



Patients with type 1 diabetes exhibit altered cerebral metabolism during hypoglycemia

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Patients with type 1 diabetes mellitus (T1DM) experience, on average, 2 to 3 hypoglycemic episodes per week. This study investigated the effect of hypoglycemia on cerebral glucose metabolism in patients with uncomplicated T1DM. For this purpose, hyperinsulinemic euglycemic and hypoglycemic glucose clamps were performed on separate days, using [1-¹³C]glucose infusion to increase plasma ¹³C enrichment. In vivo brain ¹³C magnetic resonance spectroscopy was used to measure the time course of ¹³C label incorporation into different metabolites and to calculate the tricarboxylic acid cycle flux (V_{TCA}) by a one-compartment metabolic model. We found that cerebral glucose metabolism, as reflected by the V_{TCA} , was not significantly different comparing euglycemic and hypoglycemic conditions in patients with T1DM. However, the V_{TCA} was inversely related to the HbA_{1c} and was, under hypoglycemic conditions, approximately 45% higher than that in a previously investigated group of healthy subjects. These data suggest that the brains of patients with T1DM are better able to endure moderate hypoglycemia than those of subjects without diabetes.

Introduction

Patients with type 1 diabetes mellitus (T1DM) experience on average 2 hypoglycemic episodes per week and 1 severe episode of hypoglycemia each year (1). Because the brain depends almost exclusively on glucose, recurrent hypoglycemia may be a threat for cognitive dysfunction and cerebral damage. Patients with T1DM are at increased risk for accelerated cognitive decline and possibly for dementia (2, 3). Interestingly, however, patients with T1DM enduring recurrent episodes of severe hypoglycemia do not appear at greater risk of developing cognitive function impairment than patients without such a history (4). This suggests that recurrent hypoglycemia can induce protective adaptations with respect to cerebral glucose metabolism or that hyperglycemia is at least as detrimental for the brain as hypoglycemia.

Recently, we showed that brain glucose metabolism in healthy subjects at glucose levels of approximately 3 mmol/l did not differ from that at normal glucose levels, as reflected by similar tricarboxylic acid (TCA) cycle rates (V_{TCA}) (5). This remarkable maintenance of normal V_{TCA} during symptomatic hypoglycemia indicates that the glucose threshold for effects on cerebral metabolism lies below 3 mmol/l, either because the brain can endure low glucose levels or because entrance of nonglucose energy substrates such as lactate compensates for the fall in glucose (6–9). Whether these findings can be extrapolated to patients with T1DM remains to be determined.

This study was therefore undertaken to investigate the effect of hypoglycemia on brain glucose metabolism in a representative group of patients with longstanding, uncomplicated, reasonably well-controlled T1DM. To do so, we used ¹³C magnetic resonance spectroscopy (MRS) of the human brain during euglycemic and hypoglycemic glucose clamps with infusion of [1-¹³C]glucose, as described previously (5, 10). With this approach, it is possible to directly assess the effects of hypoglycemia on cerebral glucose metabolism and therefore to compare V_{TCA} under euglycemic and hypoglycemic con-

ditions. In addition, we were able to compare the results acquired in the patient group to data previously obtained in subjects without diabetes, who had undergone a similar protocol (5).

Results

To assess the metabolic effects of hypoglycemia in patients with T1DM, a total of 8 hyperinsulinemic euglycemic and 8 hypoglycemic clamps were performed. Six patients completed both clamps (on separate days), 2 patients completed only the euglycemic clamp, and 2 other patients completed only the hypoglycemic clamp.

The clamps started with infusion of a 30 ml 100% [1-¹³C]glucose bolus to rapidly increase plasma ¹³C enrichment for steady-state conditions. As a consequence, plasma glucose and glucose ¹³C enrichment initially peaked (Figure 1). Thereafter, plasma glucose was maintained at 5.0 ± 0.2 mmol/l or 2.9 ± 0.2 mmol/l, as intended. Plasma glucose ¹³C enrichment stabilized at around 30% during both euglycemic and hypoglycemic clamps.

Hypoglycemia stimulated the release of the glucose counterregulatory hormones, adrenalin, noradrenalin, cortisol, and growth hormone (Figure 2), but not that of glucagon, as is characteristic for patients with longstanding T1DM. Although not formally addressed by questionnaires, all patients correctly identified the hypoglycemic clamp, indicating normal hypoglycemic awareness.

