^{13}C MRS during [3-^{13}C]lactate infusion under hyperinsulinemic-hypoglycemic conditions reveals compartmentalized lactate metabolism in human brain

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Introduction

The brain relies on glucose as its primary energy substrate but is also able to use alternative substrates to fuel its metabolism. Blood lactate may be an important brain fuel under elevated conditions, such as during intense exercise as well as under hypoglycemic conditions. Previously we performed ^{13}C MRS studies during infusion of [3-^{13}C]lactate to determine the concentration dependence of plasma lactate uptake in human brain (1). We found that blood lactate could be a significant substrate for brain metabolism. However due to label from [3-^{13}C]lactate being scrambled into glucose by the liver we were not able to determine what fraction of the lactate was consumed in the neurons versus the astroglia. Here we present preliminary results from ^{13}C MRS studies during [3-^{13}C]lactate, [1-^{13}C]glucose and [2-^{13}C]acetate infusion to determine the relative neuronal and glial consumption of blood lactate versus blood glucose. Infusion of [3-^{13}C]lactate during hyperinsulinemic hypoglycemia to inhibit liver gluconeogenesis, resulted in significant labeling of brain metabolites. In addition, comparison of the labeling patterns of brain metabolites during [1-^{13}C]glucose and [2-^{13}C]acetate infusion and simplified two-compartment metabolic modeling (2) indicate that lactate is metabolized primarily in the neuronal compartment, with a similar neuronal/glial distribution as for glucose.

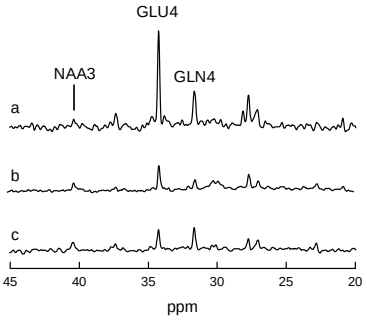

Figure 1: ^{13}C MR spectra averaged over the last ~ 60 min of the infusion of a) [1-^{13}C]glucose, b) [3-^{13}C]lactate and c) [2-^{13}C]acetate. Spectra are processed similarly and scaled on the NAA3 peak height. GLU=glutamate; GLN=glutamine; NAA=N-Acetyl aspartate.

Materials and Methods

MRS acquisition

MR spectra were acquired using a 4T whole body magnet equipped with a Bruker console (Bruker Instruments, Billerica, MA). 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 for imaging, shimming, polarization transfer and decoupling. Following scout imaging, shimming was performed using the FASTERMAP procedure (3) and decoupling power was calibrated. ^{13}C MR spectra were acquired using a polarization transfer sequence (3) (TR=2500ms, 128 averages) in combination with 3D ISIS localization and outer volume suppression in a 900 ml voxel located in the occipital-parietal lobe, before and during the infusion of the ^{13}C-labeled substrate of interest. Blood samples were collected every 5-10 minutes for determination of plasma glucose, lactate and acetate concentration and fractional ^{13}C enrichments. In total 18 healthy subjects were studied during infusion of [3-^{13}C]lactate (n=4), during [1-^{13}C]glucose infusion (n=8) and during [2-^{13}C]acetate infusion (n=6). Both the [3-^{13}C]lactate and [2-^{13}C]acetate studies were carried out during steady state hypoglycemia ([plasma glucose]: ~3.1 mM) induced by hyperinsulinemia.

Spectral processing and analysis

Spectra were manually phase corrected and Lorentzian (-2Hz) and Gaussian (6 Hz) apodization and baseline correction up to 2nd order were applied. Following correcting for detection efficiency, peak heights were determined for the creatine C2 peak (in an overall averaged spectrum) and for glutamate (GLU4) and glutamine (GLN4) C4 peaks in a spectrum averaged over the last 11 FIDs of the time series. Relative fractional enrichment of GLU4 (fGLU4) and GLN4 (fGLN4) were determined assuming concentrations for glutamate (9.1 mmol/kg)(4), glutamine (4.1 mmol/kg)(5) and creatine (6.9 mmol/kg) (6**)**. Relative contributions of lactate and glucose to total oxidation during steady state were considered as functions of fGLU4 and fGLN4 as given by equations 1 and 2 for neuronal and astroglial compartments, respectively. Values for V_{cyc}, $V_{ox,n}$, and $V_{ox,a}$ were assumed identical as previously reported (7) from combined datasets of [2-^{13}C]acetate and [1-^{13}C]glucose experiments, measured with the same experimental set-up and methods as used in the present study: V_{cyc} = 0.16, $V_{ox,n}$ = 0.53 and $V_{ox,a}$ = 0.13 mmol·kg^{-1}·min^{-1}. V_{gs} was considered 1.1· V_{cyc} and $V_{ox,total} = V_{ox,n} + V_{ox,a}$.

