



Effect of Exercise-Induced Lactate Elevation on Brain Lactate Levels During Hypoglycemia in Patients With Type 1 Diabetes and Impaired Awareness of Hypoglycemia

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Since altered brain lactate handling has been implicated in the development of impaired awareness of hypoglycemia (IAH) in type 1 diabetes, the capacity to transport lactate into the brain during hypoglycemia may be relevant in its pathogenesis. High-intensity interval training (HIIT) increases plasma lactate levels. We compared the effect of HIIT-induced hyperlactacidemia on brain lactate during hypoglycemia between 1) patients with type 1 diabetes and IAH, 2) patients with type 1 diabetes and normal awareness of hypoglycemia, and 3) healthy participants without diabetes ($n = 6$ per group). All participants underwent a hypoglycemic (2.8 mmol/L) clamp after performing a bout of HIIT on a cycle ergometer. Before HIIT (baseline) and during hypoglycemia, brain lactate levels were determined continuously with J-difference-editing $^1\text{H-MRS}$, and time curves were analyzed using nonlinear mixed-effects modeling. At the beginning of hypoglycemia (after HIIT), brain lactate levels were elevated in all groups but most pronounced in patients with IAH. During hypoglycemia, brain lactate decreased $\sim 30\%$ below baseline in patients with IAH but returned to baseline levels and remained there in the other two groups. Our results support the concept of enhanced lactate transport as well as increased lactate oxidation in patients with type 1 diabetes and IAH.

Impaired awareness of hypoglycemia (IAH) affects 25–30% of patients with type 1 diabetes, is characterized by the

suppression of symptoms during hypoglycemia (1,2), and considerably increases the risk for severe hypoglycemia (1). IAH results from habituation to prior hypoglycemia (3), but the underlying mediators have yet to be fully revealed.

One likely mediator is brain lactate (4–6). We have previously shown that brain lactate levels fall in response to hypoglycemia in patients with type 1 diabetes and IAH, whereas lactate levels remain unaltered in patients with normal awareness of hypoglycemia (NAH) and in healthy participants (6). This fall in brain lactate likely reflects increased cerebral lactate oxidation, which may preserve brain metabolism and interferes with the brain's capacity to detect hypoglycemia.

This concept is supported by the finding that intravenous administration of lactate has brain glucose-sparing effects under euglycemic conditions (7), suppresses counter-regulatory hormone and symptom responses to hypoglycemia, and preserves cognitive function (8,9). We observed similar, albeit less pronounced, effects when plasma lactate levels were endogenously raised during hypoglycemia after a bout of high-intensity interval training (HIIT) in patients with type 1 diabetes and NAH (10).

Using isotopically enriched lactate infusions, De Feyter et al. (5) observed increased brain lactate concentrations during hypoglycemia in patients with IAH compared with volunteers without diabetes but no increase in cerebral lactate oxidation. This finding contrasts with observations that endogenous hyperlactacidemia after exercise enhances

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both brain lactate uptake (7,11,12) and oxidation (7,13,14), at least under euglycemic conditions. The effect of endogenously elevated plasma lactate levels on brain lactate concentrations during hypoglycemia is currently not known. Therefore, we used a single bout of HIIT to raise endogenous plasma lactate levels, and used ^1H -MRS to assess cerebral lactate levels during subsequent hypoglycemia in patients with type 1 diabetes with and without IAH and healthy participants.

RESEARCH DESIGN AND METHODS

Participants

We enrolled six patients with type 1 diabetes and IAH, six patients with type 1 diabetes and NAH, and six healthy participants. Patient assignment was initially based on the Dutch modified version of the Cox questionnaire (15), but one patient initially classified as having IAH switched groups because of intact hormonal and symptomatic responses to hypoglycemia. Patients with type 1 diabetes were excluded from participation if their HbA_{1c} levels exceeded 75 mmol/mol (9.0%) or if they had microvascular complications, except for background retinopathy. Other exclusion criteria were as follows: age >40 years, BMI >30 kg/m², cardiopulmonary disease, contraindications for MRI examination, and a history of brain injury. The study was approved by the institutional review board of the Radboud University Medical Center, and all participants gave written informed consent.

