The Feasibility of Detecting Neuropsychologic and Neuroanatomic Effects of Type 1 Diabetes in Young Children

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OBJECTIVE—To determine if frequent exposures to hypoglycemia and hyperglycemia during early childhood lead to neurocognitive deficits and changes in brain anatomy.

RESEARCH DESIGN AND METHODS—In this feasibility, cross-sectional study, young children, aged 3 to 10 years, with type 1 diabetes and age- and sex-matched healthy control (HC) subjects completed neuropsychologic (NP) testing and magnetic resonance imaging (MRI) scans of the brain.

RESULTS—NP testing and MRI scanning was successfully completed in 98% of the type 1 diabetic and 93% of the HC children. A significant negative relationship between HbA_{1c} and Wechsler Intelligence Scale for Children (WISC) verbal comprehension was observed. WISC index scores were significantly reduced in type 1 diabetic subjects who had experienced seizures. White matter volume did not show the expected increase with age in children with type 1 diabetes compared with HC children (diagnosis by age interaction, $P = 0.005$). A similar trend was detected for hippocampal volume. Children with type 1 diabetes who had experienced seizures showed significantly reduced gray matter and white matter volumes relative to children with type 1 diabetes who had not experienced seizures.

CONCLUSIONS—It is feasible to perform MRI and NP testing in young children with type 1 diabetes. Further, early signs of neuroanatomic variation may be present in this population. Larger cross-sectional and longitudinal studies of neurocognitive function and neuroanatomy are needed to define the effect of type 1 diabetes on the developing brain.

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ype 1 diabetes affects the developing brain. Although the mechanism is not known, those diagnosed with onset of type 1 diabetes before 5 years have poorer intellectual performance (1,2), particularly in memory and attention, visual-perceptual function, and fine motor speed and coordination (3). Hypoglycemia is often implicated as the cause

of these deficits (4–7). However, long-term exposure to hyperglycemia has also been hypothesized to have deleterious effects on the brain (8–11).

Young children with type 1 diabetes have excursions in blood glucose (BG) values often resulting in multiple episodes of hypoglycemia and hyperglycemia. Inconsistent eating patterns as well

as the decreased ability to recognize and report symptoms make it difficult to achieve tight glycemic control in this age group. Therefore, we hypothesized that frequent exposure to hypoglycemia and hyperglycemia during childhood may lead to neurologic deficits. Young children's brains undergo dynamic changes, such as myelination and modification of synapses, which can make their brains vulnerable to these glycemic excursions and potentially lead to neurocognitive deficits (12). We report the initial results of neuropsychologic (NP) testing and magnetic resonance imaging (MRI) studies of the brain in young children with type 1 diabetes compared with healthy age- and sex-matched control (HC) children.

RESEARCH DESIGN AND METHODS
—Children between 3 and 10 years of age with type 1 diabetes for at least 6 months and HC subjects were recruited. All procedures were approved by the institutional review board. All parents or guardians signed informed consents, and children older than 7 years also signed informed assents.

Diabetes history
Detailed information about the subject's diabetic history was collected by parental interviews and from review of hospital records, clinic visit notes, HbA_{1c} values, and laboratory records. A time-weighted average of a subject's HbA_{1c} values was calculated using the trapezoid rule. From the time of enrollment to the time of NP testing and MRI scan, data from BG meters and insulin pumps were downloaded and reviewed. In particular, parents were asked about the occurrence and frequency of seizures related to severe hypoglycemia. Severe hyperglycemic events were defined as admission and treatment for diabetic ketoacidosis.

NP testing
An age-appropriate battery of NP testing was administered by trained personnel.

To assess general intellectual function, the Wechsler Preschool and Primary Scale of Intelligence (WPPSI, 3rd Edition) was used for subjects aged 3 to younger than 5 years old, and the Wechsler Intelligence Scale for Children (WISC, 4th Edition) was administered to participants 5 to 10 years old. In addition, The NEPSY (A Developmental NEuroloPSYcological Assessment) was used to evaluate executive function, memory/attention, motor, and visual-spatial domains (Supplementary Appendix 1). BG levels were measured before NP testing in the subjects with type 1 diabetes to ensure that levels were between 80 and 250 mg/dL before NP testing. Those administering the tests were aware of signs of hypoglycemia during the procedure and were instructed to stop testing if signs were noted, treat, and continue only after achieving BG within the above parameters.

