

# Alterations in White Matter Structure in Young Children With Type 1 Diabetes

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## OBJECTIVE

To investigate whether type 1 diabetes affects white matter (WM) structure in a large sample of young children.

## RESEARCH DESIGN AND METHODS

Children (ages 4 to <10 years) with type 1 diabetes ( $n = 127$ ) and age-matched nondiabetic control subjects ( $n = 67$ ) had diffusion weighted magnetic resonance imaging scans in this multisite neuroimaging study. Participants with type 1 diabetes were assessed for HbA<sub>1c</sub> history and lifetime adverse events, and glucose levels were monitored using a continuous glucose monitor (CGM) device and standardized measures of cognition.

## RESULTS

Between-group analysis showed that children with type 1 diabetes had significantly reduced axial diffusivity (AD) in widespread brain regions compared with control subjects. Within the type 1 diabetes group, earlier onset of diabetes was associated with increased radial diffusivity (RD) and longer duration was associated with reduced AD, reduced RD, and increased fractional anisotropy (FA). In addition, HbA<sub>1c</sub> values were significantly negatively associated with FA values and were positively associated with RD values in widespread brain regions. Significant associations of AD, RD, and FA were found for CGM measures of hyperglycemia and glucose variability but not for hypoglycemia. Finally, we observed a significant association between WM structure and cognitive ability in children with type 1 diabetes but not in control subjects.

## CONCLUSIONS

These results suggest vulnerability of the developing brain in young children to effects of type 1 diabetes associated with chronic hyperglycemia and glucose variability.

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Careful regulation of insulin dosing, dietary intake, and activity levels are essential for optimal glycemic control in individuals with type 1 diabetes. However, even with optimal treatment many children with type 1 diabetes have blood glucose levels in the hyperglycemic range for more than half the day and in the hypoglycemic range for an hour or more each day (1). Brain cells may be especially sensitive to aberrant blood glucose levels, as glucose is the brain's principal substrate for its energy needs.

Research in animal models has shown that white matter (WM) may be especially sensitive to dysglycemia-associated insult in diabetes (2–4). Specifically, animal studies have shown that hyperglycemia but not hypoglycemia affects brain structure and cognition (5). Early childhood is a period of rapid myelination and brain development (6) and of increased sensitivity to insults affecting the brain (6,7). Hence, study of the developing brain is particularly important in type 1 diabetes.

WM structure can be measured with diffusion tensor imaging (DTI), a method based on magnetic resonance imaging (MRI) that uses the movement of water molecules to characterize WM brain structure (8,9). Results are commonly reported in terms of mathematical scalars (representing vectors in vector space) such as fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD). FA reflects the degree of diffusion anisotropy of water (how diffusion varies along the three axes) within a voxel (three-dimensional pixel) and is determined by fiber diameter and density, myelination, and intravoxel fiber-tract coherence (increases in which would increase FA), as well as extracellular diffusion and interaxonal spacing (increases in which would decrease FA) (10). AD, a measure of water diffusivity along the main axis of diffusion within a voxel, is thought to reflect fiber coherence and structure of axonal membranes (increases in which would increase AD), as well as microtubules, neurofilaments, and axonal branching (increases in which would decrease AD) (11,12). RD, the mean of the diffusivities perpendicular

to the vector with the largest eigenvalue, is thought to represent degree of myelination (13,14) (more myelin would decrease RD values) and axonal "leakiness" (which would increase RD). Often, however, a combination of these WM characteristics results in opposing contributions to the final observed FA/AD/RD value, and thus DTI scalars should not be interpreted globally as "good" or "bad" (15). Rather, these scalars can show between-group differences and relationships between WM structure and clinical variables and are suggestive of underlying histology. Definitive conclusions about histology of WM can only be derived from direct microscopic examination of biological tissue.

To date, four studies have used DTI to investigate WM structure in type 1 diabetes (16–19). However, only one study (18) used DTI to investigate WM structure in an exclusively pediatric type 1 diabetes cohort. In that small study, significant differences in AD values were observed in children with type 1 diabetes compared with control subjects, and higher lifetime HbA<sub>1c</sub> values were positively associated with RD values, suggesting possible brain insult to myelin induced by hyperglycemia in children with type 1 diabetes.

In the current study, we investigated WM structure in a much larger sample of young children with type 1 diabetes using DTI compared with sex- and age-matched control subjects. We also sought to identify potential links between WM microstructure, blood glycemic indices, and standardized measures of cognition. We used continuous glucose monitors (CGMs) to obtain a more detailed measure of short-term glucose levels and variability than is provided by HbA<sub>1c</sub> data (20). Based on past findings in humans (18) and animals (2), we hypothesized that there would be significant reduction of AD in children with type 1 diabetes compared with nondiabetic control subjects and that hyperglycemia severity, early age of diabetes onset, and longer duration of type 1 diabetes would be associated with increased RD.

