

# In vivo MRS studies of metabolic changes induced by recurrent antecedent hypoglycemia probed by 3- <sup>13</sup>C-lactate

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## Introduction

Diabetes complications can be reduced by tight control of blood glucose level via intensive insulin therapy. However, this bears the risk of increased incidence of severe hypoglycemia, which may lead to significant brain injury. Repeated exposure to hypoglycemia may lead to blunting of the central counter regulatory response to low blood glucose (counter regulation failure), resulting in a state of 'hypoglycemia unawareness', representing the loss of higher cognitive function. The blunting of hormonal counterregulatory responses to hypoglycemia observed in type 1 diabetic patients exposed to frequent hypoglycemic episodes are reproduced in animal models of recurrent antecedent insulin-induced hypoglycemia (1), suggesting that such models may offer insights into the metabolic adaptations observed in the clinical setting. When brain is repeatedly deprived of glucose, its capacity to take up and utilize alternate fuels such as monocarboxylic acids may be increased. This view is supported by a recent MRS study that demonstrated an increase in acetate metabolism in type 1 diabetic patients with hypoglycemia unawareness (2). The present study was undertaken to test whether lactate utilization is increased following exposure to recurrent hypoglycemia in a rat model. [3-<sup>13</sup>C]-lactate was infused intravenously into animals subjected to 3 days of antecedent recurrent hypoglycemia (RH3D) and controls under conditions of euglycemia or acute hypoglycemia. The time courses of brain glutamate and glutamine <sup>13</sup>C labeling were measured *in vivo* using <sup>1</sup>H-<sup>13</sup>C NMR spectroscopy and metabolic fluxes were estimated by fitting a two-compartment (astrocyte and neuron) metabolic model to the time course data (3).

## Materials and methods

Sprague-Dawley rats (270±30g) were used in these experiments. Recurrent hypoglycemia was induced by daily i.p. injection of regular insulin (10U/kg) to produce 3h of hypoglycemia (blood glucose 1.7-2.2 mM) on 3 consecutive days (RH3D). NMR experiments were conducted on the day 4. Rats were anesthetized with isoflurane, tracheotomized and ventilated with 30% O<sub>2</sub>/70% N<sub>2</sub>O. The left femoral artery was catheterized for blood sampling and monitoring of blood pressure. The left femoral vein was catheterized for infusion of insulin and glucose (via Y-connector) to clamp the glucose at the desired level while a catheter in the right femoral vein was used to infuse [3-<sup>13</sup>C]-Lac. Body temperature was maintained at ~37°C with recirculated warm water pad. In vivo MRS was conducted on a 9.4T horizontal magnet equipped with a 9 cm diameter gradient coil insert and interfaced to a Bruker ADVANCE console. Localized <sup>1</sup>H-[<sup>13</sup>C] NMR spectra were obtained from a volume of 180μL (6x5x6 mm<sup>3</sup>), centered in the middle of the cortex. Spectra were collected with a repetition time of 2.5s. At the end of the experiment, animals were removed from the magnet and brains were frozen *in situ* using liquid nitrogen with continued mechanical ventilation to preserve labile metabolites. Ethanol extracts were made of the frontal cortex of the frozen brain. Metabolite concentrations and enrichments in the brain extract were determined using high resolution <sup>1</sup>H-<sup>13</sup>C-NMR spectroscopy at 11.7 T (Bruker Avance). Four groups of animal were studied: (1) RH3D at euglycemia, (2) RH3D under hypoglycemic clamp, (3) Controls euglycemia, (4) Controls under hypoglycemic clamp. Metabolic fluxes were determined by fitting the two compartment model of neurons and astrocytes. Mass and isotope balance were expressed in coupled differential equations and solved within CWave 3.0 software. Error distributions were calculated by Monte-Carlo analysis of group average data sets.

## Results

Antecedent recurrent hypoglycemia led to large differences in neuronal lactate utilization compared to control animals. At euglycemia neuronal lactate utilization (V<sub>LacN</sub>) in RH3D animals was 62% lower than in control animals and dilution flow from unlabeled sources (V<sub>pdnN</sub>) was 30% lower. When animals were made acutely hypoglycemic, V<sub>LacN</sub> and V<sub>pdnN</sub> were greatly enhanced in RH3D animals compared to euglycemia, in clear contrast to control animals, which showed no significant difference between eu- and hypoglycemia (Fig. 1B). Furthermore, RH3D animals at hypoglycemia also showed >2x increase in glutamine synthesis (V<sub>gln</sub>) during [3-<sup>13</sup>C]lactate infusion, indicating that glutamate/glutamine cycling between neurons and astrocytes was also enhanced. Because blood lactate levels in RH3D animals at euglycemia (2.2±1.2 mM) and hypoglycemia (2.0±0.7) were similar, the increase in V<sub>LacN</sub> seen in the RH3D animals during acute hypoglycemia may be due to increased lactate transport resulting from increased expression of monocarboxylic acid transporters.

**Conclusions:** Our results suggest that exposure to repeated hypoglycemic episodes results in increased capacity of neurons to oxidize lactate and possibly other alternative substrates. A better understanding of the regulation of neuronal plasticity to hypoglycemia could provide new therapeutic avenues to reduce the risks of CNS injury in Type-1 diabetes.

**References:** (1) Jacob RJ, et al. *Diabetes* 48:141-145, 1999; (2) Mason GF et al. *Diabetes* 55:929-34, 2006; (3) Patel AB et al. *Proc Natl Acad Sci U S A.* 102(15):5588-93, 2005. (4) Mason GF et al. *Brain Res Brain Res Protoc* 10:181-190, 2003.

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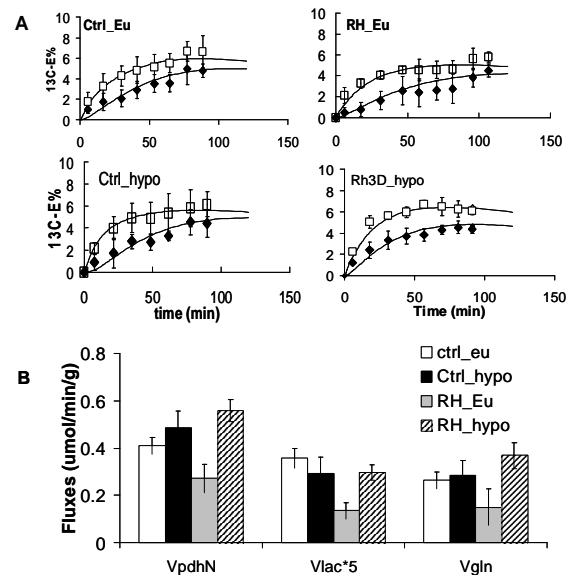


Figure 1(A). Time-courses of Glu4 and Gln4 during 3-<sup>13</sup>C-lactate infusions for different groups: Control-euglycemia, Control-hypoglycemia, RH-euglycemia, RH-hypoglycemia. □ represents <sup>13</sup>C- Glu4 enrichment, ♦ represents <sup>13</sup>C- Gln4 enrichment. (B) Metabolic fluxes for four groups of rats.