MRI

Subjects were prepared for the MRI scan after their NP assessment. The children and their parents were both interviewed to determine if the subjects would be able to stay still for the MRI scan. In addition, the subjects and the parents participated in a simulated MRI protocol designed to lessen anxiety and improve compliance. They were also asked to view *1*) a 6-min video that demonstrates a child experiencing the sights and sounds of a scan; *2*) a 20-min narrated CD that contains a collection of assorted MRI sounds; and *3*) illustrated written materials that include instructions for activities or "games" that reduce head motion and increase the understanding of the imaging procedure. BG levels measured before the MRI scans in the subjects with type 1 diabetes were between 100 and 250 mg/dL. The research assistants and parents were nearby the children to monitor for signs of hypoglycemia.

The children underwent high-resolution structural MRI scans using a General Electric Signal 1.5-Tesla imaging system (Waukesha, WI). Coronal three-dimensional volumetric spoiled grass gradient recalled (SPGR) series were acquired with the following scan parameters: repetition time, 35 msec; echo time, 6 msec; flip angle, 45°, 1.5-mm thick; 0-mm gap; number of excitations, 1; field of view, 24 cm; and a 256 × 192 matrix size for 124 contiguous slices (scan time, 14 min; Supplementary Appendix 2).

Imaging data were imported into the software BrainImageJava (BIJ) for visualization, processing, and quantification (http://cibsr.stanford.edu/tools). Non-brain tissues were removed from the images, instrument-related image artifacts were corrected, and brains were positionally normalized with a six-parameter rigid body transformation. Next, segmentation of tissue components (gray matter [GM], white matter [WM], cerebrospinal fluid) and parcellation of anatomically distinct regions (cerebral lobes, cerebellum, subcortical regions) were performed on each brain according to well-established protocols (13). Amygdala and hippocampal (HP) delineation procedures were adapted from previously published protocols (14). One experienced, blinded rater traced the exterior boundaries of each of these structures.
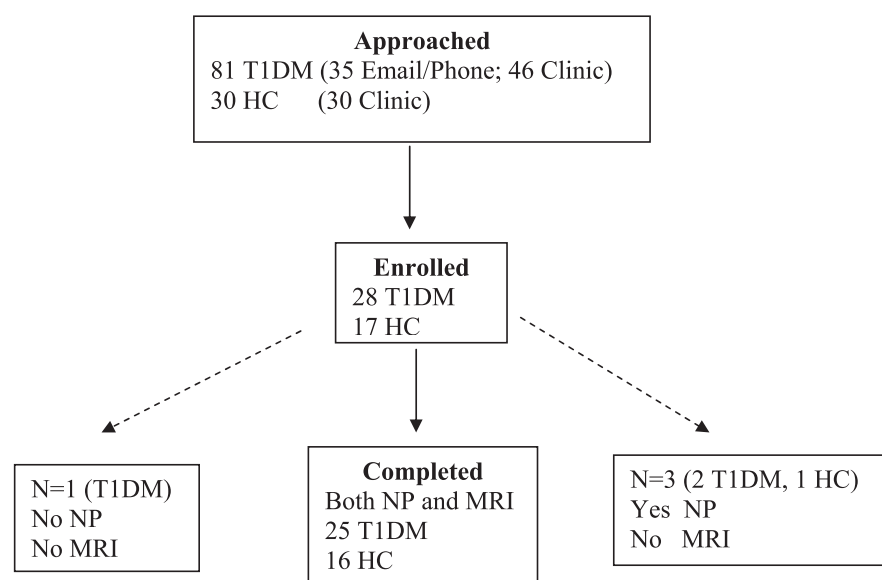
Statistical methods

Statistical analysis was performed using SPSS 13 software (SPSS Inc., Chicago, IL). Scores were compared between type 1 diabetic and HC subjects using *t* tests. For within-group analysis of those with type 1 diabetes, linear regressions were performed to examine the strengths of the relationship between WISC test scores and HbA_{1c} as well as between WISC test scores and seizure occurrence. Results were divided into core domains for each assessment measure. We used univariate ANOVA, controlling for age and sex, to determine relationships between total GM, WM, and HP volumes and diabetes, age, HbA_{1c}, and history of seizures.

