Steady-State Brain Glucose Concentrations During Hypoglycemia in Healthy Humans and Patients With Type 1 Diabetes

Kim C.C. van de Ven,¹ Marinette van der Graaf,¹,² Cees J. Tack,³ Arend Heerschap,¹ and Bastiaan E. de Galan³

The objective of this study was to investigate the relationship between plasma and brain glucose levels during euglycemia and hypoglycemia in healthy subjects and patients with type 1 diabetes mellitus (T1DM). Hyperinsulinemic euglycemic (5 mmol/L) and hypoglycemic (3 mmol/L [1-13C]glucose clamps were performed in eight healthy subjects and nine patients with uncomplicated T1DM (HbA₁c 7.7 ± 1.4%). Brain glucose levels were measured by 13C magnetic resonance spectroscopy. Linear regression analysis was used to fit the relationship between plasma and brain glucose levels and calculate reversible Michaelis-Menten (MM) kinetic parameters. Brain glucose values during euglycemia (1.1 ± 0.4 μmol/g vs. 1.1 ± 0.3 μmol/g; P = 0.95) and hypoglycemia (0.5 ± 0.2 μmol/g vs. 0.6 ± 0.3 μmol/g; P = 0.52) were comparable between healthy subjects and T1DM patients. MM kinetic parameters of combined data were calculated to be maximum transport rate/cerebral metabolic rate of glucose (Tmax/CMRglc) = 2.25 mmol/g vs. 1.16 mmol/g vs. 0.66 mmol/g when plasma glucose levels lie anywhere between 0 and 5 mmol/L (3–6).

Hypoglycemia frequently complicates (intensive) insulin treatment in patients with type 1 diabetes mellitus (T1DM). On average, T1DM patients experience 2-3 hypoglycemic events every week and one hypoglycemic event complicated by loss of consciousness or seizures, reflecting severe brain dysfunction, every 1 to 2 years (1). Knowledge about glucose transport over the blood–brain barrier during hypoglycemia is important because the brain is dependent on continuous supply of glucose as its principal source of energy.

Glucose transport over the blood–brain barrier takes place through facilitated diffusion mediated by the glucose transporter GLUT1 (2). Cerebral glucose content depends on the plasma glucose concentration, transport of glucose in and out of the brain, and the cerebral metabolic rate of glucose (CMRglc). Several studies using magnetic resonance spectroscopy (MRS) have shown that over a range of plasma glucose from 4.6 to 30 mmol/L, brain glucose content is linearly related to the plasma glucose level (3–5).

RESEARCH DESIGN AND METHODS

Subjects. We enrolled eight healthy nondiabetic volunteers and nine patients with T1DM. Data from healthy volunteers have partly been reported before (5) but were reanalyzed for this study. Patients with T1DM were excluded if they had a history of repeated severe hypoglycemia, a severe hypoglycemic incident in the past 6 months, or evidence of hypoglycemia unawareness on the Clarke’s questionnaire (9,10). Patients with signs of autonomic neuropathy, peripheral neuropathy, proliferative retinopathy, or micro- or macroalbuminuria by review of medical records or physical examination were also excluded from participating. The study protocol was approved by the institutional review board of the Radboud University Nijmegen Medical Centre, and all volunteers gave written informed consent. For subjects participating in both euglycemic and hypoglycemic study protocols, experiments were scheduled in random order and at least 2 weeks apart. In females, a 4- or 8-week interval was chosen to avoid influences from the menstrual cycle. All nondiabetic volunteers and three T1DM patients had data available from both experiments. For six patients, data were only available from either the hypoglycemic clamp (n = 2) or the euglycemic clamp (n = 4).
Hyperinsulinemic glucose clamps. Hyperinsulinemic (60 ml/min/m²), euglycemic (5.0 mmol/L), or hypoglycemic (3.0 mmol/L) glucose clamps were conducted, as described previously (7,8). Briefly, the brachial artery was cannulated for blood sampling, and a contralateral antecubital vein was cannulated for administration of insulin and glucose 20% to maintain plasma glucose at the pre-determined level for at least 50 min. Exogenous glucose was given in the form of [1-13C]glucose 20% weight for weight at variable enrichments as described earlier (8) to increase plasma 13C enrichment to stable levels during both euglycemic and hypoglycemic experiments. Arterial blood was sampled every 5 min for immediate determination of plasma glucose levels and for later determination of 13C isotopic enrichment of glucose by nuclear magnetic resonance (1H-NMR) (11,12).

