# Quantification of the [1-$^{13}$C]Glycogen Signal in the Human Brain: The Turnover of Bulk Brain Glycogen is Very Slow

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## Introduction

Detection of human brain glycogen by $^{13}$C NMR utilizing an OVS-based, non-echo localization method was recently reported (1). This study also described a low amount of $^{13}$C label incorporation into glycogen from infused glucose indicating a synthesis rate of human brain glycogen that was much slower than in the rat brain (2). These results were based on the quantitation of brain glycogen using the commonly employed external reference method (3). However, a reliable quantitation of the C1 glycogen signal is challenging due to its complicated lineshape (4, 5). It has been suggested that integration limits over large ppm ranges be used (4) which is not feasible in the presence of glucose resonances *in vivo*, e.g. during [1-$^{13}$C]-glucose administration. Conditions permitting a reliable measurement of brain glycogen thus need to be assessed. The aim of the current study was **(a)** to follow the wash-out of $^{13}$C label from C1 glycogen in healthy subjects over 2 days after administration of [1-$^{13}$C]glucose in order to estimate glycogen turnover times in the human brain and **(b)** to assess the reliability of brain glycogen quantification.

## Methods and Subjects

*In vivo* measurements were performed on a 4 T/90 cm magnet (Oxford/Varian). A quadrature 14 cm $^{1}$H surface coil with a 9 cm diameter linear $^{13}$C coil was used. The localization was achieved by 3D outer volume suppression (OVS) combined with 1D ISIS (1). Two healthy volunteers (1 male, both 50 years old) were studied after an overnight fast by administering a total of 80g of [1-$^{13}$C]glucose, which maintained the isotopic $^{13}$C enrichment of plasma glucose above 50% for the first 5 hours of the study. Following the glucose infusion the subjects continued their daily activities and were scanned 3 more times, the evening of the infusion, and the morning and evening of the next day.

*In vitro* measurements were performed on a 14.1 T vertical bore spectrometer (Varian) using aqueous solutions of oyster, bovine liver and rabbit liver glycogen (Sigma). A reference compound such as $^{13}$C formate or glucose was also included in the samples in order to correct for loading differences at different temperatures. Spectra were obtained with a pulse-acquire sequence without NOE enhancement, with and without WALTZ-16 decoupling and under fully relaxed conditions (90° pulse, TR > 5×$T_1$) after determination of $T_1$ relaxation times of both glycogen and the reference compound by inversion recovery at each temperature. Signal integration was done both by peak fitting software provided by the spectrometer and integration over a range of limits.

## Results and Discussion

When assessing the conditions of the external reference used to quantitate the *in vivo* glycogen signal, a temperature dependence of the glycogen linewidth and area was demonstrated *in vitro* at 14.1T. The linewidth and area changes were present to the same extent both in decoupled and coupled $^{13}$C NMR spectra (Fig. 1). The effect did not depend on glycogen concentration (in the range 0.1-0.5M) and was observed in all 3 types of glycogen investigated (Fig. 1). The temperature dependence of the glycogen integral and linewidth was also observed at 4 and 9.4T (not shown). Therefore, when quantitating *in vivo* glycogen signals the linewidth cannot a priori be assumed to be constant and the temperature control of the external reference is important.

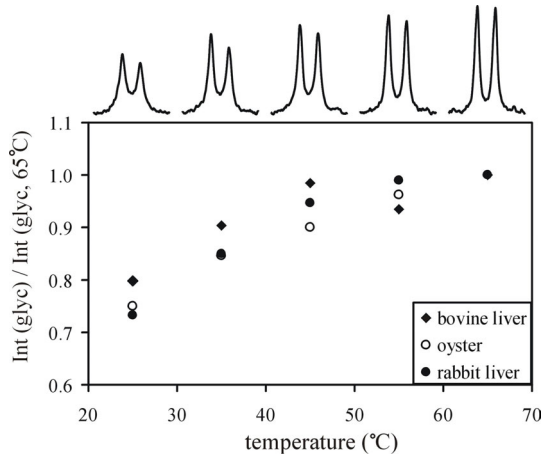


**Fig. 1.** Temperature dependence of the coupled C1-glycogen signal integral. All integrals were normalized to those at 65°C. Data from 3 different commercial sources are shown, as well as representative spectra from rabbit liver glycogen above the plot.

Quantification of the brain glycogen signal in 2 subjects with a glycogen reference solution at body temperature (Fig. 2) resulted in label incorporation rates consistent with the previous measurement of human brain glycogen synthesis, which also utilized a warmed reference phantom (1). Furthermore, the [1-$^{13}$C]glycogen concentration remained above 0.2 µmol/g after 34h, indicating a very slow label wash-out rate. Assuming a total brain glycogen concentration of a few µmol/g, these data imply that the turnover time of human brain glycogen is on the order of several days to one week. This slow turnover indicates that any increases in brain glycogen content, such as the supercompensation observed in the rat brain after a single episode of hypoglycemia (6), can persist for several weeks, which is generally the time required to reverse hypoglycemia unawareness in patients with type 1 diabetes (7) and therefore supports a role for brain glycogen in the development of this syndrome (1, 6).

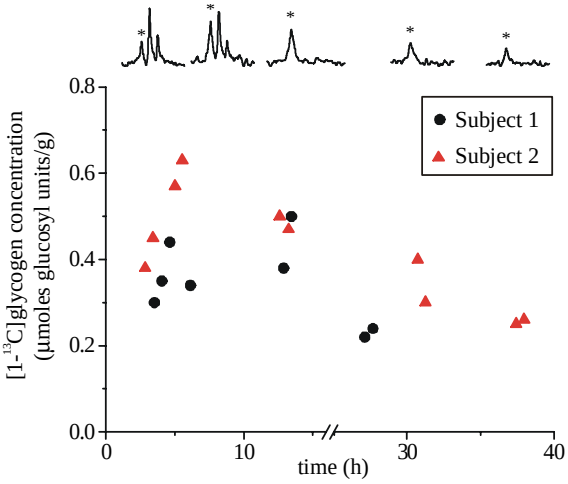


**Fig. 2.** Quantification of $^{13}$C label incorporation into and wash-out from glycogen C1 over time. Administration of labeled glucose was from t=0-5h. Each data point represents 25 min averaging (VOI=210ml). Spectra on top are from one subject acquired at the respective time points. *: glycogen C1 peak at 100.5ppm. The other two peaks in the first two spectra are β and α C1 glucose.

## References

**1.** Oz G et al, *Neurochem Int*, 43, 323, 2003. **2.** Choi IY et al, *J Neurochem*, 73, 1300, 1999. **3.** Gruetter R et al, *MRM*, 20, 327, 1991. **4.** Overloop K et al, *MRM*, 36, 45, 1996. **5.** Bergans N et al, *NMR Biomed*, 16, 36, 2003. **6.** Choi IY et al, *J Neurosci Res*, 72, 25, 2003. **7.** Cryer PE, *Am J Physiol Endocrinol Metab*, 281, E1115, 2001.
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