

## Measuring ABSOLUTE glycogen concentration *in vivo* and over time

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**Objective:** 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. The goals of this study was (a) to quantify *total* brain glycogen concentration under conditions of hypoglycemia or normoglycemia using biochemical methods and to compare it with previous  $^{13}\text{C}$ -NMR data, and (b) to implement a strategy to enable NMR measurement of direct glycogen concentration changes over time.

**Methods:** Rats were fasted overnight with free access to water and then anesthetized using isoflurane. Vascular access was secured for administration of  $\alpha$ -chloralose, glucose, insulin and/or somatostatin, as well as for blood sampling (blood gases, glucose etc.). Rats were sacrificed using a focused microwave fixation device (1.4 s @ 4 kW) thereby minimizing possible *in vitro* glycogen loss. For NMR experiments, the rats were “pre-labeled” by getting [1- $^{13}\text{C}$ ]-labeled glucose as drinking solution instead of regular food.  $^{13}\text{C}$  enrichment in NAA was measured *in vivo* using  $^1\text{H}\{^{13}\text{C}\}$  spectroscopy (9.4 T).  $^{13}\text{C}$  enrichment in NAA and glycogen was further determined *in vitro* by high field spectroscopy at 600 MHz.

**Results:** Biochemical results showed that metabolism of brain glycogen was glucose- and insulin-sensitive and that insulin-induced hypoglycemia promoted a gradual glycogenolysis ( $\sim 1.5 \mu\text{mol/g/h}$ ). The data confirmed that previous interpretation of  $^{13}\text{C}$  NMR data accurately reflected changes in *total* brain glycogen content. Finally, the study showed that after extended administration of  $^{13}\text{C}$ -labeled-glucose, glycogen is substantially labeled, and that the degree of labeling can be inferred as being twice ( $2.2 \pm 0.2$ ) the *in vivo* enrichment of NAA (a NMR-measurable metabolic product of glutamate and ultimately acetyl-CoA).

**Conclusions:** These results show that *in vivo* NMR spectroscopy allows over time measurement of ABSOLUTE brain glycogen concentration changes. Moreover, achievement of near steady-state glycogen enrichment using long-term  $^{13}\text{C}$ -glucose administration allows to match  $^{13}\text{C}$ -enrichment of subsequent glucose infusion, thereby minimizing turnover as a potential confound.