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Brain Lactate Concentration Falls in Response to Hypoglycemia in Patients With Type 1 Diabetes and Impaired Awareness of Hypoglycemia

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Brain lactate may be involved in the development of impaired awareness of hypoglycemia (IAH), a condition that affects approximately 25% of patients with type 1 diabetes and increases the risk of severe hypoglycemia. The aim of this study was to investigate the effect of acute hypoglycemia on brain lactate concentration in patients with IAH as compared with those with normal awareness of hypoglycemia (NAH) and healthy control subjects (*n* = 7 per group). After an overnight fast, all subjects underwent a two-step hyperinsulinemic euglycemic (5.0 mmol/L)–hypoglycemic (2.8 mmol/L) glucose clamp. Brain lactate concentrations were measured continuously with ^{1}H-MRS using a specific lactate detection method. Hypoglycemia generated symptoms in patients with NAH and healthy control subjects but not in patients with IAH. Brain lactate fell significantly by ∼20% in response to hypoglycemia in patients with type 1 diabetes with IAH but remained stable in both healthy control subjects and in patients with NAH. The fall in brain lactate is compatible with increased brain lactate oxidation providing an alternative fuel source during hypoglycemia, which may contribute to the impaired detection of hypoglycemia.

Approximately 25% of patients with type 1 diabetes have lost the capacity to timely detect hypoglycemia, a condition referred to as impaired awareness of hypoglycemia (IAH) (1). IAH increases the risk for severe, potentially hazardous hypoglycemia up to sixfold (2) and is usually the end result of a process of habituation to recurrent hypoglycemia (1).

Although the precise mechanisms underlying IAH remain to be revealed, there may be a pivotal role for the alteration in the brain's handling of energy substrates other than glucose (3). Indeed, using ^{13}C-MRS, we found that brain metabolism was largely preserved during hypoglycemia in both subjects without diabetes and subjects with type 1 diabetes, despite a similar fall in brain glucose availability (4–6). These observations indicate that metabolism of a nonglucose carbohydrate energy source may be involved.

Several observations suggest that this nonglucose energy source is lactate. Lactate is a valuable energy source for the brain during euglycemia (7–9) and may be critical to maintaining brain function during severe hypoglycemia (10). Administration of lactate during hypoglycemia impairs hypoglycemic symptoms, attenuates counterregulatory hormone responses, and preserves cognitive function, mirroring the changes seen in subjects with IAH (11,12). Finally, brain lactate transport capacity through monocarboxylic acid transporters was found to be increased during hypoglycemia in patients with IAH (13,14).

The brains of patients with type 1 diabetes and IAH may have been conditioned to use lactate under glucopenic conditions to maintain brain function, thereby simultaneously impairing hypoglycemia sensing. We therefore

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hypothesized that brain lactate levels would fall during hypoglycemia in people with type 1 diabetes and IAH. To test this hypothesis, we measured brain lactate under hypoglycemic conditions with a dedicated ^{1}H-MRS method optimized for lactate detection (15).

RESEARCH DESIGN AND METHODS

Subjects

We recruited seven patients with type 1 diabetes and IAH, seven patients with normal awareness of hypoglycemia (NAH), and seven healthy subjects without diabetes. Awareness state was based on the Dutch modified version of the Cox questionnaire, where scores of 0–1 out of 5 indicate normal awareness and scores ≥3 indicate impaired awareness (16,17). Patients were eligible if they had an HbA_{1c} <9.0% (75 mmol/mol) and were free from microvascular complications, except for background retinopathy. Exclusion criteria were contraindications for MRI examinations, a history of brain injury or cardiovascular events, and the use of drugs other than insulin interfering with glucose metabolism. The institutional review board of the Radboud university medical center approved the study, and all subjects gave written informed consent.

