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Brain glucose concentrations in poorly controlled diabetes mellitus as measured by high-field magnetic resonance spectroscopy

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Abstract

Hyperglycemia and diabetes alter the function and metabolism of many tissues. The effect on the brain remains poorly defined, but some animal data suggest that chronic hyperglycemia reduces rates of brain glucose transport and/or metabolism. To address this question in human beings, we measured glucose in the occipital cortex of patients with poorly controlled diabetes and healthy volunteers at the same levels of plasma glucose using proton magnetic resonance spectroscopy. Fourteen patients with poorly controlled diabetes (hemoglobin A_{1c} = 9.8% ± 1.7%, mean ± SD) and 14 healthy volunteers similar with respect to age, sex, and body mass index were studied at a plasma glucose of 300 mg/dL. Brain glucose concentrations of patients with poorly controlled diabetes were lower but not statistically different from those of control subjects (4.7 ± 0.9 vs 5.3 ± 1.1 μmol/g wet wt; *P* = .1). Our sample size gave 80% power to detect a difference as small as 1.1 μmol/g wet wt. We conclude that chronic hyperglycemia in diabetes does not alter brain glucose concentrations in human subjects.

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1. Introduction

The effects of chronic hyperglycemia on the brain have been difficult to define. Reduced cognitive function, particularly with respect to memory and attention, has been identified in patients with poorly controlled diabetes [1-3], and epidemiological studies demonstrate a link between dementia and a diagnosis of diabetes [4,5]. Investigators have identified specific abnormalities in central nerve conduction that are more common in diabetes [6-8], but it is unclear if these abnormalities are linked to metabolic changes that occur as a result of chronic hyperglycemia or are caused by the vascular disease so common in patients with diabetes.

Overall levels of glycemia have been proposed to play a role in determining brain glucose uptake and metabolism [9]. Because normal glucose sensing in the brain

relies in part on rates of brain glucose uptake and metabolism, abnormalities in glucose sensing should be expected in subjects with aberrations in glucose homeostasis. Observations made in human subjects with diabetes suggest that these anticipated changes in glucose sensing do indeed occur. Subjects with recurrent hypoglycemia demonstrate a decrease in the blood glucose concentration at which they develop symptoms of hypoglycemia or mount a counterregulatory response to hypoglycemia [10-13]. Conversely, chronic hyperglycemia has been associated with an increase in the blood glucose concentration at which the symptoms of hypoglycemia (increased heart rate, release of counterregulatory hormones, etc) occur [14]. One plausible mechanism that has been proposed and investigated is that brain glucose uptake is changed in such a way as to compensate for the altered glucose delivery to the brain [9]. Glucose transport at the blood-brain barrier is predominantly mediated by the ubiquitous glucose transporter, GLUT1 [15-18]. In the setting of chronic hypoglycemia, glucose transporters at the blood-brain barrier have been found to be increased by some [19,20] but not all [21] investigators. After chronic hyperglycemia, glucose transporters

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at the rodent blood-brain barrier have been found to be reduced [19,22–24]. Together, these studies suggest that glucose transport may be one mechanism through which the brain can compensate for alterations in global glucose metabolism.

If altered expression of GLUT1 occurs as a result of aberrations in glucose homeostasis, brain glucose concentrations in subjects with such aberrations should be different from those in control subjects with chronic normoglycemia. Assuming a fixed glucose transport capacity on a per GLUT1 molecule basis, the decrease in transporter density that has been shown to occur in chronic hyperglycemia [19,22–24] would be expected to lead to a decreased permeability surface area, which in turn would lead to a decreased apparent maximal glucose transport rate, T_{\max} . Without a major adaptive change in brain energy metabolism, which almost entirely relies on blood glucose and its transport across the blood-brain barrier, a decrease in T_{\max} would be expected to lead to decreased brain glucose content at a given plasma glucose concentration, which would imply that glucose transport could become rate limiting for metabolism at higher plasma glucose concentrations. Regardless of the mechanism, it would appear that a change in brain glucose concentration at a given plasma glucose concentration is central to the hypothesis that brain glucose transport is a critical element underlying how the brain copes with changes in glucose availability.