¹³C MRS was performed to assess the tissue concentrations of ¹³C-labeled cerebral metabolites derived from glucose during the hyperinsulinemic clamps. Metabolic fluxes were calculated by fitting time courses of these metabolite concentrations with the one-compartment model, as presented elsewhere (5). This model was used to enable quantification and comparison of cerebral glucose metabolism under the two glycemic conditions. Input factors for the model included (a) plasma concentrations and plasma ¹³C isotopic enrichment of glucose and lactate and (b) the time courses of ¹³C incorporation into glutamate C4 (Glu₄) and glutamate C3 (Glu₃), derived from the ¹³C magnetic resonance (MR) spectra of the brain (Figure 3) that were measured sequentially, with a time resolution of 2.5 minutes.

Conflict of interest: The authors have declared that no conflict of interest exists.

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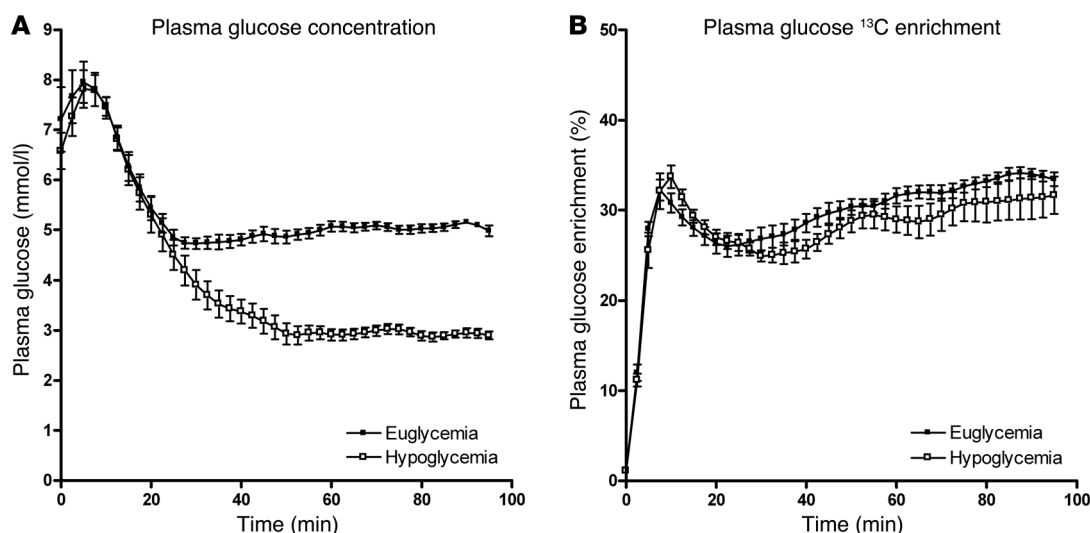


Figure 1

Plasma glucose concentration and ^{13}C enrichment during clamp studies. **(A)** Plasma glucose levels and **(B)** glucose ^{13}C enrichment during euglycemic and hypoglycemic clamps. All data are expressed as mean \pm SEM.

Plasma lactate concentrations and lactate ^{13}C enrichment increased over the first 20 minutes of the clamp. Thereafter, plasma lactate levels stabilized at around 1.42 ± 0.03 mmol/l. Plasma lactate ^{13}C isotopic enrichment continued to increase during the euglycemic clamp and tended to stabilize during hypoglycemia (Figure 4).

The time courses of isotope incorporation into glucose metabolites in the brain tissue showed greater increase of ^{13}C Glu₄ than of Glu₃, consistent with Glu₄ being labeled during the first turn of the TCA cycle and Glu₃ being labeled during the second, irrespective of the glycemic condition. The concentrations of both Glu₄ and Glu₃ were lower during the hypoglycemic clamp than during the euglycemic clamp (Figure 5), as was expected from the slightly lower plasma glucose ^{13}C enrichment values during hypoglycemia.

The free flux parameters, V_{TCA} , V_{dil} (representing the dilution of lactate), and V_{gln} (representing the efflux of glutamine), of the metabolic model were adjusted to best fit the time courses of Glu₄ and Glu₃ levels. Note that the model was applied to fit all individual data sets but that, for reasons of clarity, Figure 5, C and D, shows the averaged fits of the time courses of Glu₄ and Glu₃ on top of the averaged data.

