

## Observation of TCA cycle metabolism in human liver by dynamic $^{13}\text{C}$ -MRS

D. E. Befroy<sup>1,2</sup>, K. F. Petersen<sup>2</sup>, P. B. Brown<sup>1</sup>, D. L. Rothman<sup>1,3</sup>, and G. I. Shulman<sup>2,4</sup>

<sup>1</sup>Diagnostic Radiology, Yale University School of Medicine, New Haven, CT, United States, <sup>2</sup>Internal Medicine, Yale University School of Medicine, New Haven, CT, United States, <sup>3</sup>Biomedical Engineering, Yale University School of Medicine, New Haven, CT, United States, <sup>4</sup>Howard Hughes Medical Institute, New Haven, CT, United States

### Introduction

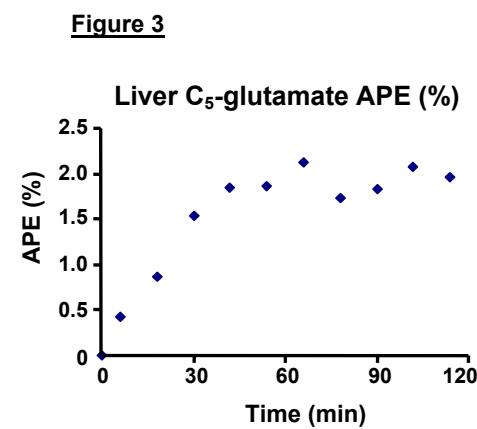
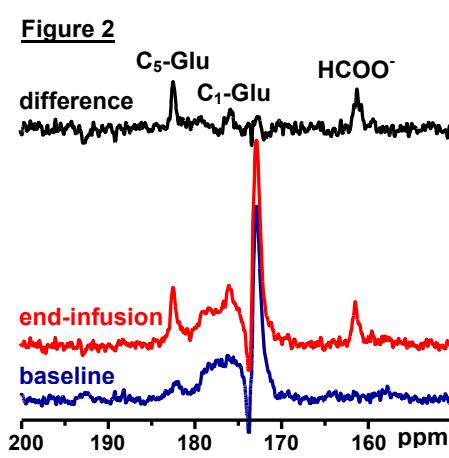
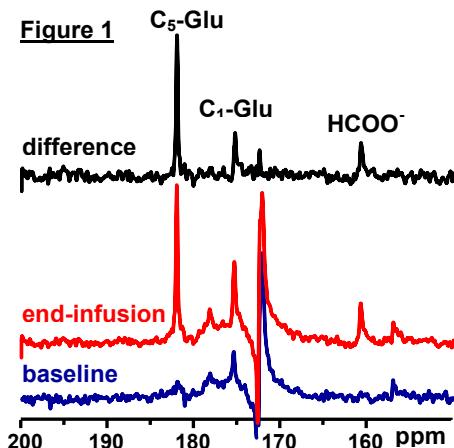
The classic  $^{13}\text{C}$ -labeling strategy to measure TCA cycle flux ( $V_{\text{TCA}}$ ) *in vivo* involves the infusion of 1- $^{13}\text{C}$  glucose or 2- $^{13}\text{C}$  acetate and monitoring enrichment at  $\text{C}_4$  and  $\text{C}_2$ -glutamate in the target tissue, using either direct  $^{13}\text{C}$ -MRS (1) or POCE detection (2). However, for tissues with significant deposition of intracellular lipid, e.g. muscle and liver, such an approach is complicated by the presence of overlapping lipid peaks which obscure the detection of natural-abundance  $\text{C}_4$ -glutamate even at 7Tesla (3). By incorporating lipid suppression,  $V_{\text{TCA}}$  may be measured using difference spectroscopy in muscle (4), but the detection of  $\text{C}_4$ -glutamate enrichment in liver has not been possible due to dominant lipid resonances. Recently, alternative labeling strategies have been demonstrated in brain whereby  $\text{C}_5$ -glutamate can be detected following the infusion of 2- $^{13}\text{C}$  glucose (5,6) or 1- $^{13}\text{C}$  acetate (7). Although primarily developed to permit the utilization of low-power decoupling schemes, this approach also has the advantage that the glutamate carbonyl peak is not obscured by overlapping lipid peaks. In this work we demonstrate that  $\text{C}_5$ -glutamate enrichment can be detected in human liver (and muscle) *in vivo* during a 1- $^{13}\text{C}$  acetate infusion with sufficient sensitivity to enable the measurement of hepatic  $V_{\text{TCA}}$ .

### Methods

Liver and muscle  $^{13}\text{C}$  spectra were acquired on a 4.0T Bruker Medspec system with dedicated, custom-built probes. Each probe consisted of a 9cm diameter  $^{13}\text{C}$  surface coil with a pair of quadrature-driven  $^1\text{H}$  coils for decoupling, either 13cm in diameter (muscle) or 14.5 x 11cm elliptical coils (liver). Liver  $^{13}\text{C}$  spectra were acquired using an adiabatic pulse-acquire sequence with WALTZ16 decoupling (peak power input during acquisition = 217W), Nuclear Overhauser Enhancement (NOE: 200 x 1ms hardpulses, 49ms interpulse delay) and oblique 1-dimensional outer volume suppression (OVS) to suppress signals arising from the chest wall. The effective  $T_R$  for this sequence was 10.1s, giving an average power input of 6.6W. Spectra were acquired in blocks of 32 scans and were obtained prior to and throughout a 120 minute infusion of 99% enriched 1- $^{13}\text{C}$  acetate at a rate of 3 mg / kg / min to obtain a time course of  $\text{C}_5$ -glutamate enrichment. Muscle spectra were acquired using the same sequence but with 3-dimensional OVS to select an ~275ml volume within the gastrocnemius and soleus muscles, and were obtained at baseline and at the end of infusion.

### Results

Labeling of the  $\text{C}_5$ -glutamate pool was observed in both muscle (Figure 1) and liver (Figure 2) due to the oxidation of 1- $^{13}\text{C}$  acetate via the TCA cycle. Enrichment at  $\text{C}_1$ -glutamate and bicarbonate were also observed as the  $^{13}\text{C}$  label traversed a 2<sup>nd</sup> turn of the cycle. Liver  $\text{C}_5$ -glutamate enrichment was estimated from the integral of the  $^{13}\text{C}_5$  peak relative to baseline, and expressed as atom percent excess (APE = enrichment - 1.1%). The progression of liver  $\text{C}_5$ -glutamate labeling during the infusion is shown in Figure 3, demonstrating that time-courses of enrichment can be obtained which will permit dynamic modeling of  $V_{\text{TCA}}$ , and other metabolic reactions.



### Conclusion

By taking advantage of an innovative  $^{13}\text{C}$  labeling strategy, substrate oxidation via the TCA cycle was observed for the first time in liver *in vivo*. Enrichment at  $\text{C}_5$ -glutamate can be observed without interference from intracellular lipid peaks which prohibit the detection of the  $\text{C}_4$ -glutamate pool in liver. Furthermore, this method will enhance established techniques to measure  $V_{\text{TCA}}$  in muscle since absolute enrichment of the glutamate pool can now be determined unambiguously.

**References:**

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