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Clinical Research Article

Impact of recurrent hypoglycemia on brain glucose transport kinetics in type 1 diabetes

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Abstract

Context **Impaired awareness of hypoglycemia (IAH) significantly impacts efforts to maintain optimal glycemia. Impaired awareness of hypoglycemia occurs when recurrent episodes of hypoglycemia (HG) occur within a short period of time, but the mechanisms are unknown. Upregulation of glucose transport may contribute to the development of IAH.**

Objective **To determine if brain glucose transport is upregulated in response to recurrent HG in people with type 1 diabetes (T1D) and normal awareness of hypoglycemia.**

Methods **Participants were recruited from a clinical registry. Inclusion criteria were diagnosis of T1D, 18-65 years, hemoglobin A1C < 8.5%, and diabetes duration of 2-25 years. Exclusion criteria included IAH on the Clarke and Gold questionnaires. Forty-five subjects enrolled and 30 completed the entire 3-day protocol. Participants underwent magnetic resonance spectroscopy scans at 3 tesla during which they were clamped at 150, 225, or 300 mg/dL before and after exposure to three 2-hour HG (target = 50 mg/dL) clamps over 2 days between December 2020 and June 2023. The primary metabolite of interest was glucose. Brain glucose transport kinetics were measured in the hypothalamus (HTL) and prefrontal cortex (PFC) during hyperglycemic clamps using reversible symmetric Michaelis–Menten model.**

Results **Brain glucose transport kinetics measured during hyperglycemic clamps was not different before vs after exposure to recurrent HG clamps.**

Conclusion **Short-term exposure to recurrent HG does not upregulate glucose transport kinetics in the setting of T1D.**

Keywords type 1 diabetes, hypoglycemia

Impaired awareness of hypoglycemia (IAH) affects as many at 25% of individuals with type 1 diabetes (T1D) at any one point in time and can significantly impact efforts to maintain the near normal glycemia required to minimize the risks of diabetes complications. Recent investigation has shown that even the use of state of the art technology like hybrid closed loop insulin pumps and continuous glucose monitors have not reduced the prevalence of IAH in adults with type 1 diabetes (1). Impaired awareness of hypoglycemia occurs when recurrent episodes of hypoglycemia (HG) occur within a short period of time. The normal counterregulatory response (CRR) to a falling blood sugar is dependent upon a reduction in serum insulin and a rise in glucagon secretion, neither of which occur sufficiently in individuals with T1D to prevent hypoglycemia. As a result, people with T1D rely on sympathoadrenal activation to recognize and manage HG, but because the glucose level that leads to activation and the magnitude of the activation is reduced by each episode of HG, the patient may not have a response before the onset of neuroglycopenia (2).

The reasons why recurrent HG in patients with diabetes leads to IAH remain uncertain, but because detection of a fall in blood and brain glucose is central to triggering the CRR, many investigators have centered on identifying the cerebral adaptations in energy metabolism that ensure the brain has adequate fuel to support

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metabolism during future episodes of HG. Our group has focused on determining if HG induces changes in brain glucose transport that enhance fuel delivery. Motivated by reports of maintenance of brain glucose uptake (as measured by the arteriovenous difference method) during HG in healthy individuals subjected to 56 hours of HG (3) and in patients with T1D and a reduced CRR to hypoglycemia (4), we were the first to demonstrate that glucose concentrations in the occipital cortex were significantly higher in patients with T1D and IAH than in healthy volunteers studied at the same blood glucose concentrations (5). Together with our observation that cortical oxidative glucose consumption was not different in patients with T1D and IAH than controls (6), this finding strongly supported the hypothesis that recurrent HG leads to an upregulation in brain glucose transport that ensures the brain has sufficient glucose for energy demands during subsequent HG and thus does not need to trigger the CRR to enhance fuel availability. Further support for the upregulated glucose transport hypothesis comes from studies of rodents subjected to chronic (7) and recurrent HG (8), our demonstration of brain glycogen supercompensation in humans following a single hypoglycemic episode (9), and some PET studies reporting regional, but not global, differences in glucose kinetics in T1D with IAH vs normal awareness of HG (NAH) (10, 11). Together, these data supported an association between higher glucose levels in brain tissue and IAH; however, whether recurrent HG causes increased cerebral glucose transport remained unknown.

In this study, we aimed to address this knowledge gap by exposing participants with T1D and NAH to recurrent hypoglycemia to induce impaired awareness in response to the recurrent hypoglycemia. We used a protocol that has been used by us (12) and others (13) to induce IAH in an experimental setting. Previously, we and others have demonstrated that a linear relationship exists between brain and blood glucose concentrations in humans and that blood glucose transport kinetics can be described using reversible Michaelis–Menten kinetics when brain glucose concentrations are measured under steady state conditions of multiple levels of glycemia (14-16). Thus, in this study, we measured brain glucose concentrations in the hypothalamus and prefrontal cortex during 3 levels of hyperglycemia by proton MR spectroscopy (^{1}H MRS) before and after the induction of IAH and used these data to calculate brain glucose transport kinetics. We selected to measure brain and blood glucose concentrations during hyperglycemia because the concentration of glucose in the brain during hypoglycemia is too low to be reliably measured, particularly in the hypothalamus. Our purpose was to determine if recurrent hypoglycemia causes changes in glucose transport that lead to the syndrome of IAH in the setting of T1D. Our primary outcome was the difference between ratio of maximal transport rate to cerebral metabolic rate of glucose (V^t_{max}/CMR_{glc}) measured before and after exposure to recurrent hypoglycemia.

Materials and methods

Participants

Participants were recruited from a registry maintained by the investigators of individuals interested in research, from the University of Minnesota Endocrinology Clinic, and from other healthcare locations in the Minneapolis/St. Paul area. The protocol was approved by the University of Minnesota Institutional Review Board. Written informed consent was obtained from all participants. Participants were enrolled between December 2020 and June 2023 and studied by the same experimental team throughout the study period, which included a shut down during the COVID-19 pandemic.