In patients with T1DM, calculated values for V_{TCA} were not different under euglycemic or hypoglycemic conditions (0.59 ± 0.19 $\mu\text{mol/g/min}$ versus 0.62 ± 0.15 $\mu\text{mol/g/min}$, euglycemia versus hypoglycemia, respectively; $P = 0.72$; Figure 6). This did not materially change when only patients for whom there were complete data sets under both glycemic conditions were included ($n = 6$; 0.57 ± 0.21 $\mu\text{mol/g/min}$ versus 0.58 ± 0.11 $\mu\text{mol/g/min}$, euglycemia versus hypoglycemia, respectively; $P = 0.90$). To explore the role of

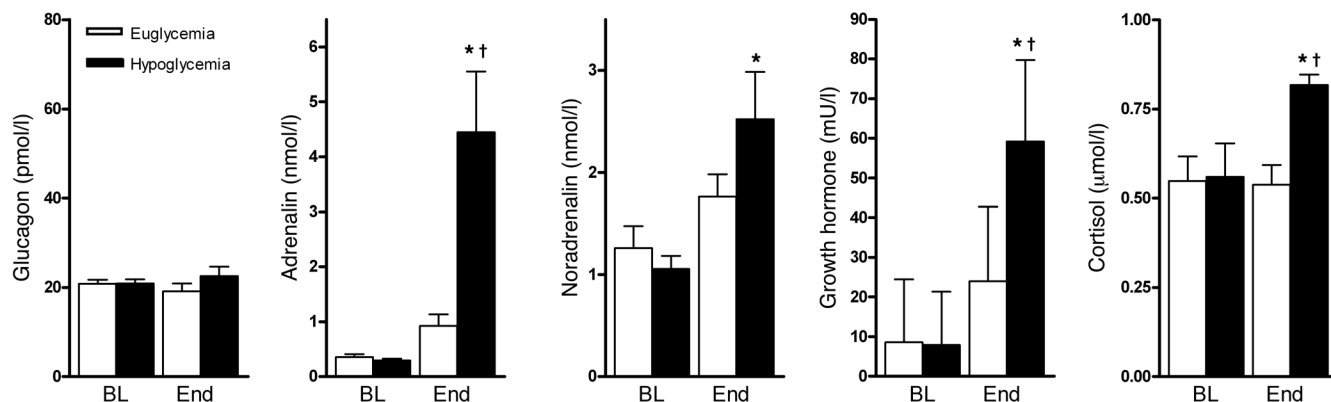
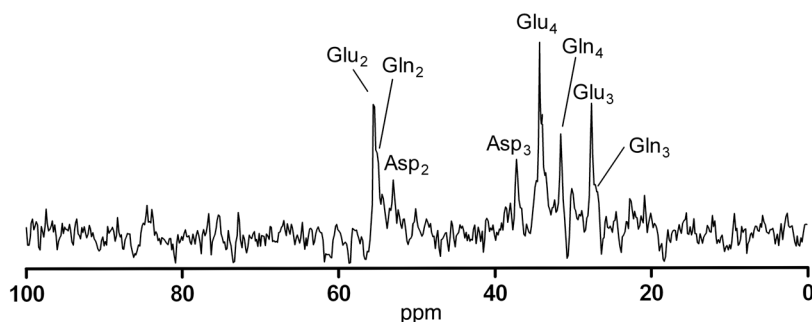


Figure 2

Counterregulatory hormone levels. Levels of plasma glucagon, adrenalin, noradrenalin, growth hormone, and cortisol at baseline (BL) and at the end (End) of the euglycemic and hypoglycemic clamp. All data are expressed as mean \pm SEM. * $P < 0.05$ compared with corresponding baseline value. † $P < 0.05$ compared with end value of euglycemic clamp.

**Figure 3**

¹³C MR spectrum. Representative MR spectrum acquired in 15 minutes after correction for baseline spectra to remove residual fat contamination (note that myo-inositol signal is also not visible because the concentration in the reference spectra is the same as in the dynamic spectra); this is an example of a spectrum fitted in jMRUI to derive concentrations of different labeled metabolites. ppm, parts per million.

glycemic control as a surrogate measure of hypoglycemic burden, we investigated the relationship between the levels of V_{TCA} and glycosylated hemoglobin (HbA_{1C}) and found that lower HbA_{1C} levels were associated with higher V_{TCA} levels ($r = 0.56$, $P < 0.01$) (Figure 7).

