

Metabolic flux analysis of acetylcarnitine turnover and mitochondrial oxidation of [2-¹³C]acetate in rat skeletal muscle *in vivo* measured by ¹³C MRS

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INTRODUCTION: Acetylcarnitine is a necessary intermediate in the mitochondrial oxidation of acetate, since it transports the acetyl moiety across the mitochondrial membrane [1]. It has only been observed using hyperpolarized methods [2,3] or with localized polarization transfer sequences [5]. Glutamate has been used as an indicator of citric acid cycle (TCA) fluxes following [2-¹³C]acetate infusion [6,7,8], however, the detection of [5-¹³C]glutamate is hindered by an overlapping [1-¹³C]acetate resonance in hyperpolarized MRS studies and the detection of the glutamate C3 and C4 resonances are challenging due to large lipid resonances. Here we used localized DEPT at high field to monitor ¹³C enrichment and isotope turnover in glutamate and acetylcarnitine in skeletal muscle *in vivo* following [2-¹³C]acetate infusion. Two different modeling approaches were evaluated to obtain metabolic fluxes of mitochondrial acetate oxidation, either with or without the ¹³C labeling of acetylcarnitine and the enzymatic fluxes of acetylCoA synthetase (ACS) and acetylcarnitine transferase (CAT).

METHODS: Overnight fasted, male Sprague-Dawley rats (n = 5, 200-250g) were anesthetized, a catheter placed in the jugular vein for substrate delivery and in the artery for blood sampling. A home built ¹H/¹³C coil was used for localized and unlocalized ¹H and ¹³C NMR data acquisition. Animals were infused with 200 umol/kg/min [2-¹³C]acetate for up to 4 hours. ¹³C NMR spectra were acquired at 14.1T using semi-adiabatic distortionless enhancement by polarization transfer (DEPT) combined with a 3D ISIS localization scheme and outer volume suppression [4]. Tissue was rapidly excised and frozen in liquid nitrogen for ¹³C isotopomer analysis of perchloric acid extracts and metabolite concentrations. The creatine concentration was measured and cross referenced with the ¹³C creatine signal *in vivo* to determine ¹³C concentrations. Spectra were analyzed in LC model and metabolic fluxes were obtained by mathematical modeling in Matlab.

MODEL: The conventional model (I) is described by a set of isotopic mass balance equations for α -ketoglutarate (OG), Glu, oxaloacetate (OAA) and acetylCoA C2 and uses the ¹³C time courses of Glu C2, C3 and C4. The extended model (II) includes the ¹³C label passage through the acetylcarnitine pool and contains additionally equations for cytosolic acetylCoA and acetylcarnitine, and uses the ¹³C labeling time course of acetylcarnitine. Model II also estimates total acetylcarnitine. The FE time course of plasma acetate is used in both models as an input function. V_{dil} constitutes the amount of unlabeled precursors entering the acetylCoA pool, V_{ANA} the loss of ¹³C label.

RESULTS AND DISCUSSION: Localization with OVS and ISIS suppressed lipid resonances and ¹³C labeling of glutamate C2,C3 and C4 and acetylcarnitine C2 were clearly observed *in vivo*. The ¹³C fractional enrichment of Glu C4, C3, C2 was 0.49 ± 0.04 , 0.36 ± 0.04 and 0.44 ± 0.02 respectively, determined in tissue extracts. Time courses of the ¹³C tissue concentrations of Glu C4, C3, C2 and acetylcarnitine C2 (Fig. 2) were used to obtain metabolic fluxes with two different mathematical models. The overall contribution of acetate to TCA cycle oxidation was 80%. The estimation of the TCA cycle flux was 20% higher using model II which additionally uses the time course of acetylcarnitine labeling. Using this model the acetylcarnitine pool size was determined to be 0.36 ± 0.01 umol/g. Although absolute fluxes are different, the relative fluxes compared to V_{TCA} are similar in both models (Table 1 and Fig. 1), with the exception of the exchange between glutamate and α -ketoglutarate, V_X .

CONCLUSION: The detection of acetylcarnitine and glutamate *in vivo* using localized DEPT allows a more complete description of the mitochondrial oxidation of acetate in skeletal muscle, unhindered by unwanted lipid resonances. A more detailed model was constructed to quantify metabolic fluxes and acetylcarnitine concentrations and could lead to an improved understanding of acetate oxidation in metabolic disorders.

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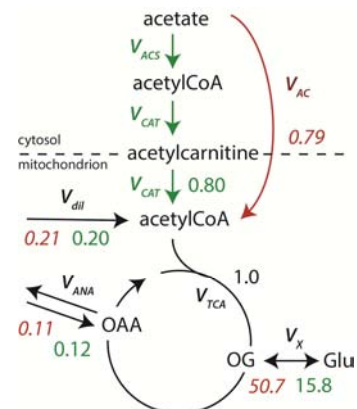


Fig. 1. Two different models were evaluated. A conventional model (red italic), and an extended model (green), which uses the dynamic ¹³C labeling of acetylcarnitine. Numbers indicate the fluxes relative to the TCA cycle flux.

Table 1.

Flux [mol/g/min]	Model I	Model II
V_{TCA}	0.14 ± 0.01	0.17 ± 0.03
V_{dil}	0.03 ± 0.00	0.03 ± 0.01
V_X	7.21 ± 6.48	2.62 ± 2.10
V_{ANA}	0.02 ± 0.00	0.02 ± 0.01

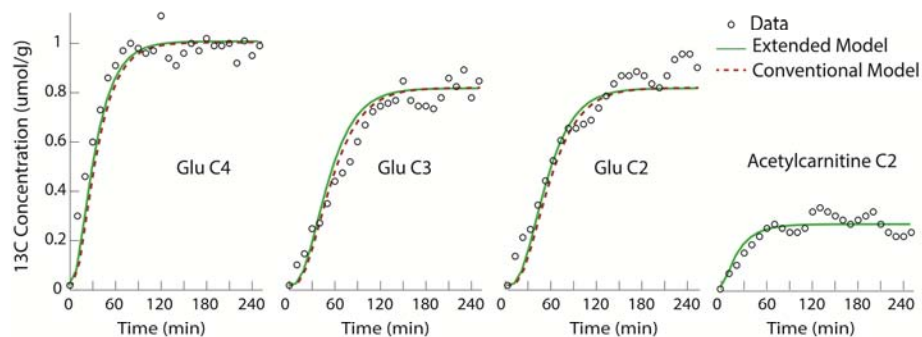


Fig. 2. Time courses of the ¹³C concentration in Glu C4, Glu C3, Glu C2 and acetylcarnitine C2 following the infusion of acetate C2 in skeletal muscle *in vivo*. Two models were fitted to determine the metabolic fluxes.