In Vivo Hyperpolarized ^{13}C-MRS Shows Abnormal Cardiac Metabolism in the PPARα Knockout Mouse

Michael Dodd[1,2], Rosalind Bray[1], Vicky Ball[1], Mark Cole[1], Kieran Clarke[1], and Damian Tyler[1]

[1]*Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, OXON, United Kingdom,* [2]*Department of Cardiovascular Medicine, Oxford University, Oxford, OXON, United Kingdom*

Introduction: During fasting, plasma free fatty acids increase and stimulate their own catabolism through increased fatty acid oxidation. This is achieved, in part, via the activation of the peroxisome proliferator-activated receptors (PPARs), a group of nuclear response elements. PPARα has a high binding affinity for fatty acids and functions to transcriptionally regulate the expression of a range of fatty acid oxidation genes. The PPARα knockout mouse has been developed to investigate the role of PPARα in metabolism and disease. The aim of this work was to assess the *in vivo* metabolic phenotype of the PPARα-KO heart using hyperpolarized magnetic resonance spectroscopy. In particular the metabolism of [1-^{13}C]pyruvate was measured in fed and fasted mice.

Methods: *Animals* - Five 12-14 month old male PPARα-KO mice and their littermate controls (129Sv, n = 5) received two hyperpolarized scans on two separate days. Before the first scan, the mice were provided with food and water *ad libitum* and scans were performed between 7 am and 11 am during the fed state. For the second scan, the mice were fasted overnight (minimum of 18 hours), with free access to water.

Hyperpolarized ^{13}C MRS Protocol - [1-^{13}C]pyruvate was hyperpolarized and dissolved as previously described [1,2]. 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° RF excitation pulse. 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]. The rate of exchange of the ^{13}C label between pyruvate and its metabolites was termed ^{13}C label incorporation. ^{13}C label incorporation into the bicarbonate pool is a sensitive measure of pyruvate dehydrogenase (PDH) flux in the rat [3].

Results: Fed PPARα-KO mice had a 41% increase in PDH flux when compared to fed control animals (figure 1, $p < 0.05$), whilst incorporation of the ^{13}C label into lactate and alanine pools was unchanged between groups. Control animals in the fasted state had a 10 x 10^{-4} s^{-1} reduction (55%) in PDH flux when compared to the same animals in the fed state ($p < 0.05$). Interestingly the fasted PPARα-KO mice also had a 10 x 10^{-4} s^{-1} decrease in PDH flux (45%) when compared to their fed state ($p < 0.001$). However, the fasted PPARα-KO mouse had a 50% higher PDH flux than control fasted animals ($p < 0.05$).

Discussion: The normal cardiac response to fasting is a greater reliance on fatty acids and a shift away from glucose oxidation. The breakdown of fatty acid stores results in elevated plasma fatty acid levels which stimulate their own catabolism through increased fatty acid oxidation. This leads to decreased PDH flux that "spares" pyruvate for oxaloacetate production and gluconeogenesis [4]. The decrease in PDH flux is partly mediated by increased expression of PDH kinase (PDK) 4, the inhibitor of PDH [5]. In fasted wild type mice, PPARα increases several key fatty acid oxidation proteins, as well as PDK4. In the fed PPARα-KO mice there is a significant increase in PDH flux that is potentially due to decreases in PDK4 expression. Fasting led to a highly significant decrease in PDH flux in both control and PPARα-KO mice. Interestingly the overall decrease in PDH flux in the control and PPARα-KO mice was the same, indicating that this alteration is PPARα independent and is a response to increased fatty acid supply and utilization. Previously it has been shown that PPARα expression decreases with age [6], so future work will assess PDH flux in younger control and PPARα-KO mice to determine whether this is an age related alteration in PDH flux control.

References: [1] Golman, K., *et al*. PNAS, 2006. **103**(30): p.11270-5 [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. [4] Huang, B., et al. Diabetes, 2002. **51**(2): p. 276-83. [5] Wu, P. *et al*., Biochem J., 1998. **329** p 197-201 [6] Iemitsu, M., et al., A.J.P Heart Circ. Physiol., 2002. **283**(5): p. H1750-60

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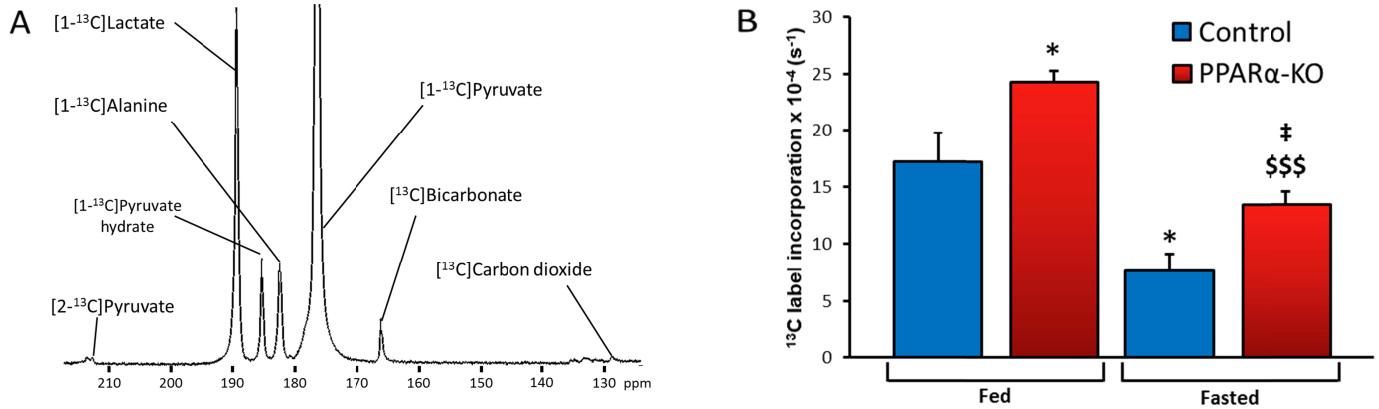

Figure 1 – **A)** Summed *in vivo* spectra from a fed PPARα-KO mouse acquired over 45 seconds. **B)** ^{13}C label incorporation into bicarbonate (PDH flux). During the fed state there is a signifcant increase in PDH flux in PPARα-KO mice. During fasting there is a singificant reduction in PDH flux in both controls and PPARα-KO mice. Values are mean data and error bars are S.E.M. Significant differences between mean values were determined by analysis of variance (ANOVA) followed by student two tailed t-test. * $p < 0.05$ compared to fed control, **$$$** $p < 0.001$ compared to fed PPARα-KO and ‡ $p < 0.05$ compared to fasted control