Magnetic resonance spectroscopy. All data were acquired on a 3T MR system (Magnetom Trio, Siemens, Erlangen, Germany) (7,8). A 13C coil was placed in a birdcage 1H coil (13), and an ISIS-DEPT sequence was used for localization and polarization transfer to increase the signal-to-noise ratio of 13C signals (14). A voxel of ~125 mL was placed in occipital brain tissue. Data were acquired dynamically with a time resolution of 2.5 min, starting at least 10 min after the glycemic target was reached.

13C MRS data processing and quantification. 13C MR spectra measured from a phantom were used to eliminate effects of MRS data processing and quantification.

MM kinetics. MM kinetic parameters were derived from the data using reversible MM kinetics, as described by Gruetter et al. (3), assuming a linear relationship between plasma glucose (Glcpl) and brain glucose (Glcbr). In this model, K denotes the MM constant for substrate concentration at half maximum transport rate, CMRglc, the consumption rate of glucose, and Vd, the physical distribution of glucose (0.77 mL/g) (3,19).

\[ \text{Glc}_{\text{br}} = \frac{\left( T_{\text{max}}/\text{CMR}_{\text{glc}} - 1 \right) \cdot \text{Glc}_{\text{pl}} - K_{i}}{T_{\text{max}}/\text{CMR}_{\text{glc}} + 1} \]

The data in this study were fitted by linear regression analysis. From this linear relationship, \( T_{\text{max}}/\text{CMR}_{\text{glc}} \) and \( K \), were calculated. The kinetic parameters were determined using a bootstrapping method implemented in Matlab (Mathworks, Natick, MA). From the original dataset, the same amount of data points is selected randomly, and this is repeated 10,000 times.

Statistical analysis. All data are expressed as means ± SD, unless mentioned otherwise. Differences in means were tested by two-tailed Student t tests; a P value < 0.05 was considered statistically significant. Statistical analyses were performed with GraphPad Prism 4 (GraphPad, La Jolla, CA) and SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Baseline characteristics are shown in Table 1. Plasma glucose values during the euglycemic clamps averaged 5.1 ± 0.3 mmol/L (coefficient of variation [CV] 4.1 ± 1.7%) and 5.0 ± 0.2 mmol/L (3.8 ± 1.8%) (P = 0.79) in healthy subjects and patients, respectively; corresponding values during the hypoglycemic clamps were 3.0 ± 0.3 mmol/L (5.7 ± 2.2%) and 2.9 ± 0.2 mmol/L (6.9 ± 3.7%) (P = 0.67). Plasma glucose 13C enrichments were also stable over the last 50 min of the experiment. In healthy subjects, 13C glucose enrichments were 35.4 ± 1.4% (CV 3.1 ± 1.7%) during euclymphemia and 29.9 ± 5.2% (6.0 ± 2.0%) during hypoglycemia. In T1DM patients, the values were, respectively, 32.2 ± 2.3% (5.2 ± 2.2%) and 30.2 ± 5.3% (8.9 ± 2.2%). In response to hypoglycemia, glucagon levels significantly increased in healthy subjects, but not in patients with T1DM. Levels of all other counterregulatory hormones (adrenaline, growth hormone end cortisol) increased significantly and to a similar extent during hypoglycemia in both groups (data not shown).

In all 13C brain MR spectra of both healthy volunteers and T1DM patients, there was a clear difference in the intensity of the glucose signal relative to the natural abundance mI signals between the euglycemic and hypoglycemic state (Fig. 1). Individual steady-state brain glucose levels as a function of plasma glucose under hypo- and euglycemic clamp conditions are presented in Fig. 2. Brain glucose values averaged 1.1 ± 0.4 and 1.1 ± 0.3 mmol/g (P = 0.95) during the euglycemic clamps in healthy subjects and T1DM patients, respectively; corresponding values during the hypoglycemic clamps were 0.5 ± 0.2 and 0.6 ± 0.3 mmol/g, respectively (P = 0.52).

