Glycemic variability and brain glucose levels in T1DM

Janice J. Hwang, MD[1*], Lihong Jiang, PhD[2*], Elizabeth Sanchez Rangel, MD[1], Xiaoning Fan, MD, Yuyan Ding, BA, Wai Lam, BA, Jessica Leventhal, APRN, Feng Dai, PhD[3], Douglas L. Rothman, PhD[2], Graeme F. Mason, PhD[2,4], Robert S. Sherwin, MD[1]

* These authors contributed equally to this work

[1] Section of Endocrinology, Yale School of Medicine, New Haven, CT

[2] Department of Radiology & Biomedical Imaging, Yale School of Medicine, New Haven, CT

[3] Yale Center for Analytical Sciences, Yale School of Public Health, New Haven, CT

[4]Department of Psychiatry, Yale School of Medicine, New Haven, CT

Corresponding Author:

Janice Hwang

The Anlyan Center, TAC 119S

PO Box 208020

New Haven, CT 06520

203-785-4107 (phone)

203-737-5558 (fax)

Janice.Hwang@yale.edu

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ABSTRACT

The impact of glycemic variability on brain glucose transport kinetics amongst T1DM individuals remains unclear. 14 individuals with T1DM (age 35±4 years, BMI 26.0±1.4 kg/m^2, HbA1C 7.6±0.3) and 9 healthy control participants (age 32±4, BMI 23.1±0.8, HbA1C 5.0±0.1) wore a continuous glucose monitor (Dexcom) to measure hypoglycemia, hyperglycemia and glycemic variability for 5 days followed by ^{1}H magnetic resonance spectroscopy scanning in the occipital lobe to measure the change in intracerebral glucose levels during a 2-hour glucose clamp (target glucose 220 mg/dl). Hyperglycemic clamps were also performed in a rat model of T1DM to assess regional differences in brain glucose transport and metabolism. Despite similar change in plasma glucose levels during the hyperglycemic clamp, individuals with T1DM had significantly smaller increments in intracerebral glucose levels (P=0.0002). Moreover, amongst T1DM individuals, the change in brain glucose correlated positively with the lability index (r = 0.67, P=0.006). Consistent with findings in humans, streptozotocin-treated rats had lower brain glucose levels in the cortex, hippocampus, and striatum compared to control rats. These findings that glycemic variability is associated with brain glucose levels highlight the need for future studies to investigate the impact of glycemic variability on brain glucose kinetics.

The Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) trials established the benefits of lowering blood glucose to “near normal” levels in patients with type 1 diabetes mellitus (T1DM) (1-3). This has led to the widespread use of more intensified insulin therapy to prevent or delay diabetes complications. However, global application of intensive insulin therapy has been limited by a higher rate of severe hypoglycemia, often occurring without warning symptoms and thus reducing the patient’s ability to take corrective action (4). Furthermore, patients with diabetes often overcorrect for hypoglycemia leading to “yo-yoing” of glucose levels and increased glycemic variability. The impact of glycemic variability on clinical outcomes remains unclear; although, a growing body of literature has shown that increased glycemic variability is associated with adverse outcomes (5-8), including increased rates of cerebrovascular events (9), white matter hyperintensities (10) and cognitive decline (11). However, the underlying mechanisms for these associations remain unclear.

In healthy individuals, circulating glucose crosses the blood-brain barrier in a nearly linear pattern within normal physiologic ranges of plasma glucose levels (12-14). Moreover, there is some evidence that glucose transport kinetics can be modulated by both hyperglycemia as well as hypoglycemia (15-20). Prior studies have shown that T1DM patients receiving intensive insulin therapy are able to maintain brain glucose uptake during acute hypoglycemia, whereas healthy control subjects rendered hypoglycemic for several days show a 20-30% decrease (20; 21), suggesting that prior hypoglycemia exposure induces the human brain to more efficiently use glucose much like that seen in rodent studies (22; 23). Furthermore, individuals with high frequency of hypoglycemia and hypoglycemia unawareness have increased cerebral glucose concentrations during acute hyperglycemia (24), again consistent with the concept that frequent hypoglycemia can increase the brain’s capacity for glucose entry. However, other recent studies have found no evidence that recurrent hypoglycemia for 3 days in healthy volunteers leads to an increase in hypothalamic glucose transport under acute

hyperglycemic conditions (200-400 mg/dl) (25; 26). Thus, whether hypoglycemia alone is sufficient to drive increased glucose transport remains unknown. Conversely, in other studies, individuals with poorly controlled T2DM have significantly decreased brain glucose entry during acute hyperglycemia (27), while another study of both T1DM and T2DM individuals showed a tendency towards blunted brain glucose levels (18). Taken collectively, these studies suggest that hyperglycemia and hypoglycemia likely have opposing effects on brain glucose transport kinetics; but none of these studies have accounted for the impact of glycemic variability on brain glucose transport. Furthermore, because many studies in this area have relied on subjective patient questionnaires to assess frequency of hypoglycemia, which may induce recall bias, we sought to obtain objective measures of hypoglycemia, hyperglycemia and glycemic variability in the period immediately preceding brain scanning.

