Hyperpolarized ketone body metabolism in the *in vivo* rat heart

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Target. Researchers interested in metabolism and hyperpolarized imaging.

Purpose. The ketone bodies (acetoacetate and β-hydroxybutyrate) are important fuel sources in starvation, and oxidation of these substrates is altered in diabetes and diabetic cardiomyopathy[1]. Dissolution-DNP can be used to study metabolic changes in real-time, but the majority of studies to date have focused on [1-^{13}C]pyruvate as a measurement of carbohydrate metabolism. In this work, we build on previous work in the *ex vivo* heart [2], and investigate the feasibility of observing metabolism of hyperpolarized acetoacetate in the *in vivo* rat heart.

Methods. Sodium [1-^{13}C]acetoacetate was produced from ethyl acetoacetate by ester hydrolysis, followed by lyophilization. A stock solution was formulated as previously described [2] by mixing 277 mg sodium acetoacetate with 9.4 mg OX063, 50 μL DMSO, and 150 μL H2O. Samples for polarization were made by mixing 33 μL of the stock solution with 3.5 μL of 10 mM Dotarem. Samples were polarized for 2 hours in a custom-made polarizer at 93.951 GHz, and dissolution was performed in 6 mL of heated and pressurized water. 2 mL of the dissolved solution was injected over 20 seconds via tail vein into male Wistar rats, and ^{13}C spectra were acquired (Agilent 7T, TR 1s, FA 15°, 15 μs hard pulse, 20 mm surface transmit/receive coil). Spatial localization was performed by placement of the surface coil over the rat heart. A slab containing the sensitive region of the ^{13}C coil was shimmed; resulting linewidths were on the order of 50 Hz. A separate phantom study was performed using a HyperSense polarizer system (Oxford Instruments) and an 11.7T MRI system to characterize the impurities present in the sample.

Results. Representative spectra from fed and fasted rats are shown in Figure 1. The impurities present in the substrate are also shown. The *in vivo* linewidth (0.5 ppm) was sufficient to resolve metabolism into acetylcarnitine as well as interconversion into the ketone body β-hydroxybutyrate. Additionally, decarboxylation of acetoacetate into bicarbonate was observed in the fed state, but not in the fasted state ($p<0.05$). Citrate, located between [1-^{13}C]acetate and [1-^{13}C]acetoacetate, was not observed. The main impurities in the substrate are [1-^{13}C] acetate and an unknown resonance upfield of β-hydroxybutyrate which overlaps with the expected chemical shift of [5-^{13}C]glutamate. In separate phantom experiments, the T_1 (30 s) of this resonance is the same as that of [1-^{13}C]acetoacetate, indicating that it is in rapid exchange with acetoacetate. *In vivo*, this resonance decays with a different time course, suggesting that the Krebs cycle product [5-^{13}C]glutamate is produced.

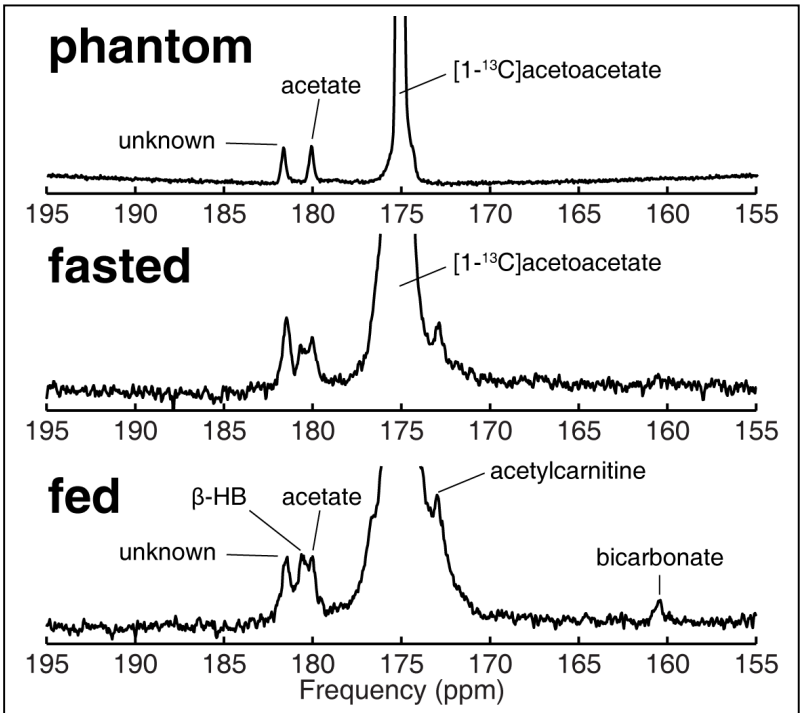

Figure 1. Spectra acquired following administration of hyperpolarized acetoacetate to the *in vivo* rat heart. The resonances shown indicate conversion of the substrate [1-^{13}C]acetoacetate into acetylcarnitine and β-hydroxybutyrate. Acetate and the upfield resonance are impurities in the substrate (top row). Decarboxylation to [^{13}C]-bicarbonate was observed only in the fed state (bottom row).

Discussion. This work has demonstrated the potential to observe *in vivo* metabolism of hyperpolarized [1-^{13}C] acetoacetate, a ketone body, in the *in vivo* rat heart. The polarization level was sufficient to observe metabolism into β-hydroxybutyrate and the Krebs cycle product acetylcarnitine. Interestingly, the modulation of the decarboxylation of acetoacetate into acetone and CO_2 by metabolic state suggests that this substrate can be used as a probe of acetoacetate decarboxylase. This is an enzyme which is expressed in blood and serves to regulate concentrations of both acetoacetate and β-hydroxybutyrate. This study builds on previous work investigating hyperpolarized ketone body metabolism in the perfused heart. Based on those results, β-hydroxybutyrate was not pursued as a probe due to its spectral position, as well as lower T_1 and polarization levels relative to [1-^{13}C]acetoacetate. In future studies, we aim to apply these results to a model of type II diabetes.

References. [1] Heather L J Mol Cell Cardiol 2011. [2] Ball DR ISMRM 2014.