In vivo ^{1}H MRS of Dynamic ^{13}C Labeling of Glutamate and Glutamine

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Target audience: Scientists and clinicians who are interested in the *in vivo* measurement of dynamic glutamate (Glu) and glutamine (Gln) turnover in the human brain.

Purpose: *In vivo* measurement of Glu and Gln turnover from intravenously infused ^{13}C labeled substrates is a powerful tool for investigations of energy metabolism and neurotransmission in the human brain. Common techniques for measuring Glu and Gln turnover either use ^{13}C magnetic resonance spectroscopy (MRS) or ^{13}C edited ^{1}H MRS because analysis of changes in ^{1}H MRS spectra due to incorporation of ^{13}C labels is complicated by severe spectral overlapping (1). Here, we propose to use a ^{1}H MRS pulse sequence to measure dynamic Glu and Gln turnover from intravenously infused [U-$^{13}C_6$]glucose by detecting Glu and Gln ^{1}H signal changes due to ^{1}H-^{13}C J-coupling at 7T.

Methods:

Pulse sequence: A TE-optimized PRESS (point resolved spectroscopy) pulse sequence modified with an inserted J-suppression pulse has been proposed to spectrally resolve and detect Glu, Gln, and GSH at 7T (2). The J-suppression pulse is a frequency selective RF pulse placed at the resonance frequency of the aspartyl CH proton of N-acetyl-aspartate (NAA) at 4.38 ppm, thereby altering the J-evolution of the NAA aspartyl CH_2 multiplet at 2.49 ppm. It was found that TE_1 = 69 ms, TE_2 = 37 ms, and J-suppression pulse flip angle = 90° resulted in minimal NAA multiplet signals at 2.49 ppm while retaining near-maximum peak amplitudes for the C4 proton resonances of Glu, Gln, and GSH (3).

Numerical simulations: The largest peaks in the ^{1}H spectra of Glu and Gln correspond to the C4 protons. Moreover, ^{13}C is incorporated into the C4 sites of Glu and Gln in the first turn of the tricarboxylic acid cycle during [U-$^{13}C_6$]glucose infusion. Hence we focused our attention on the C4 proton signals to see if spectrally resolved ^{13}C-labeled Glu and Gln C4 proton signals can be reliably observed during the infusion. The effects of carboxylic and amide ^{13}C's were negligible. Density matrix simulation programs were developed using the GAMMA C++ library to compute spectra of Glu, [4-^{13}C]Glu, Gln, and [4-^{13}C]Gln using ^{13}C chemical shift values and ^{1}H-^{13}C J-coupling constants reported in Ref. (4). An ideal excitation pulse and two experimental refocusing pulses along with 2D spatial localization using 201 × 201 spatial points were used to simulate the spectra accurately and efficiently.

In vivo experiments: Two female volunteers were recruited for the ^{13}C labeled glucose infusion experiments following procedures approved by our local institutional review board. Before scanning, two antecubital veins of the subject were cannulated, one for infusing [U-$^{13}C_6$] glucose and the other for withdrawing blood to monitor glucose levels. A baseline MRS scan was performed before the infusion using the following sequence parameters: TR = 2.5 s, TE_1 = 69 ms, TE_2 = 37 ms, J-suppression pulse flip angle = 90°, spectral width = 4000 Hz, number of data points = 2048, and number of transients = 128. Water suppression was accomplished using eight RF pulses of ~350 Hz bandwidth. The baseline scan lasted ~6 minutes. The infusion of [U-$^{13}C_6$]glucose (20% w/w) started after the baseline scan at a bolus infusion rate of 900 ml/h, followed by an exponential decay to the rate of 100 ml/h at the 15th minute of infusion. The subsequent infusion rate was adjusted to keep glucose levels at 160-200 mg/dL. MRS scans were performed repeatedly, and between scans, the resonance frequency was adjusted on the scanner to correct for small frequency drifts.

