Hippocampal Volumes in Youth With Type 1 Diabetes

Tamara Hershey,[1,2,3] Dana C. Perantie,[1] Jenny Wu,[1] Patrick M. Weaver,[1] Kevin J. Black,[1,2,3,4] and Neil H. White[5,6]

OBJECTIVE—Hippocampal neurons in adult animals and humans are vulnerable to severe hypoglycemia and hyperglycemia. Effects are hypothesized to be exacerbated during development, but existing studies on developing human brains are limited. We examined whether hypoglycemia or hyperglycemia experienced during brain development in humans affects hippocampal volumes.

RESEARCH DESIGN AND METHODS—We analyzed T1-weighted magnetic resonance images in 95 youth with type 1 diabetes and 49 sibling control subjects aged 7–17 years. Youth with diabetes were categorized as having 0 (n = 37), 1–2 (n = 41), or 3 or more (3+; n = 17) prior severe hypoglycemic episodes. Hyperglycemia exposure was estimated from median lifetime A1C, weighted for duration of diabetes. Stereologic measurements of hippocampal volumes were performed in atlas-registered space to correct for whole brain volume.

RESULTS—Greater exposure to severe hypoglycemia was associated with larger hippocampal volumes (F [3,138] = 3.6, P = 0.016; 3+ larger than all other groups, $P < 0.05$). Hyperglycemia exposure was not associated with hippocampal volumes (R^2 change = 0.003, F [1,89] = 0.31, P = 0.58, semipartial r = 0.06; one outlier removed for high median A1C), and the 3+ severe hypoglycemia group still had larger hippocampal volumes after controlling for age of onset and hyperglycemia exposure (main effect of hypoglycemia category, F [2,88] = 6.4, P = 0.002; 3+ larger than all other groups, $P < 0.01$).

CONCLUSIONS—Enlargement of the hippocampus may reflect a pathological reaction to hypoglycemia during brain development, such as gliosis, reactive neurogenesis, or disruption of normal developmental pruning. ***Diabetes*** **59:236–241, 2010**

Neuropathological data from adult animals suggest that severe hypoglycemia may preferentially harm neurons in the medial temporal region, including the hippocampus (1,2). The degree of hippocampal damage increases with duration of hypoglycemia and with the presence of seizures (3–5). In contrast, the cerebellum appears to be relatively spared by hypoglycemia (6). This pattern of selective vulnerability has been reported in neuropathological studies of adult animals and humans (3,4,6) and on visual inspection of clinical brain scans of adult humans with type 1 diabetes (2,7). Cell death during hypoglycemia is thought to be caused by an *N*-methyl-D-aspartate (NMDA) receptor–mediated excitotoxic process; hippocampal sensitivity may be (8) explained by the relatively high proportion of NMDA receptors in that region (8). However, there may be other mechanisms by which hypoglycemia affects neurons in the hippocampus, such as apoptosis (9).

Animal studies of hypoglycemic effects on the developing hippocampus have been limited. However, neither of two relevant studies found increased neuronal death in the hippocampus after moderate hypoglycemia in young rats (10,11). Notably, these studies did find significant cell death in cortical regions, which could correspond to the regional cortical volume loss observed in voxel-wise neuroimaging analyses of youth with type 1 diabetes (12). Although it is possible to measure the volume of the hippocampus reliably and detect even subtle atrophy in this structure in children with temporal lobe epilepsy, anoxia, and other medial temporal lobe insults (13), examinations of the effects of hypoglycemia on the hippocampus using quantitative neuroimaging methods have been limited. One recent study reported an increased incidence of medial temporal sclerosis in diabetic children with early hypoglycemia (before 6 years of age) compared with those with later hypoglycemia (6 years or older), but not compared with those with no hypoglycemia history. In addition, no differences were found in absolute hippocampal volumes across these small groups. However, no correction for whole brain volume was performed and no control group was assessed (14). Interestingly, studies on children with temporal lobe seizures have found that early exposure to seizures can cause greater long-term memory deficits (15) and greater hippocampal damage (16–18) than seizures that occur during adulthood.

The possibility of acute severe hypoglycemia occurs within a context of chronic hyperglycemia, which may have its own effects on brain integrity. In fact, studies have found that hyperglycemia is related to decreased hippocampal gray matter in adults with type 2 diabetes (19) and possibly decreased hippocampal function in adult animal models (20,21). However, previous studies have not been able to disentangle the effects of severe hypoglycemia and chronic hyperglycemia exposure during development on the hippocampus in humans with type 1 diabetes. Further understanding of the effects of exposure to glycemic extremes on the hippocampus in development may provide critical information for minimizing risks and maximizing clinical benefits over a patient's childhood. In this study, we acquired structural magnetic resonance imaging (MRI) and glycemic history from a large sample of youth with type 1 diabetes and nondiabetic siblings who have already been characterized cognitively (22). We com-

From the [1]Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri; the [2]Department of Neurology, Washington University School of Medicine, St. Louis, Missouri; the [3]Department of Radiology, Washington University School of Medicine, St. Louis, Missouri; the [4]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri; the [5]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri; and [6]St. Louis Children's Hospital, St. Louis, Missouri.