Hyperinsulinemic Glucose Clamps

All participants presented at 8:00 A.M. after an overnight fast having abstained from caffeine, alcohol, and smoking for 24 h and from strenuous exercise for 3 days. Subjects with diabetes were instructed to adjust their basal insulin dose the evening before the clamp to prevent nocturnal hypoglycemia and to omit their morning prandial insulin dose. The brachial artery of the nondominant arm was cannulated under local anesthesia for frequent blood sampling. An intravenous catheter was inserted into the antecubital vein of the contralateral arm to administer glucose 20% (Baxter, Deerfield, IL) and insulin (insulin aspart; Novo Nordisk, Bagsværd, Denmark). After cannulation and baseline measurements, the subjects were positioned in the MR scanner, and a two-step hyperinsulinemic (60 mU/m^2/min) euglycemic (5.0 mmol/L)–hypoglycemic (2.8 mmol/L) glucose clamp was initiated. During the clamp, arterial plasma glucose and lactate levels were determined every 5 min (Biosen C-Line; EKF Diagnostics, Cardiff, U.K.). Counterregulatory hormone and insulin levels were determined at the end of each glycemic phase. Insulin levels were also measured at baseline. Subjects completed an 18-item semiquantitative symptom questionnaire just prior to initiating the glucose clamp and at the end of the hypoglycemic phase in which symptoms were scored from 0 (none) to 6 (severe).

Analytical Methods

Plasma insulin was assessed by an in-house radioimmunoassay (18). Plasma adrenaline was measured by high-performance liquid chromatography combined with fluorometric detection (19).

MRS Protocol

MRS measurements were performed at 3T (Tim MAGNETOM Trio; Siemens, Erlangen, Germany) using a 12-channel receive-only head coil. First, an anatomical image was acquired (T1-weighted MPRAGE; 256 × 256 mm^2 field of view, 256 slices, 1 mm^3 isotropic voxels). Subsequently, ^{1}H-MRS data were acquired from a 25 cm^3 voxel (Fig. 1*A*) in data blocks consisting of two consecutive acquisitions to determine tissue concentrations of brain lactate and of the other major brain metabolites, respectively. Brain lactate concentrations were determined using an interleaved J-editing semi-LASER sequence (20) optimized for lactate detection (15) (echo time (TE) 144 ms; repetition time (TR) 3,000 ms; 32 averages; total duration of acquisition

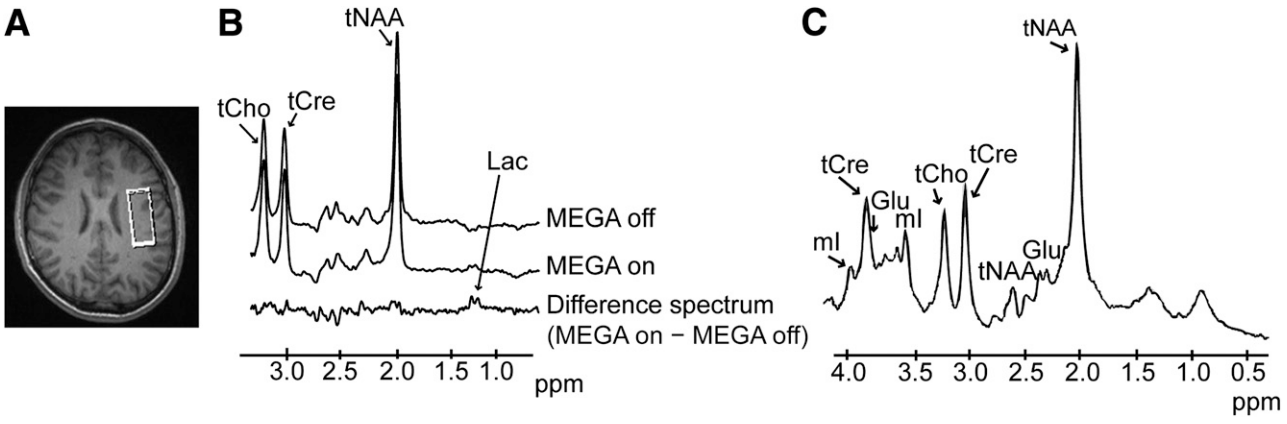

Figure 1—Representative MRS data from one healthy subject. *A*: T1-weighted anatomical image with typical location of the voxel (2.0 × 5.0 × 2.5 cm) for the acquisition of the MRS data. *B*: MEGA off, MEGA on, and difference spectra of one subject. J-editing was performed with MEGA pulses centered on the lactate quartet at 4.1 ppm (MEGA on) and subsequently at −3 ppm (MEGA off). As a consequence, the lactate doublet at 1.3 ppm is inverted in the MEGA off spectrum and upright in the MEGA on spectrum. Subtracting the MEGA on spectrum from the MEGA off spectrum results in the difference spectrum, which contains only the positive lactate doublet, removing the signals from all other metabolites in the spectrum. *C*: MR spectrum recorded with a TE of 30 ms. Glu, glutamate; mI, *myo*-inisitol; tCre, total creatine; tCho, total choline; tNAA, total *N*-acetylaspartate; Lac, lactate.

