



Glycemic Variability and Brain Glucose Levels in Type 1 Diabetes

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The impact of glycemic variability on brain glucose transport kinetics among individuals with type 1 diabetes mellitus (T1DM) remains unclear. Fourteen individuals with T1DM (age 35 ± 4 years; BMI 26.0 ± 1.4 kg/m²; HbA_{1c} 7.6 ± 0.3) and nine healthy control participants (age 32 ± 4 ; BMI 23.1 ± 0.8 ; HbA_{1c} 5.0 ± 0.1) wore a continuous glucose monitor (Dexcom) to measure hypoglycemia, hyperglycemia, and glycemic variability for 5 days followed by ¹H MRS scanning in the occipital lobe to measure the change in intracerebral glucose levels during a 2-h glucose clamp (target glucose concentration 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 a 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, among individuals with T1DM, 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 with 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) study established the benefits of lowering blood glucose levels 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 patients with T1DM receiving intensive insulin therapy are able to maintain brain glucose uptake during acute hypoglycemia, whereas healthy control (HC) 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 a high frequency of hypoglycemia and hypoglycemia unawareness have increased cerebral glucose concentrations during acute hyperglycemia

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(24), which, again, is consistent with the concept that frequent hypoglycemia can increase the capacity of the brain for glucose entry. However, other recent studies (25,26) 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). 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), whereas another study of both T1DM and T2DM individuals showed a tendency toward 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 the 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 among individuals with T1DM by using hyperglycemic clamp techniques coupled with *in vivo* MRS. 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

Thirty-one participants were recruited for this study, and 23 participants (9 HC subjects and 14 individuals with T1DM) completed the study and were included in the analysis. The HC 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 the last 3 months, or an 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

Five to 7 days prior to MRS scanning, all participants with T1DM underwent placement of a continuous glucose monitor (Dexcom G4). All participants received instructions and training on the proper use of the continuous glucose monitoring (CGM) system. 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 (Supplementary Table 1). For calculation of the lability index, the last 576 glucose values (representing the 48 h immediately prior to scanning) were imported into Easy Glucose Variability (EasyGV) software (www.phc.ox.ac.uk/research/technology-outputs/easygv) (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/h for the duration of the study in order to minimize the risk of ketosis. HC subjects arrived on the morning of the scan. All groups were fasting. Hyperglycemia (target plasma glucose 220 mg/dL, 2 h) was maintained using a variable 20% dextrose infusion adjusted every 5–10 min, as previously described (27). Insulin levels were measured at 0, 60, and 120 min.

¹H MRS Scanning

Participants were positioned supine in a 4.0-T whole-body magnet interfaced with 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 localization (32) in $30 \times 20 \times 30$ mm³ voxels in the occipital lobe for 20 min at baseline and then every 10 min for at least 2 h of hyperglycemia. The sequence parameters were repetition time = 2,000 ms, echo time = 15 ms, mixing time = 10 ms, bandwidth = 5,000 Hz, and sampling points = 2,048. 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 to 3.54 parts per million and scaled back to total intensity from 3.10 to 3.96 parts per million in the free glucose spectrum (1:2.9), which contains 11 protons. The concentration of glucose was then compared with the creatine methyl peak in the *in vivo* spectrum, which was assumed to be 10 mmol/kg, and which represents three protons.

Calculation of the maximum rate of blood-brain glucose transport (T_{\max})/brain glucose utilization rate (CMR_{glucose})

was derived from known reversible Michaelis-Menten models of glucose transport into the brain (13,14):

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

where V_d is the brain water space (33), 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.

Laboratory Analysis

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

Statistical Analysis

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

Human Subjects Study Approval

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

Animal Studies

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

Two groups of rats were studied: normal control and streptozotocin (STZ)-induced diabetes. Diabetes was induced using a single injection of 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 the STZ group, the animals underwent aseptic surgery for vascular catheter placement into the left carotid artery for blood sampling, and into the 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 level during this period for all rats was 531 ± 69 mg/dL. In addition, the diabetic animals were treated with PZI Bovine Insulin (BCP, Houston, TX).