Corresponding author: Tamara Hershey, tammy@wustl.edu.

Received 29 July 2009 and accepted 22 September 2009. Published ahead of print at http://diabetes.diabetesjournals.org on 15 October 2009. DOI: 10.2337/db09-1117.

See accompanying commentary, p. 4.

pared diabetic and control groups and examined the association of hippocampal volumes, measured with validated and unbiased stereologic methods, with previous histories of severe hypoglycemia and chronic hyperglycemia exposure.

RESEARCH DESIGN AND METHODS

Subjects. Children aged 7–17 years with type 1 diabetes and nondiabetic siblings (control subjects) were recruited from the Pediatric Diabetes Clinic at Washington University in St. Louis and St. Louis Children's Hospital. Subjects were excluded for mental retardation, chronic disease other than type 1 diabetes (e.g., hypothyroidism), significant neurological history not due to diabetes, diagnosed psychiatric disorder, current use of psychoactive medications, prematurity (less than 36-week gestation) with complications, and contraindications to MRI (e.g., metal implants). Diabetic subjects had to have been on insulin for at least 2 years prior to study entry. Handedness was assessed with a modified Edinburgh Handedness Inventory (23). Procedures were approved by the Washington University School of Medicine's Human Studies Committee, and all participants and their parents or guardians signed informed consents. Data from this cohort of children have been published previously (12,22). Hippocampal analyses reported here have not been published previously except in abstract form.

Clinical variables. History of severe hypoglycemia was collected by simultaneous parental and child interview. All incidents described were counted, regardless of who remembered the details. Severe hypoglycemia was defined similarly to Diabetes Control and Complications Trial criteria and our previous reports (12,22,24). This definition states that hypoglycemic events are severe when there are symptoms consistent with hypoglycemia and neurological dysfunction including seizure, loss of consciousness, or inability to arouse from sleep or individuals need assistance to treat their hypoglycemia and there is a documented response to oral or parenteral glucose or glucagon treatment (24). Due to the skewed distribution of the number of hypoglycemic events, we categorized individuals as having 0, 1–2, or 3 or more (3+) severe hypoglycemic events in their past. The age at first severe hypoglycemic event was noted. Those with a first episode occurring when they were 5 years or younger were considered "early" and those with a first episode occurring at older than 5 years were "late," consistent with other work (25).

Chronic exposure to hyperglycemia was calculated by collecting all available A1C test results from medical records at Pediatric Diabetes Clinic at Washington University in St. Louis and St. Louis Children's Hospital. The amount of time represented by the A1C tests was calculated by multiplying the number of tests by 3 months, the time frame reflected in each test, and dividing by duration of the individual's diabetes in months. To minimize the effect of incomplete data and to eliminate outliers from analyses, participants with A1C "coverage" less than 30% of their duration of diabetes ($n = 10$) were excluded. To account for duration of exposure to hyperglycemia, a "hyperglycemia exposure score" was calculated. Median A1C and duration variables were transformed to z scores, and each patient's pair of z scores was summed (A1C z score + duration z score). This method of calculation resulted in a near-normal distribution of hyperglycemia exposure scores, with higher scores indicating more overall exposure to hyperglycemia; however, these scores can be interpreted to indicate hyperglycemia exposure only relative to this sample.

Image acquisition. Structural MRIs were acquired for each subject on a Siemens Sonata 1.5 Tesla imaging system with a standard Siemens 30-cm circularly polarized radiofrequency (RF) head coil. For each subject, three to five scans consisting of 128 contiguous 1.25-mm sagittal slices were acquired using magnetization prepared rapid gradient echo (MPRAGE; repetition time [TR] = 1,900 ms, echo time [TE] = 3.93 ms, flip angle = 15°, matrix = 256 × 256 pixels, voxel size = 1 × 1 × 1.25 mm, single scan time = 7 min, 7 s). Subjects with movement or other artifact were excluded from further analyses ($n = 10$). Images with suspected anatomical abnormalities were referred to a neuroradiologist for review; three subjects were excluded for confirmed brain abnormalities (two were benign anomalies but discarded due to possible difficulty with registration).

Image preprocessing. The three highest quality images were coregistered by an automated, validated technique (26,27), averaged for each subject and atlas transformations computed. We used a target image composed of 24 brains—one for every age and sex between the ages of 7 and 18 years—made to represent the atlas of Talairach and Tournoux (28) as modified by Lancaster et al. (29). The coregistered, averaged, atlas-transformed data were resampled to a standard, 0.5-mm cubic voxel volume in which the long axis of the hippocampus was perpendicular to the coronal plane (30). Stereology proceeded as described below and previously (31) except that the anatomy was stretched in conformity with the atlas, making the measurements "relative" volumes (i.e., corrected for whole brain volume). Because the atlas transformation is affine, measured (relative) versus absolute volumes are related by a known multiplicative factor (the determinant of the transform matrix or atlas scaling factor [ASF]) that is constant over the entire volume. Thus, we can compute the absolute volume of the hippocampus from the relative volumes after it has been measured. Previous work has shown that the ASF is equivalent to manual measures of total intracranial volume and that computed absolute hippocampal volume is equivalent to direct measurement of the hippocampus in native space (32). Performing stereologic measures in a standard space avoids errors due to inconsistent structure boundary selection (e.g., amygdala/hippocampus) that otherwise occur due to variability of brain size and head orientation (33).