RESULTS—We approached 81 subjects with type 1 diabetes, of whom 35 received an e-mail or a telephone message (there was no response from 25 potential participants), and another 46 were directly approached in the clinic. One child was excluded for a history of a neurologic condition independent of diabetes. We enrolled 28 with type 1 diabetes. Likewise, we approached 30 HC children in person, mostly siblings or friends of our subjects with type 1 diabetes and enrolled 17 HC subjects. One was excluded for having an oral metal appliance. The most common reasons for not participating in the study were time commitment and not wanting to undergo an MRI scan. Of the 45 enrolled, only one 3-year-old child was unable to complete both the NP testing and the MRI scanning (Fig. 1). Another three subjects, two with type 1 diabetes (4 years old, 5 years old) and one HC child (6.5 years old), completed the NP testing but not the MRI scanning. Two additional subjects (6-year-old with type 1 diabetes and an 8-year-old HC child) were able to complete the MRI scanning but had only partially usable data because of movement artifact. The final groups were closely matched in age (type 1 diabetes, 7.0 ± 1.4; HC subjects, 7.2 ± 1.6 years; Table 1).

NP testing

NP testing was successfully completed in 98% of participants: 19 with type 1 diabetes and 13 HC children completed the WISC battery of testing, and 6 with type 1

Approached
81 T1DM (35 Email/Phone; 46 Clinic)
30 HC   (30 Clinic)

Enrolled
28 T1DM
17 HC

N=1 (T1DM)
No NP
No MRI

Completed
Both NP and MRI
25 T1DM
16 HC

N=3 (2 T1DM, 1 HC)
Yes NP
No MRI

Figure 1—*Recruitment and enrollment. Subjects were more likely to participate in the study when approached in person in the clinic. Most of the subjects with type 1 diabetes mellitus (T1DM) and the HC participants were able to complete both NP testing and the MRI.*

Table 1—Demographics

Variable	Type 1 diabetes	Control subjects
Mean age (years)	7.0 ± 1.4 (4.7–9.2)	7.2 ± 1.6 (4.2–9.5)
Sex		
Male	13	11
Female	14	7
Handedness		
Right	26	17
Left	1	1
HbA_{1c} (%)		
Mean	7.6 ± 0.9	NA
Median	7.6 ± 1.0	NA
Mean time-weighted HbA_{1c} (%)	8.0 ± 0.6	NA
Mean age of onset (years)	3.5 ± 1.9	NA
Mean duration of type 1 diabetes (years)	3.6 ± 1.9	NA
Seizure occurrence		
Yes	8	NA
1 seizure	4	
2 seizures	0	
>2 seizures	4	
No	19	

Mean and median data are presented with the SD (range); categoric data are presented as *n*. NA, not applicable.

diabetes and 4 HC subjects completed the WPPSI battery of testing based on age. Results from the two age groups were very similar, and only the WISC data are shown (Fig. 2). The full-scale mean ± SD IQ scores were 109.4 ± 13.5 for subjects with type 1 diabetes and 115.8 ± 14.8 for HC subjects. No statistically significant differences were noted in general intellect, executive function, memory/attention, motor, and visual-spatial domains between the type 1 diabetes and HC subjects ($P = 0.1$–0.9; Fig. 2).

A statistically significant negative relationship between HbA_{1c} and WISC verbal comprehension scores was observed (linear regression $R^2 = 0.318$, $F_{1,15} = 6.99$, $P = 0.018$). Interestingly, there was no significant relationship between seizure occurrence and WISC verbal comprehension scores ($R^2 = 0.108$, $F_{1,16} = 1.938$, $P = 0.183$). However, all other index scores (WISC processing speed, FSIQ, working memory and perceptual reasoning) showed statistically significant differences in functioning between those type 1 diabetes subjects who had seizures and those who did not. Seizure event was most robustly associated with the WISC processing speed score ($R^2 = 0.505$, $F_{1,16} = 16.310$, $P = 0.001$).

