Quantitative Assessment of Glucose Metabolism in Rat Brains using *In Vivo* Deuterium Magnetic Resonance

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Introduction Cerebral glucose metabolism is of importance for brain function since glucose is the major fuel for energy production in the form of ATP, which is essential to maintain electrophysiological activity including neuronal firing and signaling. Simultaneous assessment of cerebral glucose consumption rate (CMR_{glc}) and major metabolic fluxes, such as the TCA cycle (V_{TCA}), α-ketoglutarate/glutamate exchange (V_x) and oxygen consumption ($CMRO_2$), is crucial to understand neuroenergetics under various physiological and pathological conditions. However, such simultaneous measurement has not been possible due to the complexity of brain glucose metabolism and the limitations of experimental measuring. For decades, *in vivo* ^{13}C NMR spectroscopy has been the unique tool to investigate brain metabolic fluxes (V_{TCA} and V_x) noninvasively by analyzing ^{13}C-labeled glutamate time courses (1) with particularly complex metabolic modeling (2). ^{18}FDG-PET imaging has been the gold standard for CMR_{glc} quantification though a radioactive agent is needed. Deuterium NMR has been applied in the study of gluconeogenesis through plasma samples (3). Mateescu *et al*. had recently demonstrated the first *in vivo* attempt of Deuterium MR (DMR) in mice for assessing mitochondrial respiration (4). In this study, a novel DMR approach having a potential to simultaneously measure CMR_{glc} and major metabolic fluxes (such as V_{TCA} and V_x) was proposed and examined in rat brains at 16.4 T. Preliminary results demonstrate the feasibility and sensitivity of this DMR method in assessing cerebral glucose metabolism.

Method Eight male Sprague Dawley rats (BW=350±47 g) were anesthetized by 2% isoflurane and prepared for *in vivo* DMR scans. The rat femoral arteries and veins were catheterized for blood sampling/physiological monitoring and deuterated glucose/morphine infusion. All MR experiments were conducted at 16.4 T/26 cm scanner (Varian/VNMRJ) using $^1H/^2H$ surface coil, which was placed over the rat brain and tuned to 107 MHz for ^2H scan. A single-pulse-acquire sequence was applied to obtain dynamic ^2H spectra from the rat brain with the following parameters: 3 kHz spectral width, 512 points for each FID, 300 ms TR with 50 averages (15 s per spectrum) and 520 repetitions (130 min). For each rat, 10 min baseline spectra were acquired followed by 2 min infusion of 400 mg D-Glucose-6,6-d_2 (d66, Sigma-Aldrich) dissolved in 2.5 mL saline. To test the sensitivity of DMR in response to altered metabolic rates, 4 of the 8 rats were switched from isoflurane inhalation to constant morphine infusion (25mg/kg/hr) before the onset of sequential DMR acquisitions. A 13-Hz linebroadening was used to enhance SNR. A spherical phantom containing 5 mM deuterated glucose was also prepared for identifying chemical shifts of water and glucose resonance peaks and validating glucose quantification. All resonance signals (except for the small lactate peak) were fitted using a MATLAB-based program for quantification. The concentrations of deuterated glucose and/or glutamate/glutamine (Glx) were quantified by normalizing their fitted peak integrals to that of the baseline water signal, which has the natural abundance ^2H concentration of 17.16 mM (=55×2×0.0156%). T_1 values of deuterated water were measured using the inversion recovery technique under fully relaxed condition in the phantom solution and rat brains. T_1 of deuterated glucose was also determined in the phantom solution.