Study Protocol

All participants presented in the morning (8:00 A.M.) after an overnight fast, having abstained from caffeine, alcohol and smoking for 24 h and from strenuous exercise for 2 days. Patients with type 1 diabetes were instructed to check their plasma glucose levels regularly in the 24 h before the experimental day to prevent hypoglycemia and to omit their morning prandial insulin dose. An intravenous catheter was inserted in the antecubital vein to administer insulin (insulin aspart; Novo Nordisk, Bagsvaerd, Denmark) and glucose 20% (Baxter, Deerfield, IL). The brachial artery of the contralateral arm was cannulated under local anesthesia for frequent blood sampling to determine plasma glucose and plasma lactate levels every 5 min (Biosen C-line; EKF Diagnostics).

After cannulations, a hyperinsulinemic (60 mU/m²/min)-euglycemic (5.0 mmol/L) glucose clamp was initiated, and baseline magnetic resonance (MR) measurements were acquired. Participants were subsequently taken out of the scanner and performed a HIIT session on a cycle ergometer, consisting of three 30-s all-out sprints interspersed with 4 min of active recovery, as described previously (10). After the HIIT session, participants were placed in the MR scanner at the earliest opportunity, and meanwhile plasma glucose levels were gradually decreased to 2.8 mmol/L and maintained there for 50–60 min.

Before the HIIT session and at the end of hypoglycemia, participants completed an 18-item questionnaire in which hypoglycemic symptoms were scored from 0 (none) to 6

(most severe), and blood samples were drawn to determine counterregulatory hormone responses and insulin levels (see Fig. 1A for a schematic overview of the study protocol).

MRS Protocol and Data Analysis

MR data were acquired using a 3T MR system (TIM Magnetom Trio; Siemens, Erlangen, Germany). Each MR session, i.e., at baseline and postexercise, started with the acquisition of an anatomical image (T1-weighted MPRAGE, 256 × 256 mm² field of view, 256 slices) for voxel localization and for later voxel content determination. The 20 × 45 × 25 mm voxel was placed in the periventricular region of the brain (Fig. 2A). During both MR sessions, brain lactate levels were determined continuously with a time resolution of ~4 min, using a J-difference-editing MEGA-semi-LASER sequence (TE 144 ms, TR 3,000 ms, and 32 averages), as described previously (6).

The J-difference-edited spectra were zero-filled from 1,024 to 2,048 points and Fourier transformed. Thereafter, spectra were phase and frequency aligned (16), subtracted pairwise, and apodized in the time domain with a 5-Hz Lorentzian. A three-point moving average filter over time was applied. The lactate doublets at 1.3 ppm in the final difference spectra were fitted with the AMARES algorithm in jMRUI (17). Cerebral lactate was quantified using the unsuppressed water signal as a reference, taking voxel composition, differences in T2 relaxation, and the contribution of plasma lactate into account, assuming a vessel volume in the (mainly white matter voxel) of 2% (18). Baseline cerebral lactate levels (before HIIT) were averaged; cerebral lactate levels acquired after HIIT were analyzed over time.

Analytical Methods

Plasma insulin was determined by an in-house radioimmunoassay (RIA) (19) and plasma glucagon by a commercially available RIA (Eurdiagnostica, Malmö, Sweden). Plasma adrenaline and noradrenaline were analyzed by high-performance liquid chromatography combined with fluorometric detection (20).

Statistical Analysis

Within-group differences were compared with two-sided Student *t* tests or Wilcoxon signed rank test for nonparametric data, and between-group differences with ANOVA followed by Bonferroni post hoc tests or with the Kruskal-Wallis test and post hoc Mann-Whitney *U* tests. The change in brain lactate from baseline to the start of hypoglycemia ($\Delta\text{Lac}_{\text{startHypo}}$), the difference in steady-state lactate levels between hypoglycemia and baseline ($\Delta\text{Lac}_{\text{plateau}}$), and the decay rate (*k*) were estimated per group using a nonlinear mixed-effects (NLME) model. The following exponential decay function was fitted to the data:

$$\text{Lac}(t) = (\Delta\text{Lac}_{\text{startHypo}} - \Delta\text{Lac}_{\text{plateau}})e^{-kt} + \Delta\text{Lac}_{\text{plateau}} \quad (1)$$

Differences in model parameters (i.e., $\Delta\text{Lac}_{\text{startHypo}}$, $\Delta\text{Lac}_{\text{plateau}}$ and *k*) were compared between groups and within groups.

