the left parahippocampal, inferior-temporal, lingual, and fusiform gyri, and hippocampus ($P < 0.001$), whereas correlations with glucose variability included clusters in the left temporal and parietal lobes and bilateral dorsal cingulate gyri ($P < 0.001$). Similarly, white matter growth in children with diabetes negatively correlated with glucose variability, particularly in the occipital area and near the splenium ($P < 0.001$) (Fig. 2). MAGE and glucose SD analyses were covaried by mean glucose levels. There were no significant correlations with percent glucose in the hypoglycemic range.

Cognitive Correlations

There were no significant correlations of change in overall IQs with change in total gray or white matter volumes in either group.

There were 51 episodes of diabetic ketoacidosis (DKA), 46 at diagnosis of T1D, in 46 subjects. The median time

Table 2—Neurocognitive metrics (*z* scores)

	Baseline					18 month				
	Diabetes		Control		*P*	Diabetes		Control		*P*
	n	mean ± SD	*n*	mean ± SD	value*	*n*	mean ± SD	*n*	mean ± SD	value*
IQ†	144	−0.19 ± 1.01	70	+0.10 ± 0.88	0.03	137	+0.09 ± 0.96	67	+0.22 ± 0.99	0.46
Verbal IQ	144	−0.17 ± 1.06	70	+0.14 ± 0.94	0.01	137	+0.06 ± 0.92	67	+0.17 ± 0.94	0.66
Performance IQ	144	−0.13 ± 0.99	70	+0.13 ± 1.00	0.26	137	+0.05 ± 0.99	67	+0.18 ± 0.94	0.36
Executive functions†	135	−0.43 ± 0.91	70	−0.23 ± 0.96	0.03	138	+0.34 ± 0.89	68	+0.46 ± 0.93	0.55
Learning and memory†	142	−0.53 ± 0.78	71	−0.50 ± 0.80	0.61	139	+0.57 ± 0.82	67	+0.57 ± 0.94	0.80
Processing speed†	140	+0.10 ± 0.98	72	0.00 ± 0.83	0.22	139	0.00 ± 1.06	69	−0.16 ± 1.02	0.08
BRIEF‡	140	+0.13 ± 0.89	68	−0.06 ± 1.07	0.24	144	0.00 ± 0.98	70	−0.23 ± 1.02	0.22
Externalizing (behavior assessment by parent)‡	144	+0.06 ± 0.93	70	−0.04 ± 0.99	0.73	142	−0.03 ± 1.00	70	−0.07 ± 0.98	0.68
Internalizing (behavior assessment by parent)‡	144	+0.28 ± 0.87	69	−0.32 ± 1.04	<0.001	142	+0.07 ± 0.98	70	−0.38 ± 0.96	0.002

BRIEF, Behavior Rating Inventory of Executive Function by Parent. **P* value uncorrected for multiple comparisons. Obtained from repeated-measure least squares regression models, adjusted for siblings from same family, age, sex, and parent IQ. †Higher scores are better. ‡Higher scores are worse.

from the DKA event to cognitive testing was 3.1 years (range 0.1–7.6 years). After controlling for age of onset and sex, a history of DKA was not related to memory scores. There were only four DKA episodes in four patients between baseline and 18 months, not enough to permit meaningful analysis of any associations with cognitive or anatomical outcomes.

DISCUSSION

In this large cohort of young children (age 4 to <10 years) with and without T1D studied longitudinally, our studies demonstrate that early-onset diabetes significantly affects the development of total and regional gray and white matter volumes, with differences between groups enhanced over time. Remarkably, in the diabetic group, the slower growth was most strongly associated with hyperglycemia and glycemic variability, as measured by several metrics, including glycated hemoglobin, and extensive quarterly data from CGM. These studies provide strong evidence that the developing brain is a vulnerable target for diabetes complications.