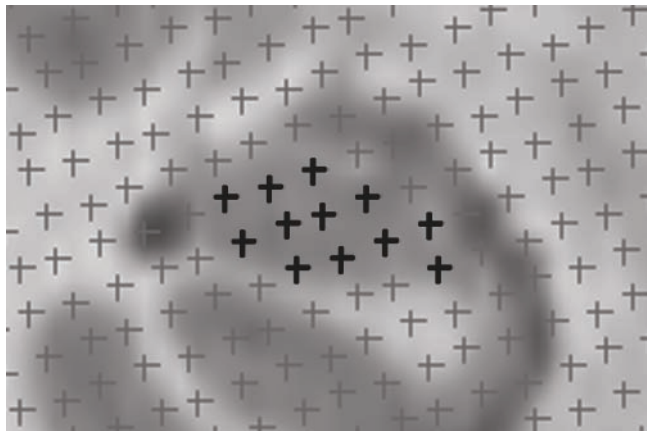

FIG. 1. Example of the stereologic method for measuring hippocampal volumes. Selected points (black) were determined to be within the left hippocampal gray matter on a coronal view (shown), while simultaneously viewed in the sagittal and transverse perspectives.

Stereologic method and reliability. Stereologic methods (34) were used to estimate hippocampal volumes using Analyze (Biomedical Imaging Resource, Mayo Foundation, Rochester, NY). In stereology, a grid of points is randomly placed on a brain slice. Points that fall within a structure of interest are selected. The number of these points times the grid dimensions (length, width, height) is mathematically proven to produce an unbiased estimate of volume (35). While selecting the points, orthogonal views of that point were examined in Analyze for better anatomical identification. Sampling parameters and grid size were set to yield ~150 selected grid points per hippocampus, a number that has previously been determined to yield reliable measurements in brain volume determination (36). Measures were performed on coronal slices every five slices (2.5 mm) within a three-dimensional MRI cubical subvolume composed of 0.5 × 0.5 × 0.5–mm voxels (Fig. 1). Starting slice was randomly determined for each measurement, an essential step in guaranteeing that the volume measurements are unbiased (34,35). The process is identical to previously published methods (31), with one exception. Instead of randomly placing the grid once per brain, we generated random grid angle and starting positions on every slice, thus taking advantage of the reduced variance (increased precision) this procedure provides (36). Raters were blind to subject identity and measured left and right hippocampal volumes separately. Mean left and right hippocampal volumes were determined from an average of four measurements of each volume, two measurements from each rater. Average measures intraclass correlations (two-way mixed, absolute agreement) between each rater pair were above 0.94 for total hippocampal volume. Anatomical boundaries of the hippocampus were defined using specific rules (37,38) and applied as previously described (31).

Analyses. To determine the relationship between hippocampal volume and diabetes, hypoglycemia, and hyperglycemia, we performed both repeated-measures general linear models and hierarchical linear regressions controlling for age and sex, and for analyses of diabetic groups only, age of onset. Hypoglycemia exposure was examined by comparing hypoglycemia frequency subgroups (0, 1–2, vs. 3+). Hyperglycemia was examined using our hyperglycemia exposure variable. The primary dependent measures were total relative and absolute hippocampal volumes and ASF as an index of total intracranial volume. For significant effects or interactions, post hoc correlations and 2-sample comparisons (t tests) were performed.

RESULTS

Type 1 diabetic subjects versus nondiabetic control subjects. Ninety-five youth with type 1 diabetes and 49 nondiabetic siblings had adequate imaging and glycemic

TABLE 1
Demographic and clinical variables

	Nondiabetic control subjects	Type 1 diabetic subjects			
		Total sample	0 hypo	1–2 hypo	3+ hypo
n	49	95	37	41	17
Age (years)	12.4 ± 2.7	12.4 ± 2.8	12.1 ± 2.4	12.5 ± 2.8	12.9 ± 3.3
Female subjects	49	43	38	39	65
Right handed	98	88	89	85	94
Age of onset (years)*	—	7.1 ± 3.2	8.5 ± 2.6	6.8 ± 3.2	4.7 ± 3.2
Duration of diabetes (years)*	—	5.4 ± 2.8	3.7 ± 1.3	5.8 ± 2.6	8.1 ± 2.9
Median A1C	—	8.3 ± 1.0	8.2 ± 1.2	8.3 ± 0.8	8.7 ± 0.9
Hyperglycemia exposure score*	—	0 ± 1.4	−0.76 ± 1.2	0.10 ± 1.1	1.48 ± 1.2
Atlas scaling factor (unitless)	0.86 ± 0.08	0.87 ± 0.08	0.89 ± 0.08	0.88 ± 0.08	0.86 ± 0.07
Total absolute	4,339 ± 607	4,417 ± 541	4,487 ± 485	4,337 ± 462	4,680 ± 578
Hippocampal volume (mm^3)					
Total relative hippocampal volume†	5,056 ± 581	5,085 ± 541	5,076 ± 492	4,965 ± 474	5,475 ± 545
Right relative hippocampal volume	2,546 ± 307	2,577 ± 286	2,599 ± 254	2,515 ± 251	2,773 ± 300
Left relative hippocampal volume	2,510 ± 285	2,507 ± 267	2,477 ± 252	2,450 ± 234	2,702 ± 285