Brain glucose concentrations can be directly measured using *in vivo* magnetic resonance spectroscopy (MRS) [25,26], whereas positron emission tomography can provide precise measurements of the glucose phosphorylation in the brain and, to some extent, of the unidirectional glucose transport from the operational parameter, k_1 . Positron emission tomography studies thus far have not reported a reduction in glucose transport or an increase in glucose metabolism in patients with poorly controlled diabetes [27,28], possibly because small but clinically significant differences may have been beyond the precision of these important studies. However, we have recently demonstrated that patients with type 1 diabetes and hypoglycemia unawareness have steady-state brain glucose concentrations that are 13% higher than those in healthy volunteers studied under the same metabolic conditions using proton MRS [29]. Proton MRS may be able to identify the small differences in brain glucose concentrations that are expected to result from an alteration in glucose transport if the hypothesis that antecedent hyperglycemia in poorly controlled patients with diabetes reduces brain glucose transport is true. Therefore, the aim of the current study was to examine in human beings whether chronic hyperglycemia alters glucose content by measuring brain glucose concentrations in patients with poorly controlled diabetes and healthy volunteers at the same levels of plasma glucose using proton MRS.

2. Methods

2.1. Study participants

Subjects with diabetes were recruited for study participation from the Endocrine Clinic of the University of Minnesota. To be included in the study, subjects were required to be older than 18 years, have a hemoglobin A_{1c} (HgbA_{1c}) of greater than 8.5% within 3 months of enrollment (for healthy subjects, the range is from 4.5% to 6.0%), have no recent episodes of hypoglycemia, and fulfill the requirements for a magnetic resonance study, which, in addition to the usual magnetic resonance exclusion criteria, precluded subjects weighing more than 300 lb. Control subjects were recruited from the University of Minnesota and Fairview-University Medical Center communities and were similar to the patients with respect to age, body mass index, and sex. The study protocol was conducted according to procedures approved by the institutional review board.

2.2. Protocol

All subjects with diabetes were asked to monitor their blood glucose values at home before each meal and at bedtime for the week before the study. Subjects with hypoglycemia (blood glucose, <70 mg/dL with or without symptoms) within 24 hours before the study were excluded from participation. Patients with self-reported diagnosis of type 1 diabetes were admitted to the General Clinical Research Center of the University of Minnesota the night before the experiment. Their evening dose of long-acting insulin was held and they were maintained on an intravenous infusion of insulin overnight to maintain their glycemia between 100 and 180 mg/dL. The insulin infusion was discontinued at 7:30 AM and the patients were then transported to the Center for Magnetic Resonance Research for the experiment. Subjects with a self-reported diagnosis of type 2 diabetes were asked to discontinue their oral medications 3 days before study and to hold their insulin the night before the experiment. Medications used alone or in combination by the 6 subjects with type 2 diabetes included troglitazone, insulin ($n = 4$), metformin ($n = 2$), glyburide ($n = 3$), pioglitazone ($n = 2$), and glipizide.

All subjects were studied in the Center for Magnetic Resonance in the morning after an overnight fast. In preparation for the experiment, an intravenous catheter was placed retrograde into one foot for the acquisition of blood samples and 2 additional intravenous catheters were placed into the upper extremities for the delivery of somatostatin, insulin, and glucose. The leg used for blood sampling was wrapped in heated towels and hot packs to arterialize the venous blood [30], and baseline samples were obtained for glucose, insulin, and ketones. A somatostatin infusion was then begun and advanced slowly to a rate of 0.16 mg/(kg min) over 30 minutes to suppress endogenous insulin secretion [31]. An intravenous insulin infusion

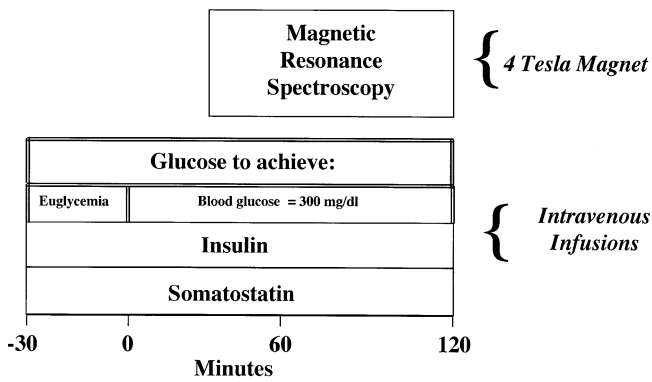


Fig. 1. Experimental protocol. Subjects were given intravenous infusions of somatostatins [$0.16 \mu\text{g}/(\text{kg min})$] and insulin [$0.5 \text{ mU}/(\text{kg min})$] starting at time 0. Glucose was then infused as necessary to maintain plasma glucose at 300 mg/dL between minutes 0 and 120. Data to measure cerebral glucose concentrations were acquired by MRS during the final 60 minutes of the study.