$$\frac{V_{substrate,n}}{V_{ox,total}} = \frac{\left[fGLU_n 4\left[1+\frac{V_{cyc}}{V_{ox,n}}\left(1-\frac{fGLN_a 4}{fGLU_n 4}\right)\right] * V_{ox,n}\right]}{V_{ox,total}} \quad (1)$$

$$\frac{V_{substrate,a}}{V_{ox,total}} = \frac{\left[fGLN_a 4\left[1+\frac{V_{cyc}}{V_{ox,a}}\left(\frac{V_{gs}}{V_{cyc}}-\frac{fGLU_n 4}{fGLN_a 4}\right)\right] * V_{ox,a}\right]}{V_{ox,total}} \quad (2)$$

Results and discussion

In Figure 1 steady state averaged spectra are depicted, showing similar labeling patterns of the glutamate and glutamine resonances during [3-^{13}C]lactate and [1-^{13}C]glucose infusion, while both are clearly different from the labeling originating from [2-^{13}C]acetate infusion. The relative fractional enrichments of GLU4 and GLN4 (Table 1) appear to be different for the various ^{13}C labeled substrates infused, but fGLU4/fGLN4 are strikingly similar for [3-^{13}C]lactate and [1-^{13}C]glucose infusions. The similar labeling patterns during [3-^{13}C]lactate and [1-^{13}C]glucose infusions indicate similar metabolic pathways and kinetics. As brain metabolism is known to be highly compartmentalized, i.e. neuronal vs. astroglial, these data suggest that lactate and glucose are oxidized in the same metabolic compartments. This latter is confirmed by the calculated relative contribution of neuronal and astroglial to total oxidation of [3-^{13}C]lactate and [1-^{13}C]glucose (Table 2), indicating that lactate is metabolized predominantly (~90%) in the neuronal compartment. The present data are in general agreement with the results of an ex vivo MRS study in rats using the same ^{13}C labeled substrates (8).

Table 1: relative fractional enrichments of GLU4, GLN4 and their ratio

	[3-^{13}C]lactate (n=4)	[1-^{13}C]glucose (n=8)	[2-^{13}C]acetate (n=6)
fGLU4 (%)	13.32 ± 8.98	22.83 ± 7.13	5.59 ± 2.52
fGLN4 (%)	8.99 ± 4.66	15.65 ± 4.60	15.68 ± 7.91
fGLN4/fGLU4	0.72 ± 0.11	0.69 ± 0.08	2.81 ± 0.60

Table 2: relative contribution of neuronal ($V_{substrate}$_n) and astroglial ($V_{substrate}$_a) metabolism of [3-^{13}C]lactate and [1-^{13}C]glucose to total brain metabolism (Vox_tot).

	[3-^{13}C]lactate	[1-^{13}C]glucose
$V_{substrate}$_n/Vox_tot (%)	90.9 ± 4.4	91.9 ± 3.6
$V_{substrate}$_a/Vox_tot (%)	9.1 ± 4.4	8.13 ± 3.6

Conclusion

In the present study we compared steady state brain metabolite labeling during infusion of [3-^{13}C]lactate, [1-^{13}C]glucose and [2-^{13}C]acetate in healthy humans. The similarity of the brain metabolite labeling patterns during infusion of [3-^{13}C]lactate and [1-^{13}C]glucose, and the preliminary analysis indicate that lactate metabolism occurs predominantly in the neuronal compartment with a similar distribution between neurons and astroglia as for blood glucose.

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