In Vivo Detection of ^{13}C Labeling of Glutamate and Glutamine Using Proton MRS at 7T

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Target audience: Physicians and researchers with an interest in dynamic glutamate (Glu) and glutamine (Gln) turnover in the human brain.

Purpose: *In vivo* measurement of Glu and Gln turnover has many applications in neuroscience research since it can provide valuable information on energy metabolism and neurotransmission in the human brain (1). In a previous study, 7 Tesla ^{1}H MRS has been shown to be capable of measuring dynamic changes in Glu and Gln signal strength during intravenous infusion of [U-^{13}C$_6$]glucose (2). In this work, the feasibility of quantifying the time-courses of [4-^{13}C]Glu and [4-^{13}C]Gln concentrations using ^{1}H MRS at 7 Tesla is evaluated.

Methods: In the ^{1}H spectra of Glu and Gln, the largest peaks come from the C4 protons. During [U-^{13}C$_6$]glucose infusion, ^{13}C is incorporated into the C4 sites of Glu and Gln in the first turn of the tricarboxylic acid cycle. Therefore, the main focus of this study was to observe and quantify the signal changes of Glu-C4 and Gln-C4. Basis spectra of Glu, Gln, and their ^{13}C isotopomers were numerically simulated using an in-house developed program based on the GAMMA C++ library.

Magnetic resonance spectroscopy (MRS) time-course data were successfully collected during [U-^{13}C$_6$]glucose infusions from eight healthy volunteers following procedures approved by our local institutional review board. Two antecubital veins of the subjects were cannulated before the subjects were brought to the scanner room, one for infusing [U-^{13}C$_6$] glucose and the other for withdrawing blood to monitor glucose levels. Before infusion started, a baseline MRS scan was performed using a TE-optimized PRESS (point resolved spectroscopy) pulse sequence modified by inserting a J-suppression pulse (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 and Gln. Other parameters for the modified PRESS sequence were: TR = 2.5 s, spectral width = 4000 Hz, number of data points = 2048, and number of transients = 128. The infusion of [U-^{13}C$_6$]glucose 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 using the same pulse sequence as the baseline scan. Each MRS scan lasted ~6 minutes.

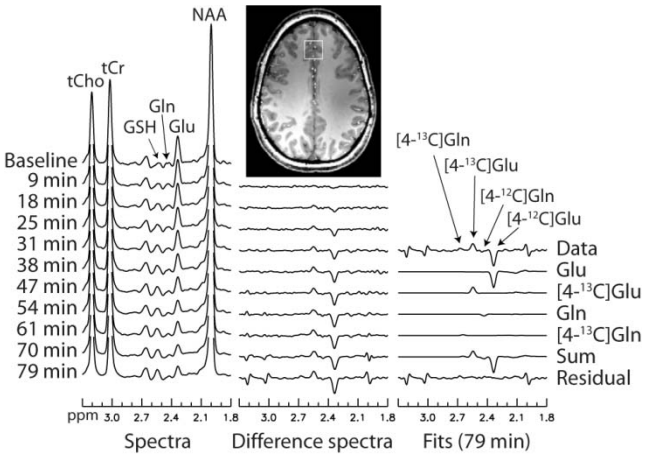

Fig. 1 Time-course spectra (left), time-course difference spectra (middle), and fitting results for the last time point (right) from a 2×2×2 cm^3 voxel in the medial prefrontal cortex of a healthy volunteer during [U-^{13}C$_6$]glucose infusion. The listed times are the mid-point times of the MRS scans after the start of infusion.