[$0.5 \text{ mU}/(\text{kg min})$, Humulin Regular, Eli Lilly and Company, Indianapolis, Ind] was also begun at time 0. During the first 40 minutes, basal euglycemia was maintained by infusing glucose (50% dextrose) at a variable rate. Thereafter, the glucose infusion was rapidly increased to clamp plasma concentration at 300 mg/dL (Fig. 1) a level we have previously shown to be high enough to yield a robust signal of the H-1 proton of α -glucose at 5.23 ppm , which was used for brain glucose quantification [32–34] (Fig. 2).

2.3. Laboratory analyses

Beginning with the start of the somatostatins and insulin infusions, blood samples were obtained every 5 minutes for determination of plasma glucose concentration in a nearby autoanalyzer (Beckman, Fullerton, Calif). Additional samples for subsequent determination of serum insulin concentrations by a Beckman Access instrument were obtained every 30 minutes. Hemoglobin A_{1c} was measured by high-performance liquid chromatography [35] in all subjects with diabetes.

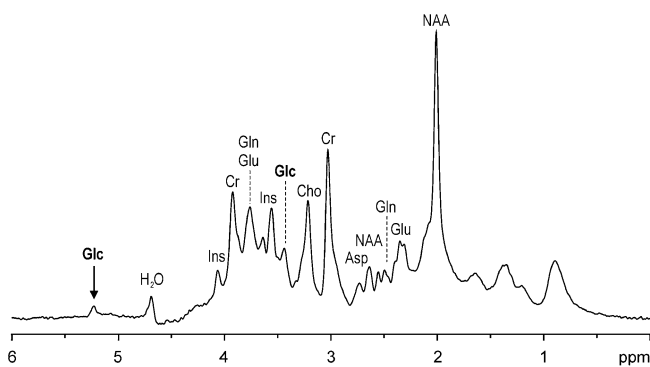


Fig. 2. In vivo ^1H magnetic resonance spectrum of a human brain during glucose infusion. In vivo ^1H MRS at 4 T was used to acquire a spectrum of a 15.6-mL volume of occipital cortex from a patient with diabetes when plasma glucose was clamped at 300 mg/dL . The peak at 5.23 ppm was used to quantify the brain glucose concentration as described in the text. Glc indicates glucose; Ins, inositol; Cho, choline; Glu, glutamate; NAA, *N*-acetylaspartate.

2.4. Magnetic resonance spectroscopy

Experiments were performed on a 4-T Siemens (Erlangen, Germany)/Oxford Magnet Technologies (Oxford, UK) magnet interfaced to a Varian console as detailed previously [32,36]. Subjects were placed supine on a bed above the surface coil and their heads were held in place by cushions. To minimize exposure to gradient noise, all subjects wore earplugs. A quadrature transmit/receive radio frequency (RF) coil consisting of 2 single-turn coils with a diameter of 14 cm was used [37]. A 16- to 27-mL nominal volume of occipital cortex was selected for study. Localization of the signals was based on anatomical landmarks identified on T1-weighted modified driven equilibrium Fourier transform (MDEFT) [38] or rapid acquisition relaxation enhancement/fast spin echo (RARE/FSE) imaging [39]. Localized shimming was performed using fast, automatic shim technique for mapping along projections (FASTMAP) [40,41], which has resulted in consistent line shapes and line widths of the water resonance of 7 to 9 Hz. For the measurements, we used $\text{TE} = 4\text{--}20 \text{ milliseconds}$, $\text{TM} = 33 \text{ milliseconds}$, and $\text{TR} = 4.5 \text{ milliseconds}$ [42]. Water suppression was accomplished by the application of four 25-millisecond gaussian pulses [43] or by applying a series of RF pulses according to the variable power and optimized relaxation (VAPOR) scheme [44]. The water suppression did not affect signal by more than 5% outside $\pm 0.4 \text{ ppm}$ of the water signal. Outer volume saturation was achieved in slices adjacent to the volume of interest using hyperbolic secant pulses with variable RF power [32,42,45]. Data were saved in 10 blocks (16 scans per block) and then corrected for frequency drift (a few hertz) and summed. In each case, the plasma glucose concentrations were maintained at steady state for at least 20 minutes before spectral data were acquired. Data were acquired over a minimum of 20 minutes during which glycemia was clamped at target level.

