

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

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Introduction

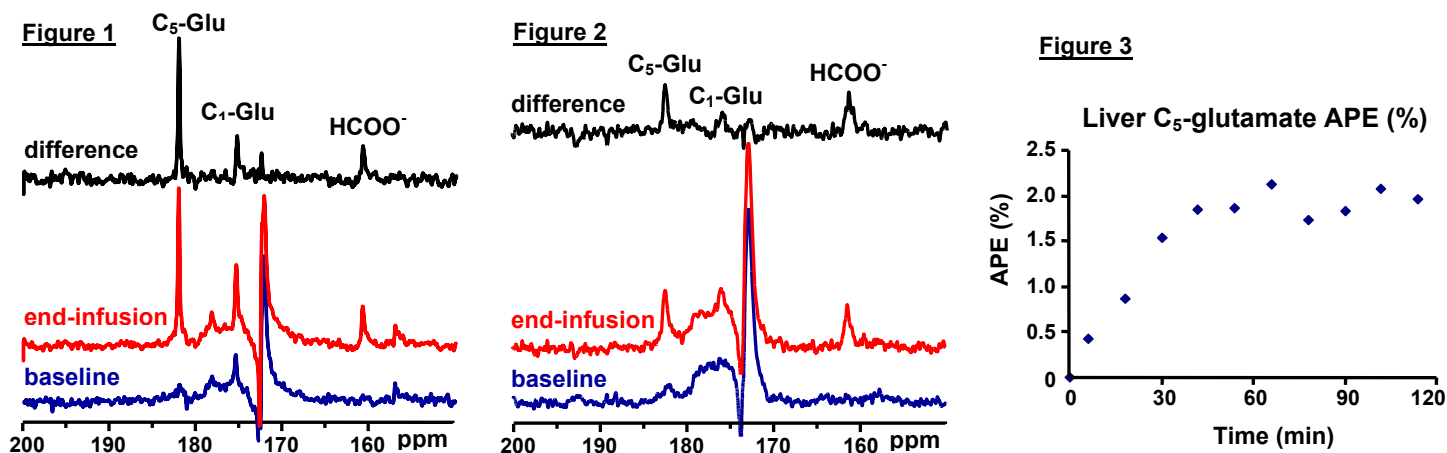
The classic ^{13}C -labeling strategy to measure TCA cycle flux (V_{TCA}) *in vivo* involves the infusion of $1\text{-}^{13}\text{C}$ glucose or $2\text{-}^{13}\text{C}$ acetate and monitoring enrichment at C_4 and C_2 -glutamate in the target tissue, using either direct ^{13}C -MRS (1) or POCE detection (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 C_4 -glutamate even at 7Tesla (3). By incorporating lipid suppression, V_{TCA} may be measured using difference spectroscopy in muscle (4), but the detection of 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 C_5 -glutamate can be detected following the infusion of $2\text{-}^{13}\text{C}$ glucose (5,6) or $1\text{-}^{13}\text{C}$ 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 C_5 -glutamate enrichment can be detected in human liver (and muscle) *in vivo* during a $1\text{-}^{13}\text{C}$ acetate infusion with sufficient sensitivity to enable the measurement of hepatic V_{TCA} .

Methods

Liver and muscle ^{13}C spectra were acquired on a 4.0T Bruker Medspec system with dedicated, custom-built probes. Each probe consisted of a 9cm diameter ^{13}C surface coil with a pair of quadrature-driven ^1H coils for decoupling, either 13cm in diameter (muscle) or 14.5 x 11cm elliptical coils (liver). Liver ^{13}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 $1\text{-}^{13}\text{C}$ acetate at a rate of 3 mg / kg / min to obtain a time course of C_5 -glutamate enrichment. Muscle spectra were acquired using the same sequence but with 3-dimensional OVS to select an $\sim 275\text{ml}$ volume within the gastrocnemius and soleus muscles, and were obtained at baseline and at the end of infusion.

Results

Labeling of the C_5 -glutamate pool was observed in both muscle (Figure 1) and liver (Figure 2) due to the oxidation of $1\text{-}^{13}\text{C}$ acetate via the TCA cycle. Enrichment at C_1 -glutamate and bicarbonate were also observed as the ^{13}C label traversed a 2nd turn of the cycle. Liver 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 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_{TCA} , and other metabolic reactions.



Conclusion

By taking advantage of an innovative ^{13}C labeling strategy, substrate oxidation via the TCA cycle was observed for the first time in liver *in vivo*. Enrichment at C_5 -glutamate can be observed without interference from intracellular lipid peaks which prohibit the detection of the C_4 -glutamate pool in liver. Furthermore, this method will enhance established techniques to measure V_{TCA} in muscle since absolute enrichment of the glutamate pool can now be determined unambiguously.

- References:**
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