Hyperglycemic Clamp in Normal and STZ Rats

On the day of the study, overnight fasted STZ rats ($n = 7$) were connected to infusion pumps ~1 h prior to the start of the experiment and then left undisturbed to recover from handling stress. After the recovery period, an insulin infusion of $25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was used to lower plasma glucose levels to between 110 and 120 mg/dL for 30 min prior to start of hyperglycemic clamp. Then, at the start of the hyperglycemic clamp the insulin infusion rate was maintained at $10 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ 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% [$1\text{-}^{13}\text{C}$] glucose (99% ^{13}C labeled) (Cambridge Isotope Laboratories). Plasma glucose was measured using a glucose analyzer (Analox Instruments USA, Lunenburg, MA) to adjust the [$1\text{-}^{13}\text{C}$] glucose infusion rate to maintain a stable 220–250 mg/dL glucose level until the end of the study at 30 min, as previously described (35–37).

Animal Studies: MRS

Rats were euthanized with microwave irradiation immediately at the end of the infusion (Microwave applicator; Muromachi Kikai Co.) operating at 5 kW for 1.4 s. 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 0.1 mol/L HCl/methanol (2:1 v/v) using an OMNI Bead Ruptor 24 (OMNI International) followed by adding 60% ethanol + 0.1 mol/L sodium phosphate buffer at pH 7.4 mixed with Omni Bead Ruptor for 3 min and spun down at 15,000g for 30 min using a high-speed centrifuge (Sorvall Legend X1R Centrifuge; Thermo Fisher Scientific). Supernatants were then lyophilized (Freezone 4.5 Plus; Labconco) and then dissolved in 5-mm nuclear magnetic resonance (NMR) tubes in H_2O 60% + 40% D_2O with 100 μL of 10 mmol/L sodium formate as an internal concentration reference. Brain extractions and plasma ^{13}C enrichments were acquired using proton observed carbon-13 edited MRS using a 500-MHz Bruker Topspin 3.2. The NMR spectra were then analyzed using the software package NMRSpecs (version 1.0; Wuhan Institute of Physics and Mathematics, Wuhan, People's Republic of China) (39).

RESULTS

Participant Characteristics

Fourteen individuals with T1DM and 9 HC subjects participated in this study. Compared with the HC group, participants with T1DM were similar in age, sex, and

BMI (Table 1). As expected, individuals with T1DM had significantly higher HbA_{1c} levels compared with HC 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 $9.0 \pm 0.9 \mu\text{U/mL}$; end insulin $104.9 \pm 26 \mu\text{U/mL}$; $P = 0.01$), which was absent among the individuals with T1DM (baseline insulin $34.8 \pm 11 \mu\text{U/mL}$; end insulin $25.5 \pm 3.7 \mu\text{U/mL}$; $P = 0.45$). Individuals with T1DM had a mean duration of diabetes of 16 ± 3 years. Ten 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, eight of the participants with T1DM reported impaired awareness of hypoglycemia. There were no differences in the change in mean brain glucose levels between individuals who had normal hypoglycemia awareness (T1DM-Aware) and those who had impaired hypoglycemia awareness (T1DM-Unaware) (T1DM-Aware $0.93 \pm 0.07 \text{ mmol/L}$; T1DM-Unaware $1.0 \pm 0.1 \text{ mmol/L}$; $P = 0.58$); therefore, all participants with T1DM 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 the rates of mild hypoglycemia (defined as glucose concentration $<70 \text{ mg/dL}$), moderate hypoglycemia (glucose concentration $<50 \text{ mg/dL}$), or the total time spent in hypoglycemia between the individuals who were aware or unaware of hypoglycemia by Clarke score. Plasma β -hydroxybutyrate (BHB) concentrations were higher among individuals with T1DM compared with HC subjects at the end of the study (mean BHB: individuals with T1DM $0.28 \pm 0.07 \text{ mmol/L}$; HC subjects $0.02 \pm 0.002 \text{ mmol/L}$; $P = 0.004$).

Plasma and Brain Glucose Levels

Using a mixed-effects regression model, plasma glucose levels were slightly higher among participants with T1DM compared with HC subjects over the course of the study (least squares mean difference at time 120 min 0.93 ; 95% CI, $0.1, 1.75$; $P = 0.03$) (Fig. 1A). However, taking the glucose values at steady state (averaging the plasma glucose levels from time 60 to 120 min), there were no differences in the change in plasma glucose levels between groups (change in plasma glucose levels: HC subjects, $7.2 \pm 0.2 \text{ mmol/L}$; participants with T1DM $7.1 \pm 0.3 \text{ mmol/L}$; $P = 0.74$) (Fig. 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 squares mean difference at time 120 min -0.61 ; 95% CI $-0.90, -0.32$; $P < 0.0001$) (Fig. 1C) and the change in brain glucose at steady state (averaged from time 60 to 120 min) also differed significantly (HC subjects $1.46 \pm 0.1 \text{ mmol/L}$; individuals with T1DM $1.03 \pm 0.07 \text{ mmol/L}$; $P = 0.006$) (Fig. 1D).