Potential participants underwent a screening visit during which a history and brief physical exam were completed to ensure they meet eligibility criteria. Inclusion criteria were a diagnosis of T1D (based on clinical or laboratory grounds), ages 18-65 years, hemoglobin A1C < 8.5%, and diabetes duration of 2-25 years. Exclusion criteria included impaired awareness of hypoglycemia on the Clarke (score of 3 or more) (17) and Gold (score of 4 or more) (18) questionnaires, uncontrolled hypertension (BP > 145/95 at screening), evidence of autonomic neuropathy (presence of orthostatic hypotension or history of gastroparesis), proliferative retinopathy, GFR < 45 mL/min, history of myocardial infarction, stroke, seizures, neurosurgical procedures, major depression requiring hospitalization within the last 5 years, current substance abuse, arrhythmias, and use of drugs that can alter glucose metabolism (other than insulin). Those with a weight >300 lbs. or who answered that they had metallic substances in their body were also excluded because they did not meet the requirements for the MRI scan. As shown in the CONSORT diagram (Fig. 1), a total of 48 individuals were assessed for eligibility. Of these, 3 had scheduling conflicts, 4 withdrew on their own, 2 were found to be ineligible, and 9 were excluded due to intravenous access failure. In total, 30 individuals with complete data were included in our analysis. The CONSORT checklist is shown in Fig. S1 (19).

Study protocol

The study protocol is shown in Fig. 2. Participants reported to the Center for Magnetic Resonance Research (CMRR) at noon on Day 1 after eating a low carb breakfast before 8 AM to participate in clamp 1. Intravenous (IV) catheters were placed, and IV infusion of insulin was started and adjusted as necessary to bring blood glucose to 95 mg/dL. The limb from which the blood was sampled was arterialized using methods previously described (20). Blood was collected every 10 minutes for measurement of glucose on a nearby Analox machine to guide adjustments in the insulin infusion rate. The experiment began when the subject's blood glucose was at 95 mg/dL. At this time, the insulin infusion rate was fixed at 0.5 mU/kg/min, samples for blood glucose were collected every 5 minutes and an intravenous infusion of glucose (20% dextrose) was administered as necessary to achieve target glycemia. Participants were then placed into the 3 tesla (T) scanner. Blood glucose was maintained at 95 mg/dL for the next ~35 minutes as baseline MRS data were acquired from the hypothalamus (HTL) and prefrontal cortex (PFC). Subjects were then given an intravenous bolus injection of glucose over 1-2 minutes using the formula of administering 2 mg glucose/kg body weight for each 1 mg/dL increase desired that we have used with success previously (14). Immediately after the bolus injection, a continuous infusion of 20% dextrose was started, and the rate of administration was adjusted to maintain the desired glucose target of 150 mg/dL, 225 mg/dL, or 300 mg/dL. Participants were assigned to their

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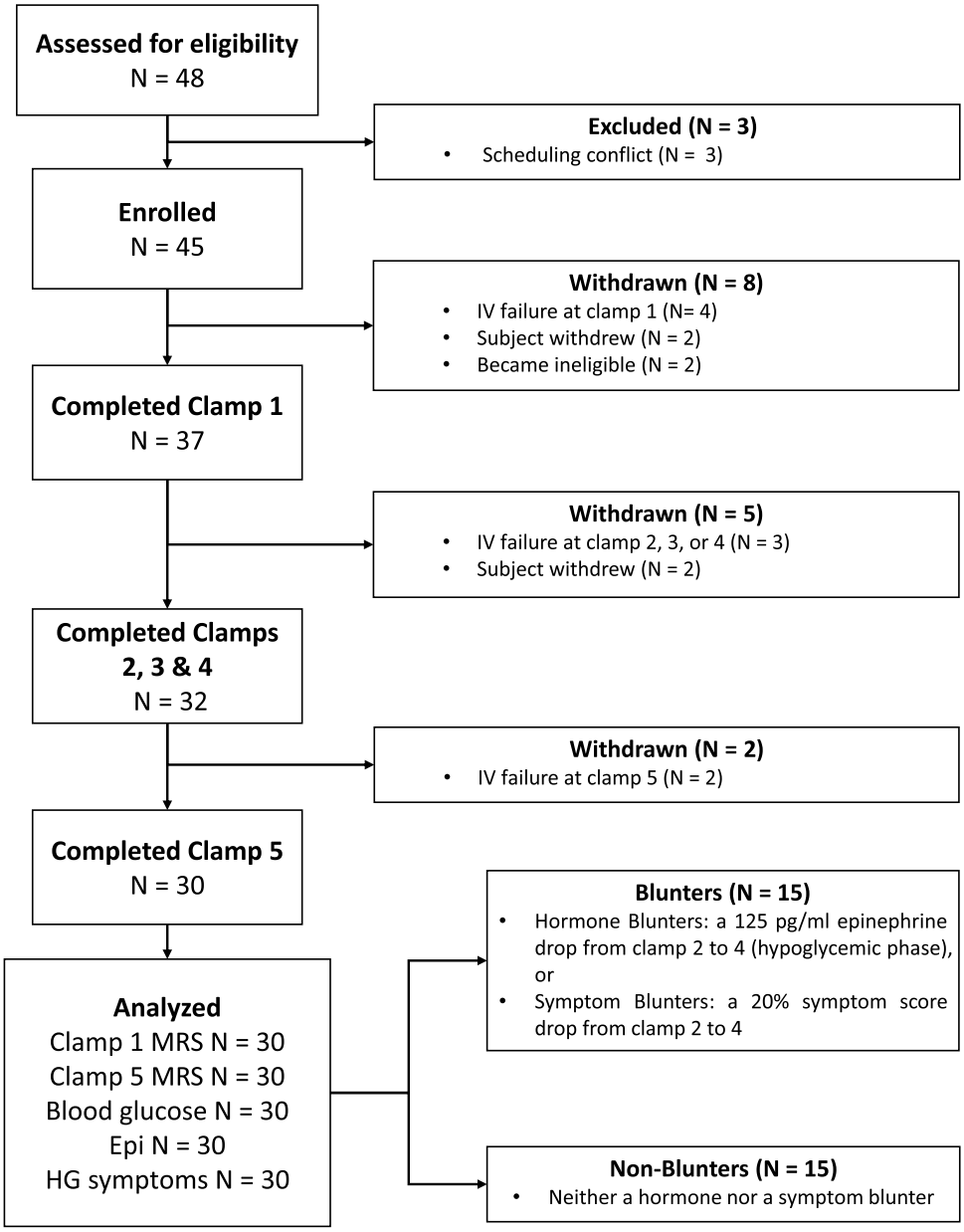

