Study of acetyl carnitine kinetics in skeletal muscle *in vivo* using hyperpolarized 1-^{13}C acetate

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Introduction: Carnitine plays a fundamental role in regulating the balance between fatty acid and carbohydrate metabolism and buffers the acetyl CoA pool by rapid conversion into acetyl carnitine via carnitine acetyl transferase (CAT) [1]. Directly monitoring acetyl carnitine kinetics is thus instrumental towards unraveling its metabolic pathways and could potentially be a valuable tool for differentiating between healthy and pathological metabolism. Recently, it has been demonstrated that the conversion of acetate into acetyl carnitine can be probed *in vivo* following the infusion of hyperpolarized 1-^{13}C acetate [2]. The conversion rates related to this transformation are directly correlated with the enzymatic activity of CAT. The aim of the present study was to characterize the dynamics of 1-^{13}C acetate metabolism in skeletal muscle *in vivo* and to deduce the associated kinetics by applying a simple metabolic model.

Materials and methods: A 300 µL sample containing 4.5 M 1-^{13}C acetate, 33 mM TEMPO, dissolved in d_6-EtOD/D_2O 1:2 mixture was turned into frozen glassy beads and subsequently polarized in a custom-designed DNP polarizer for 2.5 h [3, 4]. The sample was then rapidly dissolved and transferred to a separator/infusion pump within 3 s, allowing automatic infusing of 2.2 mL 0.2 M hyperpolarized acetate solution into the femoral vein within 9 s. Wild type male Sprague Dawley rats (300 g) were anesthetized with 1.5% isoflurane and their physiology was monitored during the experiment. Measurements were carried out on a 9.4T/31 cm actively shielded animal scanner (Varian/Magnex). Localization was achieved by placing a quadrature ^{1}H/^{13}C carbon surface coil with a 10 mm diameter on top of the rat leg skeletal muscle. Series of single pulse acquisitions were recorded 12 s after dissolution using 30° adiabatic RF pulses applied every 3 s with proton decoupling during the acquisition. The data was analyzed in Matlab. A single compartment non-steady state metabolic model for one-site exchange was used (fig. 2) to fit the hyperpolarized time courses and determine the kinetic (k) and decay rate constants of acetate ($R_{1,A}$) and acetyl carnitine ($R_{1,C}$). The model includes the correction for the effect of 30° pulses on the signal intensities.

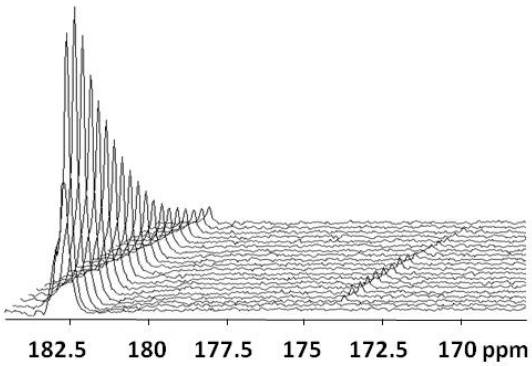

Fig. 1: Example of the time evolution of hyperpolarized acetate (182.6 ppm) and acetyl carnitine (174.0 ppm) in skeletal muscle in vivo. Spectra were acquired every 3 seconds following single 30° pulses.

Results and discussion: The time evolution of the precursor acetate and its metabolic product, acetyl carnitine, was detected in the skeletal muscle *in vivo* (n=3) (fig. 1). Typically, the line width of acetate was 15 – 20 Hz and 10 – 15 Hz for acetyl carnitine. The signal-to-noise ratio of acetyl carnitine was ~20 times lower than that of acetate. The acetate signal started decaying 3 s after the end of infusion and the acetyl carnitine signal increased to reach its maximum value 12 s after infusion. Due to the high concentration of acetate following bolus injection, the conversion rate from labeled acetate to acetyl carnitine was likely maximal. The model (fig. 2) reflects the role of acetyl carnitine as an energy buffer in resting skeletal muscle. The kinetic rate constant k reflects CAT activity, acetyl CoA synthetase activity and membrane transport of acetate, and is therefore an "apparent" kinetic rate constant. The decay rate constant of acetate $R_{1,A}$ does not correspond to its spin-lattice relaxation rate because it is in fact a superposition of the inflow of hyperpolarized acetate and the spin-lattice relaxation rate. The peak intensities were fitted to the solutions of the modified Bloch equations starting at the onset of acetyl carnitine formation (fig 3). The kinetic rate constant was $k = 0.09 \pm 0.03$ s^{-1}, the characteristic decay rate $R_{1,A} = 0.012 \pm 0.002$ s^{-1} and $R_{1,C} = 0.093 \pm 0.006$ s^{-1}.

$$\text{Acetate} \xrightarrow{k} \text{Acetyl Carnitine}$$

$$\frac{dM_A}{dt} = -R_{1,A}\left[M_A - M_{A,eq}\right] - kM_A$$

$$\frac{dM_C}{dt} = kM_A - R_{1,C}\left[M_C - M_{C,eq}\right]$$

Fig. 2: Modified Bloch equations for one site exchange. Acetate (A), acetyl carnitine (C).

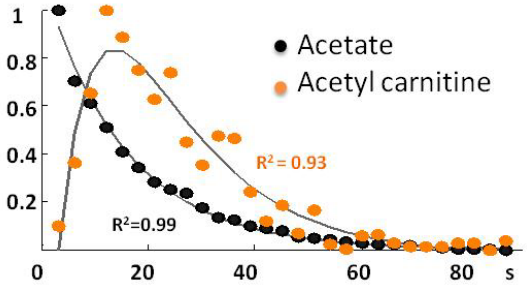

Fig. 3: Normalized signal amplitudes of the acetate and acetyl carnitine spectra showed in fig. 1 are reported as a function of time starting at the onset of acetyl carnitine formation. The data were fitted with the model presented in fig. 2 to extract the exchange and the decay constants. Time point t=0 depicts the end of infusion.

Conclusion: We deduced the apparent relaxation rates for acetate and acetyl carnitine, and the kinetic rate constant for the conversion of acetate into acetyl carnitine in healthy skeletal muscle *in vivo* using the metabolic model described herein. The conversion of acetate to acetyl carnitine has been shown to reflect disturbed mitochondrial bioenergetics in several tissues [2]. Therefore, local instantaneous measurements of the conversion rate have the potential to distinguish healthy and pathological tissues and offer insight in the kinetics of altered metabolic states.

References:[1] F. Stephens et al., *J Physiol* (2007) [2] P. Jensen et al., *JBC* (2010) [3] A. Comment et al., *Concept Magnetic Res* (2007) [4] S. Jannin et al., *J Chem Phys,* (2008)

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