**TABLE 1**

| Subject characteristics | Healthy control | T1DM |
|-------------------------|-----------------|------|
| Male/female             | 4/4             | 4/5  |
| Age (years)             | 23 ± 3          | 32 ± 8* |
| BMI (kg/m²)             | 23.9 ± 4.5      | 23.0 ± 3.6 |
| Duration T1DM (years)   | —               | 17 ± 9 |
| HbA1c (%)               | —               | 7.7 ± 1.4 |

Data presented as number or as mean ± SD. *P < 0.05 versus healthy control subjects.
FIG. 2. Data of healthy subjects (open squares) and patients with T1DM (closed circles) together with the best fit of the data and 95% CIs. \( R^2 = 0.59; P < 0.001 \).

The plasma versus brain glucose relation was fitted with linear regression analysis to determine the reversible MM kinetic parameters. The linear fit of the total data set in Fig. 2 shows that, with 95% CI (\( R^2 = 0.59; P < 0.0001 \)), cerebral glucose levels become undetectable within a plasma glucose range of \(-0–2 \text{ mmol/L}\). The MM parameters were calculated for the whole group of healthy subjects and diabetic patients to be: \( T_{\text{max}}/\text{CMR}_{\text{glc}} = 2.25 \pm 0.32 \) and \( K_t = 1.53 \pm 0.88 \text{ mmol/L} \) (Table 2). There was no indication that MM parameters differed between the two groups: \( T_{\text{max}}/\text{CMR}_{\text{glc}} = 2.43 \) and \( K_t = 2.20 \text{ mmol/L} \) for healthy subjects and \( T_{\text{max}}/\text{CMR}_{\text{glc}} = 2.08 \) and \( K_t = 0.93 \text{ mmol/L} \) for T1DM patients.

**DISCUSSION**

In this study, brain glucose levels were measured by \(^{13}\text{C}\) MRS under hypoglycemic conditions in T1DM patients and nondiabetic control subjects. Previous studies conducted under hyperglycemic conditions reported a linear relationship between brain and plasma glucose values. Our findings measured under hypoglycemic conditions are consistent with such a relationship and provide evidence for linearity up to \(-3 \text{ mmol/L}\). There was neither a difference in cerebral glucose content or in the MM kinetic parameters for cerebral glucose transport between T1DM patients and control subjects.

Previously calculated values for reversible MM kinetic parameters in humans were based on data obtained under euglycemic and hyperglycemic conditions and had rather large SD (Table 2). This lack of data made it impossible to draw firm conclusions with regard to brain glucose transport under hypoglycemic conditions. Knowledge about cerebral glucose transport during hypoglycemia is important because of the brain's dependency on glucose supply. The values we present for the MM kinetic parameters were assessed under hypoglycemic conditions and well within the SD of previously published data in humans (3,4,6) and in rats (5). Our data thus substantiate that the linear relationship between plasma and brain glucose extends well into the hypoglycemic range. Assuming continuation of this linear relationship between plasma and brain glucose, our data predict that brain glucose approaches zero at a plasma glucose level of \(-1.2 \text{ mmol/L} \) (Fig. 2).

The current study demonstrated similar cerebral glucose levels for T1DM patients and healthy subjects under euglycemic or hypoglycemic conditions. This is in accordance with previous findings using MRS obtained under clamped hyperglycemic conditions (20) and using positron emission tomography under hypoglycemic conditions (21). Two studies reporting higher brain glucose levels in patients with T1DM than in healthy control subjects (22,23). However, it should be acknowledged that plasma glucose levels were uncontrolled and therefore also much higher in the patients than in control subjects. Another study reported increased brain glucose levels measured by \(^{1}H\) MRS during a hyperglycemic clamp in T1DM patients with hypoglycemia unawareness, which the authors interpreted as a compensatory response to recurrent hypoglycemia (24). Because we examined patients with normal hypoglycemic awareness, we can neither confirm nor refute this suggestion. However, another study using positron emission tomoography reported no differences in cerebral glucose content during either euglycemia or hypoglycemia between T1DM patients with and without hypoglycemia awareness (25).