## RESEARCH DESIGN AND METHODS

### Recruitment and Exclusion Criteria

Study participants between 4.0 and <10.0 years of age were recruited at five clinical centers in the DirecNet consortium (Nemours Children's Clinic, Jacksonville; Stanford University; University of Iowa; Washington University in St. Louis; and Yale University). The studies were reviewed and approved by the five centers' institutional review boards and a National Institutes of Health–designated Data Safety Monitoring Board. Written informed consent was obtained from the parent or legal guardian of all participants, and assent was obtained from the participant as per local guidelines. All participants were screened for past medical history to exclude for disorders that could impair neurologic development, history of intellectual disability or significant learning disabilities, history of psychiatric treatment, premature birth (<34 weeks' gestation), low birth weight (<2,000 g), and MRI contraindications. Participants with type 1 diabetes were at least 6 months of age at diagnosis and were receiving insulin therapy for at least 1 month. For those diagnosed before age 1 year, positive antibody testing (GAD65, islet cell antigen-512, and micro-insulin autoantibody) was required. Requirements for nondiabetic control subjects included HbA<sub>1c</sub> <6.0% (42 mmol/mol), fasting glucose <110 mg/dL, and no history of abnormal glycemic control. Sibling control subjects of participants with type 1 diabetes had negative antibody testing within the prior year.

### Participants

The study recruited 144 children with type 1 diabetes and 72 control subjects. T1-weighted (structural) scans and diffusion-weighted scans were acquired. Results from the T1-weighted analyses are described elsewhere (21). Diffusion-weighted imaging data from 127 children with type 1 diabetes (mean age 7.1 ± 1.6 years [range 4.0–9.9]; 48% females) and 67 control subjects (mean age 7.0 ± 1.7 years [4.0–9.9]; 49% females) were of sufficient quality to be included in the analyses. There were no significant differences in age ( $P = 0.97$ )

or sex ( $P = 0.87$ ) between the two groups. All participants underwent a physical and neurological exam and had no significant clinical findings.

### Diabetes Exposure and CGM

All available history of severe hypoglycemia and hyperglycemia events prior to enrollment in the study was recorded. Most participants also had HbA<sub>1c</sub> measurements since diagnosis, which were used to determine a lifetime hyperglycemic index by computing the area under the curve  $>6.0\%$  (HbA<sub>1c</sub>AUC<sub>6%</sub>). An HbA<sub>1c</sub> baseline value was recorded at study enrollment. A CGM was used to obtain glycemic data (minimum requirement of inclusion was 72 h of data of which at least 24 h of data were acquired at night). Primary measures used in regression analyses in our study included (1) glucose AUC  $>180$  mg/dL (AUC<sub>180</sub>), a measure of hyperglycemia calculated from CGM data that reflects both percentage and severity of glucose values in the hyperglycemic range (2); SD of glucose values (gluSD), a measure of glucose variability; and (3) AUC  $<70$  mg/dL (AUC<sub><70</sub>), a measure of hypoglycemia that reflects both percentage and severity of glucose values in the hypoglycemic range. Secondary measures included HbA<sub>1c</sub> at baseline for the study, lifetime HbA<sub>1c</sub>, percentage of glucose measures  $>250$  mg/dL (glu<sub>250</sub>) and 300 mg/dL (glu<sub>300</sub>), mean amplitude of glucose excursions (MAGEs) (22), the coefficient of variation (CV) (gluSD divided by the mean), and percentage of glucose values  $<70$  mg/dL and  $<60$  mg/dL.

### MRI Scan Acquisition

Scanning sites at the five participating clinical centers used a Siemens 3T Tim Trio MRI scanner with a 12-channel head coil along with standard Siemens product sequences. A single scanning protocol was prepared and distributed to all sites. Diffusion-weighted echo planar scans were performed using the following parameters: axial planes, 30 directions; TR = 8,800 ms, TE = 99 ms, with  $b$  value 1,000 s/mm<sup>2</sup>; 64 slices, slice thickness = 2 mm (isotropic); and voxel size 2 × 2 × 2 mm. Duration of the scan was 4:59 min, repeated twice, with more scans added to obtain good image quality if necessary.

All participants underwent a sedation-free brain MRI after subject training. For children with type 1 diabetes, a finger-stick blood glucose was required to be between 70 and 300 mg/dL within 60 min prior to the scan and rechecked after the scan ended.

For multisite quality assurance, two human phantoms traveled each year to every site to be scanned in order to assess the calibration and replicability of measurements across sites. Replicability was compared using the average FA over the anterior forceps of the corpus callosum (23). For this study, the CV for FA, where CV = root mean<sup>2</sup>/mean of the measurements, was  $< 2.9\%$  across sites, which is comparable with other acceptable intersite values of DTI replicability. The CV was stable across 3 years of testing, suggesting minimal within-site temporal or multisite variability in the current study.