(TA) 1.40 min). J-editing was performed with MEGA pulses with a bandwidth of 75 Hz. Spectra with a shorter TE with water suppression were acquired to determine the tissue concentrations of other major brain metabolites (sLASER, TE 30 ms, TR 3,000 ms, 32 averages, TA 1.40 min). Lastly, spectra acquired without water suppression were used for quantification of the metabolite concentrations (TE 30 ms; TR 5,000 ms; 8 averages).

Analysis of MRS Data

After zero-filling (from 1,024 to 2,048 points) and Fourier transformation, all J-edited spectra from each subject were phase and frequency aligned with the first spectrum recorded by maximizing the scalar product between this so-called reference spectrum and the other spectra. Difference spectra were apodized with a 5-Hz Lorentzian function, and moving averaging with a sliding window of three scans was performed. In the final difference spectra, the lactate doublet was fitted with the AMARES algorithm in jMRUI (21).

The spectra acquired with a TE of 30 ms were analyzed with the LCModel software to quantify the other major brain metabolites, including total *N*-acetylaspartate, total choline, total creatine, *myo*-inositol, aspartate, glutamine, glutamate, *scyllo*-inositol, and taurine. Only metabolites with a Cramér–Rao lower bound <20% were considered to be reliably quantified and included in further analyses (22). All metabolite concentrations were calculated taking voxel composition (determined by segmenting the T1-weighted anatomical images using SPM8) and differences in T2 relaxation of metabolite spins into account.

Statistical Analyses

Within-group differences were compared with two-sided Student *t* tests. Between-group differences were analyzed by ANOVA followed by pairwise Bonferroni post hoc tests between all groups. All data are expressed as mean ± SEM unless otherwise indicated. A P value <0.05 was considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics 20.

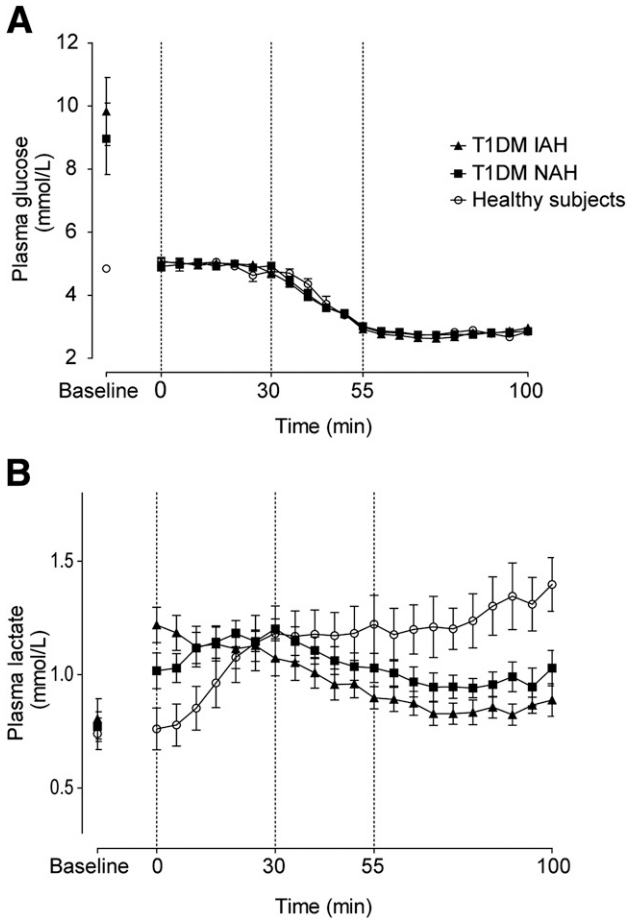

Figure 2—Time courses of plasma glucose (*A*) and plasma lactate (*B*). The dashed lines represent the beginning of the euglycemic phase, the end of the euglycemic phase, and the beginning of the hypoglycemic phase, respectively. Baseline values represent the sample obtained upon arrival at the research facility. Black triangles, patients with type 1 diabetes mellitus (T1DM) and IAH; black squares, patients with T1DM and NAH; open circles, healthy subjects.