Although both groups showed brain growth over 18 months, the growth rate was significantly less in children with diabetes than control subjects, with faster growth in younger children, as expected. Within the diabetic group, change in total gray matter was negatively correlated with increase in glycated hemoglobin across time points, implicating high glucose concentrations as a plausible mechanism for these observations. Our results are unique and represent the most comprehensive assessment to date

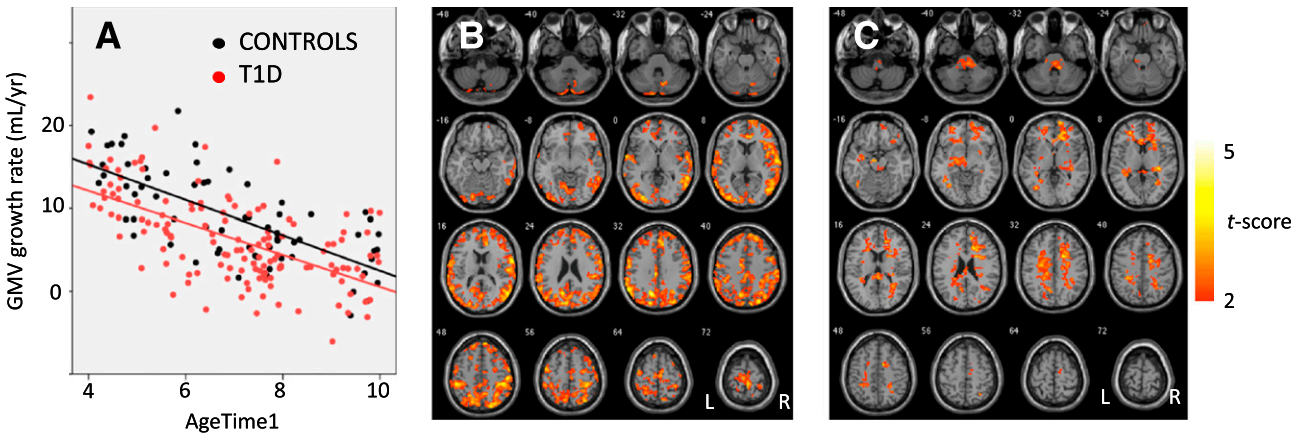

Figure 1—Longitudinal brain growth. Brain regions where the T1D group had significantly reduced growth compared with control subjects. *A*: Growth rate of total gray matter volume decreased with age ($P < 0.001$) and was significantly smaller for the diabetic group (red dots) vs. control subjects (black dots). $P < 0.001$. *B*: Regions of gray matter with significantly less growth for T1D than control subjects ($P < 0.001$). *C*: Regions of white matter with significantly less growth for T1D than control subjects ($P < 0.001$). *B* and *C* are subtraction images, so the more orange in a region, the less growth in the T1D group compared with the control group. L, left; R, right; yr, years.

Table 3—Anatomic-glycemic correlations at 18 months

Model	Covariates	Regions contained in cluster	Peak voxel (x,y,z)	Peak *t* score	Volume (voxels)	*P* value (correlation)*
Between groups difference in growth						
Control > diabetes	GMV	Most cortical gray matter, with peaks near left precuneus, left parietal and temporal lobes, right temporal, frontal, and parietal lobes	−12, −66, 32	5.52	97,608	<0.001
Control > diabetes	WMV	Right hemisphere white matter, including superior parietal, splenium, superior frontal, anterior forceps	20, 7, 30	4.39	22,683	<0.001
Control > diabetes	WMV	Left hemisphere white matter, including superior parietal, splenium, superior frontal, anterior forceps	0, −33, 21	3.57	12,388	<0.001
Correlation of glycemic exposure with brain growth for T1D						
$HbA_{1c}AUC_{6\%}$ (−)	GMV, avgHbA_{1c}	Left fusiform, parahippocampal, lingual, inferior temporal gyri, left hippocampus, left cerebellum	−33,−47,−4	3.67	8,476	<0.001 (−0.39)
MAGE (−)	GMV, gluMean	Bilateral dorsal cingulate and medial parietal lobe	12, −6, 47	4.46	14,555	<0.001 (−0.26)
MAGE (−)	GMV, gluMean	Left primary motor and temporal lobe	−45, −23, 5	4.23	8,631	0.001 (−0.30)
MAGE (−)	WMV, gluMean	Left postcentral and angular gyrus regions, splenium	−39, −41, 5	4.48	11,612	<0.001 (−0.27)
MAGE (−)	WMV, gluMean	Right postcentral and angular gyrus regions, splenium	36, −42, −4	4.29	17,025	<0.001 (−0.29)
SD (−)	GMV, gluMean	Bilateral dorsal cingulate and medial parietal lobe	0, −15, 54	4.08	15,781	<0.001 (−0.26)
SD (−)	GMV, gluMean	Left temporal lobe and primary motor	−60,−45,−6	3.73	7,294	0.003 (−0.24)
SD (−)	WMV, gluMean	Bilateral postcentral and angular gyri regions, splenium	−40, −32, 2	4.24	24,798	<0.001 (−0.29)
avgHbA_{1c} (−)	GMV	Left cerebellum, fusiform, parahippocampal gyrus	−20, −51, −24	3.57	3,770	0.08 (−0.31)
gluMean (−)	GMV	Left cerebellum, fusiform, parahippocampal gyrus	−20, −51, −24	3.43	3,420	0.12 (−0.34)
AOC70 (−)	GMV	Bilateral dorsal cingulate and medial parietal lobe, right cuneus	−3, 3, 50	3.53	3,680	0.10 (−0.30)