Thus, the current study was undertaken to investigate the factors that may modulate brain glucose transport and metabolism amongst individuals with T1DM by using hyperglycemic clamp techniques coupled with in vivo magnetic resonance spectroscopy. Furthermore, we also conducted a series of animal experiments to more deeply investigate the implications of altered brain glucose transport kinetics in T1DM.

RESEARCH DESIGN and METHODS

Participants

31 participants were recruited for this study, and 23 participants (9 healthy control and 14 individuals with T1DM) completed the study and were included in the analysis. The healthy control subjects were also included as part of another unrelated study (27). Exclusion criteria included smoking, illicit drug or recent steroid use; known psychiatric or neurological disorders, active infection, malignancy, abnormal thyroid function, cerebrovascular disease (defined as any history of stroke or transient ischemic attack), cardiovascular disease, hepatobiliary disease or

weight change in last 3 months, or inability to enter the MRI. Women who were breastfeeding, seeking pregnancy, or shown to be pregnant by urine test were also excluded.

Continuous glucose monitoring (CGM)

Five to 7 days prior to MRS scanning, all T1DM participants underwent placement of a continuous glucose monitor (Dexcom G4). All participants received instructions and training on proper use of the CGM. Frequency of mild hypoglycemia (plasma glucose < 70 mg/dl), moderate hypoglycemia (plasma glucose<50 mg/dl) and time spent in hypoglycemia were calculated using the last 5 days of CGM data immediately preceding scanning. For calculation of the lability index, the last 576 glucose values (representing the 48 hours immediately prior to scanning) were imported into Easy Glucose Variability (EasyGV) software (www.easygv.co.uk) (28) using a method adapted from Chan *et al* (29).

Scanning Protocol

On the evening prior to MRS scanning, individuals with T1DM were admitted to the Yale University Hospital Research Unit and placed on a standard hospital protocol insulin drip overnight to normalize plasma glucose levels. T1DM participants received an average of 9.7±2.1 units of insulin overnight and mean plasma glucose levels were 135±3 mg/dl overnight. The insulin infusion was maintained until the start of the scanning. At the start of scanning, the insulin infusion rate was fixed at 1 unit/hour for the duration of the study in order to minimize the risk of ketosis. Healthy control subjects arrived the morning of the scan. All groups were fasting. Hyperglycemia (target plasma glucose 220 mg/dl, 2 hours) was maintained using a variable 20% dextrose infusion adjusted every 5-10 minutes, as previously described (27). Insulin levels were measured at 0, 60, and 120 minutes.

^{1}H MRS Scanning

Participants were positioned supine in a 4.0 T whole-body magnet interfaced to a Bruker ParaVision 6.0 spectrometer (Bruker Instruments) with the head immobilized using foam inserts on top of a radiofrequency probe with a 1H circular surface coil as previously described (27; 30; 31). After tuning, calibration, and acquisition of scout images for anatomical localization, intracerebral glucose concentration signals were obtained using stimulated echo acquisition mode (STEAM) localization (32) in 30 X 20 X 30 mm3 voxels in the occipital lobe for 20 minutes at baseline and then every 10 minutes for at least 2 hours of hyperglycemia. The sequence parameters were TR = 2000 ms, TE/TM = 15 ms/10 ms, bandwidth = 5000 Hz, sampling points = 2048. Spectra were acquired with B0-lock and retrospective -frequency adjustment for motion correction.

Baseline spectra were subtracted from subsequently obtained spectra to eliminate overlap from other brain metabolites as previously described (9, 10). Changes in glucose levels were measured by peak integration referenced to creatine in the baseline spectra as a concentration reference. Glucose integrals were obtained by integrating the large peak from 3.32-3.54 ppm and scaled back to total intensity from 3.10-3.96 ppm in the free glucose spectrum (1:2.9), which contains 11 protons. The concentration of glucose was then compared to the creatine methyl peak in the in vivo spectrum, which was assumed to be 10 mmol/kg, and which represents 3 protons.

Calculation of $T_{max}/CMR_{glucose}$ was derived from known reversible Michaelis Menten models of glucose transport into the brain (13; 14). T_{max} is the maximum rate of blood-brain glucose transport, CMR_{gl} is the brain glucose utilization rate, V_d is the brain water space (33), and Δ_i is the difference between the steady-state brain glucose concentration during the infusion and baseline, and Δ_o is the analogous difference for the plasma glucose.

$$\frac{T_{max}}{CMR_{glucose}} = \frac{V_d + \frac{\Delta_i}{\Delta_o}}{V_d - \frac{\Delta_i}{\Delta_o}}$$

149

150 *Laboratory Analysis*

151 Plasma glucose levels were measured via glucose oxidase (YSI Inc.). Plasma free
152 insulin was measured by double antibody radioimmunoassay (Millipore).