Figure 1 CONSORT flowchart illustrating participant inclusion and exclusion throughout the study period.

glucose target randomly using block randomization. MRS data collection was continued until subjects had been at their target level of hyperglycemia for at least 20 minutes to assure that the brain glucose had come into equilibrium with the blood glucose, as we previously described (20). At the completion of the study, the infusions were discontinued; the participant resumed their usual insulin regimen and was fed a meal. They were discharged from the CMRR when their blood glucose values had normalized.

On the mornings of Days 2 and 3, participants reported to the Clinical Research Unit (CRU) in the fasting state to complete the induction protocol. After placement of IV lines into the arms, they underwent hyperinsulinemic hypoglycemic clamp studies between 8 and 10 AM on Day 2 (clamp 2), 2-4 PM on Day 2 (clamp 3), and 8-10 AM on Day 3 (clamp 4). In each circumstance, an IV infusion of insulin was given at 1.0 mU/kg/min and blood sugar was first clamped at 95 mg/dL. Blood was collected every 10 minutes for measurement of glucose on a nearby Analox machine to guide adjustments in the insulin infusion rate. A continuous infusion of 20% dextrose was used as needed to ensure the participant remained at euglycemia. Potassium phosphate was infused at 4 mEq/hour as long as the insulin was infused. The experiment began when blood glucose was 95 mg/dL. At time zero for each clamp, the insulin infusion rate was increased to 2.0 mU/kg/min, samples for blood glucose were collected every 5 minutes and blood glucose was allowed to fall. The rate of the 20% dextrose infusion was adjusted to ensure the participant was clamped at 50 mg/dL. Blood samples were collected at baseline and every 30 minutes once target hypoglycemia was achieved for later measurement of epinephrine. Hypoglycemic symptom scores were assessed at these same time points using a standardized questionnaire (21). At minute 120 in the morning clamp on Day 2, the insulin infusion rate was reduced to

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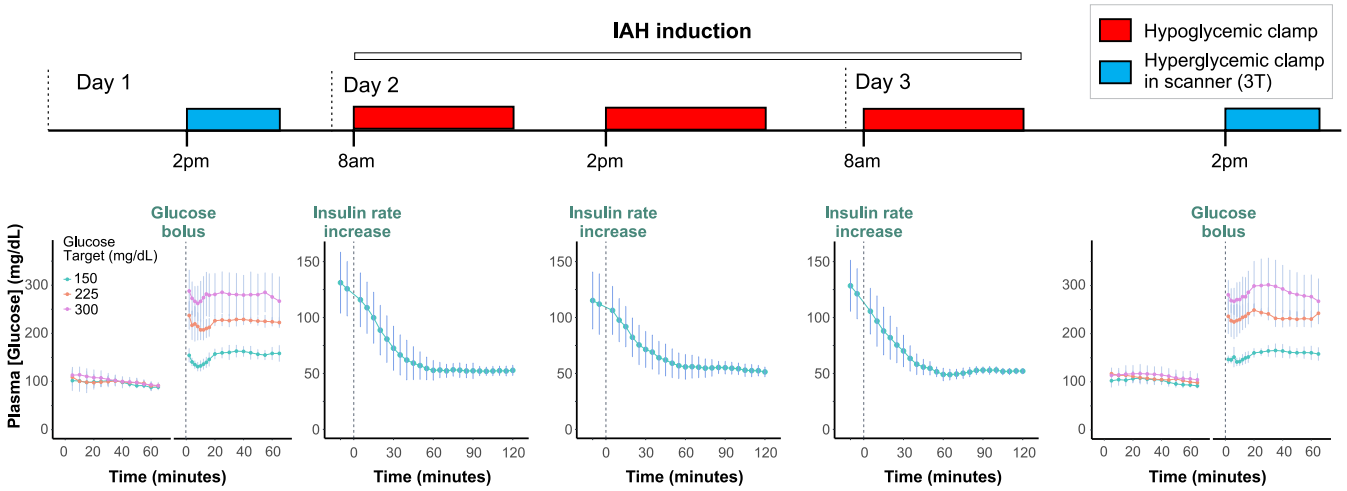

Figure 2 Study protocol and glycemic levels achieved during clamp studies. On Day 1, participants underwent a hyperinsulinemic hyperglycemic clamp study after randomized to a glucose target of 150, 225, or 300 mg/dL. During the induction of impaired awareness of hypoglycemia (IAH) on Days 2 and 3 participants underwent 3 hyperinsulinemic hypoglycemic clamps. On the afternoon of Day 3, the hyperglycemia clamp done on Day 1 was repeated using the targets assigned by randomization on Day 1. Plasma glucose values measured during each clamp study are shown. MR data was collected during the afternoon clamps on Days 1 and 3. See Fig. 2 for depiction of the MRS protocol used.

1.0 mU/kg/min and the subject's blood sugar was allowed return to euglycemia. Participants were fed a snack containing 30 gm of carbohydrate, and 20% dextrose was infused as necessary to maintain euglycemia. Three hours later, participants underwent a second hyperinsulinemic hypoglycemic clamp using the same methodology. At the completion of the second clamp, the infusions were discontinued, the participant resumed their usual insulin regimen, and was fed a meal. They were discharged from the CRU when their blood glucose values had normalized with instructions to return the following morning for a third hyperinsulinemic hypoglycemic clamp.