### Cognitive and Behavioral Assessment

A complete assessment of cognitive function in the current sample of children with type 1 diabetes has previously been described (24). All subjects were assessed using either the Wechsler Preschool and Primary Scale of Intelligence for children  $<6$  years of age or the Wechsler Intelligence Scale for Children, fourth edition for children  $>6$  years of age. For subjects with type 1 diabetes, a blood glucose level was required to be between 70 and 300 mg/dL within 60 min prior to the testing session. The Wechsler Abbreviated Scale of Intelligence was used to assess parental full-scale intelligence quotient (FSIQ).

### Data Analysis

Diffusion-weighted images were inspected for artifacts using DTIstudio ([www.mristudio.org](http://www.mristudio.org)). Diffusion-weighted scans were rated as usable if a visual review revealed no artifacts or if artifacts were small and did not affect surrounding images (in which case the images with artifacts were removed from further calculations).

Usable DTI scans were processed using DTI studio ([www.mristudio.org](http://www.mristudio.org)) and the FMRIB Software Library (FSL) ([www.fmrib.ox.ac.uk/fsl/](http://www.fmrib.ox.ac.uk/fsl/)) to generate whole-brain maps of FA, AD, and RD. Whole-brain voxel-wise analyses were then performed in FSL using Tract-Based

Spatial Statistics (25). General linear models were created to investigate differences between the type 1 diabetes group and control subjects, within-group associations (regression analyses) with clinical and cognitive measures, and between-group differences in WM structure/cognitive correlations. All within-group regression analyses were covaried for age unless noted otherwise. Statistical analyses of the data were performed using Threshold-Free Cluster Enhancement and permutation analyses implemented in FSL ("randomise") (26). All significant statistical analyses are reported for  $P < 0.05$  and were individually corrected using family-wise error.

Regression analyses focused on associations between DTI metrics and glycemic indices or cognitive function were based on preexisting hypotheses regarding the putative effects of type 1 diabetes on WM. It was anticipated that associations of DTI with similar clinical measures (e.g., AUC<sub>180</sub>, glu<sub>250</sub>, and glu<sub>300</sub>) would give similar results, and thus statistical significance was not corrected for the number of regression analyses.

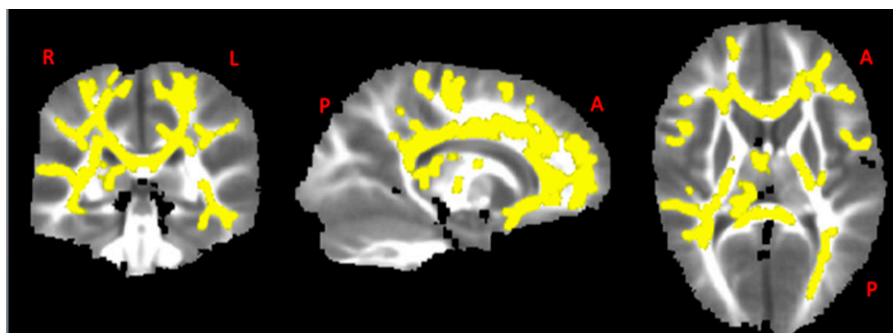
## RESULTS

### Between-Group Differences in WM Structure

Children with type 1 diabetes had significantly reduced AD values compared with the control group ( $P < 0.05$ , Cohen  $d = 1.28$ ) (Fig. 1). These reductions were widespread and were observed throughout the brain in the frontal, temporal, parietal, and occipital lobes and involved multiple WM pathways. There were no significant between-group differences for FA and RD values.

### Associations Within Type 1 Diabetes of WM Structure with Age, Age of Onset, and Duration of Diabetes Age

Consistent with previous studies in healthy control subjects (27–29), both the type 1 diabetes group and the control group had significant negative associations between age and AD [type 1 diabetes:  $t(127) = 1.72$ ,  $P < 0.05$ ; controls:  $t(67) = 1.76$ ,  $P < 0.05$ ] or RD [type 1 diabetes:  $t(127) = 2.23$ ,  $P < 0.05$ ; controls:  $t(67) = 1.73$ ,  $P < 0.05$ ] values



**Figure 1**—WM regions of significantly reduced AD values in the type 1 diabetes group compared with the control group (shown in yellow). Results are overlaid on an average FA image generated from the full sample.

and significant positive associations between FA values and age [type 1 diabetes:  $t(127) = 1.9$ ,  $P < 0.05$ ; controls:  $t(67) = 1.6$ ,  $P < 0.05$ ]. There was no significant age-by-group interaction for WM changes with age.

#### Age of Diabetes Onset

Age of diabetes onset was significantly positively correlated with age of subjects ( $R = 0.39$ ,  $P < 0.001$ ), and negatively correlated with duration of illness ( $R = -0.65$ ,  $P < 0.001$ ). Covaried for age, the mean age of onset of type 1 diabetes ( $4.1 \pm 1.9$  years) was negatively associated with RD in widespread regions of the brain [ $t(127) = 1.67$ ,  $P < 0.05$ ].