Using a reversible Michaelis-Menten model for glucose transport kinetics (12,14,41), we calculated the ratio

Table 1—Participant characteristics

Demographics	HC group	T1DM group	<i>P</i> value
<i>N</i> (M/F)	9 (5/4)	14 (6/8)	0.80
Age (years)	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 of diabetes (years)		16 ± 3	

Data are presented as the mean \pm SEM. F, female; M, male.

of T_{max} to $\text{CMR}_{\text{glucose}}$. Based on these calculations, the $T_{\text{max}}/\text{CMR}_{\text{glucose}}$ ratio was also significantly lower among individuals with T1DM compared with HC subjects (HC subjects 1.74 ± 0.09 ; individuals with T1DM 1.49 ± 0.04 ; $P = 0.01$) (Fig. 2).

Relationships Among 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 among the rates of mild hypoglycemia (glucose concentration $<70 \text{ mg/dL}$) or moderate hypoglycemia (glucose concentration $<50 \text{ mg/dL}$), percentage of time spent in hypoglycemia or percentage of time spent in hyperglycemia (glucose concentration $>180 \text{ mg/dL}$), and brain glucose levels among individuals with T1DM. Next, we examined the relationship between glycemic variability metrics obtained from the continuous glucose monitor and brain glucose. Notably, among individuals with T1DM, the lability index of blood glucose levels correlated significantly and positively with a change in brain glucose levels ($r = 0.67$, $P = 0.006$) as well as the $T_{\text{max}}/\text{CMR}_{\text{glucose}}$ ratio ($r = 0.68$, $P = 0.008$) (Fig. 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 whether there are regional differences in brain glucose transport in response to acute hyperglycemia. Two groups of rats, normal control and STZ-induced diabetic (STZ) rats, underwent a hyperglycemic clamp for 30 min. There were no differences between groups in the plasma glucose levels immediately before the experiment (control rats $113 \pm 4 \text{ mg/dL}$; STZ rats $118 \pm 6 \text{ mg/dL}$; $P = 0.58$) and after the experiment (control rats $300 \pm 20 \text{ mg/dL}$; STZ rats $293 \pm 11 \text{ mg/dL}$; $P = 0.75$). Similar to our observations in the occipital lobe of the human brain, STZ rats had significantly decreased absolute concentrations of glucose in the CO (control rats $1.49 \pm 0.2 \mu\text{mol/g}$; STZ rats $0.90 \pm 0.1 \mu\text{mol/g}$; $P = 0.03$), HI (control rats $2.08 \pm 0.3 \mu\text{mol/g}$; STZ rats $1.05 \pm 0.3 \mu\text{mol/g}$; $P = 0.04$), and ST (control rats $1.75 \pm 0.2 \mu\text{mol/g}$; STZ rats $1.06 \pm 0.08 \mu\text{mol/g}$; $P = 0.005$) compared with normal control rats (Fig. 4A).

