Increased Brain Lactate Concentrations without Increased Lactate Oxidation during Hypoglycemia in Type 1 Diabetic Individuals

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Abstract

Previous studies have reported that brain metabolism of acetate is increased more than two-fold during hypoglycemia in type 1 diabetic (T1D) subjects with hypoglycemia unawareness. These data support the hypothesis that upregulation of blood-brain barrier monocarboxylic acid (MAC) transport may contribute to the maintenance of brain energetics during hypoglycemia in subjects with hypoglycemia unawareness. Plasma lactate concentrations are approximately ten-fold higher than acetate concentrations, making lactate the most likely alternative MAC as brain fuel. We therefore examined transport of [3-^{13}C]-lactate across the blood-brain barrier and its metabolism in brain of T1D patients and non-diabetic control subjects during a hypoglycemic clamp using ^{13}C magnetic resonance spectroscopy. Brain lactate concentrations were more than fivefold higher ($P<0.05$) during hypoglycemia in the T1D subjects compared to the control subjects. Surprisingly, we observed no increase in the oxidation of blood-borne lactate in the T1D subjects, as reflected by similar ^{13}C fractional enrichments in brain glutamate and glutamine. Taken together these data suggest that in addition to increased MCA transport at the blood-brain barrier there may be additional metabolic adaptations that contribute to hypoglycemia unawareness in patients with type 1 diabetes.

Despite the increased availability of improved methods for managing glycemic control (i.e. continuous glucose monitoring), failing counter-regulation and hypoglycemia unawareness still present a real burden in the daily life of type 1 diabetes and advanced (insulin deficient) type 2 diabetes patients (1,2). Recurrent episodes of hypoglycemia are considered to induce both the failure in counter-regulatory hormone release and hypoglycemia unawareness, a concept known as hypoglycemia-associated autonomic failure (HAAF) (3,4).

While the exact mechanisms of hypoglycemia unawareness are still unknown, studies have predominantly focused on adaptations related to nutrient transport into the brain and changes in brain energy metabolism. For example, changes in the transport of plasma glucose across the blood-brain barrier and consequently the brain glucose levels have been the topic of various studies (5-10). Other studies have focused on glycogen supercompensation, a hypothesis suggesting increased storage of glucose in astroglial glycogen following recurrent hypoglycemic events (11-13). The increased astroglial glycogen would function as a glucose reserve during hypoglycemia. However, during a 50 hour wash-in and wash-out study of [1-^{13}C]-glucose, control subjects showed higher levels of newly synthesized brain glycogen than hypoglycemia unaware T1D subjects (11). Oz *et al*. consequently concluded that glycogen supercompensation did not contribute to hypoglycemia unawareness in type 1 diabetes patients (11).

Previously we have reported that brain transport and metabolism of acetate is increased more than two-fold in intensively treated T1D subjects with hypoglycemia unawareness (14). These data support the hypothesis that upregulation of blood-brain barrier monocarboxylic acid (MCA) transport via the monocarboxylic acid transporter 1

(MCT1) (15,16) may be a hallmark of hypoglycemia unawareness in T1D patients. In contrast to acetate, which circulates in plasma at relatively low concentrations (~0.1 mM), plasma lactate concentrations are approximately ten-fold higher during hypoglycemia (17) making it a primary candidate for an alternative brain fuel (18-21).

Lactate metabolism can play a central role in neuroenergetics as suggested by the astrocyte-neuron lactate shuttle (22). The astrocyte-neuron lactate shuttle models the compartmentalized metabolism of glucose in astrocytes and neurons. It describes how glucose is metabolized through glycolysis in astrocytes, producing lactate. Lactate is then shuttled to neighboring neurons where it is oxidized. The astrocyte-neuron lactate shuttle is analogous to the intercellular lactate shuttle that was proposed earlier and describes skeletal muscle lactate metabolism (23).

We have shown in healthy subjects that there is sufficient lactate transport activity to supply approximately 10% of the brain's energy needs at these physiological lactate concentrations (24). Increased blood-brain barrier transport capacity of MCA's, and thus lactate, could contribute to the maintenance of brain energetics during hypoglycemia providing the brain with an increased influx of alternative substrates (14). However, to our knowledge there is no direct evidence of increased brain transport and oxidation of plasma lactate in T1D patients. We therefore examined transport of lactate over the blood-brain barrier and its metabolic fate in healthy T1D patients and non-diabetic control subjects during a hypoglycemic clamp by measuring ^{13}C label incorporation from intravenously administered [3-^{13}C]-lactate into brain lactate, glutamate and glutamine by ^{13}C magnetic resonance spectroscopy (MRS).

