Hyperpolarized [1-^{13}C]octanoate: a probe of myocardial β-oxidation

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Background: The heart normally derives most of its energy from the oxidation of long-chain fatty acids. Myocardial metabolism can be monitored non-invasively by MRS using hyperpolarized (HP) ^{13}C-labelled compounds; however, most reported studies used HP pyruvate, which does not directly measure fatty acid catabolism. The myocardial metabolism of HP [1-^{13}C]butyrate and [1-^{13}C]acetate has been reported, but the conversion of these short-chain fatty acids to acetyl-CoA does not involve successive rounds of β-oxidation, as is the case for longer chain fatty acids. In this study we examined the applicability of hyperpolarized [1-^{13}C]octanoate, a medium-chain fatty acid, as a probe of myocardial metabolism.

Methods: [1-^{13}C]octanoic acid (4 M in DMSO, doped with stable trityl radical) was polarized by microwave irradiation (196.8 GHz) at 7 T & 1 K. After dissolution and neutralisation with an equivalent of NaOH and heated buffered D_2O, ~0.04 mmol was infused via a femoral vein catheter into anesthetized Wistar rats, either fed *ad libitum* or fasted 12 h, in a 9.4 T horizontal bore scanner (Varian). A series of single pulse (BIR-4, 30º, TR ~3 s) gated ^{13}C MRS acquisitions was performed with a surface coil positioned over the heart. To aid metabolite identification, HP [1-^{13}C]acetate and/or ^{13}C-urea (δ 165.48 ppm, HMDB) were co-infused in several experiments. To quantitate the metabolite signals, the spectra with octanoate signal were summed and the spectral peaks integrated with baseline correction.

Results and Discussion: The polarization level of [1-^{13}C]octanoate (δ 185.4 ppm) at the time of injection was ~11% and its T_1 relaxation rate in solution was 29 ± 3 s. *In vivo*, the octanoate signal decayed rapidly and was no longer measurable 20-36 s after the start of infusion. Interactions with blood proteins such as serum albumin are likely responsible for the rapid loss of signal, and the T_1 in blood *ex vivo* was ~9.6 ± 0.5 s. One metabolite peak at 175.4 ppm, was consistently observed and was assigned to [1-^{13}C]acetylcarnitine. This was confirmed by infusing HP [1-^{13}C]acetate and observing [1-^{13}C]acetylcarnitine with the same chemical shift, as well as coinfusing HP [1-^{13}C]acetate and [1-^{13}C]octanoate and observing a single metabolite peak for [1-^{13}C]acetylcarnitine (Figure 1).

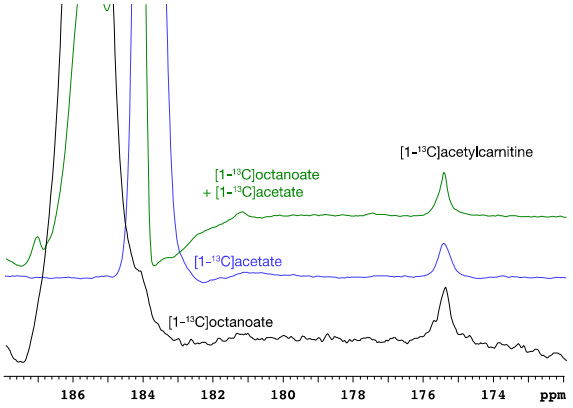

Figure 1. Assignment of metabolite produced from infused hyperpolarized [1-^{13}C]octanoate in the rat heart *in vivo* to [1-^{13}C]acetylcarnitine. Co-infusion with polarized [1-^{13}C]acetate yields the same main metabolite peak previously assigned to [1-^{13}C]acetylcarnitine.

The signal of observed metabolites was generally low, but it was possible in some experiments to detect signals from other metabolites expected from fatty acid metabolism and the TCA cycle, including [5-^{13}C]glutamate (183.9 ppm) and [5-^{13}C]citrate (181.1 ppm) as well as the ketone body [1-^{13}C]acetoacetate (177.3 ppm) (Figure 2).

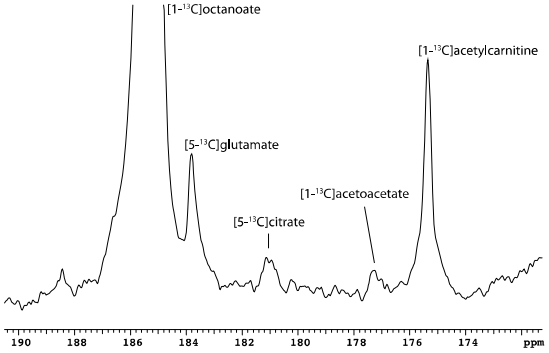

Figure 2. Metabolites of hyperpolarized [1-^{13}C]octanoate in the rat heart. [5-^{13}C]glutamate was resolved with narrow octanoate linewidth. Traces of [5-^{13}C]citrate and [1-^{13}C]acetoacetate were occasionally observed.

In experiments (n=7) with fed rats, the acetylcarnitine signal was on average 1.9 ± 0.3% of the octanoate signal, whereas in experiments (n=6) with fasted rats, it was 1.4 ± 0.6% (Figure 3). The acetylcarnitine signal in fasted rats, while more variable, was generally lower. Possible explanations include label dilution of hyperpolarized octanoate by the elevated free fatty acid levels in the blood during fasting and greater acetyl-CoA flux into the TCA cycle.

Conclusion: This study demonstrates that *in vivo* dissolution-DNP metabolic experiments can be performed with ^{13}C-labelled medium-chain fatty acids. Sufficient ^{13}C polarization in octanoate survives circulation, tissue uptake, mitochondrial transport and conversion by β-oxidation to acetyl-CoA to be detectible as acetylcarnitine, glutamate, citrate and acetoacetate. HP octanoate can be used to directly probe the β-oxidation of metabolically important fatty acids in the heart.

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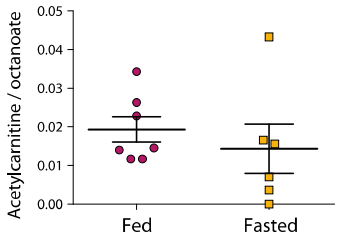

Figure 3. [1-^{13}C]acetylcarnitine and infused polarized [1-^{13}C]octanoate peak ratios from summed spectra. Fasted rats had lower and more variable level of conversion to acetylcarnitine.