Optimisation of Murine Cardiac Hyperpolarized Magnetic Resonance Spectroscopy Using Dynamic Nuclear Polarization

M. S. Dodd[1], B. Schuler[1], V. Ball[1], D. Ball[1], G. K. Radda[1], H. Ashrafian[2], H. Watkins[2], K. Clarke[1], and D. J. Tyler[1]
[1]Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom, [2]Cardiovascular Medicine, Oxford University, Oxford, United Kingdom

Introduction: Alterations in cardiac metabolism and substrate selection underlie many diseases of the heart. The advent of cardiac hyperpolarized magnetic resonance spectroscopy (MRS), via dynamic nuclear polarization (DNP), has enabled a greater understanding of the *in vivo* metabolic changes seen as a consequence of myocardial infarction, hypertrophy and diabetes [1]. However, all studies performed to date have focused on rats and larger animals, whereas more information could be gained through the study of transgenic mouse models of heart disease. Translation from the rat to the mouse is challenging, due in part to the reduction in heart size ($1/10^{th}$) and the 50% increase in heart rate. In this study, we demonstrate for the first time a slice selective approach to investigate the *in vivo* metabolism of [1-^{13}C]pyruvate in the murine heart. To validate the sensitivity of the method to detect alterations in PDH flux, mice were fasted overnight, as this has previously been shown to reduce *in vivo* PDH flux in rats [1].

Method: *Animals* - Four male Balb/c mice (~25g) were examined to determine the effects of fasting on *in vivo* PDH flux in the heart. The mice received two hyperpolarized scans on two separate days, with all scans being performed between 7 am and 1 pm. Before the first scan, the mice were provided with food and water *ad libitum* and were scanned in the fed state. For the second scan, the mice were fasted overnight, whilst still having free access to water, with food removed at 5pm on the evening before the scan.

Slice selective sequence - Using a ^{1}H volume RF coil (Rapid Biomedical), a sagittal FLASH localizer image was obtained (TR/TE, 3.67/1.63 ms; flip angle, 24°; Averages, 16; slice thickness, 2 mm; matrix, 256 x 256; field of view, 65 x 65 mm). Using this image, a 10 mm axial slice was planned to include the heart and avoid the liver, as seen in Figure 1. To insure the 10 mm axial slice was aligned with the centre of the ^{13}C RF surface coil, a point phantom was positioned underneath the centre of the coil and a 1.5 mm axial slice obtained (Figure 1 insert).

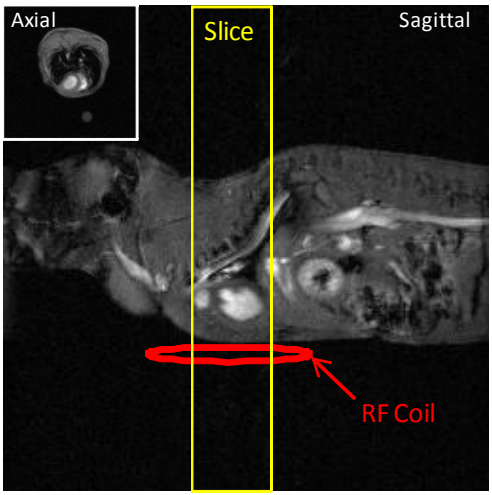

Figure 1: Sagittal proton image of the mouse, showing the position of the ^{13}C home built RF coil and planned axial slice. (256 x 256 matrix, FOV 65 x 65 mm) Insert: axial proton image at the centre of the slice.

Hyperpolarized ^{13}C MRS Protocol - [1-^{13}C]pyruvate was hyperpolarized and dissolved as previously described [2]. An aliquot of 0.2 ml of 80 mM hyperpolarized [1-^{13}C]pyruvate solution was injected over 10 s via a tail vein catheter into an anaesthetised mouse positioned in a 7 T MR scanner. Spectra were acquired for 1 min following injection with 1 s temporal resolution, using a 10° sinc RF excitation pulse. Signal was localised to the heart using a home built ^{13}C RF surface coil and the 10 mm slice, as described above. Quantified peak areas were input into a kinetic model described by Atherton *et al* and plotted against time in Microsoft Excel [3]. This model fits the spectral peak areas as a function of time and accounts for several factors, including rate of injection, rate of signal decay for pyruvate and metabolites, and time of arrival for pyruvate and metabolites. This model determines the rate constant for pyruvate to bicarbonate exchange ($k_{pyr\text{-}bic}$, s^{-1}), which is a measure of ^{13}C label incorporation into the bicarbonate pool, and can be used as a measure of PDH flux [3].

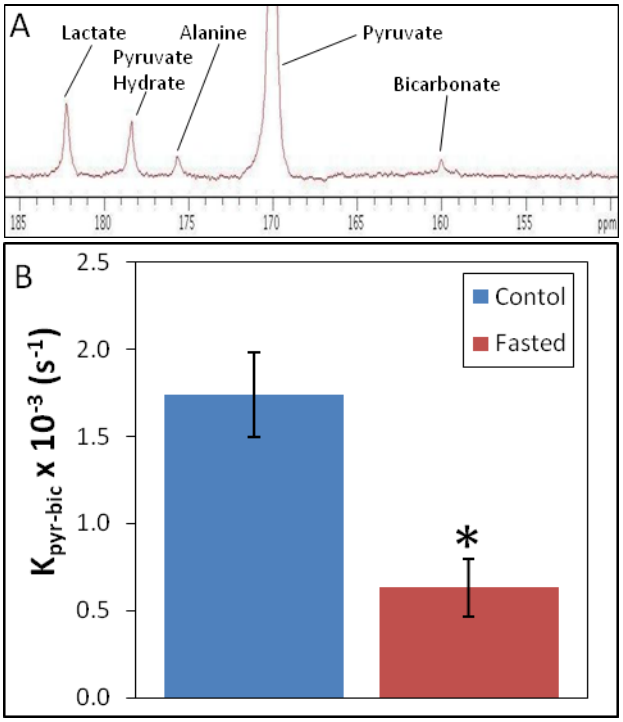

Figure 2: A) First spectrum from the fed mouse heart, showing lactate, pyruvate hydrate, alanine, pyruvate and bicarbonate. B) Fasting significantly reduces PDH flux ($K_{pyr\text{-}bic}$) in the *in vivo* mouse heart (* $p < 0.05$).

Results: Compared with control (Wistar) rats, there was ~50% lower PDH flux in control mice [1]. Overnight fasting produced a 64% reduction in PDH flux, as assessed by a decrease in the rate of pyruvate to bicarbonate exchange ($k_{pyr\text{-}bic}$, s^{-1}). No significant difference was detected in the rate of exchange into either lactate or alanine pools.

Discussion: Here we have shown the first use of a slice selective hyperpolarized MRS technique for the assessment of cardiac metabolism in the *in vivo* mouse heart. In this study we have validated the method by demonstrating the sensitivity of the technique to alterations in PDH flux by reproducing results previously seen in the rat heart. This offers the potential to use hyperpolarized MRS to investigate transgenic mouse models of cardiac diseases, providing a novel tool to assess genetic alterations seen in the diseased heart.

References: [1] Schroeder, M.A., *et al.* Proc Natl Acad Sci U S A, 2008. **105**(33): p. 12051-6. [2] Golman, K., *et al*. Proc Natl Acad Sci U S A, 2006. **103**(30): p.11270-5. [3] Atherton, H.J., *et al.* NMR Biomed. 2010 Aug 26.

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