RESEARCH DESIGN AND METHODS

Subjects

Five healthy type I diabetic patients (Age: 34±5 years, BMI: 23.0±1.5 kg/m^2) and six healthy control subjects matched for BMI (Age: 24±1 years, BMI: 23.5±0.9 kg/m^2) were recruited for this study. The type I diabetic subjects all were in well to moderate glycemic control (Hb_{A1C}: 7.6±0.9%).

The T1DM subjects were selected on the criteria of having experienced frequent hypoglycemic events based up on the Clarke questionnaire (25) with subject scores ranging from 5 to 3 with an average of 4.3±0.9. The control subjects had normal fasting plasma glucose concentrations (4.9±0.1 mM), Hb_{A1C}: 5.3±0.1% and they were not taking any medications except for birth control pills. The purpose, nature, and potential complications of the studies were explained and written consent was obtained from each subject. The protocol was approved by the Yale University Human Investigation Committee.

Hypoglycemic clamp studies

All subjects presented at 7 A.M. the morning of the study in the Yale Magnetic Resonance Research Center after an overnight fast. Subjects with diabetes were instructed to take their usual evening dose of insulin and to abstain from their morning insulin dose. After intravenous catheters were inserted into each antecubital area for blood collection and for infusions basal blood samples were collected for determination of plasma glucose, lactate, β-hydroxybutyrate, insulin, glucagon, and catecholamine concentrations.

At 8:00 A.M., the subjects were positioned in the supine position in the 4 Tesla MRS scanner. A primed-continuous infusion of insulin was initiated and kept constant at 40 mU/(m^2-min) while plasma glucose concentrations were measured every 5 min and allowed to decrease to 3.1 mM and kept constant at this level with a variable infusion of 20% dextrose. The head of each subject was positioned over the ^{13}C transmit/receiver coil and the bed was slid into the MR scanner. A primed-continuous infusion of [3-^{13}C]-L-lactate (Isotech, Miamisburg, OH) was started and continued for 90–120 min at a rate of 10 μmol/(kg-min) (Fig. 1). Magnetic resonance (MR) spectra were acquired continuously throughout the study and blood samples were drawn at intervals of 5–10 min for the determination of plasma substrate and hormone concentrations and for determination of the enrichment of plasma [^{13}C]-lactate.

Measurement of metabolites and hormones.

Plasma glucose and lactate concentrations were measured every 5 min using a YSI 2700 STAT Analyzer (Yellow Springs Instruments, OH). Samples for hormones were taken every 15 min. Plasma concentrations of insulin and glucagon were measured with the use of double-antibody radioimmunoassay kits (Linco, St. Charles, MO). Plasma epinephrine and norepinephrine concentrations were measured with a three-step procedure that consisted of adsorption onto alumina (pH 8.6), elution with dilute acid, and analysis by high-pressure chromatography.

Fractional enrichments of plasma [^{13}C]glucose and [^{13}C]lactate were measured by GC-MS using a Hewlett-Packard 5890 gas chromatograph (HP-1 capillary column; 12 x 0.2 x 0.33-mm film thickness; Hewlett Packard, Palo Alto, CA) interfaced to a Hewlett-

Packard 5971A mass selective detector operating in chemical ionization (CI) mode with isobutane as the reagent gas. Glucose was analyzed by GC-MS as the glucose-pentaacetate. ^{13}C isotopic enrichments of singly- and multiply-labeled isotopic isomers (m+1, m+2, m+3, m+4, and m+6) of glucose were determined using CI and monitoring ions 331 through 337. Singly-labeled glucose was calculated from the ratio of the m+0 signal (m/z 331), and the m+1 signal (m/z 332). Lactate was analyzed by GC-MS as the n-butyl ester-trifluoroactate derivative. ^{13}C isotopic enrichment (m+1) of lactate was determined using CI mode and monitoring ions 243 and 244.

MRS acquisition

MR spectra were acquired using a 4 Tesla whole body magnet equipped with a Bruker console (Bruker Instruments, Billerica, MA) as previously described (24). The RF-coil setup was a combination of a circular ^{13}C coil (∅ 8.5 cm) for acquisition and two quadrature ^{1}H surface coils (∅ 15 cm) for imaging, shimming, polarization transfer and ^{1}H decoupling. Following scout imaging, shimming was performed using the FASTERMAP procedure (25) and decoupling power was calibrated. ^{13}C MR spectra were acquired using a polarization transfer sequence optimized for detection of C4 of glutamate (Glu) and glutamine (Gln) (26)(TR=2500ms, 128 averages), in combination with 3D ISIS localization and outer volume suppression. The volume of interest was a 90 ml voxel centered on the midline in the occipital-parietal lobe during the infusion of [3-^{13}C]-lactate.

