# Investigating the Metabolic Effects of Heart Failure Progression *In Vivo* using Hyperpolarized Magnetic Resonance

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## Introduction

Heart failure (HF) is a progressive disease that affects more than 2% of the U.S. population. Causes of HF include myocardial infarction (MI), ischaemic heart disease, hypertension and cardiomyopathy, however all these causes are accompanied by changes in cardiac energy metabolism [1]. The rat ischaemia-reperfusion model of MI provides a powerful tool for investigating HF progression and can be used in conjunction with magnetic resonance spectroscopy (MRS) to obtain novel information regarding the metabolic deficits associated with HF progression. Unfortunately, the poor sensitivity of MRS has prevented *in vivo* study, meaning multiple experiments in the same animal throughout HF progression have not previously been possible. Instead, *ex vivo* experiments in the perfused heart have been used to provide evidence of perturbed metabolism at selected time points post MI [2,3]. The recent development of hyperpolarized MRS, a novel, non-invasive technique which enhances the SNR available from $^{13}$C-MRS experiments by more than 20,000-fold, has enabled the visualisation of real time metabolism *in vivo* [4]. The aim of this study was to investigate the temporal metabolic effects associated with HF progression in individual animals *in vivo,* up to 3 months post MI.

## Methods

*Myocardial Infarction*: The left anterior descending (LAD) coronary artery of female Wistar rats (~200 g, n = 16) was occluded approximately 2 mm from the origin. Following anaesthesia and thoracotamy, the pericardium was removed and a 5-0 prolene suture was placed under the LAD. The suture was tied around a small piece of plastic tubing, occluding the LAD, and the chest was then closed. After 50 minutes, the chest was re-opened and the tubing removed to allow reperfusion. Cardiac metabolism was assessed using the MRS protocol, described below, at 2 days, 2 weeks, 6 weeks and 12 weeks post surgery. At each time point heart function was also assessed using echocardiography and animals were classified according to ejection fraction (EF; > 65 % = control, < 45 % = infarct, n = 6 per group).

*MRS*: [1-$^{13}$C] and [2-$^{13}$C]pyruvate were hyperpolarized and dissolved as previously described [4]. One millilitre of each 80 mM hyperpolarized pyruvate solution was injected over 10 s via a tail vein catheter into an anaesthetised rat positioned in a 7 T MR scanner with ~45 minutes between injections. The order of [1-$^{13}$C] and [2-$^{13}$C]pyruvate injections into each animal was randomised. Spectra were acquired for 1 min following injection with 1 s temporal resolution and signal was localised to the heart with the use of a surface coil. Spectra were quantified and the maximum signal from each metabolite, normalized to the maximum $^{13}$C-pyruvate signal, was recorded.

## Results

The results of this study are summarized in *Fig.1*. The signals from $^{13}$C-labelled bicarbonate, acetyl carnitine, glutamate and citrate were low at day 2 relative to later time points in all animals. At 2 days, PDH flux was reduced in infarcted animals as assessed using $^{13}$C-bicarbonate/[1-$^{13}$C]pyruvate ($p<0.05$). The signal from [1-$^{13}$C]acetyl carnitine, a buffer for excess acetyl CoA, was also reduced. At 2 weeks post MI, PDH flux was comparable between control and infarcted animals and remained so up to 12 weeks. In contrast, at 6 weeks, levels of [1-$^{13}$C]acetyl carnitine, [5-$^{13}$C]glutamate and [1-$^{13}$C]citrate were significantly decreased, and signals from acetyl carnitine and glutamate remained significantly reduced relative to control levels at 12 weeks. When normalised to PDH flux (to account for potential differences in pyruvate perfusion caused by scarring), the amount of [1-$^{13}$C]acetyl carnitine and [5-$^{13}$C]glutamate generated was still significantly lower in infarcted hearts at 12 weeks (*Fig. 1F*). [1-$^{13}$C]lactate was reduced in infarcted animals at all time points analysed ($p<0.05$).

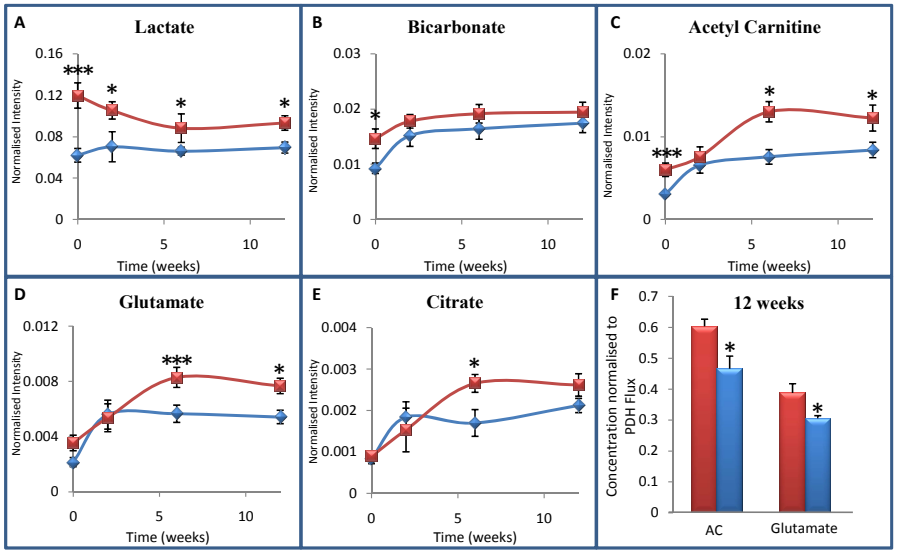


***Fig 1**. (**A-E**) $^{13}$C-metabolite levels normalised to $^{13}$C-pyruvate at a range of time points post myocardial infarction, (**F**) ratio of [1-$^{13}$C]acetyl carnitine to $^{13}$C-bicarbonate (Key: **red**- control (EF>65%), **blue**- infarct (EF<45%); * p< 0.05, *** p< 0.001)*

## Discussion

Using hyperpolarized MR we investigated the metabolic effects of myocardial infarction *in vivo* up to 12 weeks post surgery. At 2 days, the metabolic abnormalities observed were probably a direct result of the surgery. In contrast, at later time points, differences between control and infarcted animals likely reflected HF onset/progression. From 2 weeks post MI, PDH flux was equivalent in control and infarcted hearts indicating that acetyl CoA production from pyruvate was maintained regardless of cardiac function. The consistent reduction in [1-$^{13}$C]lactate in the infarcted hearts may have been caused by decreased glucose uptake and/or glycolysis [3]. At 6 weeks and 12 weeks, despite normal PDH flux, the signal from [1-$^{13}$C]acetyl carnitine was reduced. This indicated reduced excess acetyl CoA production by PDH, suggesting more pyruvate derived acetyl CoA was processed via the Krebs cycle in infarcted hearts than in control hearts. However $^{13}$C-labelling of citrate and glutamate was also reduced at later time points. This indicated that either the pool sizes of these molecules within the mitochondria of infarcted hearts were reduced, or that infarcted hearts more rapidly metabolised these $^{13}$C-labelled substrates. Both effects could be explained by a reduction in fatty acid oxidation, a well characterised effect of HF which could contribute to the energy deficit observed in failing hearts [2]. In summary, this study shows for the first time the metabolic changes associated with HF progression *in vivo*. The results highlight the importance of being able to non invasively assess metabolism at multiple time points and demonstrates the potential of hyperpolarized MRS for investigating the metabolic effects of progressive diseases such as HF.

## References

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