In vivo pyruvate dehydrogenase flux measured by hyperpolarized magnetic resonance correlates with *ex vivo* pyruvate dehydrogenase activity

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Introduction: Pyruvate, the end product of glycolysis, is transported and irreversibly catalysed to acetyl-CoA by the enzyme pyruvate dehydrogenase (PDH). PDH is dynamically regulated via a phosphatase/kinase cycle and can therefore be rapidly turned on and off in response to the cellular environment [1]. PDH kinase (PDK), which deactivates PDH by phosphorylation, is stimulated by an excess of acetyl-CoA, NADH and ATP, all products of a plentiful energy supply, such as from an increase in β-oxidation [1]. It has recently been shown that hyperpolarized [1-^{13}C]pyruvate, generated using dynamic nuclear polarization (DNP), provides an *in vivo* measurement of the [^{13}C]bicarbonate/[1-^{13}C]pyruvate ratio which has been proposed to be a measure of PDH flux [2]. The aim of this study was to investigate the correlation between the *in vivo* [^{13}C]bicarbonate/[1-^{13}C]pyruvate ratio, measured using hyperpolarized [1-^{13}C]pyruvate, and the enzymatic activity of PDH evaluated *ex vivo* using mechanisms known to modulate PDH activity, namely high fat feeding (HFF) and dichloroacetate (DCA) infusion. Four weeks of HFF is known to decrease PDH activity, via stimulation of PDK, due to increased levels of fatty acid derived acetyl-CoA [3]. DCA, a pyruvate analogue, is a potent inhibitor of PDK and is known to increase PDH activity.

Methods: *High fat feeding (HFF)* - Fourteen male Wistar rats were studied to determine the effects of high fat feeding on *in vivo* PDH flux. One group (n = 7) were fed for 4 weeks on a standard chow diet, while another group (n = 7) were fed on a high fat diet, where 55% of the calories were obtained from saturated fat. Following this feeding regime, the rats were scanned using the hyperpolarized [1-^{13}C]pyruvate protocol described below. Rats were sacrificed after 24 h and the hearts were removed for PDH activity analysis.

Dichloroacetate (DCA) - Eleven male Wistar rats were studied to determine the effect of DCA, on *in vivo* PDH flux. The first group (n = 5) received a hyperpolarized scan and within 15 min were sacrificed and the hearts removed for PDH activity analysis. In the second group (n = 6), DCA was administered (30 mg/ml, 1 ml bolus injection followed by 0.5 ml infusion over 15 min) via a tail vein catheter, followed by a hyperpolarized scan. The rats were then sacrificed within 15 min and hearts were removed for PDH activity analysis.

PDH activity analysis - PDH activity was assessed using a spectrophotometric method as previously described [4]. Data were quantified and the ratio between the total and active PDH portions was used as a measure of the PDH enzymatic activity.

Hyperpolarized ^{13}C MRS Protocol: [1-^{13}C]pyruvate was hyperpolarized and dissolved as previously described [5]. One millilitre of 80 mM hyperpolarized [1-^{13}C]pyruvate solution 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 signal was localised to the heart via the use of a surface coil. Spectra were quantified and maximum [^{13}C]bicarbonate/[1-^{13}C]pyruvate was used as a direct measure of PDH flux.

Results: Following 28 days on a high fat diet, a significant decrease was observed in PDH flux, as measured by hyperpolarized ^{13}C MRS, which was not detected using the *ex vivo* PDH activity assay (Figure 1). It was also observed that there was a highly significant ($p < 0.0001$) 2.5 and 3 fold increase in both PDH flux and PDH activity, respectively, after acute DCA treatment. Using both *in vivo* hyperpolarized ^{13}C MRS and *ex vivo* PDH activity analysis, it was observed that a positive correlation ($R^2 = 0.567$) exists between the [^{13}C]bicarbonate/[1-^{13}C]pyruvate ratio and PDH activity (Figure 2).

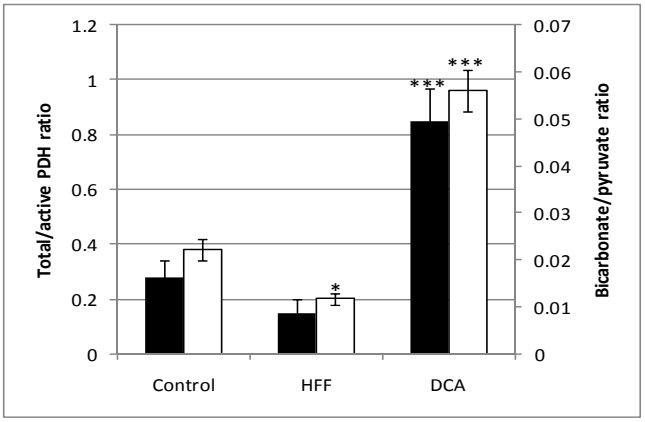

Figure 1: Total / active PDH ratio (black) verses [^{13}C]bicarbonate / [1-^{13}C]pyruvate ratio (white) in control, high fat fed (HFF) and dichloroacetate (DCA) treated hearts. *p <0.01 & ***p<0.0001.

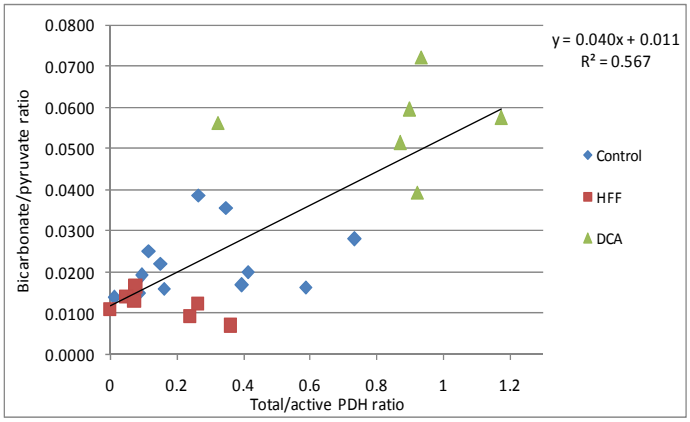

Figure 2: Positive correlation between the total / active PDH ratio and the [^{13}C]bicarbonate / [1-^{13}C]pyruvate ratio in control, high fat fed (HFF) and dichloroacetate (DCA) treated hearts.

Discussion: This study has shown that *in vivo* PDH flux, as measured by hyperpolarized ^{13}C MRS, significantly correlated with *ex vivo* PDH activity. This demonstrates that the [^{13}C]bicarbonate/[1-^{13}C]pyruvate ratio can be used as a direct measure of *in vivo* PDH activity. The PDK inhibitor, DCA, caused a significant 152% and 203% increase in PDH flux and PDH activity, respectively. Interestingly, hyperpolarized ^{13}C MRS showed that there was a significant decrease in PDH flux after 4 weeks on a high fat diet, whilst no significant change was evident using the *ex vivo* PDH activity assay. This suggests that hyperpolarized ^{13}C MRS may provide a more sensitive measurement for detecting small changes in cardiac metabolism as it maintains the *in vivo* metabolic environment and prevents the need for destructive tissue preparation methods used in *ex vivo* analysis.

References

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