Data are means ± SD or percent unless otherwise indicated. *All hypoglycemia subgroups are different from each other ($P < 0.05$). †3+ hypoglycemia group is different from all other subgroups ($P < 0.05$).

data for the planned analyses (Table 1). Across all subjects, there was no relationship between age and the ASF (indicating total intracranial volume [32]) ($r = 0.13$, $P = 0.11$) or age and absolute total hippocampal volume ($r = -0.04$, $P = 0.66$). There was a very modest but nonsignificant correlation between age and relative hippocampal volume ($r = -0.16$, $P = 0.06$). Absolute hippocampal volumes ($t = -2.77$, $P = 0.006$) and ASF ($t = -7.38$, $P < 0.001$) were greater for males ($n = 79$) compared with females ($n = 65$). However, males had smaller relative hippocampal volumes than females (males, mean volume = 4,994 mm^3, females mean volume = 5,195 mm^3; $t = 2.24$, $P = 0.03$). To ensure that age and sex would not influence our other results, we covaried both factors from subsequent analyses.

Youth with type 1 diabetes and nondiabetic siblings did not differ significantly in sex distribution ($\chi^2 = 0.44$, $P = 0.51$) or mean age ($t = 0.01$, $P = 0.99$). The diabetic group had proportionally more left-handed or ambidextrous subjects than the nondiabetic group ($\chi^2 = 3.85$, $P = 0.05$). Because almost all of the non–right-handed individuals were within the diabetic group (11/95 vs. nondiabetic 1/49), we examined significant effects with and without these individuals. Results were the same regardless of the inclusion or exclusion of non–right-handed individuals. Controlling for age and sex, groups did not differ in whole brain volumes (F [1,140] = 1.06, $P = 0.31$) or total hippocampal volume (absolute, F [1,140] = 1.22, $P = 0.27$; relative, F [1,140] = 0.34, $P = 0.56$).

Hypoglycemia and hyperglycemia. Within the group of 95 youth with diabetes, 37 subjects had no severe hypoglycemia in the past (0 hypo), 41 experienced 1–2 severe hypoglycemic episodes (1–2 hypo; mean number of episodes = 1.2, SD = 0.4), and 17 had three or more severe hypoglycemic episodes in the past (3+ group; mean number of episodes = 9.2, SD = 11.2; mean without individual with 50 episodes = 6.7, SD = 2.8). Mean age did not differ across these groups or compared with nondiabetic siblings (F [1,140] = 0.32, $P = 0.81$). Sex ($\chi^2 = 4.3$, $P = 0.23$) and handedness ($\chi^2 = 5.1$, $P = 0.17$) distributions were also similar across subgroups (Table 1).

The total number of hypoglycemic events characterized as severe was 205 (within subjects with any past hypoglycemia, mean = 3.5, SD = 6.9, range = 1–50; excluding the individual with 50 episodes, mean = 2.7, SD = 3.1, range = 1–15). All episodes involved the inability to self-treat and successful response to glucose or glucagon treatment (20 or 9.8% with glucagon shots, 16 or 7.8% with intravenous glucose administration, and the rest with food or drink). Most (179 or 87.3%) episodes had reported neurological symptoms (e.g., incoherence, hallucinations, hemiparesis, disorientation, failure to arouse, impaired speech, discoordination, seizures [79 or 38.5%], or loss of consciousness [91 or 44.4%]). Those episodes without reported clear neurological symptoms had verified low glucose levels or other somatic symptoms including vomiting, dizziness, shakiness, and slowed breathing.

Hypoglycemia frequency subgroups did not differ from each other or from nondiabetic siblings in ASF (F [3,138] = 0.57, $P = 0.64$). However, groups did differ in absolute (F [3,138] = 2.9, $P = 0.036$) and relative (F [3,138] = 3.6, $P = 0.016$) total hippocampal volumes. Because this effect was consistent for both relative and absolute hippocampal volumes and relative volumes are corrected for whole brain size, we report from here forward on relative hippocampal volumes only. Post hoc comparisons for relative hippocampal volumes revealed that the 3+ subgroup had significantly larger relative hippocampal volume than all other groups, including sibling control subjects ($P < 0.02$) (Fig. 2*A*). When considering only subjects with diabetes and additionally controlling for age of onset, this effect was strengthened (F [2,89] = 6.7, $P = 0.002$; 3+ larger than 0 and 1–2 hypo groups, $P < 0.006$). This effect was consistent across left (effect of group, F [2,89] = 6.7, $P = 0.002$; 3+ greater than 0 and 1–2 hypo groups, $P < 0.003$) and right (effect of group, F [2,89] = 6.2, $P = 0.003$; 3+ greater than 0 and 1–2 hypo groups, $P < 0.02$) hippocampal volumes (Table 1).