All analyses also include age, sex, and time span as covariates. gluMean is the mean glucose level over the 18-month interval. No correction was made for multiple diabetes-specific variables analyzed. AOC70, area over the curve glucose <70 mg/dL; GMV, gray matter volume; WMV, white matter volume. *All *P* values are cluster extent corrected for family-wise error and nonstationary smoothness.

of brain growth in the youngest cohort of children with diabetes in whom extensive quarterly glucose data from CGM for 18 months are also available.

Although previous studies in older children and adults with T1D suggest that effects of hyper- and hypoglycemic exposure on brain structure are widely distributed, frontal and parietal-occipital cortical regions appear most vulnerable, particularly in individuals with early age of onset (2,14,28). We previously observed significant cross-sectional differences at baseline in gray and white matter volumes (20) and white matter microstructure (18) in this same cohort of children. However, regional assessment of gray and white matter structures using voxel-based morphometry 18 months later now exposes slower growth of specific brain areas in the diabetic group compared with control subjects. In gray matter, the left precuneus extending to parietal and posterior regions; right temporal, frontal, and parietal lobes; and the right medial-frontal cortex showed lesser growth in diabetes. These are brain areas critical in visual-spatial processing (occipital and precuneus), spatial and working memory (parietal), auditory and object processing, and integration of information from multiple sensory systems (temporal/parietal). There were also widespread differences in white matter with slower growth in T1D versus control subjects, including the splenium of the corpus callosum, bilateral superior-parietal lobe, bilateral anterior forceps, and inferior-frontal fasciculus, with the strongest effects in the right anterior-frontal lobe near the corpus callosum, tracts pivotal for visual-spatial processing and communication between hemispheres. In aggregate, the affected areas are associated with executive functions, interoceptive awareness, and auditory and language processing as reported by others (29–31). These between-group differences were observed in brain regions previously linked to hypo- and hyperglycemia in older children and in adult diabetic populations (2,14,28,32).