After the morning clamp on Day 3 (clamp 4), the insulin infusion rate was reduced to 1.0 mU/kg/min and the participant's blood sugar was allowed return to euglycemia. Participants were fed a snack containing 30 gm of carbohydrate and 20% dextrose was infused as necessary to maintain euglycemia. Participants were transported to the CMRR for assessment of cerebral glucose transport and metabolism using ^{1}H MRS using the same procedures as were used on Day 1. They were studied at the same randomized blood glucose concentration as on Day 1. At the completion of clamp 5, the infusions were discontinued, and participants were fed a meal.

Laboratory analyses

Plasma glucose concentration was measured in duplicate during the clamps and MR scanning using an Analox machine (Analox Instruments, Stourbridge, UK). Plasma epinephrine was measured by high-performance liquid chromatography (Dionex Corp, Sunnyvale, CA) in the Vanderbilt University Diabetes Research Center Core facility.

MRI data acquisition and analysis

Spectra were acquired from the frontal cortex (LR × AP × FH = 24 × 30 × 12 mm^3) and hypothalamus (LR × AP × FH = 13 × 12 × 10 mm^3) on a 3T Siemens Prisma scanner (Erlangen, Germany), using methods described before (12, 22). Specifically, we used an optimized semi-LASER sequence (23), with echo time (TE) = 28 ms, repetition time (TR) = 5 seconds, acquiring 128 transients for the frontal cortex and 256 transients for the hypothalamus, corresponding to acquisition times of 11 and 22 minutes, respectively. T_1-weighted MPRAGE (TR/inversion time (TI)/TE = 2530/1200/3.65 ms) structural images (1 × 1 × 1mm^3 resolution) were collected prior to ^{1}H MRS measurements for prescription of MRS volumes-of-interest (VOIs) and estimation of tissue fraction for metabolite quantifications. B_0 and B_1 parameters were carefully adjusted for both VOIs at the beginning of the scanning session and were saved for reloading as acquisition began from each VOI, avoiding the need for repeated calibrations during target hyperglycemia. MRS data were continuously acquired from the frontal cortex for dynamic modeling (16). Only steady-state hyperglycemia data were acquired from the hypothalamus for the estimation of V^t_{max}/CMR_{glc} because the signal-to-noise ratio of spectra obtained from the hypothalamus is not sufficient to allow reliable dynamic modeling that allows the estimation of all 4 kinetic parameters of glucose transport and metabolism (12, 22). An atlas-based automated VOI positioning algorithm (24, 25) and real time voxel tracking and shim/frequency updates were utilized.

A subset of the MRS data collected during the euglycemic portion of the clamp studies was previously analyzed and published as part of a magnetic field comparison study (26). The quality control (QC) criteria described in that manuscript were used to evaluate spectra for inclusion in the quantitative analyses. Methods to quantify the neurochemical profiles using LCModel (27), and to assess the reliability of metabolite concentrations based on Cramér-Rao lower bounds, were identical to those described before (12, 22). The primary metabolite of interest was glucose. We used the LCModel output of the sum of glucose and taurine, in accordance with LCModel guidelines that recommend reporting the sum of metabolites with strong negative cross correlations ($r < -0.5$). We have previously demonstrated

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Table 1 Cohort characteristics

Demographics	Entire group, N = 30[a]	Blunter, N = 15[a]	Nonblunter, N = 15[a]	*P*-value[b]
Age (years)	30.4 ± 9.3	33.2 ± 10.1	27.5 ± 7.7	.10
Gender				.46
Female	16 (53%)	9 (60%)	7 (47%)	
Male	14 (47%)	6 (40%)	8 (53%)	
Duration of diabetes (years)	16.2 ± 7.2	17.3 ± 8.1	15.1 ± 6.3	.40
Hemoglobin A1c (%)	6.8 ± 0.7	6.6 ± 0.7	6.9 ± 0.7	.23
BMI	27.9 ± 6.2	27.8 ± 6.2	28.1 ± 6.5	.92
Treatment modality		8 HCL, 3 CSII, 4 MDI/inhaled	9 HCL,2 CSII, 3 MDI	

Abbreviations: CSII, continuous subcutaneous insulin infusion; HCL, hybrid closed loop insulin pump; MDI, multiple daily injections.
[a]Mean ± SD; n (%).
[b]Welch 2 sample t-test; Pearson's Chi-squared test.

that Glc is the main contributor to [Glc + Tau] and that the measured changes in [Glc + Tau] arise from changes in [Glc] (22).

Metabolic modeling

A kinetic model of glucose transport through the blood-brain-barrier (BBB) via reversible symmetric Michaelis–Menten kinetics and irreversible utilization in brain tissue was utilized. This model allows us to examine the impact of recurrent hypoglycemia and glucose transport across several levels of glycemia. Since it is known that there is a linear relationship between brain and blood glucose in humans (14), this approach provides insights into how recurrent hypoglycemia alters brain glucose concentration at any blood glucose level (14, 28). The ratio of maximal transport rate to cerebral metabolic rate of glucose (V^t_{max}/CMR_{glc}) was estimated for both VOI by steady-state modeling of [Glc_{brain}] as a function of [Glc_{plasma}], as we described previously (12, 14). In addition, the kinetics of glucose transport into and utilization in the frontal cortex was assessed using dynamic modeling, as we described previously (16), to extract the Michaelis–Menten constants and the maximal rate for glucose transport and utilization, K^t_M, V^t_{max} and K^{util}_M and V^{util}_{max}.

Statistical analyses

The primary outcome measure was the difference in the ratio of maximal transport rate to cerebral metabolic rate of glucose (V^t_{max}/CMR_{glc}) measured before and after exposure to recurrent hypoglycemia. Our secondary outcomes were the change in epinephrine levels and symptom scores collected during the second and fourth clamps. Participants were categorized into those who demonstrated a blunted response to HG during Clamp 4 compared to Clamp 2 (Blunters), and those who did not demonstrate a blunted response to HG during Clamp 4 compared with Clamp 2 (Nonblunters). A blunter was defined as a participant who experienced either a drop of 125 pg/mL in their average epinephrine measurements from the hypoglycemic portion of Clamp 2 to the hypoglycemic portion of Clamp 4 or who experienced a 20% reduction in their total symptom score from the hypoglycemic portion of Clamp 2 to the hypoglycemic portion of Clamp 4, or both. These parameters were extrapolated from the changes in counterregulatory responses observed in individuals with T1D and IAH who had successful islet transplantation and thus avoided hypoglycemia for 6 months (29).