To determine whether other characteristics of hypoglycemia besides frequency category (0, 1–2, 3+ episodes) were also associated with total relative hippocampal volumes, we explored the impact of timing (whether any hypoglycemia episodes occurred before or after age 5 years) and severity (whether any episodes involved seizures or loss of consciousness) on volumes. Within participants who had experienced a severe hypoglycemic

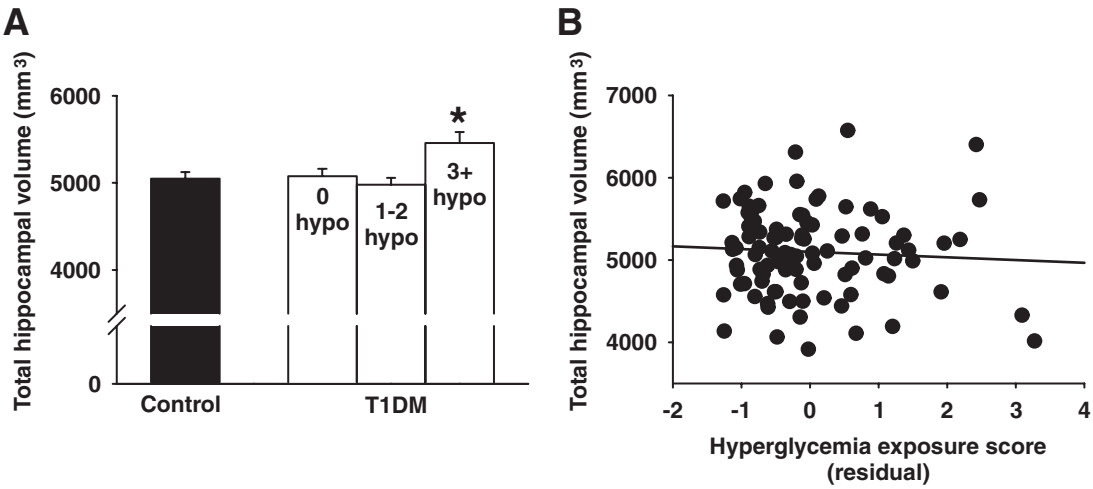

FIG. 2. *A*: **Effects of repeated severe hypoglycemia on mean ± SEM total relative hippocampal volumes (corrected for age and sex). The 3+ group had larger hippocampal volume than all other groups ($P < 0.05$).** *B*: **Hyperglycemia exposure did not correlate with hippocampal volumes across the entire type 1 diabetes group after controlling for age, sex, and age of onset ($P = 0.58$). T1DM, type 1 diabetes.**

episode in the past, there was no effect of age of first episode (younger than 5 years vs. older than 5 years; F [1,51] = 0.06, $P = 0.81$) or interaction between age of first episode category and frequency category (F [1,51] = 0.06, $P = 0.81$) on total relative hippocampal volume. Likewise, the presence of seizures or loss of consciousness did not influence hippocampal volume (F [1,51] = 0.99, $P = 0.32$), and there was no interaction between presence of seizures or loss of consciousness and frequency category on hippocampal volume (F [1,51] = 0.23, $P = 0.63$). Finally, a history of diabetic ketoacidosis did not influence hippocampal volumes ($P = 0.53$), and controlling for history of diabetic ketoacidosis did not alter the effect of hypoglycemia frequency on hippocampal volumes ($P = 0.025$).

To address the possibility of sex confounding the hypoglycemia frequency subgroups (nonsignificantly higher proportion of females in the 3+ group; Table 1), a secondary analysis within each sex category was performed separately. The effect of hypoglycemia subgroup (controlling for age and age of onset) was still significant in both the female-only subset (F [2,36] = 3.4, $P = 0.04$) and the male-only subset (F [2,49] = 3.6, $P = 0.03$), despite lower sample sizes and reduced power.

After excluding subjects with A1C coverage of <30%, the remaining subjects had a normally distributed distribution of coverage, with an average of 68% (SD = 14) of their duration of diabetes covered by existing A1C values. Hyperglycemia exposure scores (median A1C weighted for duration of diabetes) differed across hypoglycemia subgroups (F [2,92] = 20.3, $P < 0.001$), and all groups were significantly different from one another ($P < 0.003$; Table 1). However, duration of diabetes (F [2,92] = 23.7, $P < 0.001$) but not median A1C (F [2,92] = 1.9, $P = 0.16$) also differed across groups in a similar fashion (Table 1), suggesting that this effect was largely, but not exclusively, driven by the earlier age of onset of the 3+ hypoglycemia group. Regardless, when each of these confounding variables (median A1C, duration, and age of onset) was covaried, the effect of hypoglycemia frequency on relative hippocampal volumes remained significant ($P < 0.005$).

Within the entire diabetic sample, hyperglycemia exposure was not associated with relative hippocampal volume after controlling for age, sex, and age of onset (R^2 change = 0.003, F [1,89] = 0.31, $P = 0.58$, semipartial r = 0.06; one outlier removed for high median A1C) (Fig. 2*B*). Among diabetic participants without any severe hypoglycemia in their past, hyperglycemia exposure also did not correlate with hippocampal volumes ($R^2 = 0.02$, F [1,31] = 0.73, $P = 0.40$).

