

***In vivo* assessment of metabolism in the hypertensive rat heart using hyperpolarized [1-¹³C] and [2-¹³C]pyruvate**

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Introduction - Spontaneously hypertensive rats (SHRs) are used as a model of hypertension and insulin resistance. In response to hypertension and several genetic defects, including loss-of-function mutations in CD36 (a long-chain fatty acid transporter), these animals develop pressure-overload concentric hypertrophy [1]. One of the cellular responses to hypertrophy is a reduction in fatty acid oxidation to reduce oxygen consumption. This reduction is further exacerbated in the SHR model by the loss of function in CD36, resulting in a hypothesised switch to a glycolytic phenotype. The aim of this work was to assess *in vivo* metabolism in the hypertensive rat heart using hyperpolarized [1-¹³C] and [2-¹³C]pyruvate and to observe whether there is a switch to a glycolytic phenotype *in vivo*.

Methods - *Animals*: Eleven male Wistar rats (Controls, 250-300g) and thirteen male SHRs (15 weeks, 250-300g) were subjected to the hyperpolarized ¹³C MRS and cine MRI protocols described below.

¹³C MRS: On two separate days cardiac metabolism was assessed following a bolus injection of either [1-¹³C] or [2-¹³C]pyruvate. The pyruvate solutions were hyperpolarized and dissolved as previously described [2]. Immediately following dissolution, 1 ml of 80 mM hyperpolarized pyruvate was injected over 10 s via a tail vein catheter into an anaesthetised rat positioned in a 7 T MR scanner. Spectra were acquired for 1 min following injection with 1 s temporal resolution and signals were localised to the heart via the use of a surface coil. Acquired spectra were quantified using jMRUI and were fit to a kinetic model as described previously by Atherton *et al* 2010 [3].

Cine MRI: All animals were imaged to assess left ventricular mass and cardiac function. Rats were positioned in an 11.7 T (500 MHz) vertical bore MR scanner. Experiments were carried out as previously described [4].

Results - Cine MRI revealed that SHR animals had a significant 63% increase in left ventricular mass compared with controls (p<0.001). There was no change in heart function as assessed by either ejection fraction or stroke volume.

Using the hyperpolarized protocol, ¹³C label flux was observed from [1-¹³C]pyruvate into [1-¹³C]lactate, [1-¹³C]alanine, ¹³CO₂ and [1-¹³C]bicarbonate. A significant 53% increase in pyruvate dehydrogenase (PDH) flux (assessed using the sum of the ¹³CO₂ and [1-¹³C]bicarbonate resonances) was detected in the SHR hearts compared with controls (p<0.01, Fig 1). No difference in flux into lactate and alanine was observed between groups.

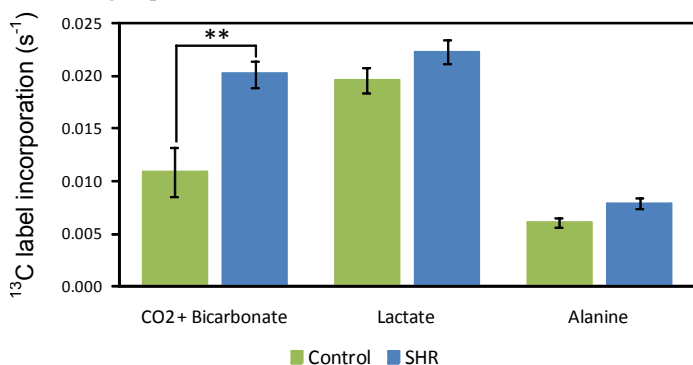


Fig 1 – Hypertensive rats show an increase in PDH flux (production of ¹³C labelled carbon dioxide + bicarbonate).

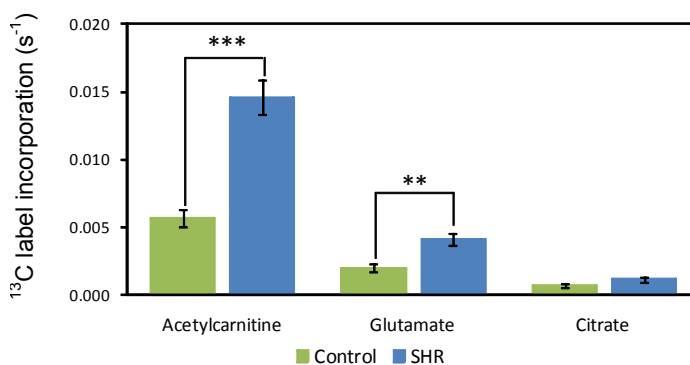


Fig 2 – Conversion of [2-¹³C]pyruvate into acetylcarnitine, glutamate and citrate monitored in the hypertensive heart.

[2-¹³C]pyruvate data was fit to the same model to assess ¹³C label incorporation into downstream metabolite pools. Incorporation into acetylcarnitine (p<0.001) and glutamate (p<0.01) pools was significantly increased within SHR hearts, while no significant change was observed in the incorporation of the ¹³C label into citrate (Fig 2).

Discussion - This study demonstrates that there is an increase in PDH flux in the hypertensive heart. A well characterized *ex vivo* enzyme activity assay was used to validate this finding and showed a significant correlation between *ex vivo* PDH activity and *in vivo* PDH flux. The increase in PDH flux was coupled with an increase in ¹³C label incorporation into acetylcarnitine and glutamate pools. However when label incorporation into these pools was normalized to flux through PDH, no change was observed in either the acetylcarnitine and glutamate pools. This implies that increases in acetylcarnitine and glutamate are derived from increased production of [2-¹³C]acetyl-CoA and represent normal flux through the Krebs cycle. This study has not shown a switch to a glycolytic phenotype, as flux into lactate remained unchanged between groups. However, it does suggest a switch to increased glucose oxidation through PDH and the Krebs cycle.

References

[1] Shimamoto, N., *et al.* Basic Res Cardiol, 1982. 77, p.359-357. [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. [4] Tyler, D.J., *et al.* J Cardiovasc Magn Reson, 2006. 8, p.327-333.

Acknowledgements - This study was supported by the British Heart Foundation, Medical Research Council and GE Healthcare