Simultaneously Measuring Glucose Transport Constants and Cerebral Metabolic Rate of Glucose by In Vivo ^{1}H MRS in the Rat Brain

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Introduction: The basal brain activity and function rely on a constant supply of glucose in the brain tissue, which is regulated by glucose transportation through the blood-brain barrier (BBB) and the glucose consumption [1]. Therefore, it is interesting to exploit *in vivo* approaches able to reliably measure the cerebral metabolic rate of glucose (CMR_{glc}) and glucose transport constants (K_T and T_{max}) simultaneously. In a previous study [2], we observed a 37% decrease in CMR_{glc} under the iso-electric (i.e., silent EEG activity) condition using high-dose pentobarbital as compared to mild isoflurane anesthesia condition. Meanwhile, we found that the brain glucose concentration also significantly reduced under the iso-electric condition which, though, only requires a minimal glucose metabolic activity for maintaining "housekeeping" power. This observation seemingly contradicted with other studies showing a decreased brain glucose concentration accompanied by the increased CMR_{glc} elevated by brain stimulation. This apparent discrepancy can be explained by the change of plasma glucose concentration, which was found to be substantially decreased under the iso-electric condition. This change had a direct effect on the brain glucose concentration regulated by glucose transportation. Another possible reason is that glucose transport constants might vary under anesthesia conditions [3]. The aim of the present study is to exploit an *in vivo* ^{1}H MRS method for measuring CMR_{glc} and glucose transport constants simultaneously.

Methods: A bolus of (non-labeled) glucose was injected into the rat vein then following a short period of glucose infusion, resulting in a hyperglycemic condition. Then, the glucose infusion was terminated, leading to dynamic decays in both brain and plasma glucose concentrations ($[G_i]$ and $[G_0]$). The time curse of $[G_i]$ decay was measured by localized *in vivo* ^{1}H MRS (PRESS sequence with 6×4×6 mm^3 voxel covering the cortical region and a portion of sub-cortical region) and quantified by the LCModel fitting. The time course of $[G_0]$ decay was measured by blood drawing and glucose analyzer. The values of CMR_{glc} and two glucose transport constants (T_{max} and K_T) were determined by the least square fitting algorithm according to the standard Michaelis-Menten glucose transport Equation [1] [1-5],

$$\frac{d[G_i]}{dt} = T_{in} - T_{out} - CMR_{glc} \quad [1]$$

$$T_{in} = \frac{T_{max}[G_0]}{([G_0] + K_T)} \qquad T_{out} = \frac{T_{max}[G_i]}{([G_i] + K_T)}$$

Results and Discussions: *In vivo* ^{1}H spectra acquired from a representative rat brain before and after glucose infusion as well as the difference spectrum between them were illustrated in Fig.1a and 1b, respectively. After glucose infusion, the glucose NMR signals across a chemical shift range of 3.0~4.2 ppm were significantly increased. The measured *in vivo* glucose resonance peaks were identical with that assigned by glucose solution spectrum (Fig. 1c). Figure 2 shows the dynamic glucose concentration decays in the plasma ($[G_0]$) and brain tissue ($[G_i]$) during post glucose infusion period and the curve fitting results from a single rat measurement. Plasma glucose concentration decay followed an exponential function, which was used to solve the differential equation of Eq. 1. Three parameters of CMR_{glc}, T_{max} and K_T were determined from 7 rats and they were 0.44±0.17 μmol/g/min, 1.35±0.47 μmol/g/min and 13.4±6.8 mM, which are consistent with the literature reports. The curve fitting errors analyzed by Mento-Carlo simulations were demonstrated in Fig. 3. It shows that the measurement error indicated by the width at 50% of maximum of gauss function for K_T is much larger than that of CMR_{glc} and T_{max}, suggesting a substantial uncertainty in measuring K_T. In contrast, the *in vivo* measurements of CMR_{glc} and T_{max} have better reliability and accuracy. Moreover, the averaged values of T_{max} and K_T were further applied to steady-state CMR_{glc} measurement approach based on a paired data of plasma and brain glucose concentration measured before the glucose infusion (euglycemia), resulting in CMR_{glc} = 0.45±0.09 μmol/g/min, which is in an excellent agreement with the result of 0.44±0.17 μmol/g/min based on the dynamic approach. Similar dynamic *in vivo* MRS approaches have been applied to measure CMR_{glc} and transport constants during the glucose infusion period [4, 5] with a technical challenge to manage glucose infusion for achieving a desired plasma glucose change. In contrast, the post-infusion protocol applied in the present study leads to a well-controlled decay of G_0 as well as G_i as demonstrated in Fig. 2. This merit improves the fitting reliability for quantifying CMR_{glc}, T_{max} and K_T. In addition, it also allows the preparation of glucose infusion outside of magnet before *in vivo* ^{1}H measurement. This approach should be useful for studying cerebral glucose metabolism associated with brain function and dysfunction.