153

154 *Statistical Analysis*

155 Analyses of every repeatedly measured variable, including intracerebral glucose and
156 plasma insulin levels, were performed using the mixed-effects regression model method, taking
157 into account the within-subject correlations of repeated measures using a combination of pre-
158 specified compound symmetry covariance matrix and an autoregressive covariance matrix. All
159 analyses were performed using SAS, version 9.4 (Cary, NC) and SPSS, version 24 (Armonk,
160 NY).

161

162 *Human subjects study approval*

163 The Yale University Human Investigation Committee approved the protocol and all
164 participants provided written informed consent prior to study participation.

165

166 *Animal studies*

167 Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 290–320 g were
168 housed in the Yale Animal Resource Center in temperature-controlled (22–23°C) and humidity-
169 controlled rooms with ad libitum access to water and normal chow (Agway Prolab 3000,
170 Syracuse, NY). Animals were acclimatized to a 12/12-h day/light cycle. The Yale University
171 Institutional Animal Care and Use Committee approved the experimental protocols.

Two groups of rats were studied: normal control and Streptozotocin-induced diabetes. Diabetes was induced using a single injection of streptozotocin (STZ) (65 mg/kg i.p. in saline) as previously described (34).

Animal Surgery

Approximately 7 days prior to the experiment and 14 days after STZ administration for STZ group, the animals underwent aseptic surgery for vascular catheter placement into the left carotid artery for blood sampling and right jugular vein for infusions. The animals were then allowed to recover for ~1 week in their home cages before undergoing the glucose clamp studies. During the ~3 weeks of diabetes, blood glucose was monitored once daily with an AlphaTRAK glucometer (Abbott Laboratories) to ensure adequate hyperglycemia. The mean glucose levels during this period for all rats was 531±69 mg/dl. In addition, the diabetic animals were treated with PZI Bovine Insulin (BCP, Houston, Texas).

Hyperglycemic clamp in normal and STZ rats

On the day of the study, overnight fasted STZ rats (n = 7) were connected to infusion pumps □1h prior to the start of the experiment and then left undisturbed to recover from handling stress. Following the recovery period, an insulin infusion of 25 $mU \cdot kg^{-1} \cdot min^{-1}$ was used to lower plasma glucose levels to between 110 and 120 mg/dl for 30 minutes prior to start of hyperglycemic clamp. Then, at the start of the hyperglycemic clamp the insulin infusion rate was maintained at 10 $mU \cdot kg^{-1} \cdot min^{-1}$ for the duration of the clamp. Overnight fasted normal SD rats (n = 6) did not receive insulin infusions during the study. At the start of the clamp, both groups underwent a primed-continuous intravascular infusion of 35% [1-^{13}C] glucose (99% ^{13}C labeled, Cambridge Isotope Lab). Plasma glucose was measured using a glucose analyzer (Analox Instruments, Lunenburg, MA) to adjust the [1-^{13}C] glucose infusion rate to maintain a stable 220-250 mg/dl glucose level until the end of the study at 30 minutes, as previously described (35-37).

Animal studies: NMR spectroscopy

Rats were euthanized with microwave irradiation immediately at the end of the infusion (Microwave applicator, Muromachi Kikai Co) operating at 5kw for 1.4 seconds. Brain regions of frontal cortex (CO), striatum (ST), Thalamus-hypothalamus (TH), Hippocampus (HI) and Cerebellum (CE) were separated and brain metabolites were extracted using a previously described ethanol extraction method (38). Brain tissues were ground with 0.1M HCl/methanol (2:1 v/v) using OMNI Bead Ruptor 24, (OMNI International, INC) followed by adding 60% ethanol+0.1M sodium phosphate buffer at pH 7.4 and mixed with Omni Bead Ruptor for 3 min and spun down at 15000g for 30 min using high speed centrifuge (Thermoscientific Sorvall legend X1R centrifuge). Supernatants were then lyophilized (Labconco Freezone 4.5 plus) and then dissolved in 5mm NMR tubes in H_2O 60%+40% D_2O with 100 µL of 10 mM sodium formate as an internal concentration reference. Brain extractions and plasma ^{13}C-enrichments were acquired using proton observed carbon-13 edited (POCE) magnetic resonance spectroscopy (NMR) using 500MHz Bruker Topspin 3.2. The NMR spectra were then analyzed using a software package NMRSpecs (version 1.0, Wuhan Institute of Physics and Mathematics, Wuhan, China) (39).