DISCUSSION

Greater exposure to severe hypoglycemia during childhood was associated with enlargement of hippocampal gray matter volume in youth with type 1 diabetes. This effect was not explained by age, sex, degree of hyperglycemia exposure, age of onset, or duration of disease and was equivalent for both hemispheres. Although the direction of the effect was unexpected, the fact that the subset of youth with three or more severe hypoglycemic episodes in their past was different from all other groups, including sibling control subjects, supports the sensitivity of the hippocampus to effects of repeated hypoglycemic episodes during brain development. These data do not support the idea that chronic hyperglycemia in childhood affects gray matter volume in the hippocampus.

These findings are in contrast to the existing, although limited, data on adults with diabetes. Case studies of adults who suffered profoundly severe hypoglycemia have reported distinct neuronal death in the hippocampus bilaterally (1,2,7,39). However, one study in adults with type 1 diabetes found that hippocampal volumes were comparable with control subjects (1) ($n = 13$). Given the small sample sizes in that study, statistical power likely limited the ability to detect differences in hippocampal volumes. In contrast, a slightly larger study of adults with type 2 diabetes ($n = 23$) found reduced hippocampal volume compared with control subjects and a correlation between hyperglycemia exposure and degree of volume loss (40). Thus, there is no precedent in the adult literature for increased volume of the hippocampus in association with diabetes, hyperglycemia, or hypoglycemia.

The single study using quantified hippocampal volumes in children with type 1 diabetes compared those with versus without hypoglycemia-related seizures and those with early versus late hypoglycemia-related seizures on whole brain gray matter volume and absolute hippocampal volume (14). No control group was assessed. They found

no differences between diabetic groups in absolute hippocampal volumes, but total gray matter volume did tend to be smaller in youth with previous seizures. Thus, if relative hippocampal volumes had been calculated, it is possible that larger relative hippocampal volumes in those with hypoglycemia-related seizures would have been observed. Notably, our study found both enlarged absolute and relative hippocampal volumes in those with multiple severe hypoglycemic episodes. Other differences in the methodology of that study, such as very strict definition of severe hypoglycemia, failure to correct for sex effects, and relatively small sample sizes, make direct comparisons with our findings difficult.

Interestingly, there is a precedent for abnormal enlargement of the hippocampus in other developmental disorders. Hippocampal enlargement has been noted in children with fragile X syndrome (41), autism, and attention-deficit hyperactivity disorder (ADHD). For example, Plessen et al. (42) found increased hippocampal volumes in children with combined type ADHD ($n = 51$; 6–18 years old) compared with control subjects ($n = 63$). However, within the ADHD group, increased symptoms were associated with smaller hippocampi volumes. The authors speculate that the enlargement could thus reflect a compensatory response to ADHD; this is supported by "abundant preclinical evidence for the presence of synaptic remodeling and neurogenesis within the hippocampus . . . in response to experiential demands." Enlargement of the hippocampus has also been reported in children (7.5–18.5 years of age) with autism, both with and without mental retardation compared with control subjects but not in adults with autism (43). The authors suggest that this could be due to "use-dependent expansion of hippocampal connections" or pathological development, perhaps because of reduced programmed cell death (44,45).

Based on these reports and the explanations that are proposed, we speculate that enlargement of the hippocampus in our study could reflect a pathological reaction to repeated severe hypoglycemia during development such as gliosis, disruption of normal developmental pruning or reactive neurogenesis, or a compensatory developmental response to injured neurons. There is little relevant experimental evidence addressing possible mechanisms of altered hippocampal volume in response to hypoglycemia. Estrada et al. (46) reported increased transient glial reactivity followed by prolonged and enhanced neurogenesis in the hippocampus after hypoglycemia, but these studies were performed in adult rats. In contrast, Yamada et al. (10) found no change in neurogenesis in the hippocampal dentate granule cell region after moderate hypoglycemia in immature rats, suggesting that this mechanism may not be responsible for our results. Further animal experimentation would be necessary to address other possible mechanisms of hippocampal enlargement in response to hypoglycemia during development.

Our data in a large sample indicate that there is no significant relationship between degree of hyperglycemia exposure and hippocampal volumes. This stands in contrast to some animal work, suggesting that neurons in the hippocampi of immature animals are more affected by hyperglycemia than hypoglycemia (47). Perhaps greater severity or duration of hyperglycemia exposure is necessary for changes in the hippocampal structure to be detected during development. Importantly, the lack of a relationship between hyperglycemia and volume does not rule out the possibility of functional effects of hyperglycemia within the hippocampus. Studies using functional MRI or other functional imaging measures would be needed to test this possibility.

These findings highlight the possibility that metabolic insults that occur during development can have a qualitatively different impact on brain structure than those occurring in a fully mature system. For example, early damage to the hippocampus and surrounding medial temporal region can produce long-term memory deficits, similar to those found in adults, but can also induce unique social and cognitive deficits (e.g., response inhibition) generally associated with prefrontal dysfunction in adults (48–50). Researchers have speculated that these effects could be due to developmental disruption of connections between the hippocampal region and the prefrontal cortex (48–50). As noted by Ennis et al. (11), differential effects of metabolic insults on the brain depending on age could be explained by the developmental stage of the cells affected or changes in the density or function of neurotransmitter receptors.