All statistical analyses were performed using R software (version 4.5.2) (30). The packages "dplyr" (version 1.1.4) (31), "ggplot2" (version 4.0.1) (32), "gtsummary" (version 2.5.0) (33), and "flextable" (version 0.9.10) (34) were used to process and visualize the data and results.

Data and resource availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Results

Cohort characteristics and data quality

Of the 45 subjects enrolled in the study, 30 completed the entire 3-day protocol (Fig. 2). The Participants were systematically assessed for harms from the study. Reasons for attrition included subject revoked consent (1), subject took long-acting insulin when instructed not to (2), physiological response to phlebotomy procedures hindering blood draw (3), and technical issues with MR scanner and motion tracking camera (4) (Fig. 1). Data included in this analysis include only those subjects who completed all parts of the study.

Participants included in the analysis comprised of a young adult cohort with equal distribution of males and females, mean diabetes duration >15 years and well-controlled glycemia (HgbA1c range from 5.7 to 7.4%) (Table 1).

Glycemic targets were consistently achieved during the hyper- and hypoglycemic clamp studies (Fig. 2). MRS data quality was high, as indicated by high signal-to-noise ratios and spectral linewidths in the excellent to adequate range (27) for 3T brain MRS, for both VOI and at both eu- and hyperglycemia (Fig. 3). No spectra were excluded based on QC criteria.

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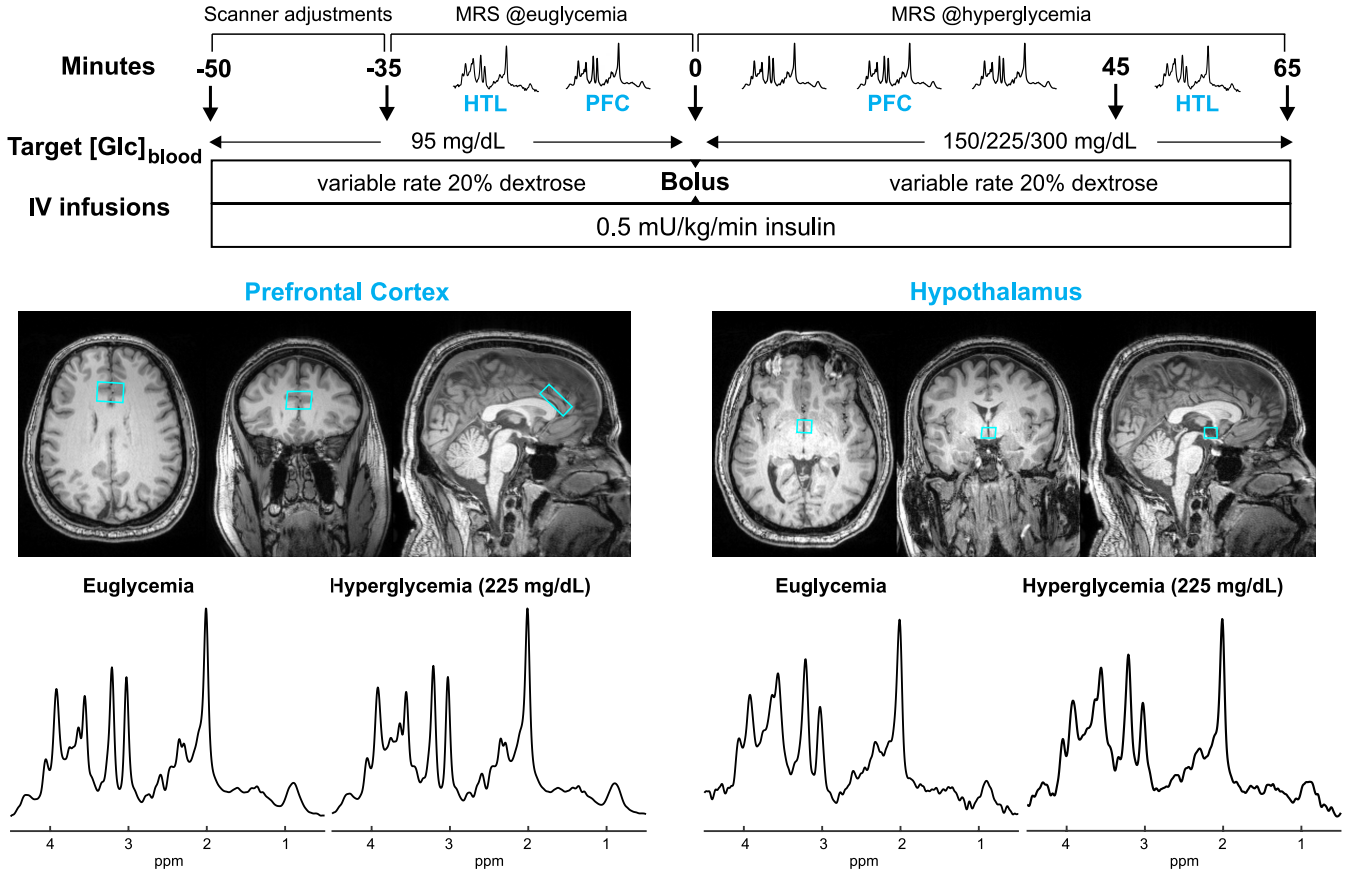

Figure 3 Magnetic resonance spectroscopy (MRS) scanning session protocol and MRS data quality. The hyperglycemic clamps were done in the 3 tesla magnet on Days 1 and 3. The sequence of data collection is shown (top row), together with the volume-of-interest (VOI) positioning on T1-weighted MR images (middle row) and spectral quality in each VOI at eu- and hyperglycemia from a participant who was clamped at 225 mg/dL (bottom row).