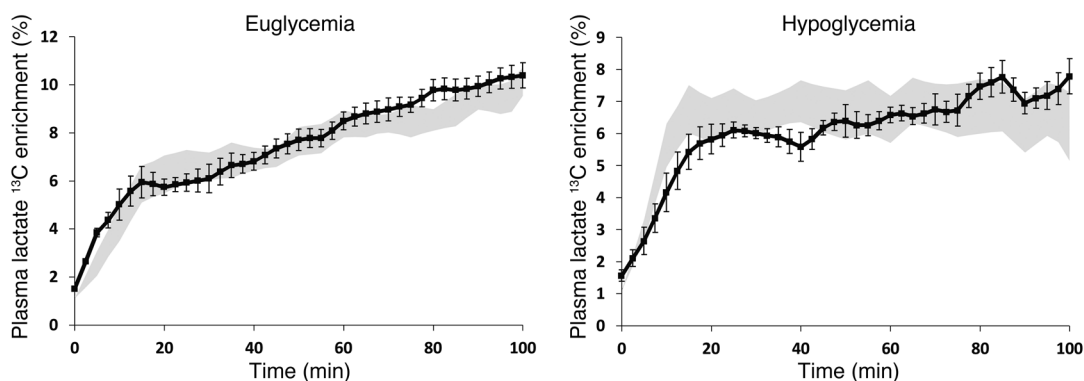
Compared with results obtained in healthy volunteers (published previously, ref. 5), the V_{TCA} in patients with T1DM was significantly higher under hypoglycemic conditions (0.43 ± 0.08 $\mu\text{mol/g/min}$ versus 0.62 ± 0.15 $\mu\text{mol/g/min}$; $P = 0.014$; Figure 6). There were no significant differences in V_{dil} or V_{gln} between healthy subjects and patients with T1DM or between glycemic states (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI62742DS1).

Discussion

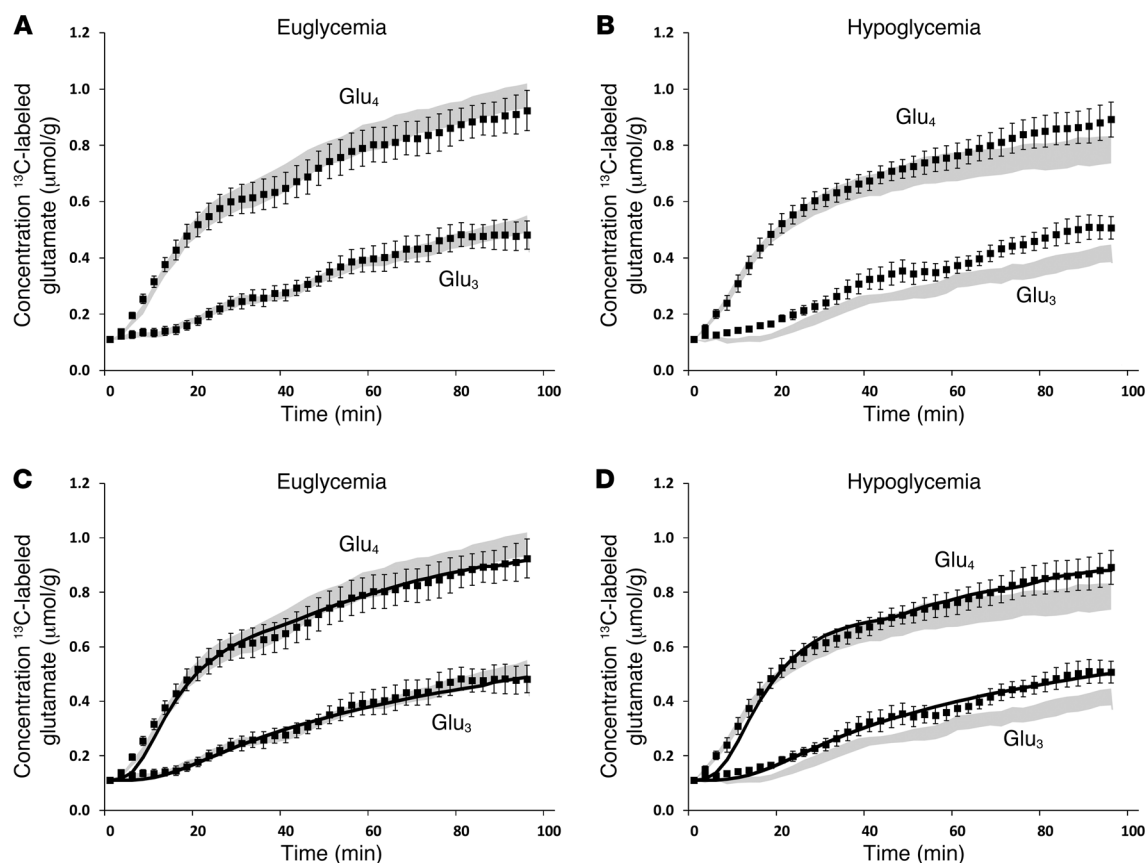
In this study, we examined the effect of hypoglycemia on brain glucose metabolism in patients with uncomplicated T1DM. The major observation of this study is that brain glucose metabolism, as reflected by the V_{TCA} , was about similar under euglycemic and hypoglycemic conditions, analogous to previous results obtained in healthy subjects (5). However, when comparing patients with T1DM with controls, we found that the V_{TCA} was approximately 45% higher in patients than in controls under hypoglycemic conditions. We also found that V_{TCA} was inversely correlated with HbA_{1C} in patients with T1DM. These findings suggest adaptations to