Spectral processing and analysis

Spectra were manually phase corrected and Lorentzian (-2Hz) and Gaussian (6Hz) apodization and baseline correction up to 2nd order was applied. Peak amplitudes were determined with an in-house software package written in Matlab® using an LC Model approach with each ^{13}C resonance having independent amplitudes (27). Basis sets for peak fitting were acquired in phantom solutions using identical MRS acquisition conditions for Glu, Gln, N-Acetyl aspartate (NAA), aspartate, creatine and lactate (Lac). Glu and Gln C4 peaks were fitted with a spectrum averaged over the last 21 min of the time series. Lac C3, and NAA C3 and C6 peak amplitudes were fitted in a spectrum averaged over the complete time course. Concentrations of ^{13}C Lac, Glu and Gln were calculated using the averaged NAA C3 and C6 peak amplitudes and assuming a concentration for NAA of 11 μmol/g (28,29). Fractional ^{13}C enrichment of Glu C4 and Gln C4 were determined assuming concentrations for Glu (9.8 μmol/g) and Gln (4.2 μmol/g) (30).

Measurement of brain lactate concentrations

Brain lactate concentrations ([brain Lac]) were determined from the measured ^{13}C concentration of $^{13}C3$ lactate ([brain LacC3]) by assuming that at steady state the fractional ^{13}C enrichment of C3 lactate (fe[brain LacC3]) was similar to that of Glu C4 (fe[GluC4]) (equation 1). This assumption is based on the lactate/pyruvate pool being the immediate precursor for acetyl-CoA, which in turn is the precursor for the Glu C4 and C5 carbons (31). A correction in the measured ^{13}C concentration of brain Lac C3 was applied for the contribution of plasma [3-^{13}C]-lactate, assuming a plasma volume of 5% relative to total brain volume (32).

$$[\text{brain Lac}] = \frac{[\text{brain LacC3}]}{\text{fe}[\text{brain LacC3}]} = \frac{[\text{brain LacC3}]}{\text{fe}[\text{GluC4}]} \quad (1)$$

Metabolic modeling analysis

Steady state metabolism of lactate was modeled using a one-compartment model as depicted in figure 2. At steady state, the inflow of plasma lactate (V_{in}) relative to the outflow from the brain (V_{out}) and lactate oxidation in the TCA cycle (V_{TCA}) was derived (equation (2)),

$$\frac{\text{fe}[\text{GluC4}]}{\text{fe}[\text{plasma LacC3}]} = \frac{\text{fe}[\text{brain LacC3}]}{\text{fe}[\text{plasma LacC3}]} = \frac{V_{in}}{2 \times CMR_{glc} + V_{in}} = \frac{V_{in}}{V_{TCA} + V_{out}} \quad (2)$$

where CMR_{glc} represents the glucose consumption rate and fe indicates fractional enrichment of the particular metabolite. Equation 2 was solved theoretically, no absolute values of CMR_{glc}, V_{TCA} V_{in} and V_{out} are derived from the present data. Previously we have described the linear relationship between plasma and brain lactate concentrations (24). To account for the possible effect of different plasma lactate concentrations between groups, we also normalized the results of equation (2) to the average plasma lactate concentration of the control group. We used the transport relationships described by Boumezbeur *et al*. (24) who showed that the relationship between plasma lactate concentration and lactate unidirectional transport is approximately linear at the concentrations of plasma lactate studied here.

Statistical analysis

Group differences between control and type 1 diabetes subjects were analyzed using two-tailed, unpaired student's *t* test, considering a *P*-value <0.05 as statistically significant. All data are presented as mean ± SEM.

