Direct validation of *in vivo* localized ^{13}C MRS measurements of [1-^{13}C] glycogen in rat brain

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Introduction

The mammalian brain contains a significant concentration of glycogen, which can be used as a source of energy supply once glucose transport becomes limiting to metabolism. Acute hypoglycemia is one of those conditions, where brain glycogen plays an important role demonstrated by *in vivo* localized ^{13}C MRS[1]. Brain glycogen metabolism had been non-invasively measured during and after acute hypoglycemia for several hours under physiological condition. *In vivo* localized ^{13}C MRS avoids the potential problems and shortfalls of invasive tissue sampling on glycogen measurement, such as postmortem effect[2]. However, achieving sufficient SNR requires the measurement following ^{13}C label administration. In addition, glycogen is a large macromolecule[3] and the complete detection of all ^{13}C glucose moieties may be difficult. While several studies have addressed the validity of measuring glycogen by ^{13}C NMR in muscle[4], heart[5] and livers[6], no study has been performed in the brain. The specific aim of this study was to directly validate *in vivo* localized ^{13}C MRS measurement of brain ^{13}C glycogen by comparison with a standard biochemical assay[2].

Subjects and Methods

To achieve a high degree of ^{13}C isotopic enrichment, brain glycogen was first reduced using hypoglycemia and then repleted with ^{13}C infusion in eleven male SD rats, as described previously[1]. Through the whole experimental period, animals were maintained under physiological conditions, verified from the blood gases measured in 5 or 35 minute intervals from the femoral arteries. After 12-14 hour of prolonged glucose infusion, *in vivo* localized ^{13}C MRS was used to measure brain ^{13}C glycogen content at 9.4-T. *In vivo* NMR ^{13}C glycogen was determined by quantifying the *in vivo* glycogen C1 signal using the external reference method at 37ºC. The integrals of glycogen C1 were Lorentzian fit using spectrometer software. Following rapid fixation (focused microwave at 4 kW within 1.4 sec) [7], biochemical analysis[2] was performed to obtain total brain glycogen content ([Glyc]) using a glucose analyzer. The isotopic enrichment of glycogen (IE_{Glyc}) was obtained from ^{1}H spectra of glucose at 14.1-T. From both [Glyc] and IE_{Glyc}, total ^{13}C glycogen in the brain tissue was determined.

Results and Discussion

In vivo quantification of the glycogen C1 signal provided a concentration range of [1-^{13}C] glycogen from 2.7 to 10.5 µmol glucosyl units/g wet weight. Biochemically determined [Glyc] ranged from 6.5 to 14.9 µmol/g. From ^{1}H spectra in the tissue extracts, isotopic enrichment of brain Glc was 85% to 93% and that of brain Glyc was 68% to 87%. Biochemically determined ^{13}C glycogen concentration ([$^{13}Glyc_1$]) was calculated as [$^{13}Glyc_1$] = IE_{Glyc}[Glyc], which ranged from 3.4 to 13.6 µmol/g wet weight. When plotting the *in vivo* NMR determined [1-^{13}C] glycogen concentration as a function of biochemical [$^{13}Glyc_1$] (solid circles in Fig. 1), an excellent correlation (R = 0.79, p = 0.0032) was obtained with a slope of 0.75 ± 0.07 (straight line in Fig. 1), which is slightly lower than the identity line (dashed line in Fig. 1). Thus, the comparison was done over a wide range of ^{13}C glycogen concentrations. Considering the experimental errors, the in vivo measurement was not substantially different from the biochemical measurement. We conclude that *in vivo* ^{13}C MRS quantifies close to 100% of ^{13}C glycogen. *In vivo* ^{13}C MRS measurement of glycogen can thus be used to non-invasively measure brain glycogen metabolism under physiological condition.

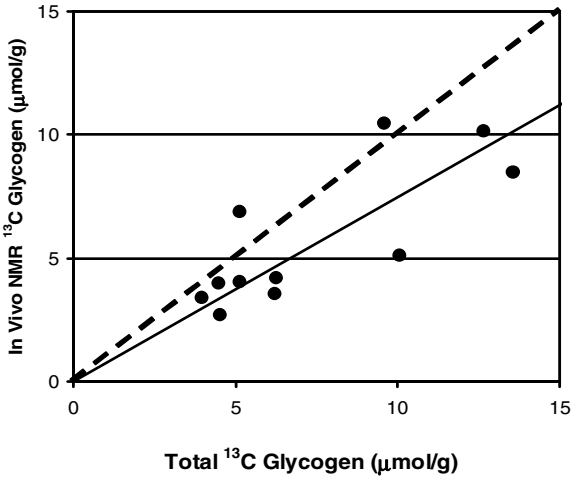

Figure 1. Correlation between biochemical ^{13}C glycogen determination (x-axis) and *in vivo* ^{13}C glycogen measurement (y-axis). Each solid circle is from one animal. The black straight line represents the best linear fit of y = 0.75x ± 0.07 (R = 0.79). The dashed line is the identity line.

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