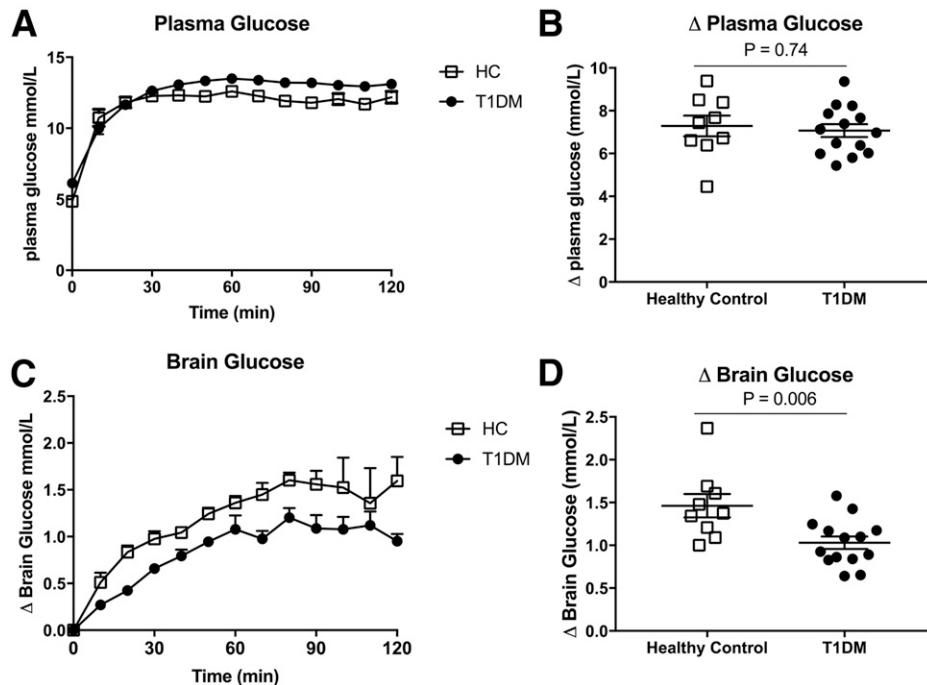


Figure 1—A: Plasma glucose during the study. B: Change in plasma glucose at steady state (baseline subtracted from average 60 to 120 min). C: Change in brain glucose level during the study. D: Change in brain glucose level at steady state (baseline subtracted from average 60 to 120 min). Squares denote HC group; black circles denote T1DM group. Data are presented as the mean ± SEM.

Moreover, the rat studies were performed using infusions of [1-¹³C]glucose, which also provided measurements of glucose oxidation rates in the various brain regions. By measuring the flow of the ¹³C label from glucose to glutamate C4 (reflecting the first turn of the tricarboxylic acid [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) (Fig. 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

Although much scientific attention has been focused on the impact of the 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 CGM 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 with HC subjects during an acute episode of modest hyperglycemia.

This finding is consistent with that of another study (18) that pooled a cohort of individuals with T1DM and T2DM and found a nonsignificant tendency toward lower brain glucose levels among patients with diabetes compared with control subjects as well as that of a recent study by our group among individuals with poorly controlled T2DM (27). These findings suggest that the brain adapts to diabetes by decreasing the capacity for glucose to enter

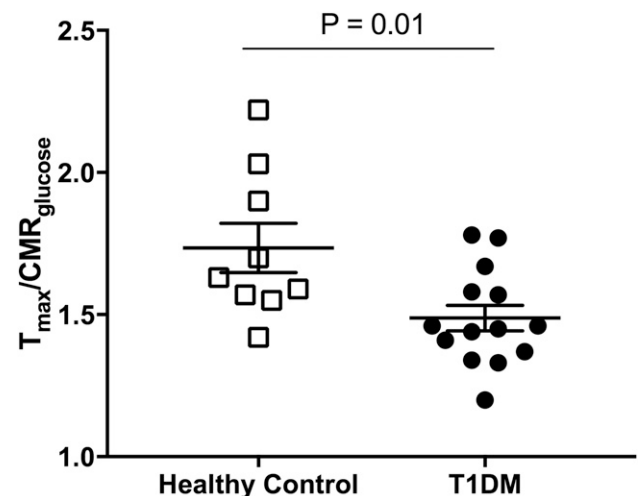


Figure 2— $T_{max} / CMR_{glucose}$ ratio between groups. Squares denote HC group; black circles denote T1DM group. Data are presented as the mean ± SEM.

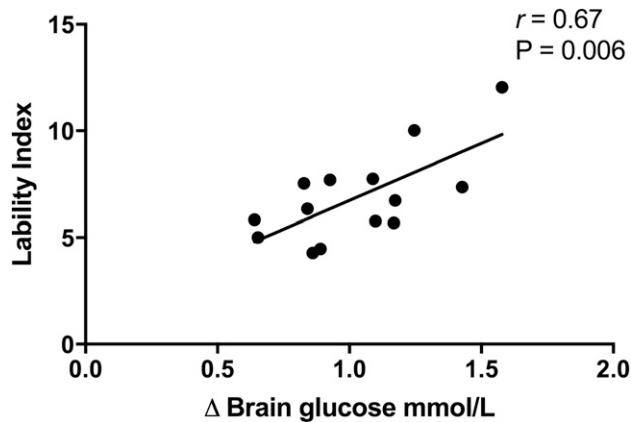


Figure 3—Relationship between lability index and change in brain glucose level at steady state (averaged between time 60 and 120 min) among individuals with T1DM.

