The Application of Hyperpolarized ^{13}C-MRS in a Cardiac Specific Fumarate Hydratase Knockout Mouse

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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 work, we demonstrate an approach to assess *in vivo* metabolism in the mouse heart using [1-^{13}C]pyruvate. To validate the sensitivity of the method to detect alterations in pyruvate dehydrogenase (PDH) flux, one set of mice were fasted overnight, whilst another set received dicholoacetate (DCA), both of which have been shown to modulate *in vivo* PDH flux in rats [2,3]. To demonstrate the use of hyperpolarized MRS on a transgenic mouse model of cardiac disease, the cardiac specific fumarate hydratase knockout (FH-KO) mouse was subsequently studied. FH-KO mice demonstrate a severe impairment of cardiac function by 12 weeks of age. In this study, FH-KO mice were initially evaluated at 6 weeks of age to assess their *in vivo* metabolic phenotype before the onset of cardiac dysfunction.

Methods: *Animals* - Male C57/Bl6 mice received [1-^{13}C]pyruvate scans, in either the fed state (n = 7) or after fasting overnight for a minimum of 14 hours (n = 7). In the fasting animals, food was removed at 16:00 h on the day before experiments, resulting in a ~10% loss of body weight. Another group of C57/Bl6 mice (n = 5) received [1-^{13}C]pyruvate scans after an infusion of sodium dichloroacetate (DCA) (30 mg/ml, in saline at pH 7.4). A bolus of 0.2 ml DCA was injected into the tail vein cannula, followed by an infusion of 0.1 ml for 10 minutes. The end of the infusion was timed to coincide with immediate injection of hyperpolarized [1-^{13}C]pyruvate. Male FH-KO mice (n = 6) and littermate controls (n = 7) were scanned using the same protocol in the fed state.

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 15^{o} RF excitation pulse (Fig 1). Signal was localised to the heart using a home-built ^{13}C RF surface coil. Quantified peak areas were input into a kinetic model described by Atherton *et al* [3]. This model fits the spectral peak areas as a function of time and accounts for rate of injection and rate of signal decay for pyruvate and its metabolites. This model determines the rate constant for pyruvate to bicarbonate exchange (s^{-1}), which is a measure of ^{13}C label incorporation into the bicarbonate pool and thus PDH flux [3].

Results: Overnight fasting produced an 80% reduction in PDH flux ($p < 0.001$, Fig 2) whilst DCA infusion resulted in a 145% increase in PDH flux ($p < 0.01$, Fig 2). Neither fasting nor DCA led to a significant difference in ^{13}C label incorporation into either lactate or alanine pools. Interestingly 6 week old FH-KO mice did not show any significant changes in ^{13}C label incorporation into any metabolite pools (Fig 3).

Discussion: We have demonstrated the use of the 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 [2,3]. This technique therefore provides a novel tool to assess genetic alterations seen in the diseased heart. Our initial assessment of the FH-KO mouse showed no significant alteration in PDH flux at 6 weeks of age despite the absence of fumarate hydratase. The aim of this early time point study was to assess the *in vivo* metabolic phenotype before the onset of cardiac complications. Later time points warrant further investigation to ascertain the relationship between functional and metabolic alterations.

References: [1] Schroeder, M.A., *et al.,* Circ, 2011. **124**(14): p. 1580-94, [2] Schroeder, M.A., *et al.* PNAS, 2008. **105**(33): p.12051-6. [3] Atherton, H.J., *et al.* NMR Biomed, 2011. **24**(2): p. 201-8.

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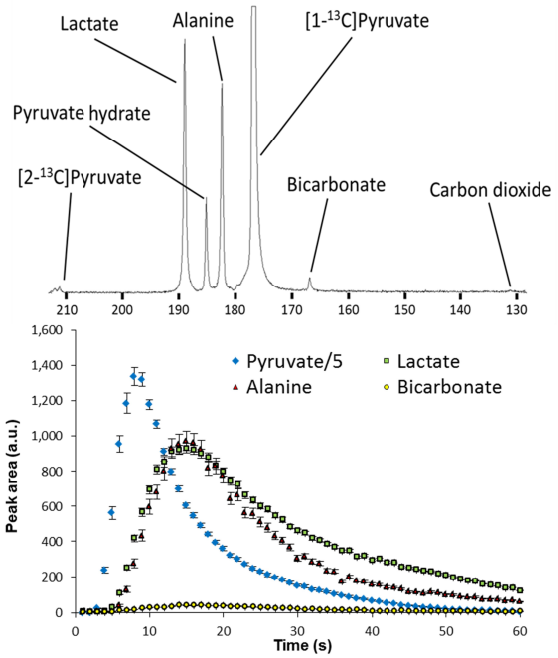

Fig 1 – Summed spectra from a control mouse (Top). Average time course for control mice (n = 4) (Bottom)

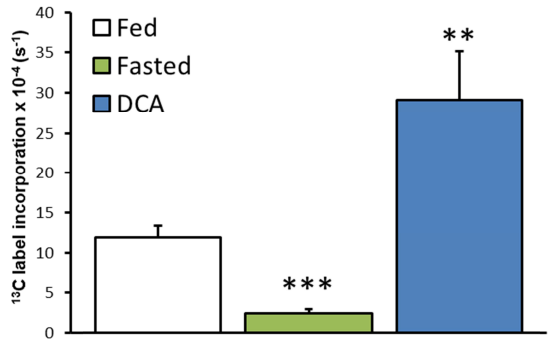

Fig 2 – ^{13}C label incorporation into bicarbonate pools in control (fed), fasted and DCA treated mice. ***$p < 0.001$, **$p < 0.01$. Students 2-tailed t-test. Data are mean values and error bars are S.E.M

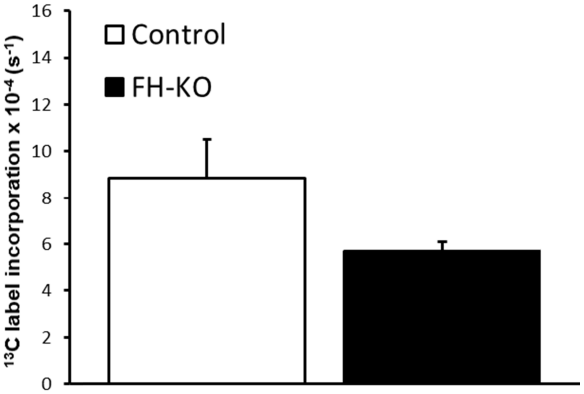

Fig 3 – ^{13}C label incorporation into bicarbonate pools in control and FH-KO mice. Data are mean values and error bars are S.E.M