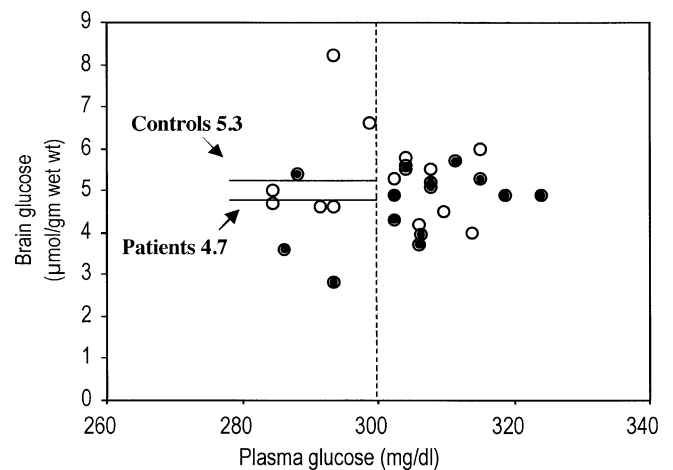


Fig. 3. Brain glucose concentrations. Fourteen subjects with diabetes and 14 matched control subjects were studied using the protocol described in the text. Mean glucose concentrations in brain and plasma for the diabetic participants (filled circles) and the healthy subjects (open circles) are shown.

Table 1
Subject characteristics (mean \pm SD)

	Age (y)	Body mass index (kg/m ²)	Sex (M/F)
Subjects with diabetes (n = 14)	43 \pm 13	28.6 \pm 6.1	5/9
Healthy volunteers (n = 14)	37 \pm 9	27.7 \pm 6.3	5/9
<i>P</i>	.161	.702	.999

2.5. Quantification of brain glucose

Free induction decays were zero filled and apodized with 2 Hz of exponential line broadening. Peak areas of the glucose (5.23 ppm), creatine (Cr; 3.03 ppm), and macromolecule (2.98 ppm) resonances were quantified using peak fitting software supplied by the spectrometer manufacturer as described previously [32,33]. In brief, the area under the glucose peak at 5.23 ppm was calculated relative to the area under the Cr methyl resonance at 3.04 ppm. The concentration of Cr was set to 10 mmol/g wet wt based on cortical concentrations of 9.6 mmol/g wet wt [46] and on contributions of 1 mmol/g wet wt γ -aminobutyric acid [47] and 1 to 2 mmol/g wet wt glutathione in this region of the brain [48,49]. Quantification of the glucose peak at 5.23 ppm was performed and validated by comparison with measurements made using ¹³C-MRS [32].

2.6. Statistical analysis

Diabetic patients and control subjects were compared by 2-sample Student *t* test as appropriate.

3. Results

Fourteen subjects with diabetes and 14 healthy volunteers were studied. As shown in Table 1, the groups were similar with respect to age, body mass index, and sex. Eight of the patients had type 1 diabetes and 6 of the subjects had type 2 diabetes; their HgbA_{1c} was 9.8% \pm 1.7% on the day of the experiment. All subjects with diabetes except one with type 2 diabetes measured blood glucose concentrations at home as requested. The mean glucose value measured during the 4 days before the experiment was 203 \pm 27 mg/dL. No correlation was found between the mean glucose values measured at home during the 4 days before the study and brain glucose concentration ($r = 0.3$; $P = \text{NS}$; $n = 13$). The relationship between glucose concentration in the brain

and HgbA_{1c} also did not achieve statistical significance ($r = -0.076$; $P = \text{NS}$; $n = 14$).

The plasma concentrations of glucose at the time of study were the same in both groups, which were accomplished using the glucose/insulin clamp technique (Table 2, Fig. 3). Serum insulin concentrations were significantly higher in the subjects with diabetes than in the control subjects ($P = .028$; Table 2, Fig. 3). Brain glucose concentrations in the subjects with diabetes were about 10% (0.6 $\mu\text{mol/g}$) lower than in the control subjects but this difference was not statistically significant ($P = .10$; Table 2). Adjusting the comparison for sex, age, or plasma glucose level gave the same finding. Our sample size gave 80% power to detect a difference in brain glucose concentration as small as 1.1 $\mu\text{mol/g}$ wet wt.