#### Duration of Type 1 Diabetes

Mean duration of type 1 diabetes was  $3 \pm 1.9$  years. As expected, there was a significant positive association between duration of type 1 diabetes and age ( $R = 0.42$ ,  $P < 0.001$ ). As stated above, duration of illness also was associated with age of onset. Covaried for age, there was a significant negative association between duration of type 1 diabetes and WM structure such that

longer duration of type 1 diabetes was associated with lower AD and lower RD values [AD:  $t(127) = 1.48$ ,  $P < 0.05$ ; RD:  $t(127) = 1.75$ ,  $P < 0.05$ ] in widespread brain regions. Duration of type 1 diabetes was positively associated with FA values in widespread brain regions [ $t(127) = 1.63$ ,  $P < 0.05$ ].

#### Glycemic Measures

Glycemic measures in our sample are described in Table 1. CGM data were collected for a median of 5.8 days (interquartile range 5.25–7.00 days). Multiple glycemic measures including HbA<sub>1c</sub> at baseline, AUC<sub>180</sub>, glu<sub>250</sub>, and glu<sub>300</sub>, and MAGE were significantly correlated with age (Table 1). To remove these age effects as well as the contributions of age to WM structure, we included age as a covariate in the initial regression analyses between WM structure and glycemic measures.

#### Associations Between WM Structure and Measures of Hyperglycemia

Measures of hyperglycemia were not significantly associated with WM structure when covaried for age. However, to investigate whether the

introduction of age as a covariate was restricting our ability to elucidate actual WM structure-hyperglycemia associations, we repeated the analyses leaving age out of the model. With this approach, the CGM measure of hyperglycemia, AUC<sub>180</sub>, was significantly negatively associated with FA values in widespread regions of the brain [ $t(127) = 1.62$ ,  $P < 0.05$ ]. Post hoc multiple regression analyses investigating individual contributions of age and AUC<sub>180</sub> on WM structure within regions derived from the whole brain analysis showed that both were significantly and independently associated with FA values (both at  $P < 0.001$ ).

With use of this same approach, glu<sub>250</sub> and glu<sub>300</sub> were also observed to be significantly negatively associated with FA values [ $t(127) = 1.99$  and  $t(127) = 1.92$ , respectively; both at  $P < 0.05$ ] in the superior longitudinal fasciculus bilaterally, corona radiata, right external capsule, inferior longitudinal fasciculus, and inferior fronto-occipital fasciculus and in cortico-spinal tracts. The most

**Table 1**—Measures of hyperglycemia and glucose variability in our sample

	Minimum	Maximum	Mean	SD	Spearman correlation with age
AUC <sub>180</sub>	3	127	42	23	$R = -0.21$ , $P = 0.02$
HbA <sub>1c</sub> at study baseline (%; mmol/mol)	6.3; 45	10.2; 88	7.8; 62	0.9; 10	$R = -0.23$ , $P = 0.01$
glu <sub>300</sub> (%)	0	53	13	10	$R = -0.21$ , $P = 0.02$
glu <sub>250</sub> (%)	1	69	25	14	$R = -0.20$ , $P = 0.02$
glu <sub>180</sub> (%)	9	94	49	17	$R = -0.16$ , $P = 0.08$
gluSD (mg/dL)	39	120	81	16	$R = -0.20$ , $P = 0.03$
gluCV (%)	26	77	42	7	$R = -0.01$ , $P = 0.87$
MAGE (mg/dL)	83	241	158	36	$R = -0.25$ , $P = 0.005$

gluCV, glucose CV.

extreme measure of hyperglycemia,  $glu_{300}$ , was also significantly positively associated with RD values [ $t(217) = 2.06$ ,  $P < 0.05$ ] in right frontal and parietal pathways including the superior longitudinal fasciculus and in the corona radiata. Post hoc analyses showed that like  $AUC_{180}$ ,  $glu_{250}$ ,  $glu_{300}$ , and age had significant independent associations with corresponding FA values (all correlations at  $P < 0.001$ ). Further, the results of post hoc analyses for the association of  $glu_{300}$  and age with RD values also were both significant (both at  $P < 0.001$ ).  $HbA_{1c}$  values at baseline were significantly negatively associated with FA values [ $t(127) = 1.55$ ,  $P < 0.05$ ], and positively associated with RD values [ $t(127) = 1.8$ ,  $P < 0.05$ ]. Post hoc multiple regression analyses showed that both baseline  $HbA_{1c}$  values and age had significant, separate contributions to FA (both at  $P < 0.001$ ) and to RD values (both at  $P < 0.001$ ). Conversely, lifetime  $HbA_{1c}$  values were not significantly associated with WM structure.