RESULTS

Participant characteristics

14 individuals with T1DM and 9 healthy control (HC) subjects participated in this study. Compared to the healthy control group, participants with T1DM were similar in age, gender and BMI (Table 1). As expected, T1DM individuals had significantly higher HbA1C levels compared to control subjects. Plasma free insulin levels were not different between groups at the start of the study (P=0.10); however, as anticipated, HC participants had a marked increase in plasma insulin levels in response to hyperglycemia (baseline insulin 9.0±0.9 µU/ml vs. end insulin

104.9±26 μU/ml, P=0.01), which was absent amongst the individuals with T1DM (baseline insulin 34.8±11 μU/ml vs. end insulin 25.5±3.7 μU/ml, P=0.45). Individuals with T1DM had a mean duration of diabetes of 16 ± 3 years. 10 of the individuals with T1DM used insulin pump therapy while the remaining 4 participants used basal/bolus regimens. Using the Clarke score (40) to assess awareness of hypoglycemia, 8 of the T1DM participants reported impaired awareness of hypoglycemia. There were no differences in the change in brain glucose levels between T1DM-Aware and T1DM-Unaware individuals (T1DM-Aware mean 0.93±0.07 mmol/l vs T1DM-Unaware 1.0±0.1 mmol/l, P=0.58), therefore all T1DM participants were analyzed together. Based on the reports from the continuous glucose monitor obtained in the 5 days prior to scanning, there were no differences in rates of mild hypoglycemia (defined as glucose <70mg/dl) or moderate hypoglycemia (glucose <50 mg/dl) or total time spent in hypoglycemia between the individuals who were aware or unaware of hypoglycemia by Clarke score. Plasma beta-hydroxybutyrate (BHB) concentrations were higher amongst T1DM individuals compared to healthy control subjects at the end of the study (BHB T1DM mean 0.28±0.07 mM vs Control 0.02±0.002 mM, P = 0.004).

Plasma and brain glucose levels

Using a mixed-effects regression model, plasma glucose levels were slightly higher amongst T1DM participants compared to HC subjects over the course of the study (least square mean difference at time 120 min, 0.93; 95% CI, 0.1, 1.75, P=0.03) (Figure 1A). However, taking the glucose values at steady state (averaging the plasma glucoses from time 60-120 minutes), there were no differences in the change in plasma glucose levels between groups (change in plasma glucose HC, 7.2±0.2 mmol/l vs. 7.1±0.3 mmol/l in T1DM, P=0.74) (Figure 1B). Despite nearly identical increments in plasma glucose levels during the study, individuals with T1DM had significantly lower increments in intracerebral glucose levels (overall between-group fixed effects, P=0.002; least square mean difference at time 120 min, -0.61; 95% CI -0.90, -0.32,

P<0.0001, Figure 1C) and the change in brain glucose at steady state (averaged from time 60-120 minutes) also differed significantly (HC 1.46±0.1 mmol/l vs T1DM 1.03±0.07 mmol/l, P=0.006, Figure 1D).

Using a reversible Michaelis-Menten model for glucose transport kinetics (12; 14; 41), we calculated the ratio of transport capacity for glucose to cerebral metabolic rate of glucose (T_{max}/CMR_{gluc}). Based on these calculations, the T_{max}/CMR_{gluc} was also significantly lower amongst individuals with T1DM compared to control subjects (HC 1.74±0.09 vs. T1DM 1.49±0.04, P=0.01)(Figure 2).

Relationships between hyperglycemia, hypoglycemia, glycemic variability and brain glucose

Based on the reports from the continuous glucose monitor obtained in the 5 days prior to scanning, there were no relationships between rates of mild hypoglycemia (defined as glucose <70mg/dl) or moderate hypoglycemia (glucose <50 mg/dl), % time spent in hypoglycemia, or % time spent in hyperglycemia (glucose > 180 mg/dl) and brain glucose levels amongst individuals with T1DM. Next, we examined the relationship between glycemic variability metrics obtained from the continuous glucose monitor and brain glucose. Notably, amongst individuals with T1DM, the lability index of blood glucose levels correlated significantly and positively with change in brain glucose (r = 0.67, P = 0.006) as well as the T_{max}/CMR_{gluc} (r = 0.68, P = 0.008) (Figure 3).

Animal experiments

Given that the spectra in human subjects were obtained in the occipital lobe, we conducted experiments using a rat model to investigate if there are regional differences in brain glucose transport in response to acute hyperglycemia. Two groups of rats, normal control and streptozotocin-induced diabetic (STZ) rats underwent a hyperglycemic clamp for 30 minutes. There were no differences between groups in the plasma glucose levels immediately before

(control 113±4 mg/dl vs. STZ 118±6 mg/dl, P=0.58) and after the experiment (control 300±20 mg/dl vs. STZ 293±11 mg/dl, P=0.75). Similar to our observations in the occipital lobe of the human brain, STZ rats had significantly decreased absolute concentrations of glucose in the cortex (Control 1.49±0.2 μmol/g v STZ 0.90±0.1 μmol/g, P=0.03), hippocampus (Control 2.08±0.3 μmol/g v STZ 1.05±0.3 μmol/g, P=0.04) and striatum (Control 1.75±0.2 μmol/g v STZ 1.06±0.08 μmol/g, P=0.005) compared to normal control rats (Figure 4A).