RESULTS

Basal plasma lactate concentrations were similar in control and T1D subjects (control: 0.80±0.03 mM; T1D: 1.06±0.21 mM, *P=0.21*). Plasma β-hydroxybutyrate tended to be higher in T1D subjects (398±184 μM) than control subjects (83±16 μM, *P=0.09*) at baseline. Following insulin infusion, plasma β-hydroxybutyrate was lower and similar in both groups at the start of the [3-^{13}C]-lactate infusion (control: 44±8 μM, T1D: 60±5 μM, *P=0.11*). After 40-60 minutes of insulin infusion plasma glucose levels stabilized at 3.6±0.1 mM in the control and 3.2±0.3 mM in the T1D subjects. M-values were not significantly different among the groups (control: 4.25+/-0.69 mg/kg-min, T1D: 3.06+/-0.59 mg/kg-min; *P=0.14*). Following the start of the [3-^{13}C]-lactate infusion, plasma lactate concentrations quickly increased to 1.8±0.4 mM in the control subjects and 1.3±0.2 mM in the type 1 diabetes subjects (*P=0.05*). The average ^{13}C fractional enrichment of plasma lactate was 26.2±4.0% in control and 31.6±6.7% in T1D subjects (*P=0.13*). Mean fractional ^{13}C enrichments of plasma glucose ([1-^{13}C]-glucose) between 60 and 90 minutes were 1.3±0.1% in the control subjects and 2.0±0.1% in the T1D subjects (*P=0.0013*).

The elevated plasma lactate levels as anticipated from previous studies led to a blunting of the counterregulatory response in control subjects with only a significant but small increase in epinephrine that was similar between groups (no difference between groups in glucagon, epinephrine, and norepinephrine concentrations). Figure 3 shows examples of ^{13}C MR spectra averaged over the last 30 minutes of [3-^{13}C]-lactate infusion from a control and a T1D subject, respectively. Glu C4 ^{13}C fractional enrichment increased quickly following the infusion of [3-^{13}C]-lactate reaching similar

steady state levels (corrected for 1.1% natural abundance ^{13}C signal) of 2.8±0.3% in the controls and 2.7±0.2% in T1D subjects (*P=0.40*). Gln C4 ^{13}C fractional enrichment was 1.9±0.5% in the controls and 2.0±0.4% in T1D subjects (*P=0.77*). The ratio of ^{13}C Gln C4/Glu C4 was 0.73±0.11 in type 1 diabetes subjects and 0.65±0.11 in control subjects (*P=0.70*) (Table 1).

The calculated brain lactate concentrations were increased by more than fivefold in the T1D subjects (1.7±0.6 μmol/g) compared to the control subjects (0.3±0.2 μmol/g, *P<0.05*)(Fig. 4a). Furthermore brain lactate concentrations normalized to the average plasma lactate concentration were more than six-fold increased in the T1D subjects (2.2±0.9 μmol/g) compared to the control subjects (0.3±0.2 μmol/g, *P<0.05*)(Fig. 4b).

Lactate influx into the brain, as a fraction of the brain TCA cycle rate and the flow of lactate out of the brain [$V_{in}/(V_{TCA} + V_{out})$], as estimated using equation (2), was similar in the control (0.11±0.02) and T1D subjects (0.09±0.01, *P=0.25*). When normalized to the average plasma lactate level of the control group $V_{in}/(V_{TCA} + V_{out})$ was still similar between the control and the T1D subjects (0.11±0.01 and 0.12±0.01, respectively, *P=0.71*).

DISCUSSION

In the present study we examined whether lactate blood-brain barrier MCA transport and subsequently oxidation of blood-borne lactate in T1D subjects were increased as compared to non-diabetic individuals during mild hypoglycemia. In support of increased MCA transport capacity in the T1D subjects we found elevated concentrations of lactate in the brain during the infusion of [3-^{13}C]-lactate. Surprisingly, despite the several-fold higher brain lactate levels in the diabetic subjects, the fractional entry of blood-borne lactate into the brain lactate pool [$V_{in}/(V_{TCA} + V_{out})$] did not appear any different from control subjects, given similar Glu C4 fractional enrichments.

The T1D group showed increased transport capacity for plasma lactate as shown by brain lactate concentrations being comparable to the levels in plasma so that the rate of lactate influx would be similar to lactate efflux ($V_{out} = V_{in}$). A lack of net lactate influx is similar to what has been measured during euglycemia (24) indicating at these mild levels of hypoglycemia the T1D subjects have not down-regulated glucose metabolism. In contrast, in the control subjects brain lactate concentration was extremely low so that almost all lactate entering the brain was being oxidized ($V_{out} \sim 0$), which indicates a reduction in glucose oxidation in the control subjects by a minimum of ~11% (Equation 2). Previous studies using PET, MRS and arterio-venous differences reported drops in glucose oxidation in control subjects between 25 and 45% (9,10,33). The actual drop in glucose oxidation in the control subjects may therefore have been considerably greater than 11%. The reduction in brain glucose metabolism in control subjects compared to the T1D subjects would also explain the similarity in the ratio [$V_{in}/(V_{TCA} + V_{out})$] despite higher unidirectional lactate transport (V_{in}) in the T1D subjects. At lower levels of

hypoglycemia it is possible that the net oxidation of lactate in the T1D subjects would increase to greater than that of the control subjects due to their increased lactate transport activity.