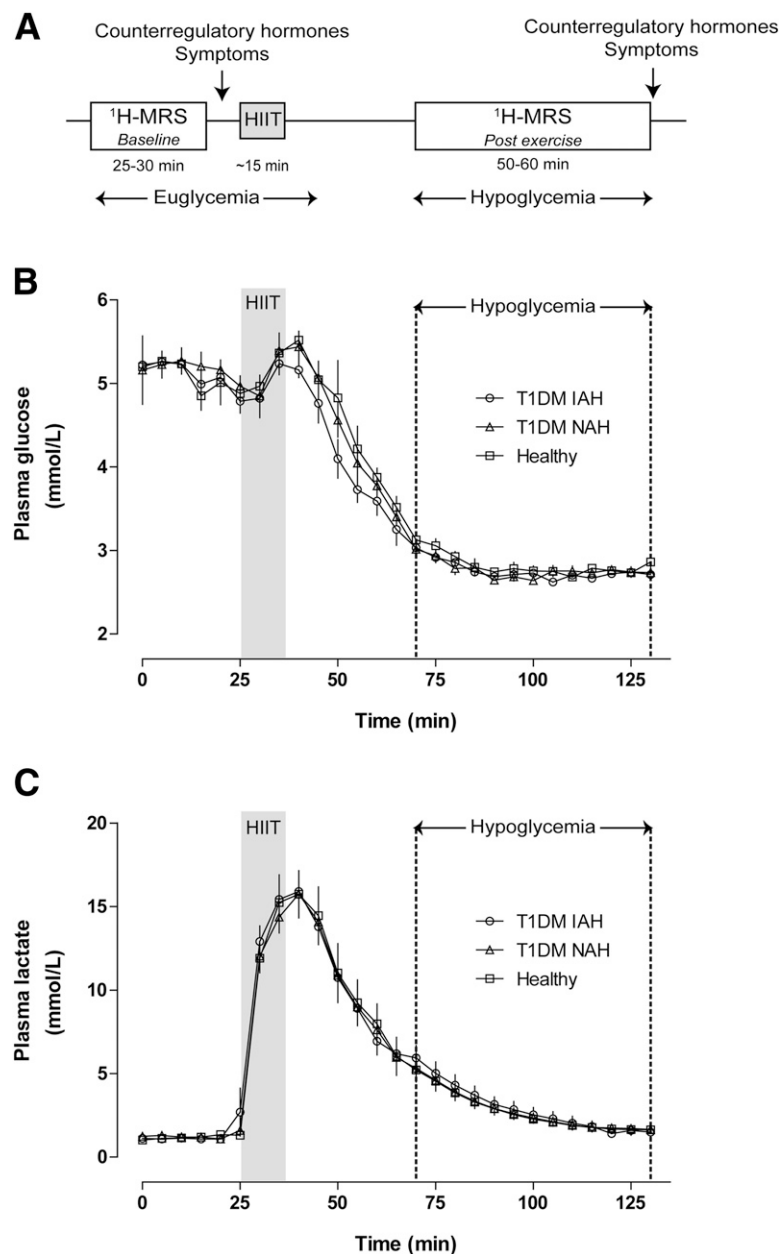


Figure 1—Study protocol and plasma glucose and plasma lactate levels. **A**: Schematic overview of the study protocol. MRS measurements were performed prior to HIIT (baseline) and during hypoglycemia. Counterregulatory hormone responses and hypoglycemic symptom score were assessed prior to the HIIT session and at the end of hypoglycemia. Time courses of plasma glucose (**B**) and plasma lactate (**C**) levels. The HIIT session is indicated by the gray area. The dashed lines represent the beginning and the end of the hypoglycemic phase. Open circles, patients with type 1 diabetes (T1DM) and IAH; open triangles, patients with T1DM and NAH; open squares, healthy participants.

All data are expressed as mean \pm SD, unless otherwise stated. $P < 0.05$ was considered statistically significant. Statistical analyses were performed in SAS 9.2 (NLMIXED procedure) or with IBM SPSS Statistics 20.

RESULTS

The study participants were well matched for relevant parameters (Table 1). Upon arrival, plasma glucose levels were elevated to a similar extent in both patient groups (7.9 ± 3.1 and 9.5 ± 2.2 mmol/L for type 1 diabetes and

IAH and type 1 diabetes and NAH, respectively). Insulin levels at baseline and during hypoglycemia did not differ between groups (data not shown). During the clamp, plasma glucose levels were maintained at 5.1 ± 0.1 mmol/L and 2.8 ± 0.1 mmol/L, without differences between study groups (Fig. 1B). Plasma lactate levels for the three groups were comparable at baseline and increased markedly and to a similar extent in response to HIIT. During hypoglycemia, plasma lactate fell gradually but remained above baseline levels (Fig. 1C).