#### **Associations With CGM Measures of Hypoglycemia**

The exposures to hypoglycemia were brief relative to the exposures to hyperglycemia. For example, the mean  $\pm$  SD percent of time our sample experienced glucose levels  $<70$  mg/dL was  $5 \pm 6\%$  compared with  $49 \pm 17\%$  time with glucose values  $>180$  mg/dL. Measures of hypoglycemia were not significantly associated with age or with any measure of WM structure (with and without covarying for age).

#### **Associations With CGM Measures of Glucose Variability**

Covaried for age,  $gluSD$  values showed significant associations with WM structure. Specifically, AD and RD values were positively associated with  $gluSD$  [AD:  $t(127) = 1.93$ ,  $P < 0.05$ ; RD:  $t(127) = 1.83$ ,  $P < 0.05$ ], whereas FA was negatively associated with  $gluSD$  [ $t(127) = 1.89$ ,  $P < 0.05$ ]. These associations were widespread involving frontal, temporal, and parietal lobes. Covaried for age, significant positive associations of MAGE with AD [ $t(127) = 1.65$ ,  $P < 0.05$ ] and RD [ $t(127) = 1.85$ ,  $P < 0.05$ ] values occurred in frontal, temporal, and parietal lobes but not occipital lobes (Fig. 2).

#### **Severe Hypoglycemia and Seizures**

Within the type 1 diabetes group, 11 (9%) experienced seizures and 23 (18%) experienced an episode of severe hypoglycemia (defined as hypoglycemia with seizures or loss of consciousness). There were no significant differences in WM structure between children with type 1 diabetes who experienced episodes of severe hypoglycemia or seizures and those who did not.

#### **Between-Group Cognitive Differences**

As reported by Hershey et al. (24), compared with control subjects, participants with type 1 diabetes had lower FSIQ scores ( $P = 0.02$ ).

#### **Within the Type 1 Diabetes and Control Groups: Associations of WM Structure and Cognitive Abilities**

There were no significant associations between WM structure and FSIQ in the control group. However, there were widespread significant positive associations between FA values and FSIQ in the type 1 diabetes group [ $t(127) = 3.22$ ,  $P < 0.05$ ]. A between-group whole-brain voxel-wise comparison of correlations between FA and FSIQ approached significance ( $P = 0.06$ ) in the right superior temporal gyrus and bilateral parietal regions (children with type 1 diabetes had a higher correlation between FA and FSIQ than control subjects) (Fig. 3).