Brain lactate originates from both the plasma lactate and from glucose metabolism through glycolysis. In ideal experimental conditions the glycolytic flux is unlabeled and would dilute the fractional enrichment of brain ^{13}C-lactate and subsequently Glu. The increased calculated brain lactate concentration in T1D subjects could consequently be the result of increased (unlabeled) glycolytic flux relative to control subjects, as explained above. However, lactate is also an important precursor in gluconeogenesis. Glucose synthesized from [3-^{13}C]-lactate will be labeled in the C1 and C6 positions. In our experiments gluconeogenesis is strongly inhibited by the infused insulin but a small metabolic flux of ^{13}C-labeled glucose needs to be considered. The levels of glucose fractional enrichment in the last 30 minutes of the study were similar across groups, small (~1.5%) compared to the fractional ^{13}C enrichment of lactate (~30%) and therefore considered negligible. In addition, when analyzing fractional enrichment of Glu C4 between 20 and 40 minutes of [3-^{13}C]-lactate infusion, (before any ^{13}C-labeled glucose could have contributed to the Glu pool), results were similar as those from the steady state analysis (control subjects: 2.4±0.6%, type 1 diabetic subjects: 2.1±0.6%, *p=0.4*). We would therefore argue that the small amounts of ^{13}C-labeled plasma glucose did not weaken our interpretation that similar Glu C4 fractional enrichments despite higher brain lactate transport indicate preservation of glucose oxidation in the T1D subjects.

At present the mechanism for the likely maintenance of brain glucose metabolism in hypoglycemic unaware subjects during hypoglycemia is unknown. Although upregulated brain glucose transport has been reported in rodent models exposed to recurrent hypoglycemia (34,35) similar findings have not been reported in humans. Positron emission tomography (PET) studies using [^{11}C]-3-O-methyl-D-glucose or [1-^{11}C]-glucose to assess glucose transport in humans have not found evidence of upregulation of glucose transport in unaware T1D or healthy subjects (5,36). Similarly, studies using ^{1}H MRS have not found definitive evidence of a metabolically significant change in glucose transport in subjects with type 1 diabetes and/or hypoglycemia unawareness under euglycemic conditions (8,37). In addition a recent study by van de Ven *et al*. did not show differences in brain glucose concentrations during both euglycemia and hypoglycemia in control and T1D subjects without hypoglycemia unawareness (7).

Recently a new role for brain lactate was proposed, acting as a volume transmitter in addition to a metabolic substrate with higher brain lactate concentrations stimulating neuronal activity and increased brain glucose metabolism (38). The mechanisms put forward include the NADH/NAD^{+} redox ratio and a cyclic AMP pathway triggered by binding of lactate to the suggested G-protein-coupled receptor 81 (GPR81) (38). GPR81 is expressed in adipose tissue and there seem to be indications of its presence in brain tissue as well (38). Future studies are required to confirm a role for lactate as volume transmitter in brain and its potential relevance in the sensing of the brain's energy status and hypoglycemia. Activation of GPR81 by increased brain lactate concentrations in hypoglycemic unaware T1D subjects could potentially be involved in

regulating brain glucose metabolism in these individuals. Besides the potential role of GPR81, various other mechanisms have been described of lactate regulating redox-sensitive pathways (reviewed in (39)).

The relative ^{13}C labeling of Gln C4 and Glu C4 following [3-^{13}C]-lactate infusion resembled closely that from providing [1-^{13}C]-glucose as a substrate (Table 1), as was shown in our previous study (24). The resemblance of the ^{13}C-labeling patterns of [1-^{13}C]-glucose and [3-^{13}C]-lactate as substrates indicates similar fractional enrichments for neuronal and glial lactate pools. This also implies that the transport rates for lactate between neurons and glia are at least similar or higher than the glucose oxidation rate, thus allowing treating the lactate pool as one, shared by neurons and glia (Fig. 2).

