Acetyl-CoA and acetyl-carnitine show organ specific distribution in mice after injection of DNP hyperpolarized $^{13}C_1$-acetate

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Introduction: Acetyl-CoA is a central metabolite and regulator in anabolic and catabolic pathways and is vital to the balance between carbohydrate and fat metabolism. Because of its high metabolic and signaling impact, the control of acetyl-CoA concentrations is crucial. Recently, carnitine has gained attention as a modulator of fatty acids and carbohydrates metabolism by means of modifying the intra mitochondrial Acetyl-CoA/CoA ratio[1]. Dynamic nuclear polarization (DNP) is a powerful emerging technique that can be used for overcoming sensitivity limitations in *in vivo* magnetic resonance spectroscopy (MRS)[2], allowing metabolism to be studied in vivo[3] in real time. In this study, we have investigated the metabolism of DNP hyperpolarized acetate in mice. It is shown that highly polarized acetate is a promising substrate to study metabolism *in vivo*. This is supported by significant differences in the metabolite ratios revealed by localized spectroscopy at liver and heart regions of the mouse.

Methods: *Sample preparation*: ^{13}C labelled acetate was prepared as its tris salt by dissolving $^{13}C_1$ acetic acid in a large volume of water together with tris and subsequently freeze-drying the mixture. The DNP sample was prepared by mixing a tris acetate solution (4 M) with a trityl radical and a DOTA based gadolinium complex. The solid state polarisation and subsequent dissolution was performed using the DNP method[2]. The liquid state polarization was 25% at the time of injection into the animal. *In vivo:* The *in vivo* MR experiments were performed on a 2.35 T Bruker Biospec Avance II system. The mice (C57/Bl6, 25 g) were anaesthetized with 2% isoflurane in a 50:50 mixture of N_2O and O_2. After *i.v.* injection of hyperpolarized $^{13}C_1$-acetate (50 mM) (Inj. Vol. and time: 175µl and 6s), ^{13}C spectra were acquired with a with a 12-mm surface coil to measure either metabolism as a function of time (30 spectra, α=30°, Tr=3 s) or to obtain mouse liver and heart specific spectra after 10 s after start of the injection (1 spectrum, α=90°). The two metabolites (acetyl-carnitine and acetyl-CoA) were identified by an *in vitro* assay using a coupled enzymatic reaction employing acetyl CoA synthetase and carnitine transferase.

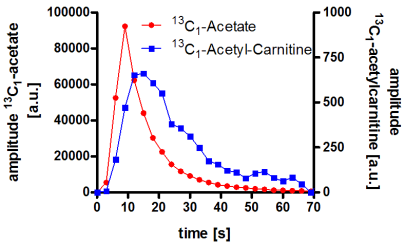

Figure 1. Dynamics of 1-^{13}C-Acetyl-Carnitine in mouse heart after injection of 1-^{13}C-Acetate.

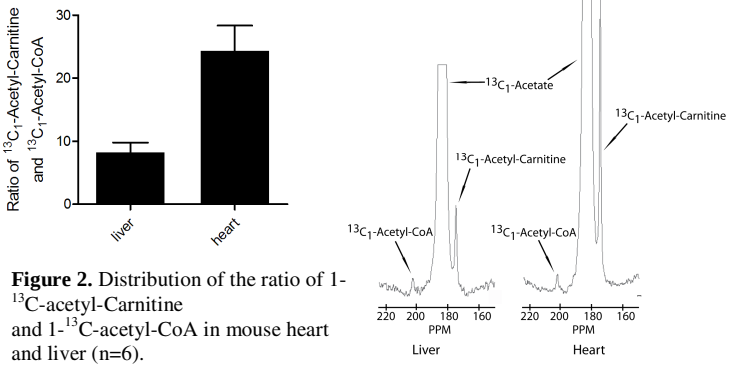

Figure 2. Distribution of the ratio of 1-^{13}C-acetyl-Carnitine and 1-^{13}C-acetyl-CoA in mouse heart and liver (n=6).

Results and discussion: The *in vivo* results showed a clear metabolite signal formed out of acetate at 174 ppm. A delayed build-up was observed for this metabolite when a series of spectra was recorded, Figure 1, indicating real metabolism. When a single 90° spectrum was acquired an additional metabolite was observed at 202 ppm. The two metabolites were identified as acetyl-CoA (202 ppm) and acetyl-carnitine (174 ppm). Significant differences in the ratio between acetyl CoA and acetyl carnitine are observed by single aquisition spectra localized over the heart or liver regions. The acetyl-carnitine level is significantly higher in the heart, Figure 2.

Conclusion: The conversion of acetate to acetyl carnitine via acetyl CoA can be visualized *in vivo* using ^{13}C-DNP NMR, is organ specific and may be a valuable diagnostic tool for fatty acid metabolism under healthy and pathological conditions.

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References: [1] R. Ramsay et al., Mol. Asp. of Med. 25 (2004): p 475-493. [2] Ardenkjaer-Larsen et al. PNAS 100:10158,2003. [3] Golman et al, PNAS, 25;103(30):11270-5, 2006