To quantify the brain glucose concentrations, we made some assumptions. First, because the \(^{13}\text{C}\) MR spectra were acquired in a rather large voxel in the occipital cortex, we assumed that 5% of the voxel contained blood vessels and corrected for this. Second, the quantification was based on the concentration of mI as internal reference, which we assumed to be stable. There is some evidence that mI levels are up to 20% increased in frontal parts of the brain.

**TABLE 2**

| Study (reference) | Subjects | Brain region | Plasma glucose levels (mmol/L) | \( T_{\text{max}}/\text{CMR}_{\text{glc}} \) | \( K_t \) (mmol/L) |
|------------------|----------|--------------|-------------------------------|--------------------------------|------------------|
| Gruetter et al. (3) | Healthy humans | Visual cortex (occipital) | 4.6–29 | 2.3 ± 0.2 | 0.6 ± 2.0 |
| Seaquist et al. (6) | Healthy humans | Periventricles (WM) | 4.4–24.5 | 2.15 ± 0.25 | 1.96 ± 2.45 |
| Seaquist et al. (6) | Healthy humans | Occipital cortex (GM) | 4.4–24.5 | 2.24 ± 0.23 | -0.98 ± 2.13 |
| de Graaf et al. (4) | Healthy humans | WM | 5–18 | 2.2 ± 0.12 | 1.7 ± 0.88 |
| de Graaf et al. (4) | Healthy humans | GM | 5–18 | 1.8 ± 0.10 | 1.1 ± 0.66 |
| Choi et al. (5) | Rats | Whole brain | 1–27 | 2.7 ± 0.13 | 3.3 ± 1.0 |
| Current study | Healthy humans + T1DM patients | Occipital | 2.5–5.3 | 2.25 ± 0.32 | 1.53 ± 0.88 |

GM, gray matter; WM, white matter.
in T1DM patients as a consequence of hyperglycemia (22). Although the occipital cortex is probably less affected (23), and the effect of acutely normalizing plasma glucose values (such as during a glucose clamp) on cerebral mI levels is unknown, recalculating MM kinetics assuming 20% higher mI levels resulted in T_{max}/CMR_{glc} = 2.44 \pm 0.44 and K_{t} = 1.70 \pm 1.18 \text{ mmol/L}. Thus, the MM kinetic parameters changed slightly when higher mI values were assumed, but they stayed within the range of data published before (3,6). Furthermore, it should be appreciated that we cannot vouch for a linear relationship between plasma and brain glucose below plasma glucose levels of \sim 3 \text{ mmol/L}. The detection limit of brain glucose levels made it unfeasible to study the effects of very low plasma glucose levels with brain MRS.

In conclusion, our data show that the linear MM relationship between plasma and brain glucose reported previously extends well into the hypoglycemic range in patients with T1DM and nondiabetic control subjects. Our data also show that brain glucose content and kinetics of brain glucose transport do not differ between healthy subjects and patients with uncomplicated T1DM under hypoglycemic conditions. Future MRS studies need to address these issues in T1DM patients with hypoglycemia unawareness.

ACKNOWLEDGMENTS
This work was financially supported by the Dutch Diabetes Research Foundation (Grant 2004.00.012), National Institutes of Health (Grant DK-006881), the framework of the Center for Translational Molecular Medicine (http://www.ctmm.nl), project Prevention and Early Detection of Cardiovascular Complications in Type 2 Diabetes Mellitus Grant 01C-104), and the Netherlands Heart Foundation and Dutch Kidney Foundation.

No potential conflicts of interest relevant to this article were reported.

K.C.C.v.d.V., M.v.d.G., and B.E.d.G. collected the data and performed data analysis. M.v.d.G., C.J.T., A.H., and B.E.d.G. designed the study. M.v.d.G. and B.E.d.G. wrote the study protocol. All authors contributed to interpreting the data, editing of the manuscript, and approval of the final version of the paper. K.C.C.v.d.V. and B.E.d.G. are the guarantors of this work and, as such, had full access to all the data, editing of the manuscript, and approval of the final version of the paper.

No potential conflicts of interest relevant to this article were reported.

