Hippocampal Volumes in Youth with Type 1 Diabetes

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Running Title: 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 controls ages 7-17. Youth with diabetes were categorized as having either 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 HbA1c, 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=.016; 3+ larger than all other groups, p<.05). Hyperglycemia exposure was not associated with hippocampal volumes (R² change =.003, F(1,89)=.31, p=.58, semi-partial r=.06; one outlier removed for high median HbA1c), 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=.002; 3+ larger than all other groups, p<.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 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, two relevant papers have both not found increased neuronal death in the hippocampus following 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 paper reported an increased incidence of medial temporal sclerosis in diabetic children with early hypoglycemia (before 6 years of age) compared to those with later hypoglycemia (6 or older), but not compared to 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 T2DM (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 MRI and glycemic history from a large sample of youth with type 1 diabetes and non-diabetic siblings who have already been characterized cognitively (22). We compared 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.
MATERIALS AND METHODS

Subjects: Children aged 7-17 with type 1 diabetes and non-diabetic siblings (controls) 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 weeks 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 DCCT 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, 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 at age 5 or before were considered “early” and those occurring after age 5 were “late”, consistent with other work (25).

Chronic exposure to hyperglycemia was calculated by collecting all available hemoglobin A1c (HbA1c) 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 HbA1c 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 HbA1c “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 HbA1c and duration variables were transformed to z scores, and each patient’s pair of z scores was summed (HbA1c 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 only can be interpreted to indicate hyperglycemia exposure relative to this sample.

Image Acquisition. Structural magnetic resonance images (MRIs) were acquired for each subject on a Siemens Sonata 1.5 Tesla imaging system with a standard Siemens 30 cm circularly polarized RF head coil. For each subject, 3 to 5 scans consisting of 128 contiguous 1.25 mm sagittal slices were acquired using magnetization prepared rapid gradient echo (MPRAGE; TR=1900 ms, TE=3.93 ms, flip angle=15 degrees, matrix = 256 x 256 pixels, voxel size = 1x1x1.25 mm, single scan time=7 min, 7 sec). 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; 3 subjects were excluded for confirmed brain abnormalities (2 were benign anomalies but discarded due to possible difficulty with registration).

**Image Preprocessing.** The 3 highest quality images were co-registered by an automated, validated technique (26;27), averaged for each subject and atlas transformations computed. We used a target image comprised of 24 brains – one for every age and gender between ages 7-18 – made to represent the atlas of Talairach & Tournoux (28) as modified by Lancaster et al (29). The co-registered, averaged, atlas-transformed data were resampled to a standard, 0.5mm 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) vs. absolute volumes are related by a known multiplicative factor (the determinant of the transform matrix or Atlas Scaling Factor [ASF]) which 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) which otherwise occur due to variability of brain size and head orientation (33).

**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 approximately 150 selected grid points per hippocampus, a number which has previously been determined to yield reliable measurements in brain volume determination (36). Measures were performed on coronal slices every 5 slices (2.5 mm) within a 3D MRI cubical subvolume composed of 0.5 x 0.5 x 0.5mm voxels (Figure 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 4 measurements of each volume, two measurements from each rater. Average measures intra-class 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, gender, 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 diabetes vs. non-diabetic controls:** Ninety-five youth with type 1 diabetes and 49 non-diabetic siblings had adequate imaging and glycemic 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=.13, p=.11) or age and absolute total hippocampal volume (r=-.04, p=.66). There was a very modest but non-significant correlation between age and relative hippocampal volume (r=-.16, p=.06). Absolute hippocampal volumes (t=-2.77, p=.006) and ASF (t=-7.38, p<.001) were greater for males (n=79) compared to females (n=65). However, males had smaller relative hippocampal volumes than females (Males, mean volume = 4994mm³; Females mean volume = 5195mm³; t=2.24, p=.03). To ensure that age and gender would not influence our other results, we covaried both factors from subsequent analyses.