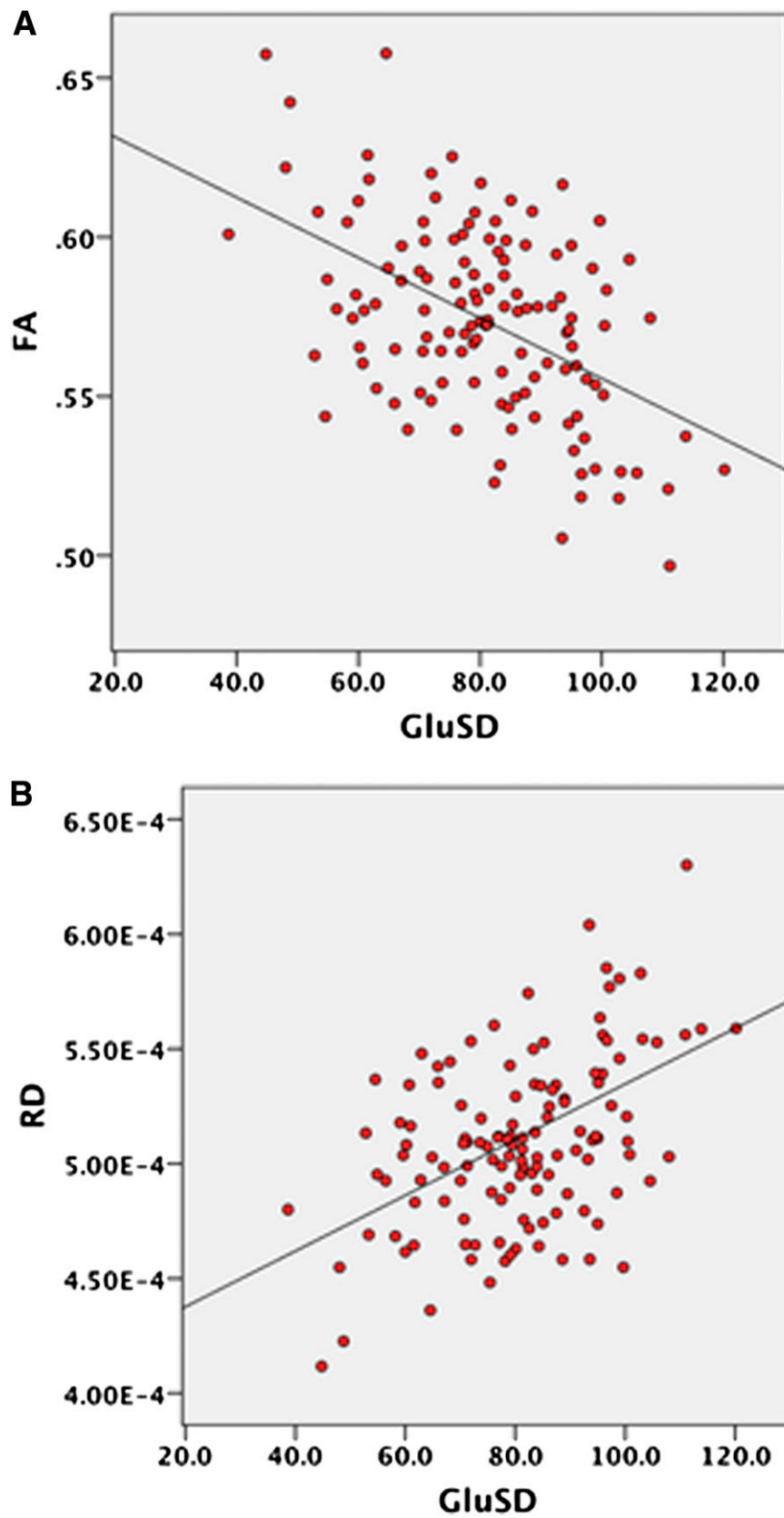
#### **CONCLUSIONS**

We investigated WM structure in a large sample of young children with type 1 diabetes compared with nondiabetic control subjects and associations within the type 1 diabetes group of WM structure with clinical characteristics such as age of onset, duration of type 1 diabetes, and measures of hyperglycemia, hypoglycemia, and glucose variability. In addition, we investigated associations between WM structure and cognitive abilities in children with type 1 diabetes and in control subjects. The current sample is almost six times larger than the previous DTI study (18) in young children with type 1 diabetes and is the largest study of its kind to date.

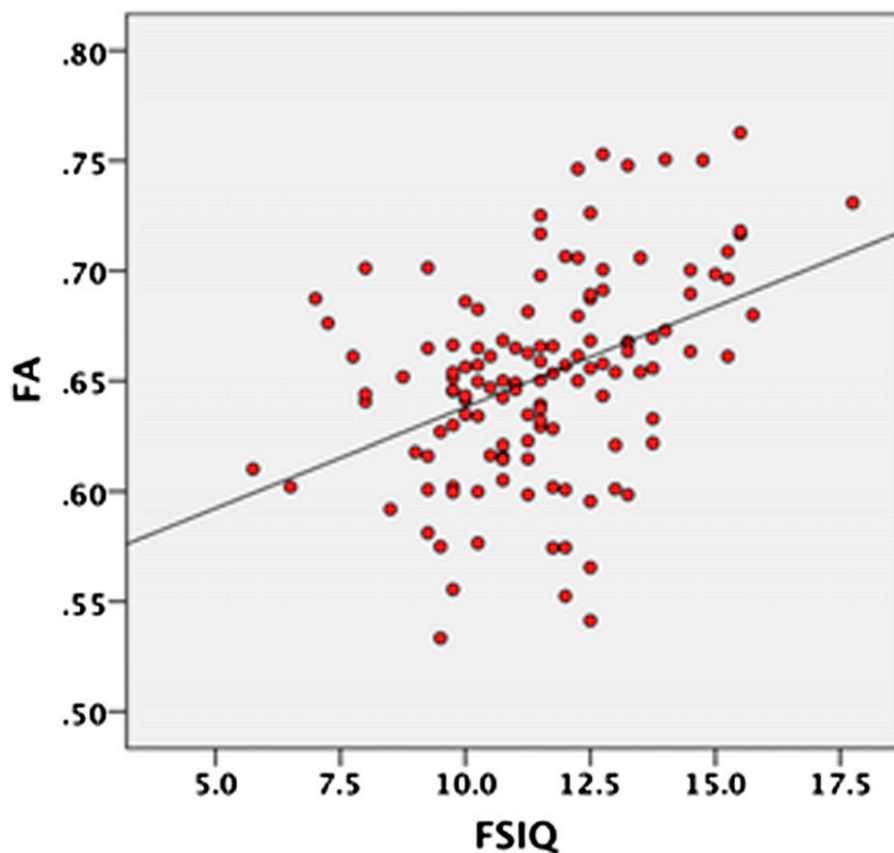
Our results show that in this very young cohort, there were significant widespread reductions in AD values in children with type 1 diabetes compared

with age-matched nondiabetic control subjects. Within the type 1 diabetes group, younger age of onset was associated with widespread lower FA and higher RD and increased duration of type 1 diabetes was associated with reduced AD, but also reduced RD, and increased FA, likely due to the dominant effect of age, which is significantly correlated with WM structure and was also significantly correlated with disease duration (28,29). In addition, measures of hyperglycemia and glucose variability were significantly associated with WM structure, although measures of hypoglycemia were not. It should be noted, however, that the hypoglycemia exposure and the number of severe hypoglycemia events may have been too small to identify statistically meaningful differences. Finally, we demonstrated a significant association between WM structure and cognitive ability in the type 1 diabetes group and a near-significant difference when comparing the level of this association between the diabetes and control groups.