the brain during acute hyperglycemia, which may be an adaptive mechanism to protect it against the adverse consequences of excess glucose, such as osmotic and oxidative stress (44). This adaptation to chronic hyperglycemia has also been proposed as one potential mechanism to explain the clinical phenomenon of “relative hypoglycemia,” first described by Wyke (45) in the 1950s, whereby symptoms of hypoglycemia at normal plasma glucose levels develop in individuals with chronic poorly controlled diabetes (46).

Furthermore, we observed that a greater degree of lability of blood glucose levels (as measured by the lability index calculated from CGM) was positively associated with greater change in brain glucose levels. However, we did not observe any relationships between change in brain glucose levels and the frequency of or duration of time spent in hypoglycemia (defined as a glucose concentration <70 mg/dL) or hyperglycemia (defined as glucose concentration >180 mg/dL) obtained from CGM in the 5 days prior to scanning. One explanation could be that the lability index takes into account all fluctuations in glucose

levels (even those between 70 and 180 mg/dL, which would not be defined as “hyperglycemia” or “hypoglycemia”). Our findings would then suggest that even relatively smaller fluctuations in glucose concentrations may have a significant impact on cerebral glucose transport and metabolism. These findings could provide one potential mechanism to explain the clinical observation that individuals who have greater variability often have greater long-term adverse consequences (5–8), particularly in the brain (9–11), in the setting where glucose levels are tightly regulated. Notably, our participants wore the CGM device for only a relatively short period of time, and it is possible that a few days of CGM is not sufficient to fully capture the effects of 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 ^1H 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 T_{max} to $\text{CMR}_{\text{glucose}}$ and found a lower ratio among individuals with T1DM. This decreased ratio could be due to decreased T_{max} or increased $\text{CMR}_{\text{glucose}}$. In our human study, we were not able to directly measure $\text{CMR}_{\text{glucose}}$. However, our rodent studies showed that under acute hyperglycemia there were no differences between control and STZ rats in the cerebral metabolic rate of glucose transport. Furthermore, one human study (47) investigating cerebral glucose metabolism under euglycemic conditions found no difference in brain glucose levels or transport kinetics between HC subjects and patients with well-controlled T1DM. The few human studies performed under hyperglycemic conditions have reported that $\text{CMR}_{\text{glucose}}$ is unchanged (48) or decreased (49,50) among individuals with T1DM compared with HC subjects. Taken together, our data raise the possibility that, under conditions of hyperglycemia, the transport capacity for

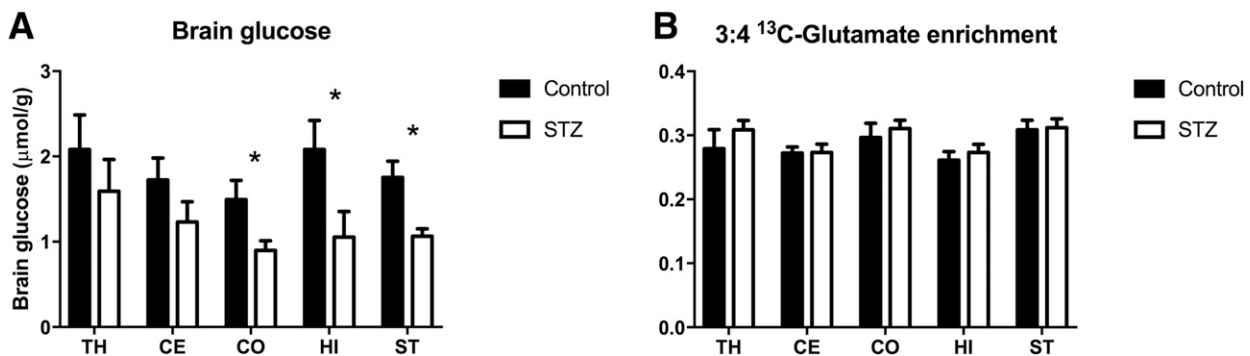


Figure 4—A: Glucose concentrations in different brain regions (CE, CO, HI, ST, and TH) between control ($n = 6$) and STZ ($n = 7$) rats after 30-min hyperglycemic infusion with $1\text{-}^{13}\text{C}$ -glucose. B: The ratio of ^{13}C enrichment in glutamate C4 (reflecting the first turn of the TCA cycle) to glutamate C3 (reflecting the second turn of the TCA cycle). * $P < 0.05$.

glucose may play a particularly important role for the differences observed between individuals with T1DM and HC subjects.

