Rates of human hepatic oxidative metabolism estimated *in vivo* using a novel ^{13}C-MRS method

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Introduction

The prevalence of metabolic diseases of the liver, including diabetes and non-alcoholic fatty liver disease, has increased rapidly and the development of methods to assess hepatic metabolism *in vivo* has become a clinical priority. We have previously demonstrated that oxidative metabolism in human liver can be observed using a novel ^{13}C-MRS technique [1]. This method took advantage of an innovative ^{13}C-labeling strategy [2,3] that enabled the detection of glutamate enrichment, which is obscured by intrahepatic lipid during traditional labeling schemes. In this work we describe the implementation of this methodology to assess rates of hepatic oxidative metabolism in healthy individuals. To accurately simulate the kinetics of ^{13}C-label turnover and generate robust estimates of these fluxes we also developed a model of hepatic metabolism that includes phenomena which distinguish the liver from other tissues.

Methods

^{13}C-MRS: Seven healthy volunteers (5 male, 2 female) were recruited from the local community; each participant underwent a natural abundance scan to determine the hepatic concentration of glutamate and an infusion study to assess the kinetics of oxidative metabolism. All experiments were performed on a 4.0T Bruker Medspec system using a custom-built probe consisting of a 9cm diameter ^{13}C surface coil with quadrature-driven, 14.5 x 11cm, elliptical ^{1}H coils for decoupling. Natural abundance ^{13}C spectra of the liver were acquired using a custom-written adiabatic pulse-acquire sequence with WALTZ16 decoupling and 3-dimensional outer-volume suppression. The hepatic concentration of glutamate was determined from the *in vivo* C_5-glutamate signal relative to that from a 25mM glutamate phantom. During the infusion study, spectra were acquired with the addition of nuclear Overhauser enhancement to increase ^{13}C sensitivity. Spectra were obtained prior to and throughout a 120 minute infusion of 99% enriched 1-^{13}C acetate at a rate of 3 mg/(kg-min) to obtain time-courses of C_5- and C_1-glutamate enrichment. Blood samples were obtained at regular intervals throughout the infusion; the concentration and $^{13}C_1$ enrichment of plasma acetate were determined by ^{1}H-NMR at 500MHz.

Metabolic Modeling: Rates of acetate infusion, plasma acetate data and time-courses of liver glutamate enrichment were fitted to a 3-compartment model of hepatic oxidative metabolism using Matlab. Discrete whole-body and hepatic blood pools were included since peripheral blood sampling does not fully represent the appearance of 1-^{13}C-acetate at the liver due to the effects of endogenous acetate production. The hepatic blood pool was subdivided into 3 regions to account for zonation of acetate metabolism within the liver [4] and each zone had a corresponding hepatocyte compartment. Oxidative metabolism within the hepatocyte was simulated using a standard model of the TCA cycle modified to incorporate the effects of gluconeogenesis, anaplerosis/pyruvate cycling and oxaloacetate-aspartate exchange which contribute significantly to hepatic metabolism.

Results

Representative liver ^{13}C spectra are shown in Figure 1. Labeling of the hepatic glutamate pools was observed during the infusion due to the oxidation of 1-^{13}C-acetate via the TCA cycle. The C_5-glutamate pool was rapidly enriched followed by labeling at C_1-glutamate (Figure 2); enrichment of both pools had plateaued by the end of the 120min infusion. The fit of the metabolic model of hepatic oxidative metabolism to the data is superimposed on the time-points in Figure 2, demonstrating that the model accurately simulates the time-courses of glutamate enrichment. Average rates (±SEM) of acetate clearance (k), endogenous acetate production (V_{SYN}), hepatic TCA cycle flux (V_{TCA}) and anaplerosis (V_{ANA}) are shown in Table 1.

Figure 1

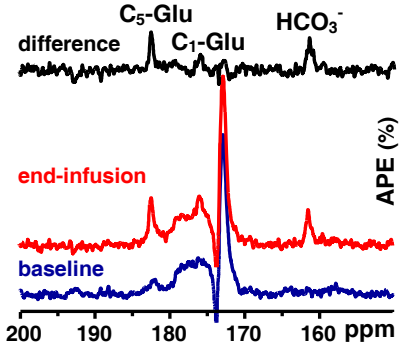

Figure 2

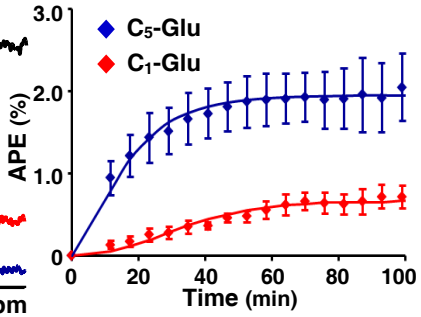

Table 1

V_{TCA}	0.42	±	0.07	µmol/g/min
V_{ANA}	0.300	±	0.070	µmol/g.min
k_{liver}	0.029	±	0.007	/min
$V_{SYN\text{-}liver}$	0.090	±	0.013	µmol/g.min
k_{body}	0.57	±	0.04	/min
$V_{SYN\text{-}body}$	0.155	±	0.017	µmol/g.min

Conclusion

We have implemented a novel ^{13}C-MRS technique to estimate rates of hepatic oxidative metabolism in healthy human subjects. These studies provide the first direct estimates of liver TCA cycle flux and anaplerosis *in vivo*. Future applications of this method will enhance our understanding of the pathogenesis of metabolic diseases of the liver including non-alcoholic fatty liver disease and diabetes, and provide a means of assessing potential therapies.

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References:

1. Befroy DE, *et al*. Observation of TCA cycle metabolism in human liver by dynamic ^{13}C-MRS. Proc ISMRM. 2011; #852.
2. Li S, *et al*. Novel Strategy for Cerebral ^{13}C MRS Using Very Low RF Power for Proton Decoupling. Magn Reson Med. 2007; 57:265–271.
3. Sailasuta N, *et al*. Clinical NOE ^{13}C MRS for Neuropsychiatric Disorders of the Frontal Lobe. J Magn Reson. 2008; 195:219–225.
4. Mittendorfer B, *et al*. Regional acetate kinetics and oxidation in human volunteers. Am J Physiol. 1998; 274: E978-983.