To date, four studies have described DTI investigations of individuals with type 1 diabetes: two in adults (17,19), one in children and young adults (ages 9–22 years) (16), and one in children aged 3–10 years (18). DTI studies in adults with type 1 diabetes indicate that structural WM differences exist between individuals with type 1 diabetes and control subjects. Specifically, Kodl et al. (19) reported significantly lower FA in type 1 diabetes compared with control subjects, and FA was negatively associated with duration of type 1 diabetes and  $HbA_{1c}$  values. This finding is in contrast to our finding of significant positive correlations between FA and duration of type 1 diabetes, likely because of the younger cohort in our study and the dominant effect of age in this age-group. Another study in an adult population investigated WM structure in individuals with type 1 diabetes and control subjects (16), and, congruent with our findings, differences in AD were observed between participants with type 1 diabetes and control subjects in this adult population. Similarly, a recent study (16) investigating a cohort of children and



**Figure 2**—A post hoc graphic representation of the association between FA (A) and RD (B) values with gluSD (FA:  $R = -0.41$ ,  $P < 0.001$ ; RD:  $R = 0.39$ ,  $P < 0.001$ ). (A high-quality color representation of this figure is available in the online issue.)



**Figure 3**—A post hoc graphic representation of the association between FA values and FSIQ scores in type 1 diabetes ( $R = 0.4$ ,  $P < 0.001$ ). Mean FA values were extracted from regions of significant correlations between FA and FSIQ in the type 1 diabetes group. (A high-quality color representation of this figure is available in the online issue.)

young adults also found lower AD values in participants with type 1 diabetes compared with their siblings who did not have type 1 diabetes.

Only one study investigated WM structure in an exclusively pediatric sample of 22 children with type 1 diabetes and 14 control subjects (18). Significant lower temporal and parietal AD values in the type 1 diabetes group were observed compared with control subjects. In addition, higher lifetime HbA<sub>1c</sub> values were positively associated with RD values in children with type 1 diabetes. There were no significant associations between WM structure and duration of type 1 diabetes or age of onset. Our current results confirm the observations of AD differences between children with type 1 diabetes and control subjects in a much larger sample of children in the same age range and further show significant associations between age of onset and disease duration and WM structure. Our present study also is the first to provide

glucose data from CGM, allowing a more detailed assessment of glucose exposure and glycemic variability. In addition to larger sample size (194 vs. 36), a notable difference between the current study and the previous DTI study in children with type 1 diabetes is that the previous study found significant associations between lifetime HbA<sub>1c</sub> values and WM structure, whereas we found only significant associations with HbA<sub>1c</sub> values at study baseline but not with lifetime HbA<sub>1c</sub> values. This discrepancy suggests that in our sample of young children with relatively short duration of diabetes, HbA<sub>1c</sub> values at baseline were more representative of recent dysglycemia-associated brain insults than the lifetime values.

The profile of reduced overall AD in type 1 diabetes observed here suggests possible axonal damage associated with diabetes (30). Reduced AD was associated with duration of type 1 diabetes suggesting that longer exposure to diabetes worsens the insult

to WM structure. However, measures of hyperglycemia and glucose variability were either not associated or were positively associated with AD values, suggesting that these measures did not contribute to the observed decreased AD in the type 1 diabetes group. A possible explanation for these observations is that several biological processes influence WM structure in type 1 diabetes. Some processes may be related to insulin insufficiency or C-peptide levels independent of glucose levels (31,32) and may affect WM coherence (and reduce AD values as observed in the between-group results). Other processes related to hyperglycemia and glucose variability may target myelin (resulting in reduced FA and increased RD) as well as reduced axonal branching (both would result in increased AD values). Alternatively, these seemingly conflicting AD observations may be due to a dominant effect of age, which could overshadow effects from dysglycemia.

Early age of onset is one of the most replicable risk factors for cognitive impairments in type 1 diabetes (33,34). It has been hypothesized that young children are especially vulnerable to brain insults resulting from episodes of chronic hyperglycemia, hypoglycemia, and acute hypoglycemic complications of type 1 diabetes (seizures and severe hypoglycemic episodes). In addition, fear of hypoglycemia often results in caregivers maintaining relatively higher blood glucose to avoid lows altogether (1), especially in very young children. However, our study suggests that this approach of aggressive hypoglycemia avoidance resulting in hyperglycemia may not be optimal and may be detrimental to WM structure in young children.

