Kinetic Analysis of Dynamic Deuterium MR Spectra for Simultaneous Assessment of Cerebral Glucose Consumption Rate and TCA Cycle Flux

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Introduction Glucose is the major fuel for brain energy production in the form of ATP, which is essential to maintain brain function and electrophysiological activity including neuronal firing and signaling. Simultaneous assessment of cerebral glucose consumption rate (CMR_{glc}) and major metabolic fluxes, such as the TCA cycling rate (V_{TCA}), is crucial to understand neuroenergetics under various physiological and pathological conditions. However, such simultaneous measurement had not been possible due to the complexity of brain glucose metabolism and the limitations of experimental measure. Recently, we developed a novel Deuterium (^{2}H) MR (DMR) approach for quantification of glucose metabolism rates in rat brain at 16.4 T (1). Specifically, following a brief injection of deuterated glucose (D-Glucose-6,6-d_2), the dynamic labeling on glucose, glutamate/glutamine (Glx), water and lactate in the brain tissue can be monitored by tracking their well-resolved resonance signals (Fig. 1a) in dynamic DMR spectra with excellent sensitivity and temporal resolution (1). In order to quantify the important metabolic rates, a new kinetic model incorporating glycolysis, TCA cycle and α-ketoglutarate/Glx exchange was developed in this study and presented here. By least-square fittings of the model with the dynamic DMR data, CMR_{glc} and V_{TCA} can be concurrently determined. Previously obtained 16.4 T data of rat brains under two different conditions (1) were analyzed with the kinetic model to evaluate the sensitivity and reliability of this novel *in vivo* DMR approach in assessing cerebral glucose metabolism and its change.

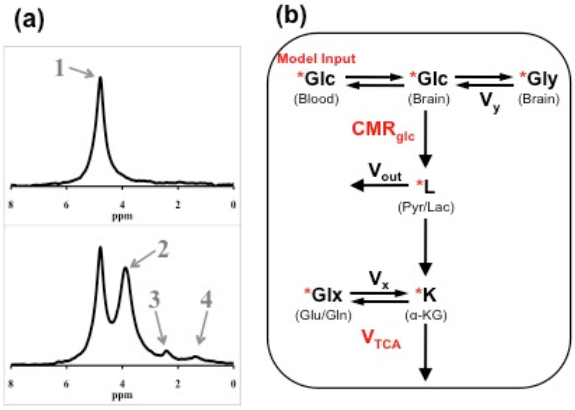

Figure 1. (a) Original *in vivo* DMR spectra from a rat brain before (top) and 5 min after (bottom) deuterated glucose infusion. Peak assignment in ppm: (1) Water (4.8); (2) Glucose (3.8); (3) Glx (2.4); (4) Lactate (1.4); **(b)** DMR modeling: Glc (glucose), Gly (glycogen), L (combined pool for Pyr (pyruvate) and Lac (lactate)), K (α-KG, α-ketoglutarate) and Glx (combined pool for Glu (glutamate) and Gln (glutamine)). V_x stands for α-KG/Glx exchange rate. V_y is the glycogen synthetic rate. V_{out} represents an efflux of lactate. A red label of '*' denotes ^{2}H-labeled metabolite.

Method Animal preparations, DMR acquisitions and spectral analysis were described previously (1). Briefly, seven male Sprague Dawley rats were scanned at 16.4 T/26 cm scanner (Varian/VNMRJ) using single-pulse-acquire sequence to obtain dynamic ^{2}H spectra from each brain with 15 s temporal resolutions for ~2 hr. Three of the seven rats were switched from isoflurane anesthesia to constant morphine infusion (25mg/kg/hr) before the onset of sequential DMR acquisitions. Each rat received a 2 min infusion (i.v.) of D-Glucose-6,6-d_2 (400 mg dissolved in 2.5 mL saline), and multiple blood samplings during the experiment. Blood glucose and plasma deuterated glucose levels were measured by glucose meter (ACCU-CHEK, Roche) and high resolution NMR (Varian/500 MHz), respectively. A new kinetic model incorporating glycolysis, TCA cycle and α-ketoglutarate/Glx exchange was developed. As shown in Fig. 1b, six metabolites including two substrates pools (blood and brain glucose) were involved in this lumped model. Both of changes in blood glucose level and plasma ^{2}H-labeled glucose concentration during the post-deuterated glucose infusion period (5 min after infusion done) were fitted based on exponential decay functions, which were then served as model inputs for further quantifications. Ten differential equations (not shown herein) from all the other five pools (two equations for each metabolite: one for total, i.e., non-labeled plus labeled; the other one for labeled only) constituted the mathematical model. By least-square fittings of the model outputs with the time course of labeled brain glucose and Glx concentrations, major metabolic fluxes such as CMR_{glc} and V_{TCA} were determined. Pool sizes and other fluxes used in this work were obtained from literatures (2-5).

Result Figure 2 demonstrates excellent model fittings of the dynamic changes in labeled glucose and Glx concentrations under both isoflurane and morphine conditions. Significantly accelerated glucose consumption and labeling accumulation in Glx were found in the morphine group (Fig. 2b) when compared with its isoflurane control (Fig. 2a). As a result, kinetic model analysis illustrated increased CMR_{glc} (0.46±0.06 vs. 0.28±0.13 μmol/g/min, under isoflurane) and V_{TCA} (0.96±0.4 vs. 0.6±0.2 μmol/g/min, under isoflurane) in the morphine treated brains, which were in response to the accelerated cerebral glucose metabolism under morphine stimulation with a higher level of neuronal activity. These values were also in good agreement with the previous results obtained through *in vivo* ^{13}C or ^{1}H MRS studies (2,5). Interestingly, we also found a significant increase in V_y under the morphine condition, indicating an elevated glycogen synthetic activity.

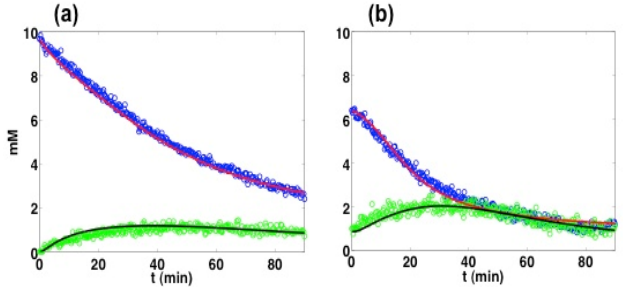

Figure 2. Time course of deuterated glucose (blue circles) and Glx (green circles) concentrations in two representative rat brains under 2% isoflurane anesthesia **(a)** vs. constant morphine infusion **(b)**. Solid lines are the model fittings of labeled glucose (red) and Glx (black) changes. Starting time point (0 min) in the x-axis means 5 min after infusion done.

Discussion & Conclusion This work demonstrates excellent spectral quality and sensitivity (Fig. 1a) of the dynamic DMR at ultrahigh field. Also when compared with ^{13}C MRS, the much shorter T_1 relaxation time of deuterated glucose (0.05±0.02 s, n=4 and measured from separated experiments) provides a substantial gain of sensitivity for detection via more signal averaging. All of these advantages make the *in vivo* application of localized DMR possible, which may eventually help the achievement of mapping regional CMR_{glc} and V_{TCA} in the brain. In summary, the results of this work indicate that *in vivo* DMR approach is robust and reliable for simultaneously assessing CMR_{glc} and V_{TCA} *in vivo* with superior sensitivity. It provides an opportunity for *in vivo* study of metabolic coupling relationship between aerobic and anaerobic glucose metabolisms as well as neuro-metabolic coupling in the animal and human brains.

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