Moreover, the rat studies were performed using infusions of [1-^{13}C]glucose, which also provided measurements of glucose oxidation rates in the various brain regions. By measuring the flow of the ^{13}C label from glucose to glutamate C4 (reflecting the first turn of the TCA cycle) and glutamate C3 (reflecting the second turn of the TCA cycle), we are able to assess the rate of the TCA cycle (42; 43) (Figure 4B). Despite changes in absolute glucose concentrations, there were no detectable differences in TCA flux during acute hyperglycemia between the control and STZ groups.

DISCUSSION

While much scientific attention has been focused on the impact of frequency of hypoglycemia (either experimentally induced or by patient self-report) on cerebral glucose transport and metabolism, fewer studies have investigated the impact of chronic hyperglycemia, and even fewer studies have directly investigated the impact of glycemic variability on brain glucose transport kinetics. With the growing use of continuous glucose monitoring technology and new integrated insulin delivery systems, understanding the impact of glycemic variability on the human brain is of critical clinical importance not only for understanding physiologic responses to diabetes, but also to inform the development of these novel therapeutics.

In this study, we show that individuals with T1DM have a blunted rise in brain glucose levels compared to healthy control subjects during an acute episode of modest hyperglycemia. This finding is consistent with another study which pooled a cohort of T1DM and T2DM

299 individuals and found a non-significant tendency towards lower brain glucose levels amongst
300 patients with diabetes compared to control subjects (18) as well as a recent study by our group
301 amongst individuals with poorly controlled T2DM (27). These findings suggest that the brain
302 adapts to diabetes by decreasing the capacity for glucose to enter the brain during acute
303 hyperglycemia, which may be an adaptive mechanism to protect it against the adverse
304 consequences of excess glucose such as osmotic and oxidative stress (44). This adaptation to
305 chronic hyperglycemia has also been proposed as one potential mechanism to explain the
306 clinical phenomenon of "relative hypoglycemia" first described by Wyke (45) in the 1950's
307 whereby individuals with chronic poorly controlled diabetes develop symptoms of hypoglycemia
308 at normal plasma glucose levels (46).

309 Furthermore, we observed that a greater degree of lability of blood glucose levels (as
310 measured by the lability index calculated from continuous glucose monitoring) was positively
311 associated with greater change in brain glucose levels. However, we did not observe any
312 relationships between change in brain glucose and the frequency of or duration of time spent in
313 hypoglycemia (defined as glucose less than 70 mg/dl) or hyperglycemia (defined as glucose
314 greater than 180 mg/dl) obtained from the CGM in the 5 days prior to scanning. One explanation
315 could be that the lability index takes into account all fluctuations in glucose levels (even those
316 between 70 – 180 mg/dl which would not be defined as "hyperglycemia" or "hypoglycemia"). Our
317 findings would then suggest that even relatively smaller fluctuations in glucose concentrations
318 may have a significant impact on cerebral glucose transport and metabolism. These findings
319 could provide one potential mechanism to explain the clinical observation that individuals who
320 have greater variability often have greater long-term adverse consequences (5-8), particularly in
321 the brain (9-11) in the setting where glucose levels are tightly regulated. Notably, our
322 participants only wore the CGM for a relatively short period of time, and it possible that a few
323 days of continuous glucose monitoring is not sufficient to fully capture the effects of
324 hyperglycemia, hypoglycemia, and variability on the brain. Nonetheless, with a growing body of

patients with diabetes using CGM devices, our observations highlight the importance of understanding the impact of glycemic variability on cerebral glucose metabolism.

In this study, we used a ^{1}H MRS scanning strategy, which maximized our sensitivity to measure changes in brain glucose, but limited our ability to measure the rate of glucose transport directly. However, using previously reported models of glucose transport into the brain, we calculated the ratio of glucose transport (T_{max}) to the cerebral metabolic rate of glucose ($CMR_{glucose}$), and found a lower ratio amongst individuals with T1DM. This decreased ratio could be due to decreased transport of glucose into the brain (T_{max}) or increased $CMR_{glucose}$. In our human study, we were not able to directly measure $CMR_{glucose}$.. However, our rodent studies showed that under acute hyperglycemia, there were no differences in the cerebral metabolic rate of glucose between control and STZ rats. Furthermore, one human study investigating cerebral glucose metabolism under euglycemic conditions found no difference in brain glucose levels or transport kinetics between healthy control subjects and patients with well-controlled T1DM (47). The few human studies performed under hyperglycemic conditions have reported that $CMR_{glucose}$ is unchanged (48) or decreased (49; 50) amongst individuals with T1DM compared to control subjects. Taken together, our data raise the possibility that under conditions of hyperglycemia the transport capacity for glucose may play a particularly important role for the differences observed between T1DM and control subjects.