Blunting of hormonal and symptom responses following recurrent hypoglycemia

The epinephrine and symptom scores collected during Clamps 2 and 4 are shown in Table 2. Fifteen participants were categorized as having a blunted CRR on Clamp 4 compared to Clamp 2 (Blunters): seven based on showing of a drop in average epinephrine measurements by 125 pg/mL and a 20% reduction in symptom scores; one based on epinephrine responses alone; and seven based on symptom responses alone. Fifteen participants did not show blunting in the epinephrine and symptom responses between Clamps 2 and 4 (nonblunters). Blunters did not differ from nonblunters with respect to age, sex, diabetes duration, hemoglobin A1c, or BMI (Table 1).

Impact of recurrent hypoglycemia on glucose transport

During hyperglycemia, brain glucose concentrations increased as a function of the target glycemia (Fig. 4). No differences were found in steady-state glucose transport kinetics before vs after exposure to recurrent hypoglycemic clamps, ie, at Clamp 1 vs Clamp 5 (V^t_{max}/CMR_{glc}, before vs after, 1.81 ± 0.14 vs 1.88 ± 0.13 in HTL and 1.94 ± 0.07 vs 1.91 ± 0.06 in PFC). In addition, no differences were found in glucose transport kinetics before vs after exposure to recurrent hypoglycemic clamps between blunters and nonblunters (Fig. 2 (35)). We also attempted to perform dynamic modeling of the glucose time courses in the PFC, similar to Shestov et al (16). However, we determined that the number of time points was insufficient for the fitting procedure to converge. The data in the 2011 study had exceptionally high SNR and temporal resolution (due to both a larger MRS voxel size and a high hyperglycemic target in all subjects) and were acquired over a longer duration (~2 hours). This was not possible in the present study because of the complexity of the study design (multiple clamps, multiple hyperglycemia targets and smaller VOIs).

Discussion

We found no difference in glucose transport kinetics in the hypothalamus and prefrontal cortex measured before and after exposure to recurrent hypoglycemia in a protocol designed to induce impaired awareness of hypoglycemia in participants with T1D diabetes. These observations agree with our previous findings of no differences in hippocampal glucose transport kinetics in people with T1D and NAH, T1D and IAH, and healthy controls (36), in hypothalamic glucose transport in healthy humans exposed to a similar protocol of recurrent hypoglycemia utilized in this report (12) and the findings of Segel et al

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Table 2 Median and interquartile ranges of blood glucose, epinephrine, and symptom scores collected during the final 30 minutes of hypoglycemia in Clamps 2 and 4

Clamp No.	Lab measurements	Entire group, N = 30[a]	Blunter, N = 15[a]	Nonblunter, N = 15[a]
Hypoglycemic Clamp 2	Blood glucose (mg/dL)	52.5 [50.2, 54.6]	52.2 [50.2, 54.1]	52.6 [50.0, 55.1]
	Epinephrine (pg/mL)	219.2 [135.0, 346.3]	323.7 [184.0, 388.0]	135.3 [96.3, 261.3]
	Total symptom score	22.3 [17.0, 30.5]	23.0 [19.5, 33.0]	20.0 [11.5, 29.5]
	Adrenergic score	11.8 [8.0, 14.0]	13.0 [10.5, 14.0]	8.5 [6.0, 14.0]
	Neuroglycopenic score	10.5 [7.5, 17.0]	12.5 [8.5, 19.5]	8.0 [5.5, 16.0]
Hypoglycemic Clamp 4	Blood glucose (mg/dL)	52.4 [51.1, 53.3]	51.8 [50.6, 53.7]	52.4 [52.0, 53.3]
	Epinephrine (pg/mL)	160.8 [62.0, 276.7]	118.0 [61.0, 203.0]	238.3 [152.0, 462.3]
	Total symptom score	18.0 [13.0, 26.0]	16.5 [11.5, 22.0]	25.0 [13.5, 35.5]
	Adrenergic score	9.5 [7.0, 14.5]	8.5 [7.0, 10.5]	13.5 [7.0, 17.5]
	Neuroglycopenic score	9.3 [5.5, 13.5]	7.0 [5.0, 13.0]	11.5 [7.5, 18.0]

[a]Median [25th, 75th percentile].