Results: Numerically simulated spectra of Glu, [4-^{13}C]Glu, Gln, and [4-^{13}C]Gln are displayed in Fig. 1. Due to magnetization transfer within the strongly coupled glutamate spin system, the proton C4 peak at 2.35 ppm in the Glu spectrum is asymmetrically shifted in the [4-^{13}C]Glu spectrum, i.e., having only one major peak at 2.56 ppm plus some small resonances around 2.14 ppm that are mixed in with the C3 proton resonances. The appearance of the [4-^{13}C]Gln spectrum is similar to the Glu case: the Gln peak at 2.45 ppm splits into a major peak at 2.66 ppm and small resonance signals at 2.24 ppm. Time-course ^{1}H spectra for one healthy volunteer during [U-$^{13}C_6$]glucose infusion are displayed in Fig. 2. The spectrum acquired before the start of infusion is plotted on top and labeled as 'Baseline'. Spectra acquired during the infusion are plotted sequentially below the baseline spectrum with their scan start time and finish time (relative to infusion start time) labeled beside the corresponding spectrum. The stack plots show that the Glu peak at 2.35 ppm ([4-^{12}C]Glu) became smaller as infusion progressed. At the end of the infusion experiment, i.e. 76 - 82 min after infusion started, the [4-^{12}C]Glu peak was less than half of the original peak in the baseline spectrum. The [4-^{12}C]Gln peak at 2.45 ppm also became smaller during infusion. From numerical simulations, we know that the major peak of [4-^{13}C]Glu is at 2.56 ppm, which overlaps the GSH peak. In Fig. 2, the compound peak around 2.56 ppm, which only contained GSH multiplet signals originally, became larger during the course of [U-$^{13}C_6$]glucose infusion. This shows that the [4-^{13}C]Glu multiplet was growing as more and more ^{12}C atoms were replaced by ^{13}C atoms at the C4 site of Glu.

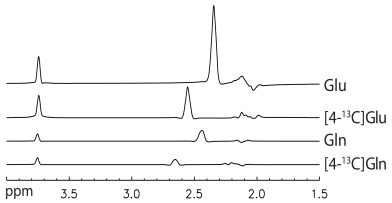

Fig. 1. Numerically simulated spectra of Glu, [4-^{13}C]Glu, Gln, and [4-^{13}C]Gln. The Gln/Glu ratio was set to 0.22. The spectra were broadened to a singlet width of 9 Hz.

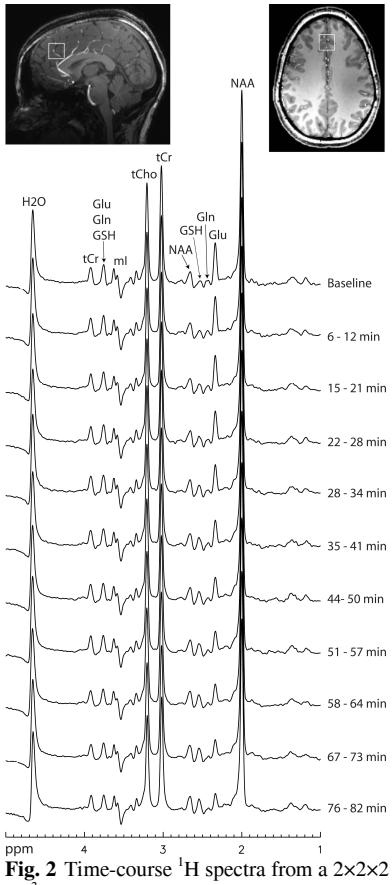

Fig. 2 Time-course ^{1}H spectra from a 2×2×2 cm^3 voxel in the medial prefrontal cortex of a healthy volunteer during [U-$^{13}C_6$]glucose infusion.

Discussion: The spectral pattern of the time-course ^{1}H spectra acquired during [U-$^{13}C_6$]glucose infusion matches our numerical simulations very well. Although a detailed and quantitative analysis of the time-course data requires the arterial blood input function, these experiments show that it is feasible to use our proposed pulse sequence to measure dynamic ^{13}C incorporation into Glu and Gln during intravenous infusion of ^{13}C labeled glucose or other substrates (e.g. the glia-specific substrate [2-^{13}C]acetate).

Conclusion: Numerical simulations and preliminary ^{13}C-labeled glucose infusion studies demonstrated the feasibility of using a proton-only pulse sequence (3) to measure dynamic Glu and Gln labeling processes during infusion of ^{13}C labeled glucose at 7T. Compared to conventional proton-observed ^{13}C-edited experiments (5), the proposed pulse sequence is much simpler in hardware and software requirements since it is a pure ^{1}H pulse sequence with no RF pulses at ^{13}C resonances.

References

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