While there is a compelling body of prior work which has shown that glucose transport can be directly modulated by exposure to both hyperglycemia and hypoglycemia (15-20), other studies have also provided evidence for other mechanisms which may play a role in maintaining cerebral glucose metabolism amongst individuals with T1DM including the increased utilization of alternate fuels (such as monocarboxylic acids like lactate or ketones (51)) or changes in cerebral glycogen stores (52; 53) which can then be a source of glucose under hypoglycemic conditions. However, the majority of these studies have focused on understanding mechanisms by which the brain can maintain metabolism under hypoglycemic conditions; thus it remains

unknown whether these findings are relevant under conditions of euglycemia or hyperglycemia. In our human study, we did not observe any relationships between plasma BHB levels at the end of the study and changes in brain glucose (P = 0.29).

The glucose spectra for our human studies were acquired from the occipital lobe in order to maximize the sensitivity to noise ratio. However, based upon our findings in rat studies, the effect of hyperglycemia to decrease brain glucose transport capacity is not limited to the occipital cortex but also occurs in the frontal lobe, the striatum as well as hippocampus, which are all brain regions that have been shown to play important roles in higher-order cognitive function related to executive control, memory and reward/motivation and have been shown to be altered amongst individuals with T1DM (54). Our animal studies are also consistent with an earlier study which also reported decreased transport capacity of glucose in the parietal cortex of rats rendered hyperglycemic (46). We did not observe differences in the cerebellum and thalamus/hypothalamus between groups. Whether these brain regions are protected from the effects of chronic exposure to hyperglycemia remains unknown. One caveat to the interpretation of our animal data is that we are measuring cerebral metabolic processes, which change dramatically upon death. Although the microwave irradiation method has been shown to be effective at stopping post-mortem glycolysis (55), even a few seconds can have a major impact on the absolute concentrations of glucose measured. We observed brain lactate concentrations above normal in both groups of animals, which indicates that the brain samples underwent some degree of post-mortem glycolysis and slightly lower levels of brain glucose than would have been seen if the concentrations had been measured in the living brain. However, the lactate concentrations did not differ between the two groups, which indicates that any post-mortem metabolism affected both groups of animals equally, so the differences in brain glucose are due to experimental condition rather than ischemic glycolysis. Furthermore, because we were not able to continuously monitor the plasma glucose levels amongst our STZ-treated rats,

we are unable to determine whether greater variability in glucose levels would have affected the measures of brain glucose and metabolism in our rodent studies.

In our human studies, plasma insulin levels were different between the two groups over the course of the study although using a mixed effects regression model, plasma insulin levels were not significantly associated with brain glucose levels (P=0.11). Whether insulin modulates cerebral glucose transport remains uncertain as some (56), but not all (41; 57), studies have found relationships between insulin and brain glucose transport. Thus, we elected not to use somatostatin and insulin infusion strategies to avoid confounding somatostatin-induced effects on the brain. Another consideration for our findings is that several (57, 58) but not all (59) studies have reported increased presence of occult cerebral small vessel disease amongst older individuals with T1DM (mean age 50-60 years), which could have an impact on measurements of cerebral glucose. While our findings should be interpreted cautiously with this in mind, our participants had no reported history of cerebrovascular disease and were significantly younger (mean age 35 years). Furthermore, we measured the change in brain glucose using a difference spectra strategy, which would reduce within person variability and minimize group effects of asymptomatic cerebral microvascular disease.

In conclusion, our data indicate that brain glucose levels are diminished amongst individuals with T1DM compared to healthy control subjects during acute hyperglycemia. Moreover, the positive correlation between brain glucose levels and glycemic variability highlight the importance of considering the impact of glycemic variability in the brain, which is an area that has received less scientific attention. Future studies will be needed to determine the relative contribution of factors such as cerebral microvascular disease, glycemic control, and glycemic variability on cerebral glucose transport and metabolism.