Although there is a compelling body of prior work that 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 that may play a role in maintaining cerebral glucose metabolism among individuals with T1DM, including the increased use of alternate fuels (e.g., 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 the 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 levels ($P = 0.29$).

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 ST, as well as the HI, 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 among individuals with T1DM (54). Our animal studies are also consistent with an earlier study (46), which also reported decreased transport capacity of glucose in the parietal cortex of rats rendered hyperglycemic. We did not observe differences in the CE and TH 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 postmortem 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 postmortem 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 postmortem metabolism affected both groups of animals equally, so the differences in brain glucose levels are due to experimental condition rather than ischemic glycolysis. Furthermore, because we were not able to continuously monitor the plasma glucose levels among 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 an increased presence of occult cerebral small vessel disease among older individuals with T1DM (mean age 50–60 years), which could have an impact on measurements of cerebral glucose. Although 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 levels using a difference spectra strategy, which would reduce the within-person variability and minimize the group effects of asymptomatic cerebral microvascular disease.

In conclusion, our data indicate that brain glucose levels are diminished among individuals with T1DM compared with HC subjects during acute hyperglycemia. Moreover, the positive correlation between brain glucose levels and glycemic variability highlights 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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analysis and interpretation of data, statistical analysis, and the writing of the manuscript. D.L.R., G.F.M., and R.S.S. contributed to the study concept and design, the analysis and interpretation of the data, and the writing of the manuscript. J.J.H. and R.S.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References