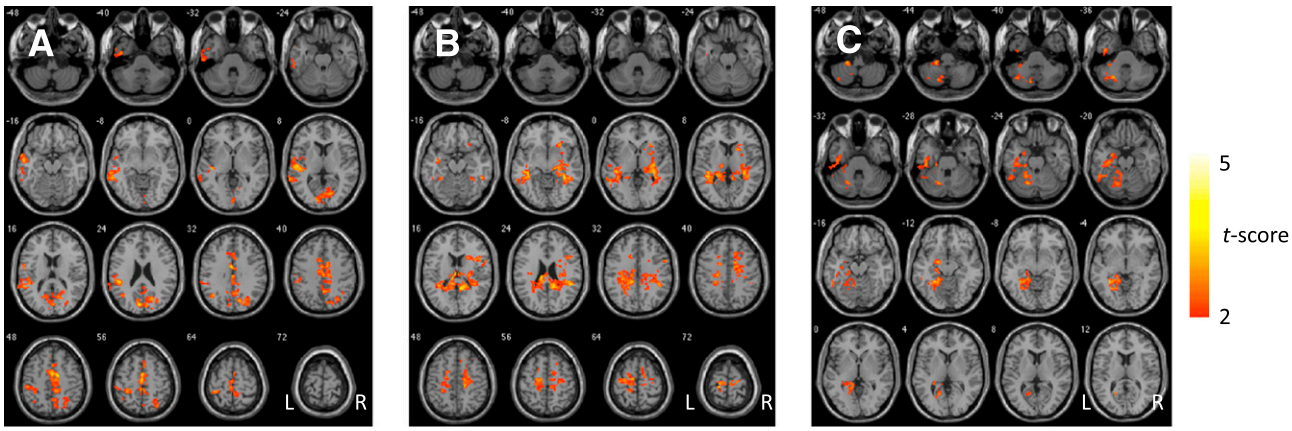

Figure 2—Effects of dysglycemia with different brain regions. Brain regions where reduced growth in the T1D group was correlated with glycemic exposure over the 18 months of study. *A*: Gray matter regions where growth was negatively correlated with average MAGE ($P < 0.001$). *B*: White matter regions where growth was negatively correlated with average MAGE ($P < 0.001$). *C*: Gray matter regions where growth was negatively correlated with high glycemic exposure (difference in $HbA_{1c}AUC_{6\%}$; $P < 0.001$). L, left; R, right.

We had postulated that brain changes would be associated with hypoglycemia, as acute hypoglycemia has deleterious effects on multiple aspects of cognition (11,33), and effects in the hippocampus and frontal cortex in both animal models and humans have been reported (5,6,34). The well-established negative impact of hypoglycemia on structural and cognitive function in diabetes is directly related to severity of hypoglycemia. However, many of the brain differences observed here were not significantly related to metrics of hypoglycemia. This may be due, in large part, to limited hypoglycemia exposure for most of the cohort, with median glucose concentrations in hypoglycemic range of only 4.6%, as reported previously (35), and severe hypoglycemia was too infrequent to perform statistical analysis, with only eight events occurring in six patients during the entire 18 months of follow-up.

However, using an index of cumulative glycemic exposure since diagnosis, $HbA_{1c}AUC_{6\%}$, we found that higher, disease-long level of hyperglycemia was associated with lower gray matter growth in multiple areas, including the left parahippocampal, inferior-temporal, lingual, fusiform gyri, and hippocampus. When correlating glucose variability, assessed by MAGE and glucose SD, gray matter growth was slower in the left temporal and frontal lobes and bilateral dorsal cingulate gyri in these young children compared with healthy, age-matched control subjects, many of them siblings. Similarly, white matter growth was negatively correlated with glucose variability particularly near the splenium. These results are remarkable given the young age of this large cohort. Increased rates of cerebral atrophy in adults with early-onset diabetes have been observed (4), but differences in total gray and white matter volume relative to control subjects (36) or regional differences (28) have only been observed in middle-aged or older adults and linked to lifetime glycated hemoglobin, disease duration, and severity of microangiopathy (28,37).

Several mechanisms for the observed slower total and regional brain growth in children with diabetes may be operative. In early childhood, increased vulnerability to brain insults is due in part to dynamic brain development, including maturation and pruning of synapses and increased myelination of white matter fiber tracts (38). Chronic hyperglycemia can lead to formation of advanced glycation end products and its receptors, nuclear factor-κB, greater increased oxidative stress, and even neurodegradation (39–42). These glycemic correlations support the notion that increased glucose variability may damage developing neurons and myelin in children with diabetes and are congruent with observations from streptozotocin-induced diabetes animal models that show in vivo degenerative changes of neurons and glia, disarrangement of myelin sheaths, and reduced myelin content with hyperglycemia (15,42,43). Changes in brain sphingolipid composition (ceramides and sphingomyelin) induced by hyperglycemia may also provoke membrane rearrangements in some cell populations, which can disturb cellular signaling and cause brain tissue damage (44). The ultimate mechanism in the observed changes is likely multifactorial.

Cognitive testing performed at baseline showed a trend toward lower scores relative to control subjects in areas of intellectual ability and executive functions after accounting for parental intelligence and parent-reported depression scores (19). However, these findings were more subtle and did not meet our stringent threshold for statistical significance after correcting for multiple comparisons. There were no differences in cognitive and executive function scores when children were reassessed 18 months later. However, others have reported that diabetes in young children can alter cognitive function (45), and compared with control subjects, those with diabetes on average have worse performance on several cognitive domains (46), especially in association with early disease

onset (46). Further, these cognitive differences can increase over time throughout puberty (47).