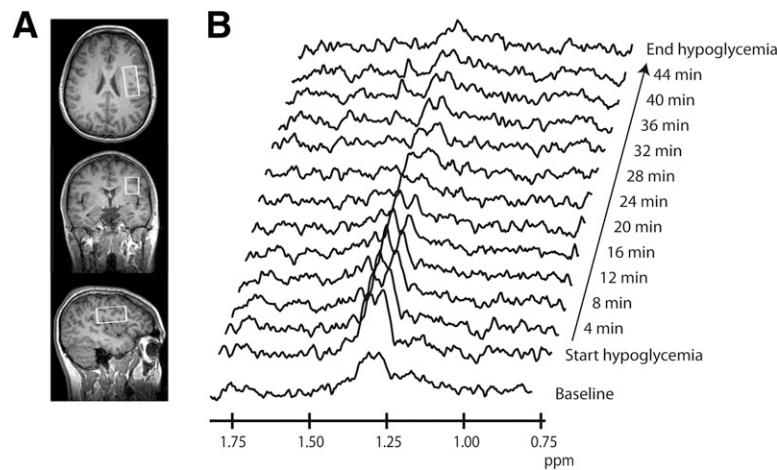


Figure 2—Example of difference spectra. *A*: Typical location of the MRS voxel, indicated by the white rectangles, (20 × 45 × 25 mm) on a T1-weighted anatomical image of one subject with type 1 diabetes and IAH. *B*: Representative difference spectra (i.e., MEGA on – MEGA off) of one subject with type 1 diabetes and IAH, depicting the lactate doublet at 1.3 ppm over time. The baseline difference spectra were recorded before HIIT. After HIIT, hypoglycemia was induced and brain lactate concentrations were measured continuously.

As expected, total symptom scores did not change in response to hypoglycemia in patients with IAH (mean increase 0.67 ± 1.26) but increased considerably in patients with NAH and in healthy participants (14.83 ± 3.61 and 10.83 ± 2.06 , respectively). The plasma adrenaline response to hypoglycemia was reduced in patients with IAH compared with patients with NAH and healthy participants (Supplementary Table 1).

Baseline brain lactate levels were similar across the three groups (Fig. 3). The first MR spectra were acquired on average 38.5 ± 5.5 min after the HIIT session, when plasma glucose levels were <3.6 mmol/L. Figure 2*B* shows a typical example of difference spectra with the evolution of the lactate doublet over time. According to the NLME model, brain lactate levels at the beginning of hypoglycemia were elevated compared with baseline levels ($\Delta\text{Lac}_{\text{startHypo}}$ in Eq. 1) by 0.20 ± 0.05 $\mu\text{mol/g}$ ($P = 0.01$) in patients with IAH, by 0.11 ± 0.05 $\mu\text{mol/g}$ ($P = 0.03$) in patients with NAH, and by 0.06 ± 0.02 $\mu\text{mol/g}$ ($P = 0.01$) in healthy participants. The increase in brain lactate after HIIT was higher in patients with IAH compared with healthy participants ($P = 0.04$). During hypoglycemia, brain lactate levels returned to and remained at baseline levels in patients with NAH and

healthy participants (0.00 ± 0.04 and -0.07 ± 0.04 $\mu\text{mol/g}$), whereas a further -0.20 ± 0.06 $\mu\text{mol/g}$ decrease below baseline ($P = 0.02$) (Fig. 3) was observed in patients with type 1 diabetes and IAH during hypoglycemia ($\Delta\text{Lac}_{\text{plateau}}$ in Eq. 1). There was no difference in the estimated exponential decay rate of brain lactate between groups ($P = 0.52$).

DISCUSSION

The current study shows that endogenously raised plasma lactate levels by a single bout of HIIT result in increased brain lactate concentrations at the start of hypoglycemia. This increase is most pronounced in patients with IAH, and this was the only group where brain lactate levels decreased below baseline values at the end of the hypoglycemic episode. These findings support altered brain lactate handling in IAH.