In vivo ^{13}C MRS can offer unique data of brain lactate transport and metabolism but are technically challenging and associated with high costs. ^{13}C MRS studies are therefore often carried out using relatively small group sizes, which is a limitation of the studies. ^{13}C MRS is also inherently limited in both spatial and temporal resolution. Our data were acquired from a relatively large volume of the brain. It is not inconceivable that smaller areas of the brain demonstrate different metabolic responses to hypoglycemia and the presence of blood-borne lactate. Similarly, the timing of the response to hypoglycemia can vary in different brain regions. Such spatial and temporal variations in metabolism cannot be detected with the method as used in the present study. Other limitations of the study are the assumptions required for the quantification and modeling of the MRS data and the slightly lower hypoglycemic glucose levels in T1D subjects compared to controls. However, the slightly lower plasma glucose levels in the T1D group makes the lack of increased lactate metabolism in those subjects even

more remarkable. Because lower plasma glucose levels equals lower brain glucose levels the T1D group experienced a somewhat more severe brain energy challenge. Nevertheless, despite higher brain lactate levels compared to controls, no increased lactate oxidation was detected in the T1D group.

In conclusion, our results showing increased brain lactate concentration in T1D subjects who experience regular hypoglycemic episodes further supporting our previous finding, using an acetate tracer, of increased MCA transport being a metabolic adaptation that may have a role in hypoglycemic unawareness. However, the present data highlight the differences between cerebral lactate and acetate metabolism, a result of brain metabolism being highly compartmentalized. The lack of increased lactate oxidation despite increased brain lactate levels in the T1D subjects is surprising and implies that other roles of lactate beyond being a metabolic fuel need to be explored. In addition, future studies using MRS should be able to answer whether under higher levels of brain activity (or lower glucose concentrations) the higher lactate transport activity becomes important for supporting metabolic demand, which we would anticipate, and to determine the degree to which glucose oxidation is relatively increased in the T1DM subjects.

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H.M.D.F., D.L.R. and K.F.P. acquired and analyzed data, G.F.M. implemented MR acquisition and quantification methods. H.M.D.F., G.I.S., D.L.R. and K.F.P. were involved in research and interpretation of the data and writing, reviewing and editing of the manuscript. H.M.D.F. and D.L.R. are the guarantors of this work and thus had full access to all the data and take responsibility for the integrity and accuracy of the data analysis.

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Table

Steady-state fractional enrichments of brain glutamate and glutamine during intravenous infusion of [3-^{13}C]-lactate in control and T1D subjects. Data are presented as mean ±SEM. P-valueds >0.05 for all group comparisons.

	Control (N=6)	T1D (N=5)
Fractional ^{13}C Enrichment Glu C4 (%)	2.80±0.30	2.68±0.18
Fractional ^{13}C Enrichment Gln C4 (%)	1.89±0.46	1.99±0.37
Fractional ^{13}C Enrichment Gln C4/Glu C4	0.65±0.11	0.73±0.11