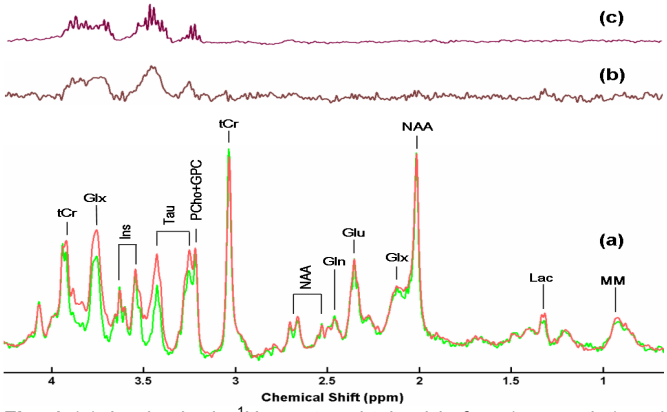

Fig. 1 (a) *In vivo* brain ^{1}H spectra obtained before (green plot) and immediately after the termination of glucose infusion (red plot) with 10 minutes of signal averaging (NS=200). (b) The difference spectrum showing a large increase in brain glucose concentration, and the *in vivo* glucose signals were very similar to the glucose phantom solution (c) with 6-Hz line-broadening.

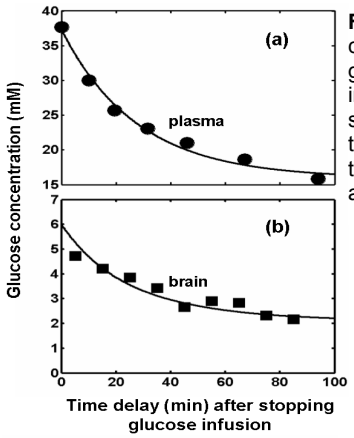

Fig. 2 (left panel) Plasma (full circles) and brain (full squares) glucose concentration changes in one representative rat after stopping glucose infusion and the best curve fitting based on the exponential decay (plasma) and Eq. 1 (brain), respectively.

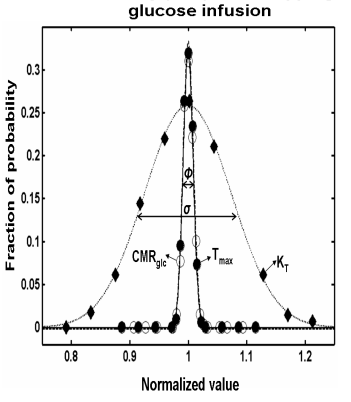

Fig. 3 (left-low panel) Quantitative descriptions of curve fitting error predicted by the Monte-Carlo simulation. The error distributions of each free parameter (CMR_{glc}, T_{max} and K_T) in the curve fitting were obtained from 1000 sets of time courses of brain glucose concentrations, which were taken from the best fitted curve of the *in vivo* data plus the random noises comparable to the curve fitting error (see Fig. 2). The error distributions were fitted by the gauss function and the results were demonstrated by the dotted (K_T) and dashed (T_{max}) as well as solid (CMR_{glc}) line, respectively. The line widths at the half maximum were indicated by the σ=0.17 for K_T and Φ=0.02 for CMR_{glc} and T_{max}.

References: 1. Lund-Andersen et al, *Physiol Rev* (1979); 2. Du et al, *MRM* (2009); 3. Gjedde et al, *J Neurochem* (1980); 4. Gruetter et al. *JCBFM* (1996); 5. Van Zijl et al, *Am J Physiol* (1997).

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