

In vivo investigation of dichloroacetate-modulated cardiac metabolism in the rat using hyperpolarized ^{13}C MRS

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Introduction

Hyperpolarized ^{13}C MRS allows the in vivo assessment of pyruvate dehydrogenase (PDH) flux, which converts pyruvate to acetyl-CoA. Hyperpolarized $[1-^{13}\text{C}]$ -pyruvate MRS has been used to measure changes in cardiac PDH flux after administration of dichloroacetate (DCA), a PDH kinase inhibitor, demonstrating an increase in ^{13}C -bicarbonate production [1]. With $[1-^{13}\text{C}]$ -pyruvate, the ^{13}C label is released as $^{13}\text{CO}_2/^{13}\text{C}$ -bicarbonate, and cannot follow acetyl-CoA into downstream metabolic steps. Hyperpolarized $[2-^{13}\text{C}]$ -pyruvate and $[1,2-^{13}\text{C}]$ -pyruvate have been used to track the ^{13}C label into the Krebs cycle and measure $[5-^{13}\text{C}]$ glutamate (generated from α -ketoglutarate in the Krebs cycle) [2,3]. This work investigates the metabolic fate of the corresponding increase in acetyl-CoA that is generated by up-regulating PDH flux with DCA in vivo in rat heart. Comparing the increase in bicarbonate from $[1-^{13}\text{C}]$ pyruvate with that of glutamate from $[2-^{13}\text{C}]$ pyruvate can provide information about the relative fraction of the acetyl-CoA produced through PDH that enters the Krebs cycle.

Methods

All measurements were performed on a GE 3T MR scanner equipped with self-shielded gradients (40 mT/m, 150 mT/m/ms) using a custom-built ^{13}C transmit/receive single-loop surface coil (inner dia=28 mm) placed over the heart (with rat supine). Healthy male Wistar rats (300-350 g, n=7) were injected in a tail vein with 4 ml of 80-mM solution of either $[1-^{13}\text{C}]$ -pyruvate (n=3) or $[2-^{13}\text{C}]$ -pyruvate (n=4). The samples were hyperpolarized via Dynamic Nuclear Polarization using HyperSense (Oxford Instruments Molecular Biotools, Oxford, UK) achieving 20-25% liquid state polarization. The time from dissolution to start of injection was ~18s.

Each animal received 2 injections of the hyperpolarized substrate followed by free induction decay (FID) dynamic MRS. A non-slice-selective pulse-and-acquire sequence with an excitation flip angle of 5.625° , spectral width of 10 kHz and 4096 points was used to acquire ^{13}C -spectra from the heart every 3 s over a 4-min period starting at the same time as the pyruvate injection. In order to manipulate the PDH flux, DCA solution (dose=150 mg/kg body weight, dissolved in saline at 30 mg/mL) was injected into the tail vein approximately 20 min before the second pyruvate injection.

The MRS data were apodized with 10-Hz Gaussian line broadening and zero-filled by a factor of two. For each spectrum a zero-order and first-order phase correction was performed and the baseline was subtracted by fitting a spline to the signal-free regions of the spectrum. Metabolite levels were measured after summing up the absorption mode spectra from time 9s to 30s after $[2-^{13}\text{C}]$ -pyruvate injection and from time 9s to 90s for data from $[1-^{13}\text{C}]$ -pyruvate injection.

Results and Discussion

Representative ^{13}C spectra (Fig. 1) for $[2-^{13}\text{C}]$ -pyruvate, acquired at baseline and post-DCA administration demonstrate the effect of the increased PDH flux on cardiac metabolism. An increase was observed in glutamate (183.8 ppm) reflecting Krebs cycle activity, in acetyl-carnitine (175.2 ppm) from fatty acid synthesis and in acetoacetate (177.3 and 212.7 ppm) indicating ketogenesis. The $^{13}\text{C}_3$ resonance of acetoacetate at 212 ppm partially overlapped with Pyr at 207.8ppm. Another product of ketogenesis, β -hydroxybutyrate at 183 ppm could not be resolved from glutamate. The conversion of pyruvate to lactate and alanine was also detected, and remained unchanged with DCA.