Youth with type 1 diabetes and non-diabetic siblings did not differ significantly in gender distribution (Chi-squared=.44, p=.51), or mean age (t=.01, p=.99). The diabetic group had proportionally more left-handed or ambidextrous subjects than the non-diabetic group (Chi-squared=3.85, p=.05). Because almost all of the non-right handed individuals were within the diabetic group (11/95 vs. non-diabetic 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 gender, groups did not differ in whole brain volumes (F(1,140)=1.06, p=.31) or total hippocampal volume (absolute, F(1,140)=1.22, p=.27; relative, F(1,140)=.34, p=.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=.4) and 17 had 3 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 to non-diabetic siblings (F(1,140)=.32, p=.81). Gender (Chi-sq=4.3, p=.23) and handedness (Chi-sq=5.1, p=.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 (eg. incoherence, hallucinations, hemiparesis, disorientation, failure to arouse, impaired
speech, dis-coordination, 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 non-diabetic siblings in ASF (F(3,138)=.57, p=.64). However, groups did differ in absolute (F(3,138)=2.9, p=.036) and relative (F(3,138)=3.6, p=.016) total hippocampal volumes. Since 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 controls (p<.02) (Figure 2A). When considering only subjects with diabetes and additionally controlling for age of onset, this effect was strengthened (F(2,89)=6.7, p=.002; 3+ larger than 0 and 1-2 hypo groups, p<.006). This effect was consistent across left (effect of group, F(2,89)=6.7, p=.002; 3+ greater than 0 and 1-2 hypo groups, p<.003) and right (effect of group, F(2,89)=6.2, p=.003; 3+ greater than 0 and 1-2 hypo groups, p<.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) and severity (whether any episodes involved seizures or loss of consciousness) on volumes. Within participants who had experienced a severe hypoglycemic episode in the past, there was no effect of age of first episode (before age 5 vs. after age 5; F(1,51)=.06, p=.81) or interaction between age of first episode category and frequency category (F(1,51)=.06, p=.81) on total relative hippocampal volume. Likewise, the presence of seizures or loss of consciousness did not influence hippocampal volume (F(1,51)=.99, p=.32) and there was no interaction between presence of seizures or loss of consciousness and frequency category on hippocampal volume (F(1,51)=.23, p=.63). Finally, a history of diabetic ketoacidosis did not influence hippocampal volumes (p=.53), and controlling for history of DKA did not alter the effect of hypoglycemia frequency on hippocampal volumes (p=.025).

To address the possibility of gender confounding the hypoglycemia frequency subgroups (non-significantly higher proportion of females in the 3+ group; see Table 1), a secondary analysis within each gender 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=.04) and the male-only subset (F(2,49)=3.6, p=.03), despite lower sample sizes and reduced power.

After excluding subjects with HbA1c 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 HbA1c values. Hyperglycemia exposure scores (median HbA1c weighted for duration of diabetes) differed across hypoglycemia subgroups (F(2,92)=20.3, p<.001) and all groups were significantly different from one another (p<.003; Table 1). However, duration of diabetes (F(2,92)=23.7, p<.001) but not median HbA1c (F(2,92)=1.9, p=.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 HbA1c, duration, age of onset) was covaried, the effect of hypoglycemia frequency on relative hippocampal volumes remained significant (p<.005).

Within the entire diabetic sample, hyperglycemia exposure was not associated with relative hippocampal volume after controlling for age, gender and age of onset (R² change = .003, F(1,89)=.31, p=.58, semi-partial r=.06; one outlier removed for high median HbA1c; See Figure 2B). Among diabetic participants without any severe hypoglycemia in their past, hyperglycemia exposure also did not correlate with hippocampal volumes (R²=.02, F(1,31)=0.73, p=.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, gender, 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 3 or more severe hypoglycemic episodes in their past were different from all other groups, including sibling controls, supports the sensitivity of the hippocampus to effects of repeated hypoglycemia 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, though 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 to controls (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 to controls 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 vs. without hypoglycemia-related seizures and those with early vs. 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 gender effects and relatively small sample sizes, make direct comparisons to 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 ADHD. For example, Plessen et al. (42) found increased hippocampal volumes in children with combined type ADHD (n= 51; 6-18
years old) compared to controls (n=63). However, within the ADHD group, increased symptoms were associated with smaller hippocampal 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 to controls 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 following hypoglycemia, but these studies were performed in adult rats. In contrast, Yamada et al. found no change in neurogenesis in the hippocampal dentate granule cell region following moderate hypoglycemia in immature rats (10), 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 fMRI 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. Due to 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 between 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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REFERENCES