Neuronal damage (reflected in altered WM structure) may affect neuronal signal transfer and, thus, cognition (35). Cognitive domains commonly reported to be affected in children with type 1 diabetes include general intellectual ability, visuospatial abilities, attention, memory, processing speed, and executive function (36–38). In our sample, even though the duration of illness was relatively short (2.9 years on average), there were modest but significant cognitive differences between children with type 1 diabetes and control subjects (24). Further, investigation of WM structure associations with overall general cognitive ability (FSIQ) revealed a significant association within the type 1 diabetes group and a trend approaching significance this group was compared with control subjects. These findings may be due to the possibility that children with type 1 diabetes with more severe insult to WM structure have lower cognitive ability than those who have intact WM structure. Future longitudinal studies of children with type 1 diabetes are thus critical for determining whether variations in WM structure early in the course of the illness predict the future trajectory of cognitive ability.

Our results are in concordance with observations from animal models of diabetes, which have shown myelin damage in rats with streptozotocin-induced diabetes. Such myelin damage

could be expected to result in reduced FA and increased RD (14). Additional abnormalities observed in rat models of type 1 diabetes include perivascular and mitochondrial swelling and fragmentation of neurofilaments, which could result in reduced AD, and loss of oligodendroglia, which would result in increased AD (2). A combination of these brain tissue insults, differentially affected by clinical characteristics between individuals, is likely to contribute to the variety of findings observed in our study.

Metabolic pathways involving intra- and extracellular products of nonenzymatic glycation (advanced glycation end products [AGEs]) have been implicated in hyperglycemic nerve damage. Extracellular AGEs interact with the receptor for AGEs to activate intracellular signaling pathways that induce transcription of proinflammatory genes and cellular oxidative stress (39,40). In addition, hyperglycemia, and thus increased flux of glucose, causes intracellular activation of the polyol pathway (sorbitol metabolism) resulting in AGEs, which in turn interact with matrix proteins, leading to functional and structural nerve and neuroglia abnormalities. Examples of detrimental glycation in neural tissue include glycation of major axonal cytoskeletal proteins such as tubulin, neurofilament, and actin (41–43), which may result in axonal atrophy and degeneration. Another mechanism of neuronal damage is nonenzymatic glycation of myelin (44). Glycated myelin is susceptible to phagocytosis by macrophages, which in turn are stimulated to secrete protease, contributing to demyelination in diabetes (44,45). Finally, increased glucose variability also may be related to oxidative damage. Specifically, in a study of adults with type 2 diabetes, oxidative stress was significantly correlated with MAGE (after adjustment for other markers of diabetes control) (46). Our findings of significant correlations between glucose variability and WM structure suggest that brain injury, perhaps due to oxidative stress, may be related to glucose variability in young children with T1D.

There are some limitations of our study. First, inferences regarding the effects of

type 1 diabetes on WM structure were potentially confounded by a significant correlation between variables of interest and age and the typical changes in WM structure associated with age. These effects are difficult to fully disentangle in statistical analyses. We addressed this issue by covarying for age; however, this approach may have been insufficient to remove the effects of age. In addition, another confounding factor is the relationship between age of onset and disease duration, which is difficult to resolve in a cross-sectional design. A third limitation is that the CGM-derived variables were acquired during a short period of time prior to the scan and thus represent only a “snapshot” rather than a robust assessment of diurnal glucose characteristics. Regular CGM evaluations as part of a longitudinal study may help address this limitation. Finally, our analyses of brain effects from (a history of) previous seizures and severe hypoglycemia may not be sufficiently sensitive to detect subtle brain differences occurring with these insults in different stages of development, since there were relatively few such events. Additional investigations with more fine-grained analyses of clinical severity, age of occurrence, and rate of these glycemia-associated complications and ideally a longitudinal design are needed in order to better understand their effects on the developing brain.

In summary, we present results from the largest study to date investigating WM structure in very young children with type 1 diabetes. We observed significant and widespread brain differences in the WM microstructure of children with type 1 diabetes compared with nondiabetic control subjects and significant associations between WM structure and measures of hyperglycemia, glucose variability, and cognitive ability in the type 1 diabetic population. Future longitudinal studies are needed to fully understand WM development in children with type 1 diabetes and whether brain structure can be used to predict cognitive outcome. A more precise understanding of brain changes in type 1 diabetes can help further our understanding of



mechanisms of injury, thereby leading to possible changes in treatment practices designed to prevent such injury.

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**Author Contributions.** N.B.-G. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. M.R. and P.M. researched data, contributed to discussion, and reviewed and edited the manuscript. M.M. researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. T.H., S.A.W., T.A., B.B., N.M., N.H.W., L.A.F., M.T., R.W.B., K.J.R., C.K., P.C., and A.L.R. 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.

## Appendix

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