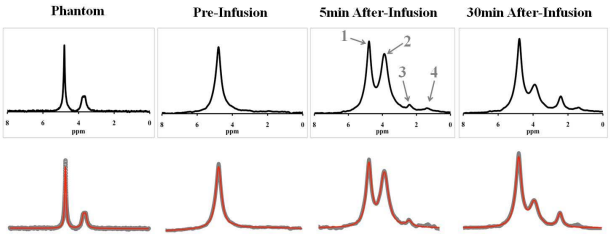

Figure 1. Original (black trace in upper row and grey trace in bottom row) and fitted (red trace in bottom row) DMR spectra from deuterated glucose phantom solution and a rat brain before, 5 min and 30 min after deuterated glucose infusion. Each *in vivo* spectrum was summed from 1 min of data acquisitions (4 spectra). Peak assignment in ppm: (1) Water (4.8); (2) Glucose (3.8); (3) Glx (2.4); (4) Lactate (1.4).

Results Figure 1 shows excellent *in vivo* spectral quality and curve fittings. Calculations based on the fitted phantom spectrum gave a deuterated glucose concentration of 5.2 mM vs. the known 5 mM, which indicates the reliability of the quantification method. As shown in Fig. 1, following the brief infusion of deuterated glucose, four well resolved resonance peaks (water, glucose, Glx and lactate) were detected in the rat brain. Thus, their changes can be monitored via the ^2H spectra during dynamic scanning. As shown in Fig. 2, the glucose consumption and labeled Glx accumulation rates were found significantly increased in the morphine group when compared with its isoflurane control. Delayed Glx appearance and onset of glucose decay were also observed in the isoflurane group. All of these observations indicate an accelerated glucose metabolism with increased CMR_{glc}, V_{TCA} and V_x under morphine stimulation, which is also consistent with the increased heart rate (416±22 vs. 340±20 bpm with isoflurane) and mean blood pressure (141±12 vs. 111±12 mmHg). *In vivo* T_1 value of deuterated water was 0.36±0.01 s, while the phantom T_1 values of deuterated water and glucose were 0.45 and 0.055 s, respectively.

Discussion and Conclusion This study demonstrates that dynamic DMR is particularly well suited for *in vivo*, quantitative assessment of glucose metabolism in the rat brain. The excellent spectral quality and sensitivity makes the *in vivo* application of localized DMR possible. When compared with ^{13}C MRS, the much shorter T_1 relaxation time of deuterated glucose (although phantom data only) provides a substantial gain of NMR sensitivity for detection via more signal averaging. Its SNR can be further improved by using a short TR and Ernst angle. Importantly, the excellent sensitivity and temporal resolution of DMR also ensures the detection of rapid change of labeled-metabolites, such as the delayed appearance of Glx under isoflurane condition in this study (Fig. 2). This observation may explain the difficulties of hyperpolarized ^{13}C technique with pruvate infusion aiming to detect the increasing glutamate and/or glutamine labeling in animal brain under anesthesia condition. The slow turnover processing for labeling carbon isotope into Glx (> 2 minutes, see Fig. 2) competes with its ^{13}C T_1 relaxation time that determines the signal detection time window with sufficient hyperpolarization and detection sensitivity, thus, missing the opportunity for detecting the hyperpolarized Glx signal *in vivo*.

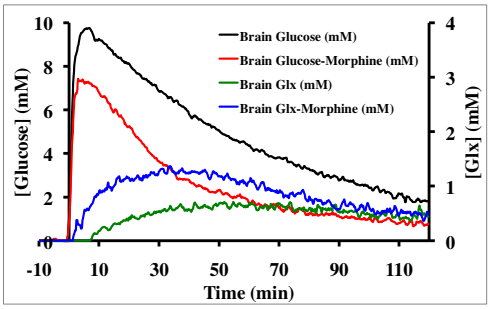

Figure 2. Time course of deuterated glucose and Glx concentrations in two rat brains under 2% isoflurane anesthesia versus constant morphine infusion.

In summary, the results of this work indicate that *in vivo* DMR approach is robust and reliable for simultaneously detecting changes in glucose and Glx concentrations in the rat brain with excellent sensitivity. When combined with metabolic modeling, the simultaneous measurement of glucose consumption rate, TCA cycle flux and α-ketoglutarate/glutamate exchange rate can be achieved in animal and human brains. It also provides an opportunity for *in vivo* study of coupling relationship between aerobic and anaerobic glucose metabolisms in the brain.

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