| Table 1. Means (SD) for demographic and clinical variables |
|---------------------------------|------------------|------------------|------------------|------------------|------------------|
|                                | Non-diabetic controls | T1DM total sample | T1DM 0 hypo | T1DM 1-2 hypo | T1DM 3+ hypo |
|                                | n                  | Age (SD)         | % Female     | % Right handed | Age of Onset * |
|                                | 49                 | 12.4 (2.7)       | 49           | 98             |
|                                | 95                 | 12.4 (2.8)       | 43           | 88             |
|                                | 37                 | 12.1 (2.4)       | 38           | 89             |
|                                | 41                 | 12.5 (2.8)       | 39           | 85             |
|                                | 17                 | 12.9 (3.3)       | 65           | 94             |
|                                | n                  | % Duration of diabetes * |       |       |
|                                | 7.1 (3.2)          | 5.4 (2.8)        | 8.3 (1.0)    | 0 (1.4)        |
|                                | 8.5 (2.6)          | 3.7 (1.3)        | 8.2 (1.2)    | -.76 (1.2)     |
|                                | 6.8 (3.2)          | 5.8 (2.6)        | 8.3 (0.8)    | .10 (1.1)      |
|                                | 4.7 (3.2)          | 8.1 (2.9)        | 8.7 (0.9)    | 1.48 (1.2)     |
|                                | n                  | Median HbA1c (SD) |       |       |
|                                | --                 | 8.3 (1.0)        | 8.2 (1.2)    | 0.10 (1.1)     |
|                                |                    | 8.3 (0.8)        | 8.7 (0.9)    | 1.48 (1.2)     |
|                                | n                  | Hyperglycemia Exposure score * |       |       |
|                                | --                 | 0 (1.4)          | -.76 (1.2)   | 0.10 (1.1)     |
|                                |                    |                    | 0.10 (1.1)   | 1.48 (1.2)     |
|                                | n                  | Atlas Scaling Factor (unitless) |       |       |
|                                | 0.86 (0.8)         | 0.87 (0.8)       | 0.89 (0.8)   | 0.88 (0.8)     |
|                                |                    | 0.86 (0.7)       | 0.86 (0.07)  | 0.86 (0.07)    |
|                                | n                  | Total absolute hippocampal volume (mm³) |       |       |
|                                | 4339 (607)         | 4417 (541)       | 4487 (485)   | 4337 (462)     |
|                                |                    | 4680 (578)       | 4487 (485)   | 4337 (462)     |
|                                | n                  | Total relative hippocampal volume (mm³) † |       |       |
|                                | 5056 (581)         | 5085 (541)       | 5076 (492)   | 4965 (474)     |
|                                |                    | 5475 (545)       | 5076 (492)   | 4965 (474)     |
|                                | n                  | Right relative hippocampal volume (mm³) |       |       |
|                                | 2546 (307)         | 2577 (286)       | 2599 (254)   | 2515 (251)     |
|                                |                    | 2773 (300)       | 2599 (254)   | 2515 (251)     |
|                                | n                  | Left relative hippocampal volume (mm³) |       |       |
|                                | 2510 (285)         | 2507 (267)       | 2477 (252)   | 2450 (234)     |
|                                |                    | 2702 (285)       | 2477 (252)   | 2450 (234)     |

* all hypoglycemia subgroups are different from each other (p<.05)
† 3+ hypoglycemia group is different from all other subgroups (p<.05)

**Figure Legends**

Figure 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.

Figure 2: A) Effects of repeated severe hypoglycemia on mean (SEM) total relative hippocampal volumes (corrected for age and gender). The 3+ group had larger hippocampal volume than all other groups (p<.05). B) Hyperglycemia exposure did not correlate with hippocampal volumes across the entire T1DM group after controlling for age, gender and age of onset (p=.58).