The HIIT-induced increase in brain lactate content is in line with previous studies using ^1H -MRS in healthy participants in response to endogenously raised plasma lactate levels by vigorous exercise, albeit under euglycemic conditions (11,12). Cerebral lactate uptake is driven by a concentration gradient from blood to brain. Interestingly, although plasma lactate levels were similar between groups, we found that the increase in brain lactate concentrations after HIIT

Table 1—Participant characteristics			
	T1DM IAH	T1DM NAH	Healthy participants
Age (years)	23.5 ± 6.1	21.5 ± 2.5	23.8 ± 3.0
Sex (male/female)	3/3	3/3	3/3
BMI (kg/m ²)	22.8 ± 1.2	22.7 ± 2.4	23.5 ± 1.6
Duration of T1DM (years)	12.0 ± 8.8	10.2 ± 4.8	—
HbA _{1c} (mmol/mol [%])	51.7 ± 10.0 [6.9 ± 0.91]	60.7 ± 8.1 [7.7 ± 0.74]	—
Self-reported exercise (h/week)	4.9 ± 3.0	4.8 ± 3.1	3.3 ± 2.0

Data are presented as number or mean ± SD. T1DM, type 1 diabetes.

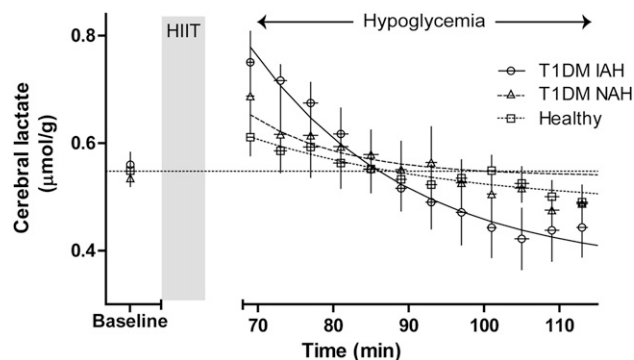


Figure 3—Brain lactate levels. Mean cerebral lactate levels before (baseline) and after HIIT during hypoglycemia. For illustration purpose only, mean (\pm SEM) brain lactate levels after the HIIT training were calculated on a 4-min time interval grid (\pm SD). Dashed lines represent the NLME model fit. Open circles, patients with type 1 diabetes (T1DM) and IAH; open triangles, patients with T1DM and NAH; open squares, healthy participants.

was most pronounced in patients with type 1 diabetes and IAH, which supports enhanced brain lactate transport capacity in patients with IAH (21,22). This difference is most visible at the beginning of hypoglycemia, when plasma lactate levels are highest.

During hypoglycemia, we showed that brain lactate levels returned to baseline values in healthy volunteers and patients with NAH but dropped to levels below baseline in patients with IAH, which could not be explained by differences in plasma lactate levels between the study groups. This decrease matches the hypoglycemia-induced fall in brain lactate in patients with IAH that we observed in a previous study, in which plasma lactate levels were not elevated (6), and may reflect enhanced brain lactate oxidation. Previous studies have found that lactate contributes substantially to cerebral energy metabolism, when its availability is high (7,23–25). Increased lactate oxidation may protect the brain by maintaining metabolism when glucose supply is low, while at the same time it could impede the brain's capacity for hypoglycemia sensing. This is in line with the observation that a prior bout of HIIT attenuated awareness of and cognitive deterioration during subsequent hypoglycemia, particularly in patients with NAH (10), which may have been mediated by brain lactate.

HIIT not only increases plasma lactate levels but also has an impact on (stress) hormones, brain activation, and physical parameters, which may have influenced our results. Because the HIIT session was performed outside the MR system, we were unable to capture early changes in brain lactate after exercise. Brain lactate levels may have been higher in the time gap between HIIT and the post-HIIT scans. All participants were young, healthy, and fit, which should be kept in mind when interpreting the results. A strength of our study is the inclusion of three clinically distinct, but well-matched, groups of participants, allowing the differentiation between the effect of type 1 diabetes and the effect of IAH. Furthermore, plasma

glucose and lactate levels were almost identical among the groups.

In conclusion, we found that brain lactate concentrations increase after a bout of HIIT, particularly in patients with diabetes and IAH. In addition, brain lactate levels fall below baseline during the subsequent hypoglycemic period in patients with IAH but not in those with NAH or in healthy participants. These results support upregulation of lactate transport capacity and increased cerebral lactate oxidation during hypoglycemia in patients with IAH. Both adaptations in cerebral lactate handling may contribute to IAH by lactate serving as an alternative nonglucose fuel for the brain during hypoglycemia.

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

Author Contributions. E.C.W. designed the study, collected the data, and analyzed the MR data. H.M.R. designed the study, recruited the participants, performed the glucose clamps, collected the data, and performed all data analysis except the MR data analysis. C.J.T. and A.H. provided input for the design of the study. H.J.M.M.G. provided input for the design of the study and was responsible for the statistical analysis. B.E.d.G. and M.v.d.G. designed the study. 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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