endure hypoglycemia in the type 1 diabetic brain that are not present in the nondiabetic brain. Since deeper hypoglycemia will eventually cause brain glucose metabolism to deteriorate, these adaptations may shift such deterioration to occur at lower plasma glucose values in patients with T1DM than in subjects without diabetes.

A few studies have directly compared cerebral glucose metabolism in patients with T1DM with that in nondiabetic controls, yet in none of these studies was cerebral glucose metabolism directly assessed under hypoglycemic conditions. Henry et al. reported no differences in V_{TCA} between patients with T1DM and hypoglycemia unawareness and healthy controls, as measured by ¹³C MRS. However, their study was conducted under hyperglycemic rather than under hypoglycemic conditions (11), clearly indicating that findings obtained during hyperglycemia cannot be extrapolated to include hypoglycemia. A PET study found no differences in brain glucose metabolism during hypoglycemia between patients with T1DM and controls (12), which is contrary to our findings. However, the measurement of glucose metabolism in this PET study was based on the rate of blood-to-brain glucose uptake, rather than on the quantification of glucose metabolites. The much milder level of hypoglycemia attained during the clamp (plasma glucose, 3.6 mmol/l) and the poor level of glycemic control of the patients enrolled (HbA_{1C} 10.1%) could explain their findings and make a direct comparison with our study difficult.

**Figure 4**

Plasma lactate ¹³C enrichment during clamps. Plasma lactate ¹³C enrichment during (A) euglycemic and (B) hypoglycemic clamps. Patients with T1DM are represented by black symbols; shaded areas represent values in healthy subjects, as reported in ref. 5. All data are expressed as mean \pm SEM.

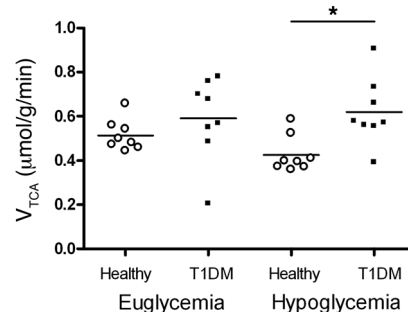
**Figure 5**

Brain glutamate ^{13}C enrichment over time. Brain Glu₄ and Glu₃ concentrations during (A) euglycemic and (B) hypoglycemic clamps, (C and D) together with the respective fits (solid lines) by the metabolic model. Patients with T1DM are represented by black symbols; shaded areas represent values in healthy subjects, as reported in ref. 5. All data were individually quantified and fitted and averaged afterward. All data are expressed as mean \pm SEM.