This highly focused study tested hypotheses about the effects of hypoglycemia and hyperglycemia during development on the gray matter of the hippocampus. Because of this focus, we cannot address possible effects in white matter of the hippocampus or other gray or white matter regions of the brain. In addition, the function of hippocampus could be affected in addition to or in the absence of structural change. Due to limitations in the resolution and methods of MRI, we cannot distinguish among possible mechanisms of structural change. Finally, our cross-sectional study would be complemented by prospective measurement of hippocampal volume and more detailed analyses of hippocampal shape or subfield volume changes over time. Future analyses may also take into account behavioral and cognitive correlates of hippocampal differences. However, our study reports on the largest sample of youth with type 1 diabetes assessed with highly reliable, unbiased, and gold standard measures of hippocampal gray matter volume. Using these powerful techniques, we found an intriguing pattern of results that must be taken into account in neurodevelopmental theories of the brain's response to insults. In addition, our findings support the idea of a special relationship between hypoglycemia and the hippocampus during development.

ACKNOWLEDGMENTS

We thank Abraham Snyder, MD, for guidance with the atlas registration and image alignment procedures and Yvette Sheline, MD, for help with the hippocampal boundaries and stereology procedures.

No potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the 68th Scientific Sessions of the American Diabetes Association, San Francisco, California, 6–10 June 2008.

REFERENCES

1. Auer RN, Hugh J, Cosgrove E, Curry B. Neuropathological findings in three cases of profound hypoglycemia. Clin Neuropath 1989;8:63–68
2. Chalmers J, Risk MT, Kean DM, Grant R, Ashworth B, Campbell IW. Severe amnesia after hypoglycemia: clinical, psychometric, and magnetic resonance imaging correlations. Diabetes Care 1991;4:922–925
3. Jones EL, Smith WT. Hypoglycaemic brain damage in the neonatal rat. In *Clinics in Developmental Medicine*. London, William Heinemann Medical Books, 1971, p. 231–241
4. Auer RN, Olsson Y, Siesjö BK. Hypoglycemic brain injury in the rat:

