Acetylcarnitine turnover in rat skeletal muscle measured in vivo using localized $^{13}$C NMR at 14.1 T

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Introduction: Acetate has been widely used as a metabolic probe for measuring TCA cycle kinetics in vivo in skeletal muscle [1,2,3]. In order to cross the mitochondrial membrane for subsequent utilization in the TCA cycle, acetate needs to be transformed into acetylcarnitine [4]. Because of the relatively small pool sizes of acetylcarnitine in skeletal muscle approximately one order of magnitude lower than glutamate [5,6] it has only been observed in skeletal muscle in vivo using hyperpolarized $^{13}$C MRS [7]. The aim of this study was to be able to detect the $[2^{-13}$C]acetylcarnitine resonance in vivo after the infusion of $[2^{-13}$C]acetate in rat skeletal muscle by conventional $^{13}$C MRS at 14.1T using localized DEPT, in order to enable improved characterization and description of the system of enzymatic reactions involved in acetate oxidation.

Materials and methods: Male Sprague Dawley rats (n = 6; 200-250g), fasted overnight, were positioned laterally and their hind limbs fixed on a custom designed holder to prevent motion. A home built $^1H/^{13}$C coil with the proton loops in quadrature mode was placed on top of the skeletal muscle for localized and unlocalized $^1H$ and $^{13}$C NMR data acquisition. Proton linewidths were adjusted to 30 ± 5 Hz in a 6x10x12 mm$^3$ voxel. Animals were infused with a dose of 200 umol/kg/min $[2^{-13}$C]acetate for up to 6 hours through the jugular vein. Arterial plasma samples were taken throughout the experiment to determine plasma acetate fractional enrichments (FE).

$^{13}$C NMR spectra (128 averages) were acquired using semi-adiabatic distortionless enhancement by polarization transfer (DEPT) combined with a 3D ISIS localization scheme and outer volume suppression [8]. $^{13}$C spectra were analyzed with jMRUI. At the end of the experiment, tissue was rapidly excised and frozen in liquid nitrogen for off all metabolites observed in vivo using localized $^{13}$C MRS spectroscopy at high field. This allows for a more detailed characterization of acetate oxidation in skeletal muscle in vivo and in studies of metabolic disorders such as diabetes where carnitine deficiency occurs.

Results and discussion: The sensitivity increase due to the high field and the use of a localized DEPT sequence allowed for the first time the observation of acetylcarnitine (AlCar) in vivo at 21.5 ppm. Other metabolites such as glutamate (C2, C3 and C4), creatine (CH$_2$ and CH$_3$), taurine (C1 and C2) and citrate were also clearly observed (Fig 1 & 2). In all experiments the FE of C2 acetate in plasma reached ~70% - 80%. The FE of C2 in acetylCoA varied between 0.5 and 0.6. NMR of tissue extracts revealed the presence of acetylcarnitine [4]. Because of the relatively small poolsizes of acetylcarnitine in skeletal muscle approximately one order of magnitude lower than glutamate [5,6] it has only been observed in skeletal muscle in vivo using hyperpolarized $^{13}$C MRS [7]. The aim of this study was to be able to detect the $[2^{-13}$C]acetylcarnitine resonance in vivo after the infusion of $[2^{-13}$C]acetate in rat skeletal muscle by conventional $^{13}$C MRS at 14.1T using localized DEPT, in order to enable improved characterization and description of the system of enzymatic reactions involved in acetate oxidation.

Conclusion: This study demonstrates for the first time, to our knowledge, the detection in vivo of acetylcarnitine using localized $^{13}$C NMR spectroscopy at high field. This allows for a more detailed characterization of acetate oxidation in skeletal muscle in vivo and in studies of metabolic disorders such as diabetes where carnitine deficiency occurs.


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Fig. 1: In vivo $^{13}$C localized spectra during $[2^{-13}$C]acetate infusion in rat skeletal muscle. Time resolution shown here is 20 min. Sum of the last 2 acquisitions is shown on top. A small residual lipid resonance is observed at ~ 30 ppm.

Fig. 2: Integrated spectral time course with a 20 min time resolution showing the $^{13}$C enrichment in C2, C3 and C4 of glutamate and C2 of AlCar.

Fig. 3: Spectrum of a tissue extract showing the glutamine, glutamate lactate and acetylcarnitine resonances.