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Study concept and design: Hwang, Jiang, Rothman, Mason, Sherwin

Acquisition of data: Hwang, Jiang, Sanchez Rangel, Lam, Leventhal, Fan, Ding

Analysis and interpretation of data: Hwang, Jiang, Dai, Rothman, Mason, Sherwin

Writing of manuscript: all authors

Statistical analysis: Hwang, Jiang, Dai

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602 **Table 1.** Participant Characteristics. Data presented as Mean±SEM
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Demographics	**HC**	**T1DM**	**P-value**
N (M/F)	9 (5/4)	14 (6/8)	0.80
Age (y)	32±4	35±4	0.62
BMI (kg/m^2)	23.1±0.8	26.0±1.4	0.14
HbA_{1c} (%)	5.0±0.1	7.6±0.3	<0.0001
HbA_{1c} (mmol/mol)	31±1.1	60±3.3	<0.0001
Duration Diabetes (y)	--	16±3	--

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607 **Figure 1.** A) Plasma glucose during the study; B) Change in plasma glucose at steady state
608 (baseline subtracted from average 60 -120 min); C) Change in brain glucose during the study D)
609 Change in brain glucose at steady state (baseline subtracted from average 60 -120 min).
610 Squares denote healthy control group, black circles denote T1DM group. Data presented as
611 Mean±SEM.
612
613

Figure 2. $T_{max}/CMR_{glucose}$ between groups. Squares denote healthy control group, black circles denote T1DM group. Data presented as Mean±SEM.

622 **Figure 3.** Relationship between lability index and change in brain glucose at steady state
623 (averaged between time 60-120 minutes) amongst individuals with T1DM.

624 **Figure 4.** A) Glucose concentrations in different brain regions (TH, thalamus/hypothalamus; CE,
625 cerebellum; CO, frontal cortex; HI, hippocampus; ST, striatum) between control (n = 6) and STZ
626 (n = 7) rats following 30 minute hyperglycemic infusion with 1-^{13}C-glucose. B) The ratio of ^{13}C
627 enrichment in glutamate C4 (reflecting first turn of TCA cycle) to glutamate C3 (reflecting
628 second turn of TCA cycle). * $P < 0.05$.
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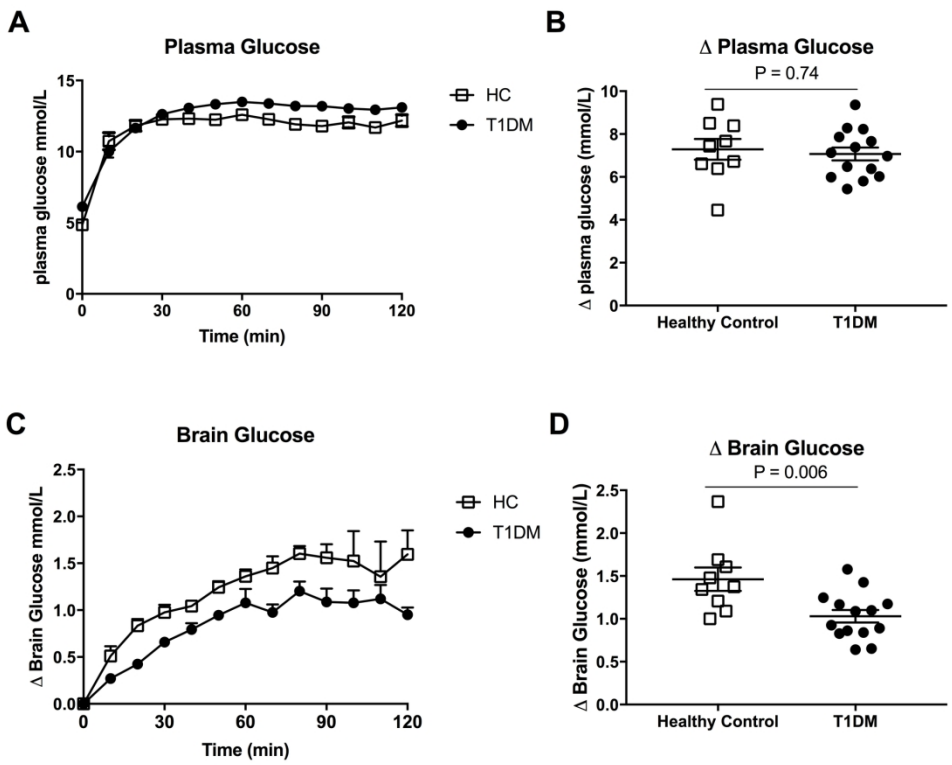