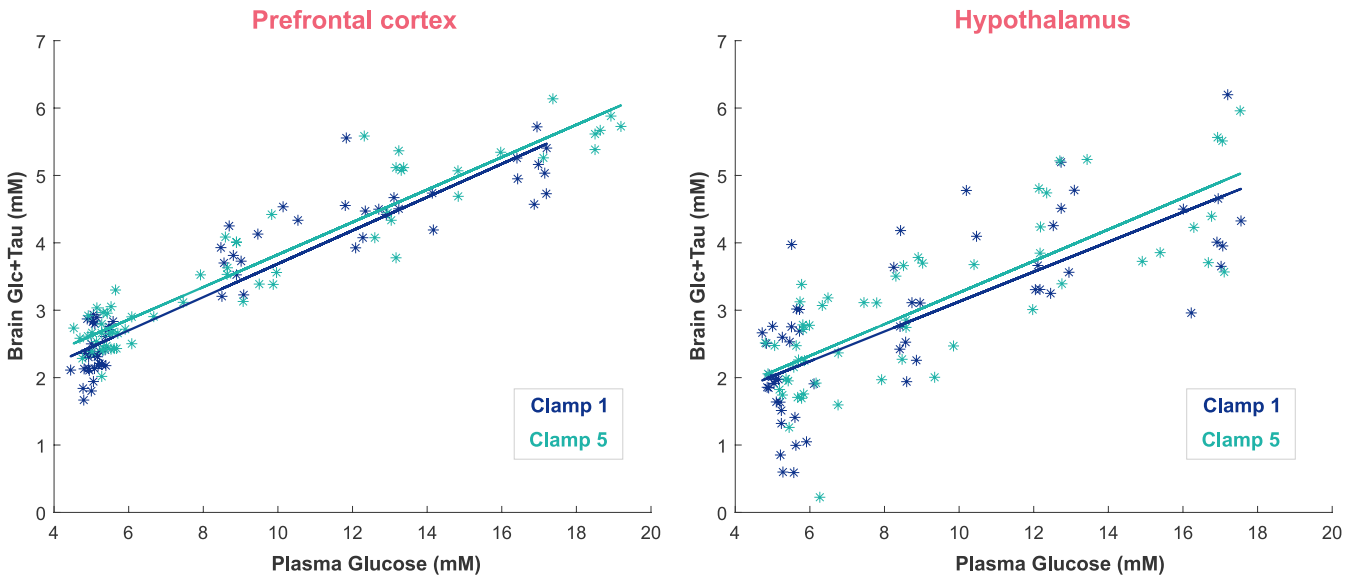

Figure 4 Steady-state blood glucose transport kinetics. Plasma glucose and brain glucose + taurine data were collected during Clamps 1 and 5. Brain tissue glucose + taurine concentrations were measured by MR spectroscopy at 3T in voxels placed in the prefrontal cortex (left) and the hypothalamus (right).

demonstrating that recurrent hypoglycemia does not alter brain glucose transport in healthy individuals using positron emission tomography (37). While it is possible we lacked the power to find a difference between these 2 groups, we must conclude that the available evidence does not support an upregulation of glucose transport in people with T1D within the short time frame during which our participants were exposed to recurrent hypoglycemia.

We measured glucose transport kinetics using a kinetic model of glucose transport through the BBB via reversible symmetric Michaelis–Menten kinetics and irreversible utilization in brain tissue. This model requires the simultaneous collection of steady-state brain and blood glucose concentrations across a range of glycemia. Glucose transport kinetics is the same at all blood glucose levels since a linear relationship between brain and blood glucose exists in humans (14, 28). Consequently, this approach allows the detection of the impact of recurrent hypoglycemia on glucose transport kinetics across a range of glycemia. We could have gained insights into the impact of recurrent hypoglycemia on glucose transport during hypoglycemia by simultaneously measuring brain and blood glucose concentrations at hypoglycemia, but the concentration of glucose in the brain during hypoglycemia is too low to be reliable measured, particularly in the small hypothalamic VOI.

Past work has suggested chronic and recurrent hypoglycemia like that experienced by individuals with impaired awareness of hypoglycemia alters brain glucose metabolism. In rodent models, exposure to chronic (7) and recurrent hypoglycemia (8) appears to upregulate brain glucose transport. In humans, we observed that glucose concentrations are higher in the occipital cortex of subjects with T1D and impaired awareness of hypoglycemia than in controls (5), which current understanding suggests could only be true if glucose transport was upregulated or glucose metabolism was reduced in these subjects with T1D and impaired awareness of hypoglycemia relative to controls.

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Subsequent to this study, we ascertained that cortical oxidative glucose consumption was not different in patients with T1D and IAH than controls (6), thereby providing further support for this hypothesis. Additional support for this concept comes from experiments from Boyle et al where brain glucose uptake was measured by the detection of arteriovenous differences in glucose concentration (3, 4). In one of Boyle's studies, people with T1D with reduced counterregulatory responses maintained brain glucose uptake during hypoglycemia while it fell in those with T1D who generated a normal CRR (4). In another study, brain glucose uptake was maintained in healthy volunteers who developed an impaired CRR to hypoglycemia after exposure to 56 hours of hypoglycemia (3). We have also observed that concentrations of glutamate decrease with hypoglycemia in the occipital cortices of healthy volunteers and individuals with T1D and NAH, but not in participants with T1D and IAH (38). We interpreted these findings to mean that the group with impaired awareness did not need to rely on glutamate for energy needs during hypoglycemia because the brain had adapted in such a way as to ensure fuel was available to meet energy needs during hypoglycemia, perhaps by upregulating glucose transport.

We employed a protocol to induce IAH that has been used previously by ourselves and others. This protocol compares the CRR obtained during the final hypoglycemic clamp with the response measured during the first. Impaired awareness of hypoglycemia was said to have occurred, that is the subject was deemed to have a "blunted" response during clamp 4 compared with Clamp 2, if they had either a drop of 125 pg/mL in their average epinephrine levels or experienced a 20% reduction in their total symptom scores from the hypoglycemic portion of Clamp 2 to the hypoglycemic portion of Clamp 4, or both. These cutoff points were selected based on the work of Rickels et al (29) who measured the CRR to hypoglycemia over time in individuals with T1D who had received an islet transplant and Rubin et al (39) who compared CRR in subjects with T1D with and without impaired awareness of hypoglycemia. This definition allows both a patient centered outcome like symptoms and an objective measure like epinephrine concentrations to support the categorization of having IAH. We have previously shown that individuals with T1D who undergo this induction protocol sometimes display no change in the CRR, display a reduction in only one outcome measure, or display a reduction in both (39). The reasons for this variability in response are not clearly known, but those subjects who failed to have a blunted response in Clamp 4 might have already been blunted in Clamp 2, perhaps by exposure to hypoglycemia in the weeks before study enrollment.

In the current experiment, we focused on measuring brain glucose concentrations in the hypothalamus, a region known to contain glucose responsive neurons that participate in the CRR, and the prefrontal cortex, a region that previously demonstrated involvement in CRR using PET imaging (11). We collected the MR signal associated with brain metabolites from these regions, the volume of which ranged from 1.5 to 8.5 mL. Our methodology allows us to measure average glucose concentrations from selected brain regions but does not allow detection from voxels smaller than 10 mm in the greatest diameter. These methods have permitted the first measurement of hypothalamic glucose concentrations in humans (22) but may not be sufficiently sensitive to detect a change in transport kinetics, especially if the only regions altering glucose transport in response to recurrent HG are the neural structures responsible for glucose sensing and triggering of the CRR. Future studies with methodologies that permit acquisition of data from individual nuclei in the hypothalamus will be necessary to conclusively demonstrate that brain glucose transport kinetics are not altered by exposure to recurrent hypoglycemia.

