# Quantification of brain glycogen concentration and turnover through localized $^{13}$C NMR of both the C1 and C6 resonance

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## Introduction

Glycogen (Glyc) is the main energy store in the brain, but its precise role remains to be fully understood. To further characterize the role of brain Glyc, its metabolic characteristics and concentration need to be determined. The only *in vivo* method available has been based on the incorporation of 1-$^{13}$C-labeled glucose (Glc) into brain 1-$^{13}$C Glyc and its detection with localized NMR. Among the challenges of this technique is the differentiation of increases in Glyc signal due to changes in $^{13}$C isotopic enrichment (IE, the percentage of the molecule that contains the $^{13}$C isotope) from changes in total Glyc concentration. In this study we present an approach based on the "pre-labeled" $^{13}$C1 position of Glyc and an acute infusion of C1,6 Glc, leading to the C1 of Glyc to represent total concentration changes and the C6 position to reflect flux through the synthesis pathway. The aim therefore was to establish the localized measurement of C6 at the same time as C1 labeled Glyc. To minimize large chemical shift displacement errors due to the 39 ppm difference between the C1 and C6 resonances, a novel sequence based on the Fourier series window (FSW,1) was implemented.

## Materials and Methods

After overnight fasting, male Sprague-Dawley rats (n=6, w = ~270 g) were given 100% IE 1-$^{13}$C Glc dissolved in water as their only source of carbohydrates for 24 h. They were then anesthetized using 1.5% isoflurane and a femoral vein was catheterized for infusion. After surgery isoflurane was switched off and α-chloralose (26.7 mg/kg/hr) was infused. Blood pressure, respiration rate and temperature were maintained within normal range. A quadrature $^{1}$H coil with a single 3-loop $^{13}$C coil was used. The animal was then inserted into a Varian Inova 9.4T 31 cm bore actively-shielded magnet.

First, IE of NAA was determined with a carbon-edited $^{1}$H STEAM sequence, which was used to calculate the IE of Glyc C1 as validated recently (2). Next, two 1 h baseline 5x5 spectroscopic imaging datasets were acquired with the FSW technique: a 3 ms BIR-4 90° pulse was used for excitation and a short time of 270 μs was used for phase encoding in two dimensions. Waltz-16 NOE and decoupling were applied at the Glyc/Glc $^{1}$H frequency, and ISIS was used for slice selection. The FSW was set to a FOV of 22x22 mm with 10 mm slice thickness. 5 coefficients were used in each direction, resulting in 121 gradient variations and an acquisition time of 58 min with TR = 1 s. Subsequently 1,6-$^{13}C_2$ Glc was infused at the previously determined IE of Glyc for 7 h, while 7 more FSW datasets were acquired. Animal physiology was maintained at a stable physiological range.

The datasets were processed in MATLAB, and the window was moved with steps of 1/16$^{th}$ of the FOV to obtain a metabolic map of 16x16 pixels (each spanning 1/5$^{th}$ of the FOV in both directions), of which one in the top of the brain was selected. Spectra were processed with 15 Hz line broadening. The AMARES routine in jMRUI was used for spectral fitting; the Glc and Glyc C6 resonances at 61.4 ppm were separated by fixing their linewidths at ~25 and ~70 Hz respectively.

The Glyc C6 time course was fitted with $S(t)=S_\infty[1-\exp(-t/\tau)]$ to obtain the turnover time τ. After the study, animals were sacrificed with 2s 4kW focused microwave fixation. Brains were taken out, homogenized and the brain Glc and Glyc concentrations were determined as previously described (2). Glc and Glyc IE of these extracts were determined in a 600 MHz high-resolution spectrometer as previously described (2).

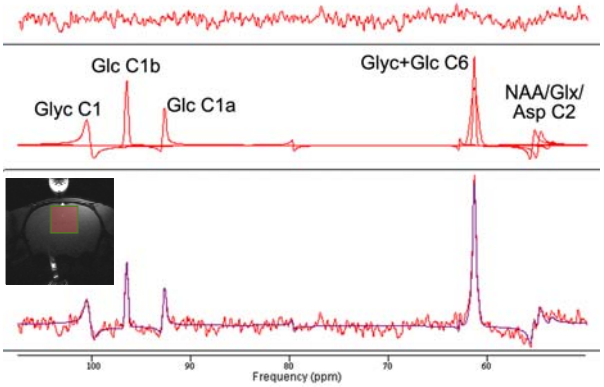


***Figure 1.*** *jMRUI fitting of a FSW brain spectrum after 6 h of infusion. Bottom: spectrum and fit. Middle: individual peaks. Top: fit residual. Inset: location of the window within the brain, with a formic acid phantom visible at the center of the detection coil.*

## Results and Discussion

Localization of the FSW sequence was robust, as judged from the lack of non-cerebral glycerol at 63 ppm in the selected spectra (Fig. 1). The time-domain based AMARES routine easily accounted for phase variation in the spectrum due to the RF pulse profile, as seen with Glyc C1 and the Glx C2 peaks in Fig. 1. The stability of the glycaemia was evidenced *in vivo* from the stable brain Glc C1 signal (● in Fig. 1).

Upon start of the infusion of 1,6-$^{13}C_2$ Glc, the C1 Glc and Glyc (▲ in Fig. 1) signal did not change and remained at an average concentration of 4.3 ± 1.0 and 5.1 ± 1.6 mM concentration (in excellent agreement with the biochemistry at 5.3 ± 1.6 mM) respectively, as expected under these experimental conditions. In contrast, the brain Glc C6 signal (▼ in Fig. 1) exhibited a step increase upon the start of infusion, validating the robust, separate quantification of the overlapping Glc and Glyc C6 peaks at 61.4 ppm.

Glyc C6 (■ in Fig. 2) gradually increased, consistent with a slow turnover rate. From its exponential fit we obtained an estimated turnover time of 8 ± 4 h ($R^2$ = 0.97), which falls into the range of the previously reported of 10 h (3). The steady state 6-$^{13}$C concentration of this fit was 3 ± 1 mM. Assuming the same C6 IE at steady state as the C1 resonance (41%, confirmed with both the NAA measurement and the brain extract spectroscopy) this gives a total glycogen concentration of 7 ± 3 mM, consistent with the value from the C1 resonance.

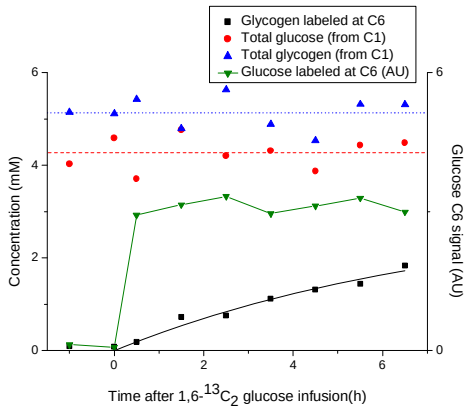


***Figure 2.*** *Time courses for brain glycogen and glucose and their fits. C1 glycogen (▲) and glucose (●) remain constant, and their average is shown. C6 glucose (▼, in arbitrary units) displays a step-like increase, while C6 glycogen (■) gradually increases.*

We conclude that (a) the FSW sequence in conjunction with constrained peak fitting can be used to simultaneously measure C1 and C6 $^{13}$C glycogen resonances with a 1 h time resolution and that (b) net IE changes due to unidirectional synthesis of brain glycogen can be derived from these data.

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## References

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