The current lack of measurable cognitive impairment in the diabetic group evaluated in our study could be attributable to several factors, perhaps most importantly, the fact that our participant cohort is still very young. Thus, progressive insult to the developing brain from dysglycemia may not have had sufficient time to overcome intrinsic compensatory mechanisms and neural plasticity. This premise is related to the well-described concept of neural (or cognitive) reserve, whereby plasticity and compensatory mechanisms can maintain cognitive performance in the face of insult to the developing (or mature) brain (48). Thus, identifiable neuroimaging abnormalities in young children with T1D might precede cognitive-behavioral deficits detectable at a later age, such as seen in neurologic disease and even in dyslexia (49). A meta-analysis of 1,393 children with T1D and 751 control subjects suggests that pediatric diabetes is associated with lower scores across most cognitive domains (46). One recurrent finding across these studies is that patients diagnosed during the preschool years may be at the greatest risk of neuropsychological deficits (47,50–54). Early age at diagnosis (<4 years) has been associated with significantly reduced attention, processing speed, and executive function (55) and an increased risk for learning disabilities that is related to episodes of severe hypoglycemia (56,57). Putative mechanisms for these adverse outcomes tend to be strongly intercorrelated (frequency of severe hypoglycemia, cumulative hypoglycemic or hyperglycemic exposure, mean daily blood glucose excursion, frequency of diabetic ketoacidosis, mild cerebral edema, and duration of diabetes); hence, specification of the specific mechanism(s) of greatest effect is difficult. Consequently, the extent of the structural changes observed in our study participants and their increasing discrepancy from control subjects over time clearly merit additional study, particularly with resting-state and task-related functional imaging and long-term assessment of cognition, adaptive behavior, and school performance (58).

Despite advances in technology, children with diabetes are still exposed to significant hyperglycemia. Data in 8–17 year olds (59) indicated children spent >40% of the time with glucose values >180 mg/dL, and in our study of 4–9 year olds (same age as this cohort) ~50% of the time (35). Yet fear of hypoglycemia is a perennial concern to parents and providers of very young children with diabetes (60), often preventing proper insulinization. The inability to avoid hypoglycemia in this young age-group led the American Diabetes Association to recommend still higher glucose therapeutic targets in very young children than in school-aged children and adolescents (61), reasoning that hyperglycemia-related damage takes many years to evolve. These recommendations should be further reexamined. Although neurocognitive testing remained comparable to control subjects over 18 months, lower total and regional brain volumes in critical areas related to learning, visual-spatial processing, and other key aspects of brain function and widening of these differences over time suggest that in the youngest patients with diabetes, glucose-related neuronal damage starts early. Whether these brain changes could improve over time with near normalization of blood sugars with closed-loop systems, for example, requires study.

In conclusion, very young children with T1D have significant differences in total brain and regional gray and white matter growth in widespread brain regions compared with healthy age-matched control subjects over 18 months. These differences are related to disease-long measures of dysglycemia and glucose variability. Although not accompanied by large cognitive differences, these data suggest that continued exposure to chronic hyperglycemia may be detrimental to the developing brain. Longitudinal follow-up of this cohort will better elucidate the neurocognitive trajectories of young children with T1D. Better glycemic control in this vulnerable age-group is warranted.

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Author Contributions. N.M. researched and analyzed data and wrote the manuscript. P.M. and A.L.R. researched and analyzed data, contributed to the discussion, and reviewed and edited the manuscript. B.B., S.W., N.H.W., E.T., T.H., A.C., T.A., L.F., A.M.A., D.W., M.T., and W.T. researched data, contributed to the discussion, and reviewed and edited the manuscript. P.C., C.K., R.W.B., K.R., D.P., and M.M. analyzed data, contributed to the discussion, and reviewed and edited the manuscript. K.K.W. provided scientific advice, contributed to the discussion, and reviewed and edited the manuscript. N.M. 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.

Appendix

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