RESULTS

The groups were well matched for relevant parameters (Table 1). Baseline plasma glucose values were elevated to a similar extent in both diabetes groups (Fig. 2*A*). During the clamp, plasma glucose levels (mean ± SD) were sequentially clamped at 5.0 ± 0.1 and 2.8 ± 0.1 mmol/L without differences between the groups (Fig. 2*A*). Insulin levels were also comparable during the clamps (data not shown).

Hypoglycemic symptom scores increased significantly in response to hypoglycemia in both healthy volunteers and in patients with NAH but not in patients with IAH

Table 1—Subject characteristics

	T1DM IAH (*n* = 7)	T1DM NAH (*n* = 7)	Healthy subjects (*n* = 7)
Age, years	24.7 ± 8.1	26.2 ± 5.8	27.6 ± 6.9
Sex, M/F	3/4	4/3	3/4
BMI, kg/m^2	23.4 ± 1.3	24.7 ± 2.9	23.5 ± 1.7
HbA_{1c}, % (mmol/mol)	7.5 ± 0.6 (58.7 ± 6.3)	7.3 ± 0.4 (56.6 ± 3.8)	—
Duration of diabetes, years	10.0 (2.5, 17.5)	10.0 (6.0, 14.0)	—
Score on modified Cox questionnaire (range)	3.7 ± 0.8 (3–5)	0.4 ± 0.5 (0–1)	—

Data are presented as *n*, mean ± SD, or median (interquartile range). F, female; M, male; T1DM, type 1 diabetes mellitus.

(mean increase 2.0 ± 0.9, 12.9 ± 3.9, and 17.4 ± 3.7 for patients with IAH, patients with NAH, and healthy control subjects, respectively). Adrenaline responses to hypoglycemia were lower in patients with diabetes than in healthy volunteers ($P < 0.05$), particularly in those with IAH, although the difference between the two patient groups was not statistically significant ($P = 0.88$) (Supplementary Table 1).

Baseline plasma lactate levels were similar across the three groups, but time courses during the clamp were different (Fig. 2*B*). During the hypoglycemic phase of the clamp, mean plasma lactate levels were significantly higher in healthy subjects than in subjects with diabetes ($P < 0.01$).

In one patient with NAH, the ^{1}H-MR spectral quality was insufficient for analysis because of head movement during data acquisition. The J-edited difference spectra of all other subjects showed a clear lactate doublet at 1.3 ppm (Fig. 1*B*). The MR voxel contained 65.5 ± 2.9% white matter, 31.2 ± 2.8% gray matter, and 3.2 ± 0.5% cerebral spinal fluid, with no differences between groups (data not shown).

Brain lactate concentration dropped from 0.52 ± 0.02 to 0.41 ± 0.02 μmol/g wet weight (ww) in response to hypoglycemia in patients with IAH ($P < 0.001$), corresponding with a fall of ~20% (Fig. 3). In contrast, brain lactate concentrations remained stable during euglycemia and hypoglycemia in both healthy subjects (0.49 ± 0.02 vs. 0.46 ± 0.01 μmol/g ww, $P = 0.12$) and patients with NAH (0.46 ± 0.03 vs. 0.45 ± 0.03 μmol/g ww, $P = 0.73$). There were no differences between groups in absolute brain lactate concentrations during euglycemia ($P = 0.17$) or during hypoglycemia ($P = 0.36$).

^{1}H-MR spectra without editing and with a TE of 30 ms (Fig. 1*C*) revealed a significant drop in brain glutamate concentrations in response to hypoglycemia in healthy subjects (from 6.0 ± 0.3 to 5.7 ± 0.3 μmol/g ww, $P < 0.01$) but not in patients with NAH (6.6 ± 0.3 vs. 6.4 ± 0.3 μmol/g ww, $P = 0.13$) or in patients with IAH (7.3 ± 0.3 vs. 7.1 ± 0.3 μmol/g ww, $P = 0.11$). There were no significant changes in response to hypoglycemia regarding other major brain metabolites.