correlation of density of brain damage with the EEG isoelectric time: a quantitative study. Diabetes 1984;33:1090–1098
5. Myers RE, Kahn KJ. Insulin-induced hypoglycemia in the non-human primate, II: Long-term neuropathological consequences. In *Clinics in Developmental Medicine*. London, William Heinemann Medical Books, 1971, p. 195–206
6. Sieber FE, Traystman RJ. Special issues: glucose and the brain. Crit Care Med 1992;20:104–114
7. Holemans X, Dupuis M, Misson N, Vanderijst JF. Reversible amnesia in a Type 1 diabetic patient and bilateral hippocampal lesions on magnetic resonance imaging (MRI). Diabet Med 2001;18:761–763
8. McCall AL. The impact of diabetes on the CNS. Diabetes 1992;41:557–570
9. Ouyang YB, He QP, Li PA, Janelidze S, Wang GX, Siesjö BK. Is neuronal injury caused by hypoglycemic coma of the necrotic or apoptotic type? Neurchem Res 2000;25:661–667
10. Yamada KA, Rensing N, Izumi Y, De Erausquin GA, Gazit V, Dorsey DA, Herrera DG. Repetitive hypoglycemia in young rats impairs hippocampal long-term potentiation. Pediatr Res 2004;55:372–379
11. Ennis K, Tran PV, Seaquist ER, Rao R. Postnatal age influences hypoglycemia-induced neuronal injury in the rat brain. Brain Res 2008;1224:119–126
12. Perantie DC, Wu J, Koller JM, Lim A, Warren SL, Black KJ, Sadler M, White NH, Hershey T. Regional brain volume differences associated with hyperglycemia and severe hypoglycemia in youth with type 1 diabetes. Diabetes Care 2007;30:2331–2337
13. Lawson JA, Vogrin S, Bleasel AF, Cook MJ, Burns L, McAnally L, Pereira J, Bye AM. Predictors of hippocampal, cerebral, and cerebellar volume reduction in childhood epilepsy. Epilepsia 2000;41:1540–1545
14. Ho MS, Weller NJ, Ives FJ, Carne CL, Murray K, Vanden Driesen RI, Nguyen TP, Robins PD, Bulsara M, Davis EA, Jones TW. Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus. J Pediatr 2008;153:385–390
15. Saykin AJ, Gur RC, Sussman NM, O'Conner MJ, Gur RE. Memory deficits before and after temporal lobectomy: effect of laterality and age of onset. Brain Cogn 1989;9:191–200
16. McMillan TM, Powell GE, Janota I, Polkey CE. Relationships between neuropathology and cognitive functioning in temporal lobectomy patients. J Neurol Neurosurg Psychiatr 1987;50:167–176
17. Zaidel DW, Esiri MM, Oxbury JM. Regional differentiation of cell densities in the left and right hippocampi of epileptic patients. J Neurol 1993;240:322–325
18. Mathern GW, Pretorius JK, Babb TL. Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. J Neurosurg 1995;82:220–227
19. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. Diabetologia 2003;46:1604–1610
20. Klein JP, Waxman SG. The brain in diabetes: molecular changes in neurons and their implications for end-organ damage. Lancet Neurol 2003;2:548–554
21. Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain Res 1998;800:125–135
22. Perantie DC, Lim A, Wu J, Weaver P, Warren SL, Sadler M, White NH, Hershey T. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. Pediatr Diabetes 2008;9:87–95
23. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113
24. The DCCT Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. Am J Med 1991;90:450–459
25. Hershey T, Perantie DC, Warren SL, Zimmerman EC, Sadler M, White NH. Frequency and timing of severe hypoglycemia affects spatial memory in children with type 1 diabetes. Diabetes Care 2005;28:2372–2377
26. Black KJ, Snyder AZ, Koller JM, Gado MH, Perlmutter JS. Template images for nonhuman primate neuroimaging, 1: baboon. Neuroimage 2001;14:736–743
27. Black KJ, Koller JM, Snyder AZ, Perlmutter JS. Template images for nonhuman primate neuroimaging, 2: macaque. Neuroimage 2001;14:744–748
28. Talairach J, Tournoux P. *Co-Planar Stereotaxic Atlas of the Human Brain*. New York, Theime Verlag, 1988
29. Lancaster JL, Glass TG, Lankipalli BR, Downs H, Mayberg HS, Fox PT. A modality-independent approach to spatial normalization of tomographic images of the human brain. Hum Brain Mapp 1995;3:209–223
30. Jack CR Jr, Theodore WH, Cook M, McCarthy G. MRI-based hippocampal volumetrics: data acquisition, normal ranges and optimal protocol. Magn Reson Imaging 1995;13:1057–1064
31. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 1996;93:3908–3913
32. Buckner RL, Head D, Parker J, Fotenos AF, Marcus D, Morris JC, Snyder AZ. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage 2004;23:724–738
33. Mega MS, Small GW, Xu ML, Felix J, Manese M, Tran NP, Dailey JI, Ercoli LM, Bookheimer SY, Toga AW. Hippocampal atrophy in persons with age-associated memory impairment: volumetry within a common space. Psychosom Med 2002;64:487–492
34. Mayhew TM, Olsen DR. Magnetic resonance imaging (MRI) and model-free estimates of brain volume determined using the Cavalieri principle. J Anat 1991;178:133–144
35. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987;147(pt 3):229–263
36. Black KJ. On the efficiency of stereologic volumetry as commonly implemented for three-dimensional digital images. Psychiatry Res 1999;90:55–64
37. Duvernoy HM. *Human Hippocampus: An Atlas of Applied Anatomy*. Munich, Germany, Bergmann Verlag, 1998, p. 1–166
38. Bartzokis G, Mintz J, Marx P, Osborn D, Gutkind D, Chiang F, Phelan CK, Marder SR. Reliability of in vivo volume measures of hippocampus and other brain structures using MRI. Magn Reson Imaging 1993;11:993–1006
39. Kalimo H, Olsson Y. Effects of severe hypoglycemia on the human brain. Neuropathological case reports. Acta Neurol Scand 1980;62:345–356
40. Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. Diabetologia 2007;50:711–719
41. Reiss AL, Lee J, Freund L. Neuroanatomy of fragile X syndrome: the temporal lobe. Neurology 1994;44:1317–1324
42. Plessen KJ, Bansal R, Zhu H, Whiteman R, Amat J, Quackenbush GA, Martin L, Durkin K, Blair C, Royal J, Hugdahl K, Peterson BS. Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 2006;63:795–807
43. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL, Amaral DG. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 2004;24:6392–6401
44. Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, Vauss YC, Rapoport JL. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4–18 years. J Comp Neurol 1996;366:223–230
45. Giedd JN, Castellanos FX, Rajapakse JC, Vaituzis AC, Rapoport JL. Sexual dimorphism of the developing human brain. Prog Neuropsychopharmacol Biol Psychiatry 1997;21:1185–1201
46. Estrada FS, Hernandez VS, Medina MP, Corona-Morales AA, Gonzalez-Perez O, Vega-Gonzalez A, Zhang L. Astrogliosis is temporally correlated with enhanced neurogenesis in adult rat hippocampus following a glucoprivic insult. Neurosci Lett 2009;459:109–114
47. Malone JI, Hanna S, Saporta S, Mervis RF, Park CR, Chong L, Diamond DM. Hyperglycemia not hypoglycemia alters neuronal dendrites and impairs spatial memory. Pediatr Diabetes 2008;9:531–539
48. Bachevalier J. Medial temporal lobe structures and autism: a review of clinical and experimental findings. Neuropsychologia 1994;32:627–648
49. Lipska BK, Halim ND, Segal PN, Weinberger DR. Effects of reversible inactivation of the neonatal ventral hippocampus on behavior in the adult rat. J Neurosci 2002;22:2835–2842
50. Bilder RM, Bogerts B, Ashtari M, Wu H, Alvir JM, Jody D, Reiter G, Bell L, Lieberman JA. Anterior hippocampal volume reductions predict frontal lobe dysfunction in first episode schizophrenia. Schizophr Res 1995;17:47–58