Hippocampal Volumes in Youth With Type 1 Diabetes

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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 (R² 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.

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-

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. Handicapped 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 parent 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 from 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 amount of time represented by the A1C tests was calculated by multiplying the years were “late,” consistent with other work (25).

**RESULTS**

Hypoglycemia exposure was examined by comparing hypoglycemia frequency for age and sex, and for analyses of diabetic groups only, age of onset. Analyses were repeated 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+). Hypoglycemia was examined using our hypoglycemia exposure variable. The primary dependent measures were total relative and absolute hippocampal volumes and A1C as an index of total intracranial volume. For significant effects or interactions, post hoc correlations and 2-sample comparisons (t tests) were performed.
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 proportionately 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 \( (11/95 \text{ vs. } 58/94) \), 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 \( (F[1,110] = 1.06, P = 0.31) \) 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 \( (179 \text{ or } 87.3\%) \) episodes had reported neurological symptoms (e.g., incoherence, hallucinations, hemiparesis, disorientation, failure to arouse, impaired speech, discoordination, seizures \( [79 \text{ or } 38.5\%] \), or loss of consciousness \( [91 \text{ or } 44.4\%] \)). 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. 2A). When considering only subjects with diabetes and additionally controlling for age of onset, this effect was strengthened \( (F[2,89] = 6.7, P = 0.002; 3+ \text{ larger than } 0 \text{ and } 1–2 \text{ hypo groups, } P < 0.006) \). This effect was consistent across left (effect of group, \( F[2,89] = 6.7, P = 0.002; 3+ \text{ greater than } 0 \text{ and } 1–2 \text{ hypo groups, } P < 0.003 \) and right (effect of group, \( F[2,89] = 6.2, P = 0.003; 3+ \text{ greater than } 0 \text{ and } 1–2 \text{ hypo groups, } P < 0.02) \) hippocampal volumes (Table 1).

To determine whether other characteristics of hypoglycemia besides frequency category \( (0, 1–2, 3+ \text{ episodes}) \) 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
After controlling for age, sex, and age of onset (P < 0.05), the 3+ group had larger hippocampal volume than all other groups (P < 0.05). Time 1 diabetes group after controlling for age, sex, and age of onset (P = 0.58). TIDM, type 1 diabetes.

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

![Diagram showing 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). TIDM, type 1 diabetes.](https://diabetes.diabetesjournals.org/DIABETES_VOL_59_JANUARY_2010_239)
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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.

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