^{1}H-[^{13}C] MRS Ex Vivo Study of Cortical Ketone Body Utilization in Awake Rats During Fasting-Induced Ketosis

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INTRODUCTION: Glucose is the primary oxidative fuel of mammalian brain under normal physiological conditions but under conditions of fasting or a high-fat (ketogenic) diet, utilization of blood-borne ketone bodies (3-hydroxylbutyrate (3-HB), acetoacetate) increase substantially. Ketone bodies administered in vitro or in vivo cannot completely replace glucose as an oxidative fuel for cerebral oxidation, indicating that glucose metabolism is likely to play a critical role in function besides oxidation. The extent to which ketone bodies and other monocarboxylates (e.g., lactate) can substitute for glucose as an oxidative fuel in neurons and astrocytes in vivo however is not clear. In this study we have measured the rates of 3-HB and glucose metabolism in cortex of awake rats *ex vivo* previously fasted to induce a state of moderate ketosis. Metabolic fluxes were determined by fitting a two-compartment (neuron-astroglia) metabolic model to time courses of ^{13}C labeling of brain glutamate and glutamine measured *ex vivo* from extracts of rats receiving timed infusions of [2,4-$^{13}C_2$]3-HB or [2-^{13}C]acetate.

METHODS: Male Sprague-Dawly rats (183±12g) were used. We studied rats under fasted conditions (36 hrs) so as to induce a significant level of ketosis. On the day of the study a tail vein was quickly (<5 min) catheterized under light anesthesia with isoflurane. The rats were allowed to recover for a period of 45 min, as evidenced by normal exploratory behavior in their cages. Rats then received timed infusions of either [2,4-^{13}C]-3-HB (7,15,30,60,100 min) or [2-^{13}C]acetate (5,120 min) using stepped infusion protocols to achieve rapid elevation and achieve constant blood enrichments. At the end of each infusion, rats were quickly sedated with isoflurane (<30s) and euthanized with a focused-beam microwave irradiation device. Heart blood was sampled for measurement of the infused substrate, glucose and lactate. The brain was rapidly removed, the cortex carefully dissected, frozen in liquid N_2, and ethanol extracts prepared (1). Metabolite concentrations and ^{13}C enrichments in blood plasma and brain extracts were measured at 11.7Tesla using ^{1}H-[^{13}C] NMR (1). Rates were calculated using CWave and a two-compartmental metabolic model.

RESULTS: Fig. 1 depicts the time courses of ^{13}C labeling of glutamate (Glu-C4 and C3), glutamine (Gln-C4), and glutamate plus glutamine (Glx-C3). ^{13}C enrichments of brain glutamate and glutamine rose rapidly during [2,4-$^{13}C_2$]-3-HB infusion, with the C4 resonances reaching >85% of their final values by 30 min of infusion. Metabolic fluxes were estimated by fitting a two-compartment (neuron-astrocyte) metabolic model (Fig 1, right) to the time course data using the ratio, V_{cyc}/V_{tcaN} as a constraint. The value of $V_{cyc}/V_{TCA(n)}$ (=0.34) was calculated from the steady-state enrichments of Glu-C4 and Gln-C4 determined at 2h during infusion of [2-^{13}C]acetate, after correction for ^{13}C labeling in blood glucose and 3-HB arising from

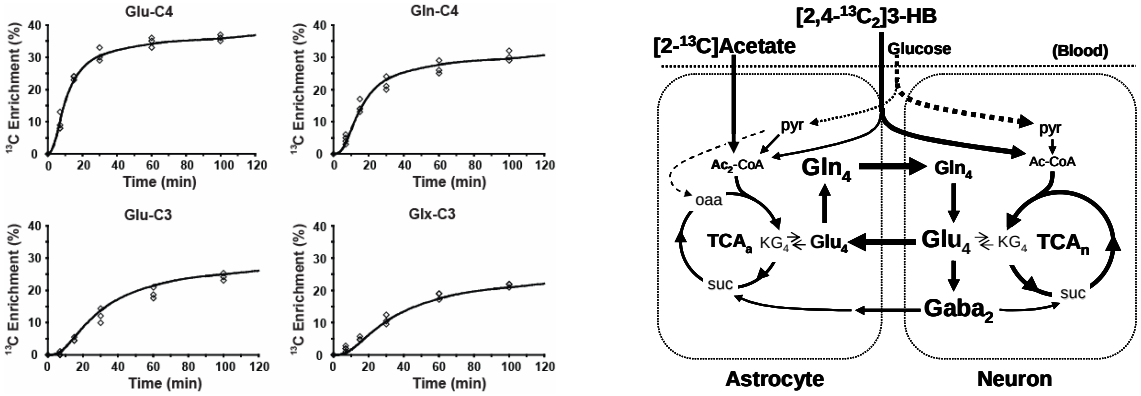

Figure 1: (Left) Time courses of Glu-C4,C3; Gln-C4, and Glx-C3 ^{13}C labeling in fasted rats during [2,4-$^{13}C_2$]-3-HB infusion. **(Right)** The metabolism of ^{13}C labeled 3-HB and acetate and the route of ^{13}C label flow between neurons and astrocytes. After transport into the brain from blood, [2,4-$^{13}C_2$]3-HB is metabolized in the mitochondria to acetyl-CoA (labeled at C2, Ac_2CoA) and enters both neuron and the astroglial TCA cycle (TCAa), whereas [2-^{13}C]acetate is metabolized in the mitochondria to acetyl-CoA (labeled at C2, Ac_2CoA) and enters the astroglial TCA cycle (TCAa), as citrate labeled at C4 after condensation with oxaloacetate (oaa). The continuous metabolism of unlabeled glucose in neurons and astroglia through pyruvate dehydrogenase serves as a constant dilution flux.

systemic acetate metabolism. Table 1 gives the calculated fluxes in the ketotic rats. 3-HB utilization comprised 37% of total neuronal TCA cycle flux in rats fasted for 36h. The results indicate that the 3-HB consumption is substantial under awake conditions.

Table 1		V_{kbN}	V_{pdhN}	V_{tcaN}	V_{cyc}
^{13}C-3HB (this study)	infused	0.55 (±0.09)	0.93 (±0.16)	1.47 (±0.21)	0.51 (±0.09)

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REFERENCES: (1) Chowdhury et al. (2007) JCBFM: 27(12):1895-1907