MRI scans
MRI scans were successfully completed in 94% of the subjects. MRI findings included similar total GM (862 ± 101 vs. 838 ± 95 mm^3), WM (377 ± 63 vs. 370 ± 57 mm^3), HP (6.3 ± 0.8 vs. 6.1 ± 0.8 mm^3) and amygdala (3.53 ± 0.31 vs. 3.41 ± 0.56 mm^3) volumes in HC subjects and type 1 diabetes subjects, respectively. Between-group analyses showed that after controlling for age and sex, there was a statistically significant diagnosis by age interaction ($P = 0.005$), such that WM volume did not show the expected rate of volume increase in children with type 1 diabetes compared with HC children (Fig. 3A and B). A similar trend was detected for HP volume (diagnosis × age: $P = 0.07$). These diagnosis-by-age interactions were specific to WM and HP volumes and were not observed for GM or amygdala volumes.

Children with type 1 diabetes who had experienced seizures showed significantly reduced GM ($F_{1,19} = 5.161$, $P = 0.035$) and WM ($F_{1,19} = 4.543$, $P = 0.045$) relative to children with type 1 diabetes who had not experienced seizures. The occurrence of seizure events did not lead to significant differences in HP volume in the type 1 diabetes subgroups ($P = 0.114$). There were no sex differences between those who did and did not have seizures. The time-weighted HbA_{1c} was not statistically associated with any of the brain volumes.

CONCLUSIONS—This study is the first to demonstrate the feasibility of NP testing and MRI studies of the brain in young children, ages 3 to 10 years, with type 1 diabetes. In fact, 40 of 45 children successfully completed NP testing and MRI scanning. No statistically significant differences were noted in general intellect, executive function, memory/attention, motor, and visual-spatial domains between those with type 1 diabetes and the HC participants. Among those with type 1 diabetes, the verbal comprehension score was lower in those with higher HbA_{1c} values. In addition, those who had type 1 diabetes and a history of hypoglycemic seizure had lower WISC processing speed, full-scale IQ score, working memory, and perceptual reasoning. The MRI findings also showed similar brain volumes between those with type 1 diabetes and the HC subjects. However, children with type 1 diabetes showed a significantly altered pattern of age-related WM development relative to HC subjects (with a similar trend for HP development) as well as reduction in GM and WM in those who experienced seizures. To our knowledge, this is the first study to report such NP and MRI findings in this age group.

Our results are consistent with previous studies on the effect of type 1 diabetes on cognition. Although there have been concerns regarding how type 1 diabetes and its treatment affect cognitive performance, neither the extent of this effect nor the putative mechanism has been elucidated. Research results are inconsistent regarding the magnitude and pattern of cognitive difficulties in children with type 1 diabetes (15).

The meta-analysis of cognitive function in children with type 1 diabetes by Gaudieri et al. (15) found mildly lower cognitive scores relative to HC participants across various domains, particularly in those children with early onset as defined by diagnosis before age 7. In this meta-analysis, the average age of diabetes onset was 6.6 years (range 4.13–11.8), with a duration of 5.23 years (0.5–8.9), whereas our average age of onset was much younger (3.5 ± 1.9 years) and duration was slightly shorter (3.7 ± 1.5 years). The meta-analysis also showed that children with type 1 diabetes had slightly lower group test scores of 1 to 3 standard score points, which are not likely to be clinically detectable. The cognitive performance of those with type 1 diabetes is generally within the normal range (15–17).