How can our current findings be reconciled with the studies mentioned above that show the brain has a metabolic adaptation to recurrent hypoglycemia? One possibility is that the heterogeneity of the counter-regulatory hormone and symptom responses to recurrent hypoglycemia, ie, the presence of nonblunters in the study, may have obscured any detectable changes in glucose transport between Clamps 1 and 5 in the whole group. However, the blunter subgroup also did not display any indication of change in glucose transport kinetics, ruling out this possibility. Another possibility is that the 3 bouts of hypoglycemia included in our protocol were not sufficient to increase glucose transport capacity but were sufficient to permit utilization of alternative fuels like lactate and glycogen to support energy needs. We have demonstrated that lactate decreases during hypoglycemia in the occipital cortex of participants with T1D and NAH but does not decrease in the brains of individuals with T1D and IAH (38). De Feyter et al (40) have also shown that brain lactate concentrations increase more during hypoglycemia in people with T1D and hypoglycemia unawareness than controls and Mason et al (41) have shown that monocarboxylic acid transport is increased during hypoglycemia in well-controlled patients with T1D compared with controls. In healthy humans, we have shown that brain glycogen is rapidly utilized during hypoglycemia and that the concentration of glycogen is higher after acute hypoglycemia than it was before (9). A similar observation was made in a rodent model (8). Taken together, the literature suggests that the brain may adapt to recurrent hypoglycemia in different stages. An early stage that involves short-term exposure to recurrent hypoglycemia may involve the use of alternative fuels like lactate and glycogen and a later stage seen after long term exposure to recurrent hypoglycemia perhaps relies on an increase in glucose transport capacity. Future studies should address this hypothesis.

An alternative explanation for the findings reported here can be found in the newly described mechanism called Glucose Sparing by Glycogenolysis (42). In a theoretical meta-analysis, Rothman et al concluded that during periods of increased cerebral demand, astrocytic glycogenolysis provides lactate that can be used to meet its fuel needs. By doing this, more glucose coming into the brain is available for neuronal utilization. Future studies using metabolic labeling may be needed to directly address this novel hypothesis.

Strengths of our study include the use of state-of-the-art MR methods to measure brain glucose concentrations, success in achieving target glycemia during all of the clamp studies, and the relatively large sample size that enrolled in and subsequently completed the study. Weaknesses include the long duration of the study, which was interrupted by the COVID-19 pandemic and technical challenges that prevented study completion in 15 enrolled participants. We originally planned to enroll as many as 50 subjects to achieve adequate power, but the constraints of the pandemic did not leave us with the resources to enroll this many participants. Another weakness was that we were unable to perform the dynamic modeling we attempted in the PFC VOI because of the insufficient number of data points

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available for modeling relative to our prior work in the occipital cortex where a larger number of data points were collected with higher temporal resolution over a longer clamp duration (16). Finally, we relied on a protocol to induce impaired awareness of hypoglycemia that was not successful in blunting the counterregulatory response in all participants. One possible reason for this finding could be that some subjects needed to experience additional episodes of hypoglycemia to show blunting of the counterregulatory response or that some study participants came into the study with impaired awareness of hypoglycemia despite their designation as aware on the Clarke and Gold surveys. If study participants had an impaired counterregulatory response on the first hypoglycemic clamp, it is uncertain if they would show additional blunting after exposure to subsequent recurrent hypoglycemia. Future studies relying on the development of hypoglycemia unawareness should consider subjecting participants to more than 3 episodes of hypoglycemia and take measures like complete avoidance of hypoglycemia for the weeks before the study to ensure participants have normal awareness at the time of study entry. The precise protocol that should be followed to uniformly induce IAH will require additional experimental work before it can be defined.

This project was developed with the goal of identifying mechanisms responsible for the development of IAH in individuals with type 1 diabetes. If changes in glucose transport kinetics were shown to be one mechanism, strategies to impact glucose transporters could be viable therapeutic options for IAH prevention. Preventing the development of IAH would be a major clinical advance for individuals with insulin treated diabetes. However, the lack of a change in glucose transport in a time frame when CRR ensues indicates that glucose transport upregulation is not responsible for IAH induction. Future investigation should therefore focus on other pathways, such as a metabolic adaptations, as potential targets for IAH prevention.

In summary, we found no difference in glucose transport kinetics in the hypothalamus and prefrontal cortex measured before and after exposure to recurrent hypoglycemia in a protocol designed to induce impaired awareness of hypoglycemia in participants with T1D. Our observations do not support upregulation of glucose transport as a causative mechanism for developing IAH in people with T1D.

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Author contributions

E.R.S. conceived and designed the study; obtained funding; collected, analyzed, and interpreted data, and drafted the manuscript. Y.W.P. collected, analyzed, and interpreted data and critically reviewed the manuscript. A.K. collected, analyzed, and interpreted data and critically reviewed the manuscript. A.A. collected, analyzed, and interpreted data and critically reviewed the manuscript. L.Z. analyzed and interpreted data and critically reviewed the manuscript. B.S. analyzed and interpreted data and critically reviewed the manuscript. L.E. designed the study, analyzed and interpreted data and critically reviewed the manuscript. A.M. collected, analyzed, and interpreted data and critically reviewed the manuscript. P.G.H. analyzed and interpreted data and critically reviewed the manuscript. G.Ö. conceived and designed the study; obtained funding; collected, analyzed, and interpreted data, and drafted the manuscript.

Disclosures

G.Ö. serves on the Scientific Advisory Board of BrainSpec Inc., which licensed the AutoVOI tool that was developed by her team and used in the current project. This research was completed while Young Woo Park, Ph.D. was a staff researcher at the Center for Magnetic Resonance Research at the University of Minnesota. The content is solely the responsibility of the authors and does not necessarily represent the official views, opinions or policies of the funding agencies, the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., the Uniformed Services University of the Health Sciences (USUHS), the Department of Defense (DoD), or the Departments of the Army, Navy, or Air Force. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. Government.

Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Guarantor statement

E.S. and G.Ö. are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Previous presentation

American Diabetes Association's Scientific Sessions in 2025.

Clinical Trial Information

NCT04387422.

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