Legends

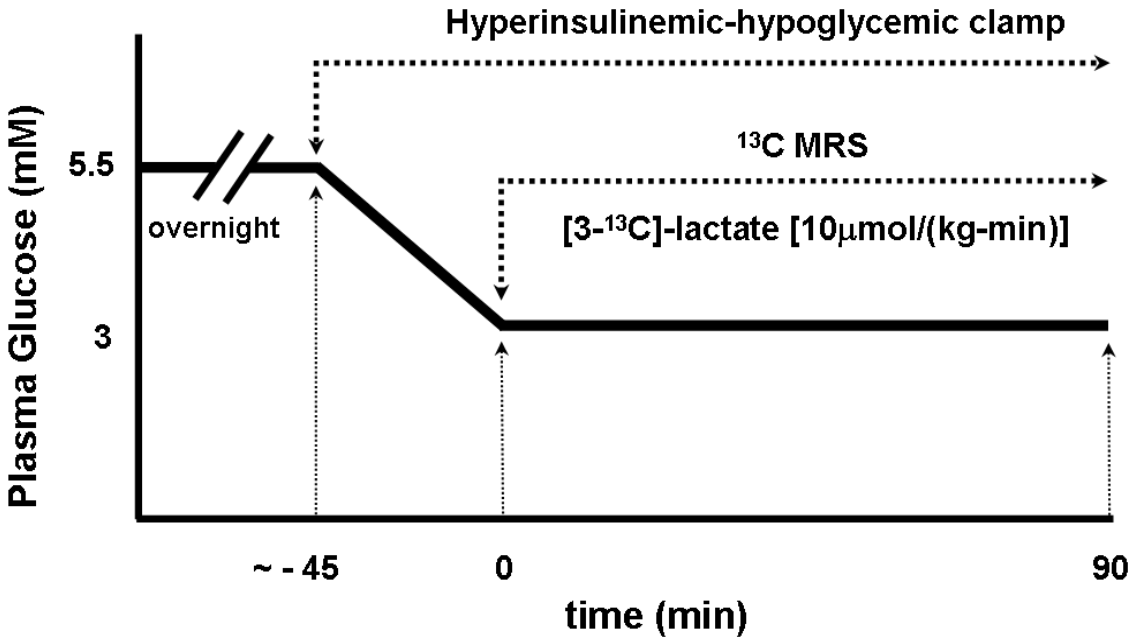
Table

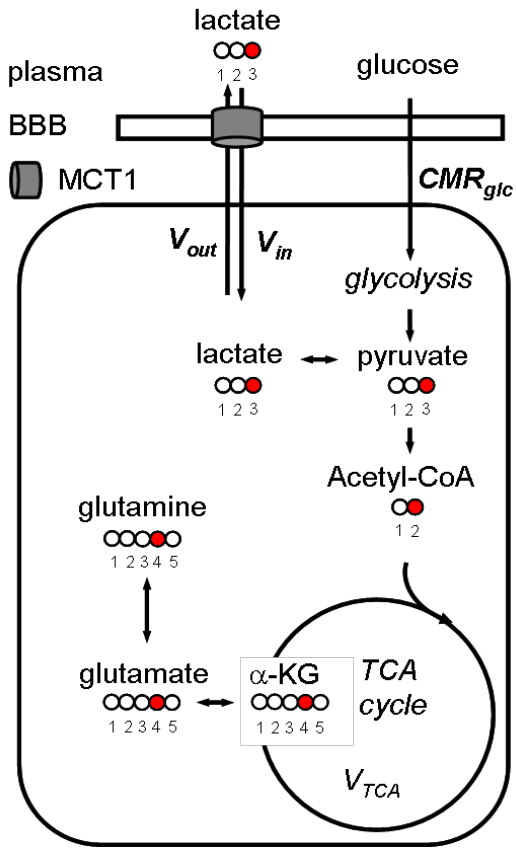
Figure 1. Schematic illustrating the time line of the hyperinsulinemic-hypoglycemic clamp, [3-^{13}C]-lactate infusion and ^{13}C MRS acquisition.

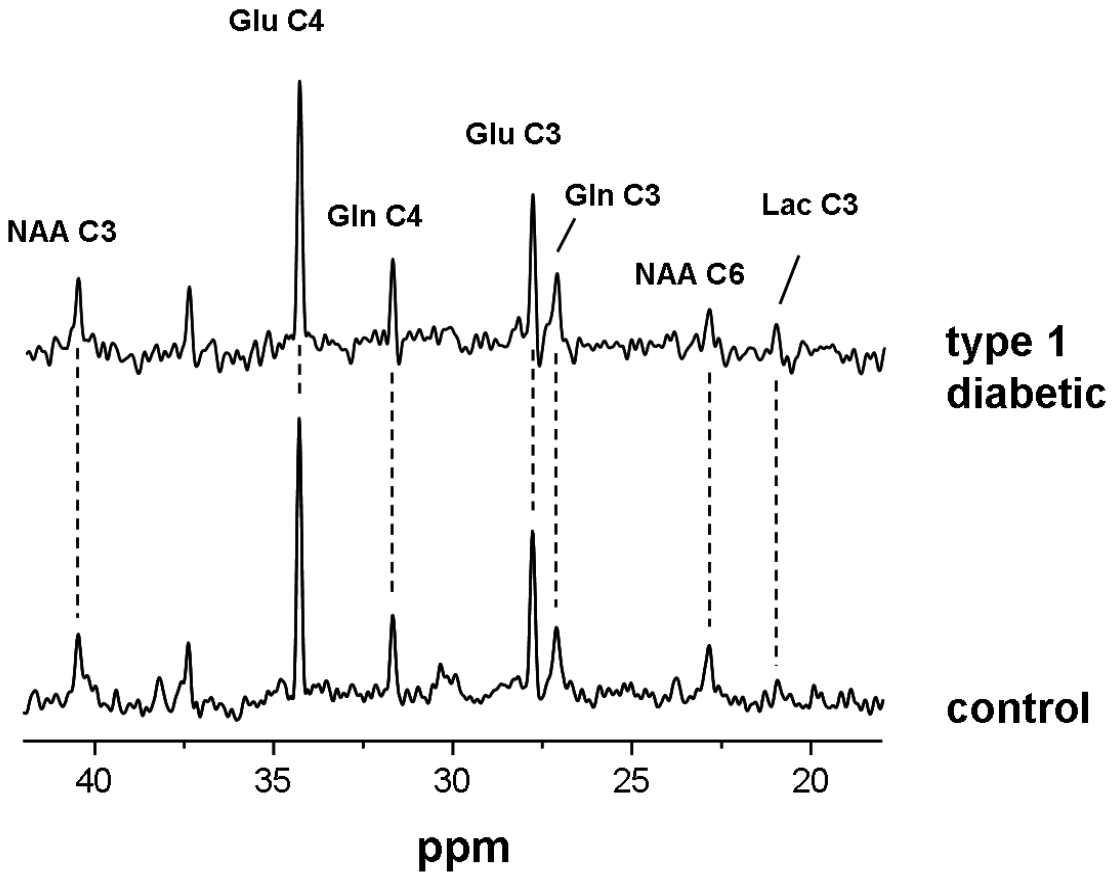
Figure 2. One-compartment model describing incorporation of ^{13}C-label from [3-^{13}C]-lactate into the brain glutamate and glutamine pools. This figure illustrates the fluxes V_{in}: lactate influx, V_{out}: lactate efflux, CMR_{glc}: glucose consumption, V_{TCA}: TCA cycle rate, that were considered to derive equation (2). α–KG: alpha-ketoglutarate, BBB: blood-brain barrier, MCT1: monocarboxylic acid transporter 1. ● represents the ^{13}C-labeled carbon position. Lactate in the neuronal and glial compartments was treated as a single pool due to the rapid transfer of lactate between these cells (23).