In a review of the effect of type 1 diabetes on cognitive impairment in those with type 1 diabetes who are younger than 18 years, Wrighten et al. (18) found that although there may be deficits in cognition in type 1 diabetes, these deficits may be mild and may not fall below the
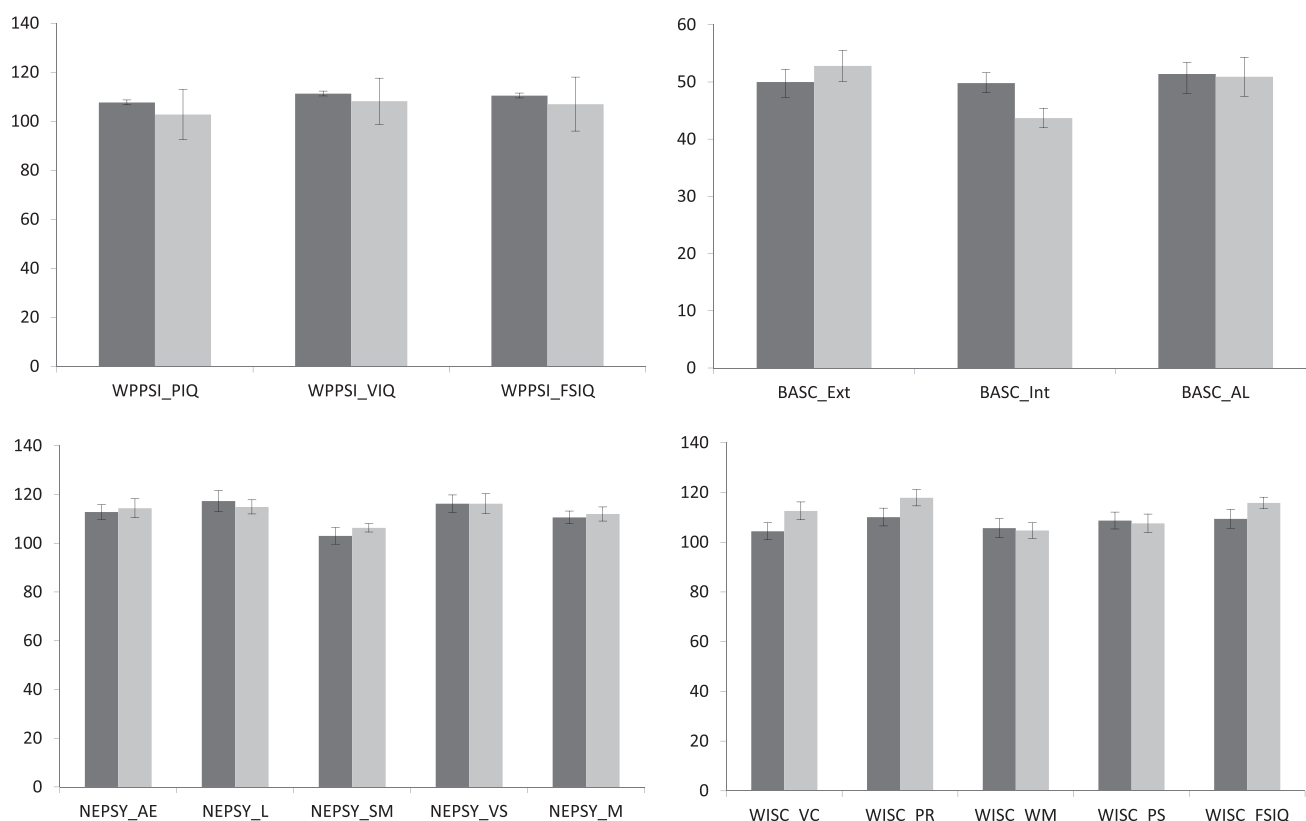
Figure 2—*Summary of NP testing. There were no statistically significant differences in mean scores between those subjects with type 1 diabetes (dark gray) and HC subjects (light gray). The error bars represent SEM. WPPSI: PIQ, performance IQ; VIQ, verbal IQ; FSIQ, full-scale IQ. WISC: VC, verbal comprehension; PR, perceptual reasoning; WM, working memory; PS, processing speed; FSIQ, full-scale IQ. NEPSY: AE, attention/executive function; L, language; SM, sensorimotor; VS, visual-spatial; M, memory. BASC: Ext, externalizing; Int, internalizing; AL, adaptive living.*

range of the general population. More specifically, a study of preschool children with type 1 diabetes observed that this group performed similarly to age-matched HC subjects, with both groups performing in the normal range (19).