The authors thank Karin Saini (Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands) and Cindy Frentz (Department of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands) for assistance during the glucose clamps, and Angelina Goudswaard (Department of Laboratory Medicine, Laboratory of Genetic Endocrine and Metabolic Diseases, Nijmegen, the Netherlands), Frederique Vermeulen (Department of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands), and Udo Engelke (Department of Laboratory Medicine, Laboratory of Genetic Endocrine and Metabolic Diseases, Nijmegen, the Netherlands) for help with the high-resolution NMR.

REFERENCES
1. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169–3176
2. Partridge WM, Boado RJ, Farrell CR. Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. J Biol Chem 1990; 265:18035–18040
3. Gruetter R, Uggulik, B. Seaquist ER. Steady-state cerebral glucose concentrations and transport in the human brain. J Neurochem 1998;70:397–403
4. 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
5. Choi IY, Lee SP, Kim SG, Gruetter R. In vivo measurements of brain glucose transport using the reversible Michaelis-Menten model and simultaneous measurements of cerebral blood flow changes during hypoglycemia. J Cereb Blood Flow Metab 2002;21:653–663
6. 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
7. van de Ven KC, van der Graaf M, Tack CJ, Klomp DW, Heerschap A, de Galan BE. Optimized [1-(15)C]glucose infusion protocol for 13C magnetic resonance spectroscopy at 3T of human brain glucose metabolism under euglycemic and hypoglycemic conditions. J Neurosci Methods 2010;186:68–71
8. van de Ven KC, de Galan BE, van der Graaf M, et al. Effect of acute hypoglycemia on human cerebral glucose metabolism measured by 13C magnetic resonance spectroscopy. Diabetes 2011;60:1467–1473
9. Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky KS. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care 1995;18:517–522
10. De Galan BE, De Mol P, Wennekes L, Schouwenberg B, Smits P, Preserved sensitivity to beta-adrenergic receptor agonists in patients with type 1 diabetes mellitus and hypoglycemia unawareness. J Clin Endocrinol Metab 2006;91:2878–2881
11. Van Den Bergh AJ, Tack CJ, Van Den Boogert HJ, Vervoort G, Smits P, Heerschap A. Assessment of human muscle glycogen synthesis and total glucose content by in vivo 13C MRS. Eur J Clin Invest 2000;30:122–128
12. Serlie MJ, De Haan JH, Tack CJ, et al. Glycogen synthesis in human gastrocnemius muscle is not representative of whole-body muscle glycogen synthesis. Diabetes 2005;54:1277–1282
13. Klomp DW, Renema WK, van der Graaf M, de Galan BE, Kentgens AP, Heerschap A. Sensitivity-enhanced 13C MRS spectroscopy of the human brain at 3 Tesla. Magn Reson Med 2006;55:271–278
14. Klomp DW, Kentgens AP, Heerschap A. Polarization transfer for sensitivity-enhanced MRS using a single radio frequency transmit channel. NMR Biomed 2008;21:444–452
15. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson 1997;129:35–43
16. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. MAGMA 2001;12:141–152
17. Ross B, Lin A, Harris K, Bhattacharya P, Schweinsburg B. Clinical experience with 13C MRS in vivo. NMR Biomed 2003;16:358–369
18. Reynaud G, Claes T, Vlerick L, et al. Age-related differences in metabolites in the posterior cingulate cortex and hippocampus of normal ageing brain: A (1)H-MRS study. Eur J Radiol 2012;81:e223–e231
19. Gjedde A, Diemer NH. Autoradiographic determination of regional brain glucose content. J Cereb Blood Flow Metab 1983;3:303–310
20. 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
21. Fanelli CG, Dence CS, Marlkham J, et al. Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. Diabetes 1998;47:1444–1450
22. Heikkinen S, Mäkimattila S. Hyperglycaemia is associated with changes in the regional concentrations of glucose and myo-inositol within the brain. Diabetologia 2009;52:534–540
23. Kreis R, Ross BD. Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy. Radiology 1992;184:123–130
24. Criado 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
25. Bingham EM, Dunn JT, Smith D, et al. Differential changes in brain glucose metabolism during hypoglycaemia accompany loss of hypoglycaemia awareness in men with type 1 diabetes mellitus. An [13C]-O-Methyl-D-glucose PET study. Diabetologia 2005;48:2080–2089