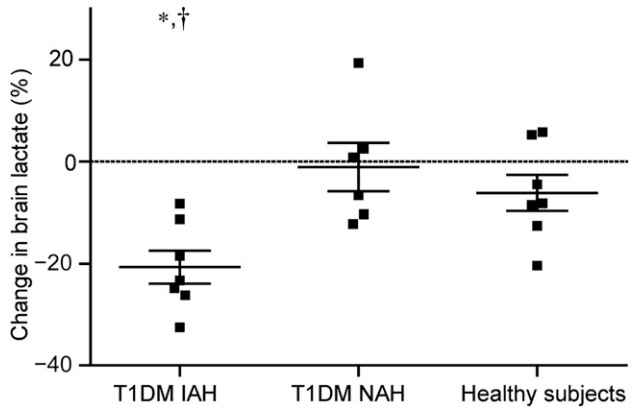

Figure 3—Hypoglycemia-induced changes in brain lactate. Mean (with SEM) group differences (horizontal bars) and individual changes (black squares) between average euglycemic and hypoglycemic brain lactate concentrations (percent change from euglycemic value) are depicted. *$P < 0.001$ for euglycemia vs. hypoglycemia and †$P < 0.05$ vs. T1DM NAH and healthy subjects.

DISCUSSION

The major finding of this study is that brain lactate concentrations decrease by ~20% in response to hypoglycemia in patients with type 1 diabetes and IAH but not in patients with type 1 diabetes and NAH or in healthy control subjects. This finding suggests that adaptations in cerebral lactate handling are involved in the etiology of IAH.

A recent ^{1}H-MRS study also reported decreased brain lactate concentrations in response to hypoglycemia, albeit this change was only significant in patients with diabetes and normal adrenaline responses to hypoglycemia (23). However, the MR methods in that study were focused on glutamate detection, and patients were stratified according to the observed adrenaline response to hypoglycemia rather than according to the awareness of hypoglycemic symptoms.

A change in brain lactate concentration reflects a change in the balance between uptake, export, production (through glycolysis), and oxidation of cerebral lactate (24). The hypoglycemia-induced reduction in brain lactate in patients with IAH most likely resulted from increased lactate oxidation as an adaptation to recurrent exposure to hypoglycemia to preserve brain metabolism when glucose supply is low. Our observation that plasma lactate levels fell in the IAH group argues against increased brain lactate export. Furthermore, it is unlikely that the lower brain lactate levels were the result of decreased cerebral lactate uptake, given that plasma lactate levels fell to a similar extent in both patient groups and that lactate transport capacity has been reported to be increased in patients with IAH (14). We cannot completely exclude that the fall in lactate reflected a decrease in glycolysis due to reduced neuronal activation (25).

In a recent ^{13}C-MRS study, De Feyter et al. (13) showed that the human brain oxidized ^{13}C-labeled lactate that was infused during hypoglycemia. Somewhat surprisingly, they found no differences in lactate oxidation between patients with diabetes and healthy control subjects, despite a higher calculated brain lactate concentration in the patients with diabetes, which seems at odds with our findings. However, inherit to their study design, infusion of ^{13}C-lactate may have resulted in greater brain lactate availability. Therefore, the physiological context (blood lactate levels and its source, pH, etc.) may be different, which renders comparison with our data difficult.

The strengths of our study include the ability to detect and quantify brain lactate concentrations in vivo in humans in a direct and optimized manner without the use of exogenous lactate and the three distinctly different groups of subjects, which enabled us to differentiate between the impact of diabetes and IAH. Although MR spectra were

recorded continuously, ^{1}H-MRS does not provide information about lactate fluxes or consumption, which is a limitation of our study.

In conclusion, we found that brain lactate concentration dropped in response to acute hypoglycemia in patients with type 1 diabetes and IAH but not in the other two groups. The fall in brain lactate is compatible with increased brain lactate oxidation during hypoglycemia in patients with IAH, and hence, the need for glucose by the brain and the consequent initiation of hypoglycemic symptoms are suppressed. Together our findings indicate that changes in brain lactate levels play an important role in the pathophysiology of IAH.

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

Author Contributions. E.C.W., H.M.R., B.E.d.G., and M.v.d.G. designed the study with input from C.J.T. and A.H. H.M.R. recruited the participants and performed the glucose clamps. E.C.W. and H.M.R. collected the data. E.C.W. and M.v.d.G. analyzed the MRS data; H.M.R. was responsible for all other data analyses. All authors discussed the results and implications and commented on the manuscript at all stages. B.E.d.G. and M.v.d.G. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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