4. Discussion

The present study found no significant difference between the brain glucose concentrations measured by MRS at 4 T in patients with poorly controlled diabetes and those in healthy volunteers studied at the same plasma glucose concentration. Although patients with poorly controlled diabetes had an average brain glucose concentration that was 0.6 $\mu\text{mol/m}$ wet wt lower than in control subjects, our study only had 80% power to detect a difference from as small as 1.1 $\mu\text{mol/g}$ wet wt. The clinical significance of any alteration smaller than 1.1 $\mu\text{mol/g}$ wet wt, particularly under conditions of euglycemia or hyperglycemia, is likely to be very small. It appears unlikely that poorly controlled diabetes has clinically meaningful effects on steady-state brain concentrations in human subjects.

Interestingly, recurrent hypoglycemia in patients with type 1 diabetes does appear to affect brain glucose transport and/or metabolism in human beings. We recently observed that brain glucose concentrations are significantly higher in patients with type 1 diabetes, recurrent and frequent hypoglycemia, and hypoglycemia unawareness than in control subjects studied under the same conditions [29], as would be expected if brain glucose transport increases and/or brain glucose metabolism decreases in response to hypoglycemia so as to ensure constant energy metabolism. Taken together, these findings may suggest that the brain is better able to compensate for conditions of glucose deficiency than for glucose excess. Alternatively, patients with type 1 diabetes and hypoglycemia unawareness may experience more prolonged or severe metabolic conditions that trigger the compensation in glucose transport and/or metabolism, as we

Table 2
Concentrations of glucose and insulin during the clamp study (mean \pm SD)

	Plasma glucose (mg/dL)	Serum insulin (pmol/L)	Brain glucose ($\mu\text{mol/g}$ wet wt)
Subjects with diabetes	306 \pm 11 (n = 14)	201 \pm 111 (n = 14)	4.7 \pm 0.9 (n = 14)
Healthy volunteers	301 \pm 11 (n = 14)	123 \pm 44 (n = 10)	5.3 \pm 1.1 (n = 14)
<i>P</i>	.253	.028	.100

previously observed, than would the patients with poorly controlled diabetes included in this report. Although poorly controlled subjects with recent hypoglycemia were excluded from participation in the experiments reported, the magnitude of hyperglycemia experienced by these subjects in the weeks before the study was variable, as evidenced by the range of HgbA_{1c} from 7.9% to 13.2%. The metabolic conditions in the previously studied patients with hypoglycemia unawareness were much more uniform in the weeks before the experiment, as demonstrated by their HgbA_{1c} of 6.9% ± 0.2% (mean ± SEM).

In considering the data presented in this report, several factors that may have contributed to an underestimation of the true effect must be considered. First, plasma glucose concentrations in the patients were on average slightly higher by 5 mg/dL ($P = .253$) compared with those in the control subjects. Because several studies have reported a linear relationship between plasma and brain glucose concentrations [33,34,50], this difference would lead to an artifactual increase in the brain glucose concentration in the patients with diabetes. If this difference in plasma glucose between the groups is of any significance, the actual difference in brain glucose concentrations between the patients with diabetes and the control subjects should be larger than what we observed. However, with such a small and nonsignificant difference in blood glucose concentrations, it is unlikely that this would alter our results. A second factor that may have led to an underestimation of the effect of poorly controlled diabetes on brain glucose concentrations is that the subjects with diabetes had a significantly higher insulin concentration during the clamp study than did the control subjects. The increased plasma insulin concentration in the patient group may have been the result of subcutaneous depots of long-acting insulin or decreased insulin clearance in these subjects. We have recently demonstrated that insulin is without significant effect on the kinetics of cerebral glucose transport and/or metabolism in healthy subjects and that pharmacological concentrations of plasma insulin do not change brain glucose concentrations [34]. Interestingly, some studies have suggested effects of insulin on brain glucose transport and/or metabolism [51,52], but all these changes implicated that the hormone should have increased brain glucose content. Therefore, if insulin were to have an (as of yet undetected) effect on brain glucose content, it would have obscured any potential difference between poorly controlled patients and control subjects.

In summary, using MRS at 4 T, we found no differences between the brain glucose concentrations in patients with poorly controlled diabetes and those in healthy subjects studied under similar levels of glycemia and insulinemia. Because we had sufficient power to detect a difference as small as 1.1 μmol/g wet wt, it is unlikely that poorly controlled diabetes alters brain glucose transport and/or metabolism in a clinically significant manner.

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