- Nathan DM, Genuth S, Lachin J, et al.; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977–986
- Gubitosi-Klug RA, Braffett BH, White NH, et al.; Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. Risk of severe hypoglycemia in type 1 diabetes over 30 years of follow-up in the DCCT/EDIC Study. *Diabetes Care* 2017;40:1010–1016
- Nathan DM; DCCT/EDIC Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes Care* 2014;37:9–16
- Diabetes Control and Complications Trial Research Group. Hypoglycemia in the Diabetes Control and Complications Trial. *Diabetes* 1997;46:271–286
- Bragd J, Adamson U, Bäcklund LB, Lins PE, Moberg E, Oskarsson P. Can glycaemic variability, as calculated from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? *Diabetes Metab* 2008;34:612–616
- Musen G, Lyoo IK, Sparks CR, et al. Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes* 2006;55:326–333
- Hirsch IB. Glycemic variability and diabetes complications: does it matter? Of course it does! *Diabetes Care* 2015;38:1610–1614
- Kim YS, Kim C, Jung KH, et al. Range of glucose as a glycemic variability and 3-month outcome in diabetic patients with acute ischemic stroke. *PLoS One* 2017;12:e0183894
- Giordani I, Di Flaviani A, Picconi F, et al. Acute hyperglycemia reduces cerebrovascular reactivity: the role of glycemic variability. *J Clin Endocrinol Metab* 2014;99:2854–2860
- Livny A, Ravona-Springer R, Heymann A, et al. Long-term variability in glycemic control is associated with white matter hyperintensities in APOE4 genotype carriers with type 2 diabetes. *Diabetes Care* 2016;39:1056–1059
- Cui X, Abduljalil A, Manor BD, Peng CK, Novak V. Multi-scale glycemic variability: a link to gray matter atrophy and cognitive decline in type 2 diabetes. *PLoS One* 2014;9:e86284
- Gruetter R, Novotny EJ, Boulware SD, et al. Direct measurement of brain glucose concentrations in humans by ^{13}C NMR spectroscopy. *Proc Natl Acad Sci U S A* 1992;89:1109–1112
- Gruetter R, Ugurbil K, Seaquist ER. Steady-state cerebral glucose concentrations and transport in the human brain. *J Neurochem* 1998;70:397–408
- de Graaf RA, Pan JW, Telang F, et al. Differentiation of glucose transport in human brain gray and white matter. *J Cereb Blood Flow Metab* 2001;21:483–492
- Pardridge WM, Triguero D, Farrell CR. Downregulation of blood-brain barrier glucose transporter in experimental diabetes. *Diabetes* 1990;39:1040–1044
- Cornford EM, Hyman S, Cornford ME, Clare-Salzler M. Down-regulation of blood-brain glucose transport in the hyperglycemic nonobese diabetic mouse. *Neurochem Res* 1995;20:869–873
- Heikkilä O, Lundbom N, Timonen M, Groop PH, Heikkinen S, Mäkimattila S. Evidence for abnormal glucose uptake or metabolism in thalamus during acute hyperglycaemia in type 1 diabetes—a ^1H MRS study. *Metab Brain Dis* 2010;25:227–234
- Seaquist ER, Tkac I, Damberg G, Thomas W, Gruetter R. Brain glucose concentrations in poorly controlled diabetes mellitus as measured by high-field magnetic resonance spectroscopy. *Metabolism* 2005;54:1008–1013
- van de Ven KC, Tack CJ, Heerschap A, van der Graaf M, de Galan BE. Patients with type 1 diabetes exhibit altered cerebral metabolism during hypoglycemia. *J Clin Invest* 2013;123:623–629
- Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ. Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 1995;333:1726–1731
- Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C. Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci U S A* 1994;91:9352–9356
- Simpson IA, Appel NM, Hokari M, et al. Blood-brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. *J Neurochem* 1999;72:238–247
- McNay EC, Sherwin RS. Effect of recurrent hypoglycemia on spatial cognition and cognitive metabolism in normal and diabetic rats. *Diabetes* 2004;53:418–425
- Criego AB, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER. Brain glucose concentrations in patients with type 1 diabetes and hypoglycemia unawareness. *J Neurosci Res* 2005;79:42–47
- Seaquist ER, Moheet A, Kumar A, et al. Hypothalamic glucose transport in humans during experimentally induced hypoglycemia-associated autonomic failure. *J Clin Endocrinol Metab* 2017;102:3571–3580
- Criego AB, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER. Brain glucose concentrations in healthy humans subjected to recurrent hypoglycemia. *J Neurosci Res* 2005;82:525–530
- Hwang JJ, Jiang L, Hamza M, et al. Blunted rise in brain glucose levels during hyperglycemia in adults with obesity and T2DM. *JCI Insight* 2017;2:95913
- Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR. Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes Technol Ther* 2011;13:921–928
- Chan CL, Pyle L, Newnes L, Nadeau KJ, Zeitler PS, Kelsey MM. Continuous glucose monitoring and its relationship to hemoglobin A1c and oral glucose tolerance testing in obese and prediabetic youth. *J Clin Endocrinol Metab* 2015;100:902–910
- Gruetter R, Novotny EJ, Boulware SD, Rothman DL, Shulman RG. ^1H NMR studies of glucose transport in the human brain. *J Cereb Blood Flow Metab* 1996;16:427–438
- Gruetter R, Rothman DL, Novotny EJ, Shulman GI, Prichard JW, Shulman RG. Detection and assignment of the glucose signal in ^1H NMR difference spectra of the human brain. *Magn Reson Med* 1992;27:183–188
- Thompson RB, Allen PS. Response of metabolites with coupled spins to the STEAM sequence. *Magn Reson Med* 2001;45:955–965
- Buschiazzo PM, Terrell EB, Regen DM. Sugar transport across the blood-brain barrier. *Am J Physiol* 1970;219:1505–1513
- Wu KK, Huan Y. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc Pharmacol* 2008;Chapter 5:Unit 5.47
- de Graaf RA, Mason GF, Patel AB, Rothman DL, Behar KL. Regional glucose metabolism and glutamatergic neurotransmission in rat brain in vivo. *Proc Natl Acad Sci U S A* 2004;101:12700–12705
- de Graaf RA, Brown PB, Mason GF, Rothman DL, Behar KL. Detection of [1,6- $^{13}\text{C}_2$]-glucose metabolism in rat brain by in vivo ^1H -[^{13}C]-NMR spectroscopy. *Magn Reson Med* 2003;49:37–46
- Mason GF, Behar KL, Rothman DL, Shulman RG. NMR determination of intracerebral glucose concentration and transport kinetics in rat brain. *J Cereb Blood Flow Metab* 1992;12:448–455
- Patel AB, de Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL. The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex in vivo. *Proc Natl Acad Sci U S A* 2005;102:5588–5593