Data from the baseline MRS scan were processed first. The time-averaged 32-channel FID signals were merged into a combined single-channel metabolite FID by a generalized least square method (3). The combined FID was Fourier transformed into the frequency domain to generate the baseline spectrum. To quantify baseline metabolite concentrations, the baseline spectrum was fitted with simulated basis sets of NAA, N-acetyl-aspartate-glutamate (NAAG), γ-aminobutyric acid (GABA), Glu, Gln, glutathione (GSH), aspartate, creatine+phosphocreatine (tCr), glycerophosphocholine+phosphocholine (tCho), and myo-inositol using an in-house developed linear combination fitting program. In the fitting process, the metabolite basis sets were scaled, apodized using a Voigt lineshape, frequency shifted, zero-order phase corrected, Fourier transformed to the frequency domain, and then combined with a spline baseline with 8 control points to fit the *in vivo* spectral data between 1.8 - 3.3 ppm. The time-course spectra during infusion were reconstructed similar to that of the baseline spectrum and were additionally line broadened by a 3 Hz Gaussian function. It is desirable to generate a series of difference spectra between the spectrum at each time point and the baseline spectrum in order to visualize the spectral changes due to the infusion of [U-^{13}C$_6$]glucose. However, the phase, frequency, linewidth, and lineshape of each spectrum may vary due to minor subject motion and system instabilities. To minimize these effects, the baseline spectrum was line-broadened using a Voigt lineshape, frequency shifted, and zero-order phase corrected to fit each time-course spectrum in the ranges of 1.7-2.1 ppm and 2.75-3.65 ppm. Each fitted baseline spectrum was subtracted from the time-course spectrum to generate a series of difference spectra. The residuals at the NAA, creatine, and choline positions in the difference spectra were mainly caused by subject motion. The difference spectra were fitted with simulated basis sets of Glu, [4-^{13}C]Glu, Gln, and [4-^{13}C]Gln in the range of 2.25-2.80 ppm with the constraints that the decrease of [4-^{12}C]Glu is equal to [4-^{13}C]Glu and the decrease of [4-^{12}C]Gln is equal to [4-^{13}C]Gln.

Results: Time-course spectra, time-course difference spectra, and fitting results for the last time point (79 min) of one healthy volunteer are displayed in Fig. 1. As the infusion progressed, the Glu peak at 2.35 ppm ([4-^{12}C]Glu) and the Gln peak at 2.45 ppm ([4-^{12}C]Gln) in the time-course spectra became smaller. As a result, the Glu and Gln peaks in the difference spectra became more negative. Meanwhile, the [4-^{13}C]Glu peak at 2.56 ppm in the difference spectra grew bigger. The concentration time courses of [4-^{13}C]Glu and [4-^{13}C]Gln averaged over all eight healthy volunteers are plotted in Fig. 2. Further metabolic modeling analysis requires the arterial input function. Work along this direction is currently in progress.

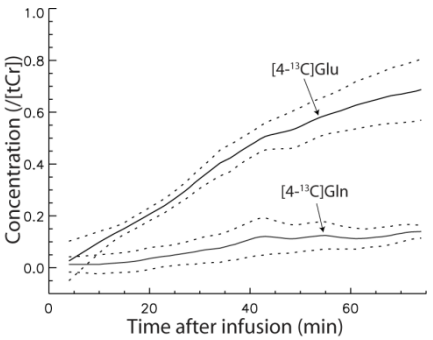

Fig. 2. Averaged concentration time courses of [4-^{13}C]Glu and [4-^{13}C]Gln during [U-^{13}C$_6$]glucose infusion. The dotted lines represent 95% confidence intervals.

Discussion and Conclusion: The time-course difference spectra clearly showed the decrease of the [4-^{12}C]Glu and [4-^{12}C]Gln signals and the increase of the [4-^{13}C]Glu and [4-^{13}C]Gln signals during the infusion, and were fitted very well using a linear combination of a numerically simulated basis set. In the future work, dynamic Glu and Gln turnover rates will be computed from these time-course data and arterial input functions. Our results demonstrated that it is feasible to quantify glutamate and glutamine turnover from intravenous infused ^{13}C-labeled glucose using proton MRS at 7 Tesla from a 2×2×2 cm^3 voxel in the prefrontal cortex. Since this method is only limited by the applicability of single voxel proton MRS, many other voxel locations are also feasible.

References

1. Rothman, DL, *et al.*, NMR Biomed:2011; 24:943–957. 2. An, L, *et al.*, MRM (2014); DOI: 10.1002/mrm.25150. 3. An, L, *et al.*, JMRI 2013; 37:1445–1450.