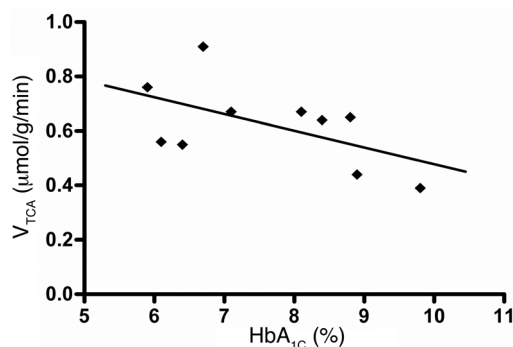
Most patients with T1DM experience hypoglycemic events on a regular basis. Many patients and care providers are concerned that structural abnormalities in white and gray matter and the increased risk of dementia and cognitive dysfunction in T1DM may (in part) be due to recurrent (severe) hypoglycemia (1, 13–15). Although severe hypoglycemia may adversely affect brain anatomy and brain function, particularly in the very young (16) and potentially in the elderly, our findings provide some reassurance for patients that moderate hypoglycemia, such as tested here, has limited effects on cerebral energy metabolism. Whether such results extend to other aspects of brain structure or function cannot be derived from our data.

Since hypoglycemia threatens brain function, it seems plausible that recurrent hypoglycemia initiates an adaptive response to protect the brain against future insults. The inverse correlation between HbA_{1c} and V_{TCA} indeed suggests a role of prior hypoglycemic exposure to the higher V_{TCA} in patients with T1DM with a similarly protective effect, although we cannot with certainty rule out an effect of diabetes per se. A variety of adaptive mechanisms have previously been suggested, including enhanced brain glucose uptake (17, 18), increased storage of glycogen in the brain (19, 20), or use of nonglucose compounds (6–9, 21, 22). However, most of these suggestions lack sufficient support from studies in humans. For instance, in contrast to

rodent data (23, 24), studies in humans have generally failed to confirm that recurrent or prolonged hypoglycemia increases brain glucose uptake during subsequent hypoglycemia (25–27). Also, the potential contribution of glycogen supercompensation as a clinically meaningful adaptation to recurrent hypoglycemia in T1DM has recently been questioned (28).

**Figure 6**

V_{TCA} and V_{TCA} during euglycemic and hypoglycemic clamps in patients with T1DM (this study) and healthy subjects (5). Individual symbols represent individual patients or healthy subjects; horizontal bars indicate the mean. * $P = 0.014$.

**Figure 7**

V_{TCA} versus HbA_{1C} . Inverse correlation between the HbA_{1C} and the V_{TCA} ($r = 0.56$, $P < 0.01$). For paired measurements, the average V_{TCA} was used. Individual symbols represent individual samples.

Lactate can serve as an alternative fuel for the brain under conditions of glucopenia. Its metabolism shares pathways downstream from pyruvate into the TCA cycle with those of glucose. Net uptake of lactate by the brain may explain why ^{13}C enrichment of plasma lactate continued to increase during euglycemia but not during hypoglycemia. Increased capacity to take up monocarboxylic acids, such as lactate, from the plasma into the brain during hypoglycemia has been observed in patients with T1DM with hypoglycemia unawareness, presumably as adaptation to recurrent hypoglycemia (29). Thus, enhanced lactate uptake may have contributed to the observed faster V_{TCA} for patients with T1DM in our study, as we previously showed that brain glucose content and transport into the brain did not differ between patients and controls during hypoglycemia (30). Interestingly, V_{TCA} was also increased in animals exposed to recurrent hypoglycemia, but only under euglycemic conditions, while it was reduced under hypoglycemic conditions and the consumption of nonglucose compounds appeared unaltered under either condition (31). The authors suggested that increased GABA levels during hypoglycemia might have inhibited neuronal metabolism (31). The discrepancy with our data may be due to differences in species but also to a different study design, since our patients were instructed to avoid hypoglycemia for at least 24 hours prior to the clamps and were exposed to less severe hypoglycemic conditions. Since patients with T1DM and hypoglycemia unawareness do not appear to have a greater V_{TCA} under hyperglycemic conditions (11), it seems that the metabolic response we observed is specific for the particular experimental conditions.

A potential benefit of increased brain metabolism under hypoglycemia may be increased ability to endure hypoglycemia. Studies in rats have indicated that exposure to recurrent (moderate) hypoglycemia protects against the development of brain damage or cognitive decline from subsequent severe hypoglycemia (32). Indirect evidence for this notion is provided by a nested case-control study showing that patients with T1DM with at least one severe episode of hypoglycemia during follow-up tended to be at lower risk of cardiovascular events, including strokes, than patients without such events (33). A recent report found that hypoglycemia caused less regional brain deactivation during working memory tasks in patients with T1DM and recurrent hypoglycemia than in healthy subjects (34). The authors interpreted this as a form of cerebral inefficiency, in that the patients needed to engage more brain regions to preserve cognitive function during hypoglycemia. However, it could also be

interpreted as greater ability to resist hypoglycemia, which would be consistent with the findings of this study. Indeed, there was an inverse correlation between HbA_{1C} and brain activation, very similar to the relationship we observed between HbA_{1C} and V_{TCA} .

