# Localized $^{13}$C NMR measurement of NAA and GSH turnover in the human brain over multiple days

P-G. Henry[1], G. Oz[1], E. R. Seaquist[2], K. Ugurbil[1], R. Gruetter[1]

[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States, [2]Department of Medicine, University of Minnesota, Minneapolis, MN, United States

## Introduction

$^{13}$C NMR spectroscopy is a powerful tool to investigate intermediary metabolism. Infusion of $^{13}$C-labeled glucose results in relatively rapid $^{13}$C labeling of amino acids such as glutamate, glutamine and aspartate [1], as well as slower labeling of NAA, glutathione and glycogen [2-4]. However, most studies in humans have been performed over only a few hours, a time frame too short to determine whether the entire pool of a metabolite with a low turnover rate is metabolically active. Recently, brain glycogen turnover was measured in the human brain [5]. In the present study we measured NAA and GSH turnover in the human brain over multiple days.

## Methods

All studies were performed on a 4 Tesla magnet interfaced to a Varian console. A linear transmit/receive $^{13}$C coil in combination with a quadrature $^{1}$H coil was used. A total of four healthy volunteers (3 M / 1 F, 34 ± 12 years old) were studied. Subjects received i.v. infusions of [1-$^{13}$C]glucose for 22 to 50 hours (50%-enriched, except for one subject who received 99%-enriched). Localized spectra from the occipital lobe (VOI 7 × 5 × 6 cm$^3$) were recorded every 6-10 hours for up to 85 hours using a $^{1}$H-localized polarization transfer sequence (TR= 3 s, NEX=128-256) [6]. Spectra were analyzed automatically using LCModel and a basis set consisting of 37 different isotopomers [7]. Quantitation of $^{13}$C label in NAA was performed relative to glutamate, assuming [Glu] = 8mM and [NAA] = 11mM, corresponding to average concentrations for a 1:1 mixture of grey and white matter.

## Results and Discussion

Spectra recorded for 50 hours during [1-$^{13}$C]glucose infusion showed progressive labeling of all three resonances of NAA (C2, C3 and C6) (Fig.1). Amino acids such as glutamate were already at isotopic steady-state at 8hr and remained stable throughout the glucose infusion. After $^{13}$C-glucose infusion was discontinued, $^{13}$C from amino acids disappeared quickly, while $^{13}$C label in NAA was washed out more slowly (Fig.1).

Analysis of spectra using LCModel allowed simultaneous quantitation of up to 37 different isotopomers, including NAA C2, C3, C6, and doublets NAAC23 and NAAC32 (Fig. 2). We also observed a resonance at 32.27ppm that was assigned to glutathione (GSH-GluC4) [3]. Although the S/N of the GSH resonance was not sufficient in most studies to measure time-resolved GSH time courses, the amount of $^{13}$C label detected was consistent with a GSH concentration of ~1mM [8].

Time courses of NAA (Fig.3) were fitted with a monoexponential to determine the rate of NAA turnover. Fitting ($R^2$ = 0.99) yielded a time constant of 31 ± 5 hours. The initial slope of the exponential fit gave (after correction for isotopic enrichment of plasma glucose) a synthesis rate $V_{NAA}$ = 0.3 ± 0.09 µmol.g$^{-1}$.hr$^{-1}$. This agreed within error with a previous study that reported the initial rate of NAA synthesis to be 0.55 ± 0.25 µmol.g$^{-1}$.hr$^{-1}$ [2].

To determine whether the entire pool of NAA is metabolically active, we compared the steady-state $^{13}$C enrichment of NAA to that of its precursor AcCoA. The steady-state NAA $^{13}$C concentration was 1.62 ± 0.16 µmol.g$^{-1}$, corresponding to a steady-state enrichment of (15.0 ± 1.5)%. This value was identical to that of AcCoA at steady-state (15.4 ± 2.1)% (calculated from glutamate C4), indicating that the entire NAA pool is metabolically active in the human brain, as it is in the rat brain [3].

