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 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.

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 ^{1}H/^{13}C coil with the proton loops in quadrature mode was placed on top of the skeletal muscle for localized and unlocalized ^{1}H 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 ^{13}C isotopomer analysis of perchloric acid extracts. FE of C2 acetylCoA was determined as described in [9].

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 off all metabolites observed *in vivo*, plus, interestingly, the resonances of glutamine (C2, C3 and C4) which were of the same order of magnitude as the glutamate resonances (Fig. 3). The resonance assigned to AlCar C2, also observed in unlocalized spectra with lipid baseline subtraction, could not be from lactate C3 since both were observed in tissue extracts at respectively 21.5 ppm and 21.0 ppm. Additionally, spectra were cross referenced with observed lactate resonances in brain studies *in vivo* from our institute and do not match with the AlCar C2 resonance.

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.

References: [1] L.Szczepaniak *et al.*, MRM (1996) [2] V.Mezzarobba *et al.*, J Exp Bio (2000) [3] L. Bertocci *et al.*, JAP (1997) [4] P. Roberts *et al.*, Am J Physiol-Endoc (2005) [5] N.Marquis *et al.*, JBC (1965) [6] M. Berger *et al.*, Diabetes (1973) [7] P. Jensen *et al.*, JBC (2010) [8] P.Henry *et al.*, MRM (2003).[9] C. Malloy *et al.*, Biochem (1990)

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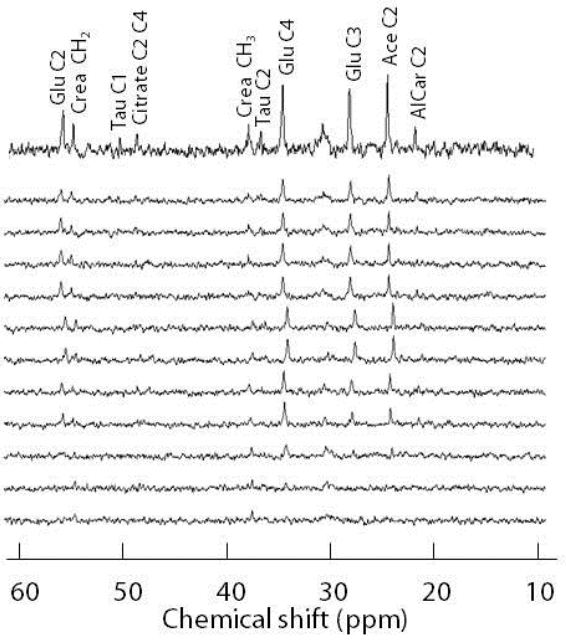

Fig. 1: In vivo ^{13}C localized spectra during [2-^{13}C]acetate infusion in rat skeletal muscle. Time resolution shown here is 20 min. Sum of the last 2 acquisitions is shown on top. A small residual lipid resonance is observed at ~ 30 ppm.

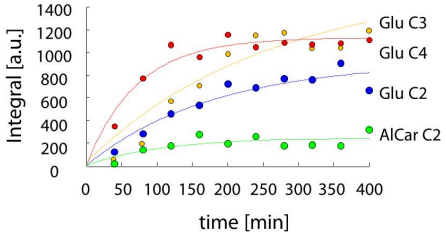

Fig. 2: Integrated spectral time course with a 20 min time resolution showing the ^{13}C enrichment in C2, C3 and C4 of glutamate and C2 of AlCar.

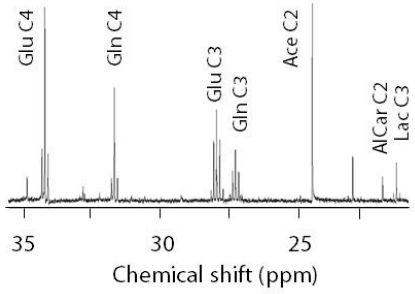

Fig. 3: Spectrum of a tissue extract showing the glutamine, glutamate lactate and acetylcarnitine resonances.