39. Liu Y, Cheng J, Liu H, Deng Y, Wang J, Xu F. NMRSpec: an integrated software package for processing and analyzing one dimensional nuclear magnetic resonance spectra. *Chemom Intell Lab Syst* 2017;162:142–148
40. Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. *Diabetes Care* 1995;18:517–522
41. Seaquist ER, Damberg GS, Tkac I, Gruetter R. The effect of insulin on in vivo cerebral glucose concentrations and rates of glucose transport/metabolism in humans. *Diabetes* 2001;50:2203–2209
42. Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny EJ. Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J Cereb Blood Flow Metab* 1995;15:12–25
43. Shen J, Petersen KF, Behar KL, et al. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo ^{13}C NMR. *Proc Natl Acad Sci U S A* 1999;96:8235–8240
44. Mäkimattila S, Malmberg-Cèder K, Häkkinen AM, et al. Brain metabolic alterations in patients with type 1 diabetes-hyperglycemia-induced injury. *J Cereb Blood Flow Metab* 2004;24:1393–1399
45. Wyke B. Electroencephalographic studies in the syndrome of relative cerebral hypoglycemia. *Electroencephalogr Clin Neurophysiol* 1959;11:602
46. Gjedde A, Crone C. Blood-brain glucose transfer: repression in chronic hyperglycemia. *Science* 1981;214:456–457
47. van de Ven KC, van der Graaf M, Tack CJ, Heerschap A, de Galan BE. Steady-state brain glucose concentrations during hypoglycemia in healthy humans and patients with type 1 diabetes. *Diabetes* 2012;61:1974–1977
48. Henry PG, Criego AB, Kumar A, Seaquist ER. Measurement of cerebral oxidative glucose consumption in patients with type 1 diabetes mellitus and hypoglycemia unawareness using (^{13}C) nuclear magnetic resonance spectroscopy. *Metabolism* 2010;59:100–106
49. van Golen LW, Huisman MC, Ijzerman RG, et al. Cerebral blood flow and glucose metabolism measured with positron emission tomography are decreased in human type 1 diabetes. *Diabetes* 2013;62:2898–2904
50. Ziegler D, Langen KJ, Herzog H, et al. Cerebral glucose metabolism in type 1 diabetic patients. *Diabet Med* 1994;11:205–209
51. Mason GF, Petersen KF, Lebon V, Rothman DL, Shulman GI. Increased brain monocarboxylic acid transport and utilization in type 1 diabetes. *Diabetes* 2006;55:929–934
52. Herzog RI, Chan O, Yu S, Dziura J, McNay EC, Sherwin RS. Effect of acute and recurrent hypoglycemia on changes in brain glycogen concentration. *Endocrinology* 2008;149:1499–1504
53. Öz G, Tesfaye N, Kumar A, Deelchand DK, Eberly LE, Seaquist ER. Brain glycogen content and metabolism in subjects with type 1 diabetes and hypoglycemia unawareness. *J Cereb Blood Flow Metab* 2012;32:256–263
54. Hwang JJ, Parikh L, Lacadie C, et al. Hypoglycemia unawareness in type 1 diabetes suppresses brain responses to hypoglycemia. *J Clin Invest* 2018;128:1485–1495
55. de Graaf RA, Chowdhury GM, Brown PB, Rothman DL, Behar KL. In situ 3D magnetic resonance metabolic imaging of microwave-irradiated rodent brain: a new tool for metabolomics research. *J Neurochem* 2009;109:494–501
56. Hertz MM, Paulson OB, Barry DI, Christiansen JS, Svendsen PA. Insulin increases glucose transfer across the blood-brain barrier in man. *J Clin Invest* 1981;67:597–604
57. Hasselbalch SG, Knudsen GM, Videbaek C, et al. No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes* 1999;48:1915–1921
58. Nunley KA, Ryan CM, Orchard TJ, et al. White matter hyperintensities in middle-aged adults with childhood-onset type 1 diabetes. *Neurology* 2015;84:2062–2069
59. Biessels GJ, Reijmer YD. Brain changes underlying cognitive dysfunction in diabetes: what can we learn from MRI? *Diabetes* 2014;63:2244–2252