## Conclusion

We conclude that the entire pool of NAA is metabolically active and that complete turnover of label in NAA requires several days. To the best of our knowledge, this is also the first reported observation of GSH labeling in the human brain.

## References

[1] Gruetter *et al.* AJP, 281, E100, 2001 [2] Moreno *et al.* J. Neurochem. 77, 347, 2001 [3] Choi *et al.* J. Neurochem. 91, 778, 2004 [4] Oz *et al.* Neurochem Int 43, 323, 2003 [5] Oz *et al,* Proc ISMRM 13, 208, 2005 [6] Henry *et al.* MRM, 50, 684, 2003. [7] Henry *et al.* NMR Biomed.16, 400, 2003 [8] Terpstra *et al.* MRM, 50, 19, 2003.

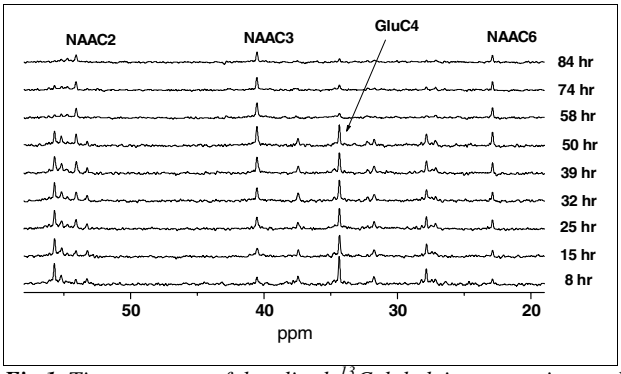


***Fig.1*** *Time courses of localized $^{13}$C label incorporation and washout from a single subject. Slight hyperglycemia with 50%-enriched [1-$^{13}$C]glucose was maintained for the first 50 hours.*

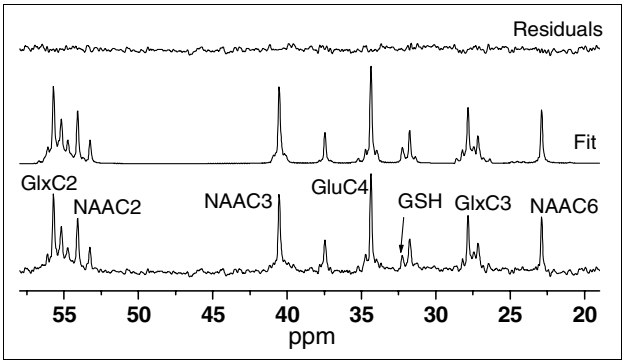


***Fig.2*** *LCModel fit of the sum of all 9 spectra from the time course shown in Fig.1. Note the glutathione peak (GSH) at 32.27 ppm.*

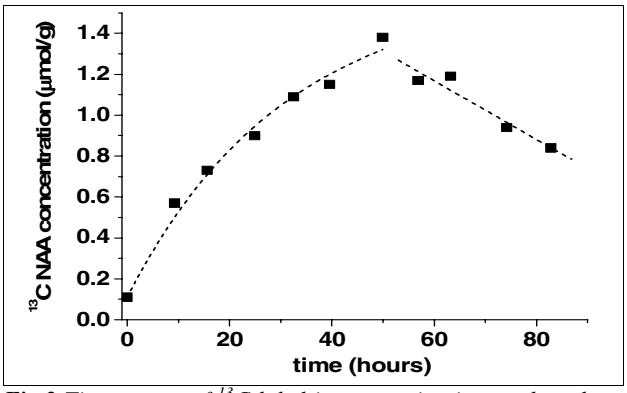


***Fig.3*** *Time course of $^{13}$C label incorporation into and washout from NAA C6. Data is the average from 3 subjects who received [1-$^{13}$C]glucose infusion for 46 ± 5 hours. Approximately 85% of NAA was turned over after two days of $^{13}$C-glucose infusion.*

## Acknowledgements

Supported by NIH P41RR08079, R01NS38672, M01RR00400 (GCRC) and the MIND Institute.