

# Pre-labeling: A novel approach towards measuring absolute glycogen concentration changes with NMR

Florence D. Morgenthaler, Sabrina Laus, Hongxia Lei and Rolf Gruetter  
Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

## Background and aims:

$^{13}\text{C}$  Nuclear Magnetic Resonance (NMR) spectroscopy has been recently established as the only method to measure brain glycogen non invasively. However, all  $^{13}\text{C}$  NMR studies of brain glycogen to date relied on observing the incorporation of  $^{13}\text{C}$  label to overcome sensitivity limitations. We previously showed that derived changes in glycogen concentration were within experimental limits in excellent qualitative agreement with biochemical measurements. Given the fact that only the  $^{13}\text{C}$ -labeled part of the glycogen molecule is measured, we sought to develop a method allowing absolute concentration measurement using NMR. The rationale for this method was to achieve near steady-state enrichment using long-term  $^{13}\text{C}$  administration, to measure this enrichment *in vivo* which can then be matched by subsequent glucose infusion, thereby minimizing turnover as potential confound.

## Materials and methods:

Male Sprague-Dawley rats (n=6) were fasted overnight with free access to water and then fed *ad libitum* for 48 h with a 50% enriched Glc solution (10% w/v) only. Then they were anesthetized using isoflurane, intubated, ventilated (for 1h45min), placed into the magnet (9.4T) and indirect measurement of  $^{13}\text{C}$  -NAA enrichment began. To minimize postmortem degradation, rats were sacrificed using a focused microwave fixation device, brains dissected and assayed for tissue glycogen and Glc concentrations using biochemical measurements. By labeling the Glc molecule in its first C position with  $^{13}\text{C}$ , we expected the enrichment of acetyl-CoA (NAA's precursor) to be half of the  $^{13}\text{C}$  -Glc enrichment. The method consisted of *in vivo* measurement of  $^{13}\text{C}$  enrichment in NAA using  $^1\text{H}$  spectroscopy.  $^{13}\text{C}$  enrichment of NAA and Glc (as well as digested

glycogen) was also evaluated *in vitro* by high field spectroscopy (14.1T, 600 MHz). Rats that did not drink a significant amount of  $^{13}\text{C}$ -Glc (n=1), resulting in  $^{13}\text{C}$ -NAA enrichment < 5%, were excluded from the analysis.

**Results:**

The biochemical measurements of brain glycogen (ave  $\pm$  SEM,  $4.8 \pm 0.1$ ) and brain Glc ( $3.8 \pm 0.3$ ) were in excellent agreement with our previous results. *In vitro* NMR measurements of  $^{13}\text{C}$ -labeled-NAA on brain extracts was within experimental error identical with the concomitant *in vivo* NMR measurements (t-test,  $P > 0.05$ ). The average  $^{13}\text{C}$ -glycogen enrichment was  $24 \pm 1\%$  and NAA was approximately half of that of glycogen (Glycogen  $^{13}\text{C}$ -enrichment / NAA  $^{13}\text{C}$ -enrichment:  $2.2 \pm 0.2$ ).

**Conclusions:**

The study demonstrates that after extended  $^{13}\text{C}$ -labeled-Glc administration, glycogen is labeled, that this degree of labeling can be inferred from the *in vivo* enrichment of NAA. Therefore, we conclude that studies where the enrichment of the infusate nearly matches that of glycogen are feasible.