The strength of our study is that we were able to measure brain glucose metabolism with ^{13}C MRS under hypoglycemic conditions in vivo in a relevant population at high risk of recurrent hypoglycemia. There are also limitations; our measurements were done in a voxel in the occipital cortex, a general limitation of brain ^{13}C MRS, so that we cannot vouch for other brain regions. Also, the metabolic modeling requires a number of assumptions on cerebral metabolite concentrations and fluxes. These assumptions were identical to those used before (5), and therefore the same limitations apply. It was not possible to assess cognitive function during the experiments, as movement artifacts would have disturbed the highly vulnerable MR measurements. Besides, it should be acknowledged that very complex tasks would have been required to detect cognitive changes during moderate hypoglycemia. No attempt was made to assess overall cognitive function in the patients or in the control subjects investigated earlier.

In conclusion, hypoglycemia does not affect brain glucose metabolism in patients with longstanding, uncomplicated T1DM, indicating that alternative sources of energy, such as lactate, can be used by the brain when glucose delivery falls. We also found that the V_{TCA} during hypoglycemia was higher in patients with T1DM than in healthy controls, which we postulate to be the result of cerebral adaptations to chronic recurrent hypoglycemic episodes.

Methods

Subjects

Ten patients with T1DM were included in this study (see Table 1 for patient characteristics). All were free from microvascular complications, except for background retinopathy, and they used no medications other than insulin, thyroxine supplementation (provided that both the dose and serum thyroxine levels were stable), or oral contraceptives. All subjects had at least finished secondary school, and all but one were in professional jobs. Hypoglycemia unawareness was excluded by a Dutch translation of the Cox questionnaire (35, 36). Patients who participated in both the euglycemic and the hypoglycemic clamp had these experiments scheduled in random order at least 2 weeks apart. Female subjects were tested at 4- or 8-week intervals to ensure that experiments took place during corresponding periods of the menstrual cycle.

Hyperinsulinemic glucose clamps

The experimental procedure has been described in detail before (5, 10). All patients came to the MR research facility at 8 AM, after an overnight fast and after having abstained from alcohol and caffeine-containing substances for 24 hours. Patients were instructed to reduce the basal insulin dose by 25% the evening before the clamp to avoid hypoglycemic incidents, to check their blood glucose level at approximately 2 AM, to omit the insulin dose on the morning of the clamp, and (if applicable) to disconnect the insulin pump at 7 AM. If hypoglycemia occurred in the 24 hours before the clamp, experiments were rescheduled. After arrival at the MR facility, the brachial artery of the nondominant arm was cannulated under local anesthesia for frequent blood sampling. An intravenous catheter was inserted in the ante-cubital vein of the contralateral arm for administration of ^{13}C -glucose and insulin. Before patients were placed in the scanner, low-dose insulin was infused to normalize plasma glucose levels (5–8 mmol/l). MR reference measurements were acquired for 30 minutes without administration of exogenous ^{13}C -labeled glucose, followed by a hyperinsulinemic (60 mU/min/m²)

**Table 1**
Subject characteristics

	Patients with T1DM
Male/female (<i>n</i>)	4/6
BMI (kg/m ²)	22.9 ± 3.1
HbA _{1c} (%)	7.6 ± 1.4
Age at first clamp (yr)	31.2 ± 7.8
Duration of diabetes (yr)	15 ± 8
Age of diabetes onset (yr)	17 ± 12
Diabetes onset before 10 years of age (<i>n</i>)	3/10 (30%)
Educational level (<i>n</i>) ^A	2/3/5

^AEducational level was split into the following groups: only secondary school/higher vocational education/university degree.