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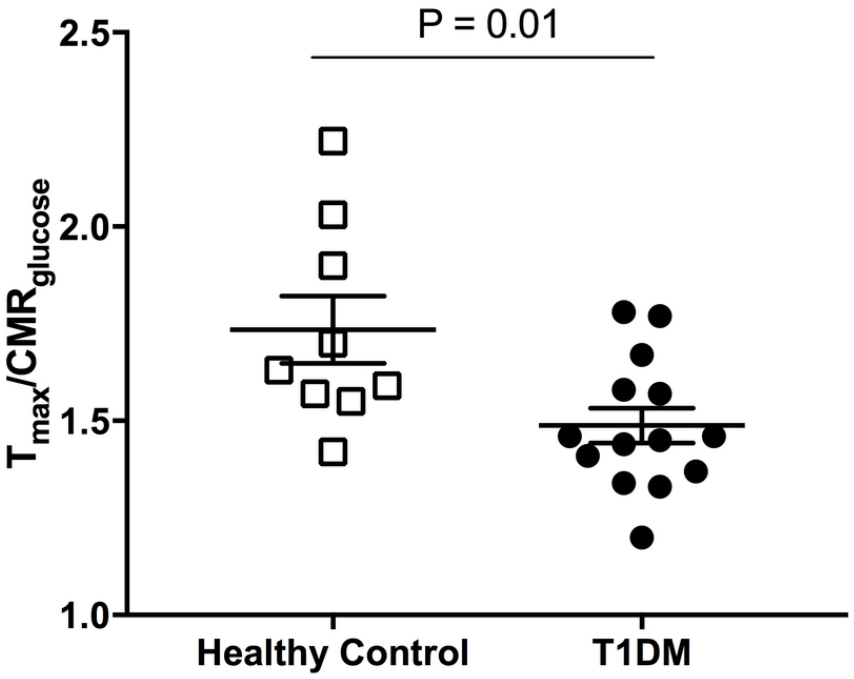

Figure 2

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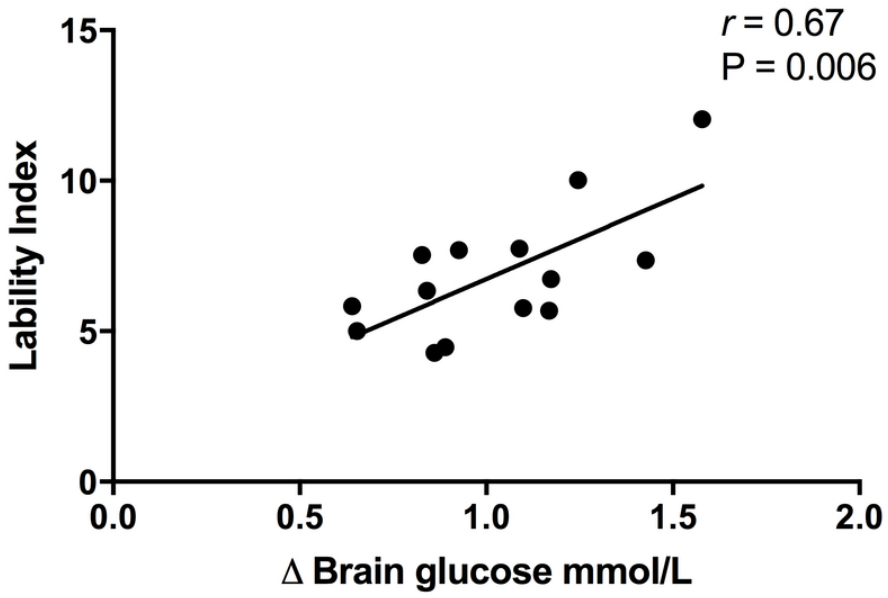

Figure 3

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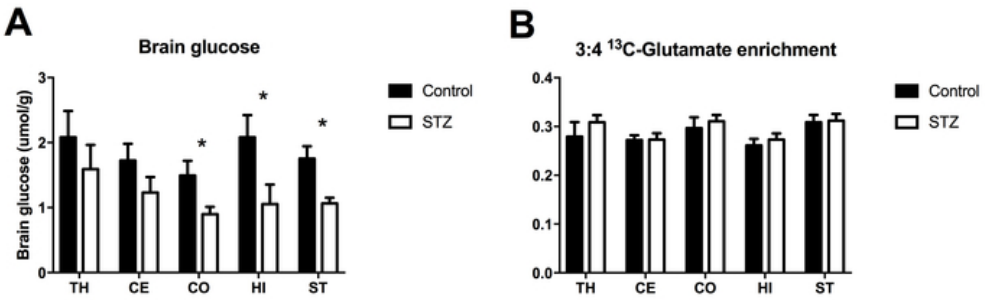

Figure 4

58x18mm (300 x 300 DPI)

Supplemental Table 1. Continuous glucose monitoring (CGM) variables calculated from the 5 days prior to MRS scanning. Data presented as Mean±SEM.

	T1DM subjects
CGM data	
% time, glucose < 70 mg/dL	5.2±0.1
% time, glucose < 50 mg/dL	0.6±0.0
% time, glucose >180 mg/dL	45.7±0.4
% time, glucose ≥ 70 mg/dL or ≤ 180 mg/dL	65.0±0.3
# of episodes, glucose < 70 mg/dL	6±1
# of episodes, glucose < 50 mg/dL	2±1