Finally, we found the verbal comprehension score was lower in those with higher HbA_{1c} values, suggesting that exposure to hyperglycemia may affect the brain, consistent with previous findings in older children with type 1 diabetes (20). This finding is similar to the Epidemiology of Diabetes Interventions and Complications study, where higher HbA_{1c} values were associated with moderate declines in function, albeit in motor speed and psychomotor efficiency (11). An interesting finding in preschool children with type 1 diabetes was that higher HbA_{1c} values were related to lower receptive language scores (19).

Some recent clinical brain imaging studies in children with type 1 diabetes have found overall changes in brain tissue composition or specific area volumes in subjects with type 1 diabetes. These studies, however, have variable patient ages, with the youngest average age of 9.4 years

(21), ages of diabetes onset, hypoglycemic events, controls, and different methods of analyses. To our knowledge, our study is the first to examine differences in GM, WM, and HP volumes between young children aged 3 to 10 years with type 1 diabetes and HC children.

Our findings are consistent with previous reports of no significant differences in total GM and WM volumes between children with type 1 diabetes and HC subjects (20); however, we did detect a significant age-by-diagnosis interaction for WM. The WM of the brain consists of the myelinated neuronal axons responsible for relaying messages between the neuronal cells. Total WM volume differences have not been associated with cognitive decline in children with type 1 diabetes. However, Kodl et al. (22) found that WM microstructural deficits were present in adults with long-standing type I diabetes and that these deficits correlated with poorer performance on selected NP tests of WM function.

The HP is an important structure for the integration of learning and memory in the mammalian central nervous system

(23) and is particularly sensitive to changes in glucose homeostasis. Ho et al. (21) recently reported an increased incidence of medial temporal sclerosis, defined as a specific pattern of hippocampal neuronal loss, (24) in children with type 1 diabetes, but it was not associated with a history of severe hypoglycemia or diabetic ketoacidosis. Furthermore, there was no control group for comparison. Hershey et al. (25) also examined the HP volume of children with type 1 diabetes (mean age 12.9 ± 3.3 years) and found that volumes were higher in those who had more than three severe hypoglycemic events compared with those who had fewer than three events and compared with HC subjects. In our study, we did not find any differences in HP volume between those children with type 1 diabetes who had seizures and those who did not. However, comparing the two studies is difficult because our subjects were much younger and only 8 of 23 of our participants with type 1 diabetes who successfully completed MRI scanning had a history of seizures, whereas 48 of 95 subjects in the Hershey et al. (25) study had severe hypoglycemia. A larger cross-sectional study of
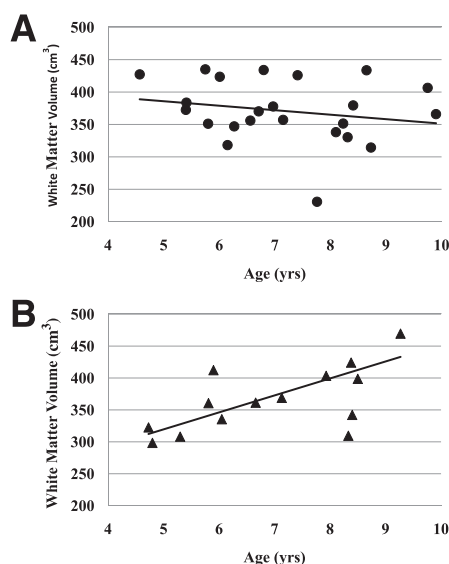
Figure 3—*WM volume did not show the expected increase in volume with age in children with type 1 diabetes (●), in contrast with HC subjects (▲) who showed the (expected) normal increase in WM volume with age* $(P = 0.005)$.

young children with type 1 diabetes perhaps will help determine the effect of type 1 diabetes and seizures on the HP in this age group. Longitudinal studies of young children with type 1 diabetes are necessary to determine if the reductions in WM and HP volumes continue to exist. Finally, our study supports the Ho et al. (21) findings that early-onset severe hypoglycemia may have an effect on GM volume.

Hypoglycemia and hyperglycemia have been associated with cognitive and neuroanatomic changes in the brain. We show that it is feasible to perform MRI and NP testing in young children with type 1 diabetes and that early signs of neuroanatomic variation may be present in this population. Larger cross-sectional and longitudinal studies of neurocognitive function and neuroanatomy to define the effect of type 1 diabetes on the developing brain are now underway.

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