Hyperpolarized Ketone Body Metabolism in the Perfused Rat Heart

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Introduction: Hyperpolarization via the Dynamic Nuclear Polarization (DNP) technique has revolutionized our ability to study metabolic changes in the heart in real time[1]. The vast majority of hyperpolarization studies have focused on the metabolism of [1-^{13}C]pyruvate as it provides a unique insight into various aspects of carbohydrate metabolism. However, alterations in the metabolic pathways of other key fuel molecules (e.g. fatty acids and ketone bodies) are a common feature of cardiovascular diseases. For example, metabolism of the ketone bodies, acetoacetate and β-hydroxybutyrate, is known to be altered in diabetes and diabetic cardiomyopathy[2]. The ability to study these changes may therefore offer a new insight into the metabolic derangements seen in diabetes and provide a new perspective on potential treatments. Building on previous work exploring hyperpolarization of the ketone bodies[3], the aim of this work was to produce a reliable method to generate hyperpolarized acetoacetate and β-hydroxybutyrate and to investigate their metabolism in the isolated perfused rat heart.

Methods: A stock solution of acetoacetate was formulated by mixing 277mg of [1-^{13}C]sodium acetoacetate with 9.4mg of OX063, 50μl of DMSO and 150μl of H_2O. Similarly, a stock solution of β-hydroxybutyrate was formulated by mixing 280mg of [1-^{13}C]β-hydroxybutyrate with 9.4mg of OX063, 50ml of DMSO and 150ml of H_2O. Samples for polarization were made by mixing 32.94μl of the appropriate stock solution with 3.5μl of a 10mM solution of Dotarem (Guerbet, Shirley, UK). Samples were polarized for ~1 hour in a HyperSense polarizer system (Oxford Instruments, Abingdon, UK) at the optimal microwave frequency. Dissolution was performed in 6ml of heated and pressurized water. The achievable liquid state polarization and T_1 relaxation time was assessed (n=3 per substrate) by injecting 2ml of the dissolved solution into an 11.7T MRI system interfaced to a Bruker Avance console. Metabolism of the hyperpolarized ketone bodies in the perfused rat heart was investigated by adding the polarized substrate directly to the Krebs-Henseleit buffer delivered to the Langendorff perfused heart (generating a 4mM concentration, n=3 per substrate), during which time ^{13}C spectra were acquired every second for one minute with a flip angle of 30°.

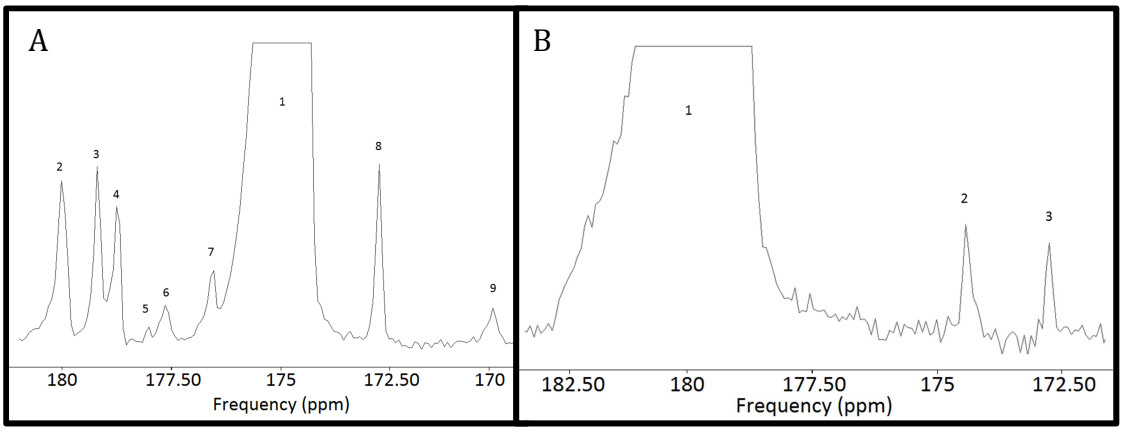

Figure 1: Summed spectrum acquired over 30s following administration of hyperpolarized acetoacetate to the perfused rat heart. (1) acetoacetate, (2) glutamate, (3) β-hydroxybutyrate, (4) unknown, (5) unknown, (6) citrate, (7) unknown, (8) acetyl-carnitine, (9) ethyl-acetoacetate. **Figure 2:** Summed spectrum acquired over 30s following administration of β-hydroxybutyrate to the perfused rat heart. (1) β-hydroxybutyrate, (2) acetoacetate, (3) acetyl-carnitine.

Results: The liquid state polarizations obtained for acetoacetate and β-hydroxybutyrate were 8±2% and 3±1% respectively. The hyperpolarized T_1's for the two substrates were 28±3s (acetoacetate) and 20±1s (β-hydroxybutyrate). Multiple downstream metabolites were observed following infusion into the perfused rat heart, both with acetoacetate (Figure A) and with β-hydroxybutyrate (Figure B). Interconversion between the ketone bodies could be seen for both substrates, whilst infusion of hyperpolarized acetoacetate also allowed for observation of metabolism into the Krebs cycle intermediates citrate, glutamate and acetyl-carnitine.

Discussion: This work has demonstrated the potential to hyperpolarize the ketone bodies acetoacetate and β-hydroxybutyrate to a level sufficient to allow the observation of their metabolism in the perfused rat heart. The higher polarization level and longer T_1 value achieved with acetoacetate, enabled observation of its rapid uptake, interconversion with β-hydroxybutyrate and downstream metabolism into the Krebs cycle. However, the additional redox step and spectral position of β-hydroxybutyrate, in addition to the lower polarization level and shorter T_1, meant that reliable detection of the resonances of citrate and glutamate was not possible. Further work will aim to transfer the study of these hyperpolarized substrates into the *in vivo* rat heart with application in a model of type II diabetes.

References: [1]Schroeder, M et al, Circulation. 2011 Oct 4;124(14):1580-94. [2]Heather, L *et al*, J Mol Cell Cardiol. 2011 Apr;50(4):598-605. [3]Kennedy, B et al, Proc. Intl. Soc. Mag. Reson. Med. 20 (2012) p4326.