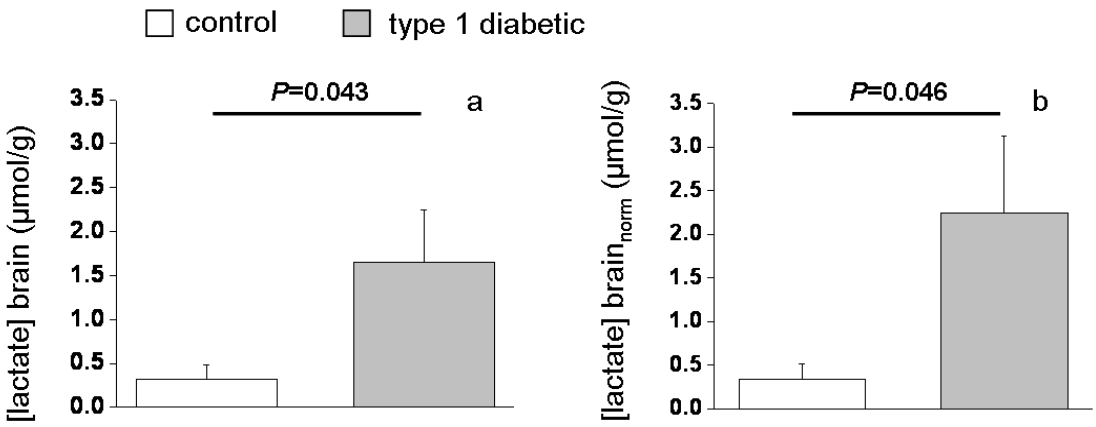
Figure 3. ^{13}C MR spectra of a T1D (top panel) and control subject (bottom panel) averaged over the last 30 min of [3-^{13}C]-lactate infusion. Peak annotations: NAA = N-Acetyl Aspartate, Glu = Glutamate, Gln = Glutamine, Lac = Lactate.

Figure 4. Total calculated lactate concentrations in brain.


Hyperinsulinemic-hypoglycemic clamp
^{13}C MRS
[3-^{13}C]-lactate [10μmol/(kg-min)]
Plasma Glucose (mM)
5.5
3
overnight
~ - 45
0
90
time (min)


lactate
1 2 3
plasma
glucose
BBB
MCT1
CMR_{glc}
V_{out}
V_{in}
glycolysis
lactate
1 2 3
pyruvate
1 2 3
Acetyl-CoA
1 2
glutamine
1 2 3 4 5
glutamate
1 2 3 4 5
α-KG
1 2 3 4 5
TCA
cycle
V_{TCA}


Glu C4
Glu C3
Gln C3
Gln C4
Lac C3
NAA C3
NAA C6
type 1 diabetic
control
40
35
30
25
20
ppm


control
type 1 diabetic
[lactate] brain (µmol/g)
P=0.043
a
[lactate] brain$_{norm}$ (µmol/g)
P=0.046
b
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5