euglycemic (5.0 mmol/l) or hypoglycemic (3.0 mmol/l) glucose clamp. A 30 ml bolus of 100%-enriched [1-¹³C]glucose (20% w/w) (Campro Scientific) was given 5 minutes after the start of insulin administration to rapidly increase plasma glucose ¹³C enrichment, followed by variable infusions of 40%- or 50%-enriched [1-¹³C]glucose (20% w/w) during the euglycemic and hypoglycemic experiments, respectively. Blood was sampled every 5 minutes for immediate determination of plasma glucose (Beckman Glucose Analyzer II, Coulter, or Biosen C-line, EKF Diagnostics) and lactate (Biosen C-line, EKF Diagnostics) and for later measurement of plasma ¹³C isotopic enrichment of glucose and lactate by high-resolution proton nuclear MR (¹H NMR) at 500 MHz (37, 38). At baseline, at *t* = 30 minutes after the start of the clamp and at the end of the clamp, additional blood was sampled for measurement of plasma insulin and counterregulatory hormones, as described previously (10). The clamps were terminated after 100 minutes.

MR protocol

MR experiments were conducted with the same protocol applied to the healthy volunteers (5) on a 3T MR system (Magnetom Trio, Siemens) equipped with a home-built ¹H volume coil, with a circularly polarized ¹³C surface coil inserted into it (39). Transversal T₂-weighted images were acquired to locate the voxel position for the spectroscopy experiment. Shimming was performed with an automatic gradient-echo-based shim sequence. ¹³C MR spectra were acquired with the ISIS-DEPT sequence (40), which combines ¹H-ISIS localization with ¹H-¹³C polarization transfer. A 45° alpha pulse was used to observe ¹³C-MR signals from CH, CH₂, and CH₃ groups simultaneously. Broadband proton decoupling (WALTZ-16; ref. 41) was applied to simplify the MR spectra. MR spectra were acquired from a voxel of approximately 125 ml in the occipital brain tissue. Each spectrum consisted of 72 scans with a TR of 2 seconds, resulting in a total duration of 2.5 minutes. Acquisition of ¹³C MR spectra started 20 minutes before [1-¹³C]glucose infusion to obtain 8 reference spectra and continued throughout the entire clamp. Visual stimulation was avoided by dimming the lights in the magnet room during the experiments.

Data analysis

¹³C MRS data processing and quantification. To remove contamination of natural abundance ¹³C MR signals, averaged reference spectra were subtracted from the dynamic spectra acquired during ¹³C-labeled glucose infusion. After application of a moving average of 15 minutes, the peaks of interest

in the spectra were fitted with the advanced MR (AMARES) algorithm (42) in the Java-based MR user interface (jMRUI) software package (ref. 43 and see Figure 3 for representative spectrum). To quantify the concentrations of ¹³C-labeled metabolites (i.e., Glu₄ and Glu₃), the natural abundance myo-inositol (mI) concentration was used, assuming that mI peaks equaled 1.1% of 6 μmol/g (44). The ratio between the mI concentration and the peak integral was derived from the sum of all spectra before baseline correction. Results from phantom measurements were used to correct for the effect of the pulse profiles on the signal intensities.

Metabolic modeling. A standard one-compartment metabolic model (11, 45, 46) was used to determine rates of metabolic fluxes representing the V_{TCA}, the loss of label through exchange with unlabeled glutamine (V_{efflux}), and the exchange of intracellular lactate with plasma lactate (V_{di}). The model was adapted to include inflow from labeled plasma lactate. A series of differential equations describe the fluxes of ¹³C labels from and to different metabolite pools. Plasma glucose and lactate concentrations and ¹³C isotopic enrichments were used as input for the metabolic model. The time courses of label incorporation into glutamate at the C4 (Glu₄) and C3 (Glu₃) positions were fitted with a nonlinear least-squares minimization algorithm in MATLAB 2008 (MathWorks). This results in values for the flux parameters. This analysis was performed on all data sets of individual measurements. Note that since the current model used the exact lactate concentration input curves, rather than approximations, the flux values in the healthy volunteers differed slightly, but not significantly, from those reported previously (5).

Statistics. All data are expressed as mean ± SD, unless mentioned otherwise. Differences in means were tested by 2-tailed Student's *t* tests, and the relationship between HbA_{1c} and V_{TCA} was derived with linear regression analysis. Graphpad Prism 4 (Graphpad) was used for statistical analysis, and a *P* value of less than 0.05 was considered statistically significant.

Study approval

All patients gave written informed consent before participating in this study, which was approved by the institutional review board of the Radboud University Nijmegen Medical Centre.

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