With DCA administration, the ratio of metabolite to pyruvate increased to 1.31 ± 0.19 (mean \pm std of post/pre-DCA) for glutamate, 1.59 ± 0.31 for acetyl-carnitine and 4.13 ± 0.60 for acetoacetate signal (at 177 ppm). The acetoacetate peak at baseline was just at/below the detection threshold, making it hard to quantify the change. In comparison, a 2.61 ± 0.13 increase in bicarbonate signal was detected with $[1-^{13}\text{C}]$ -pyruvate (data not shown), which matches the 2.6-fold increase in $k_{\text{pyr-bic}}$ in [1]. The change in ratios between the 2 injections was significant for all metabolites (paired t-test $p < 0.037$). The metabolite ratios for all rats are plotted in Fig. 2.

This work demonstrates in vivo measurement of changes in cardiac metabolism with changes in PDH flux using hyperpolarized $[1-^{13}\text{C}]$ pyruvate and $[2-^{13}\text{C}]$ pyruvate MRS, and provides information about the relationship between PDH-mediated oxidation of pyruvate and its subsequent incorporation into Krebs cycle vs. other metabolic pathways. The relatively lower change in glutamate than in bicarbonate suggests that the increase in PDH flux is not matched by the increase in Krebs cycle activity, as shown by the increase in metabolic products of fatty acid synthesis and ketogenesis.

Though hyperpolarized $[1,2-^{13}\text{C}]$ -pyruvate or co-polarization of $[1-^{13}\text{C}]$ -pyruvate and $[2-^{13}\text{C}]$ -pyruvate could allow simultaneous measurement of changes in bicarbonate and glutamate, the acetyl-carnitine peak at 175 ppm and acetoacetate at 177 ppm would likely be obscured by the $^{13}\text{C}_1$ resonances of pyruvate at 173 ppm and alanine at 177 ppm, making quantification difficult.

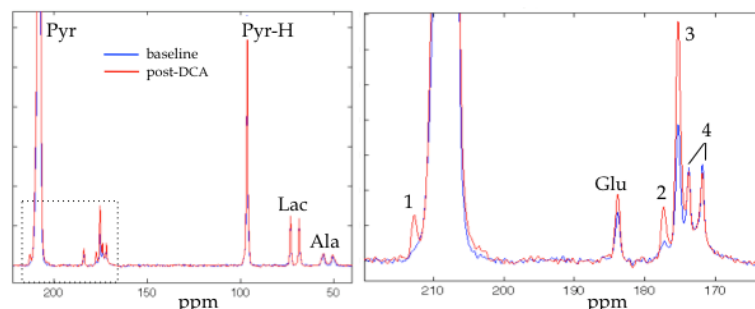


Figure1: (left) ^{13}C spectra from heart after $[2-^{13}\text{C}]$ pyruvate bolus, normalized to pyruvate signal. (right) The zoomed-in spectra show the increase in glutamate (Glu), acetoacetate (peaks 1 and 2) and acetyl-carnitine (3) with increased PDH flux. Peak 4 represents the natural abundance $[1-^{13}\text{C}]$ pyruvate doublet.

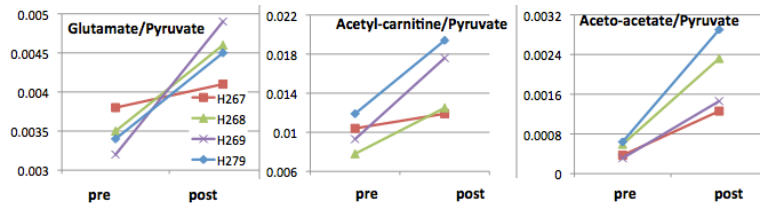


Figure2: Metabolite to pyruvate ratios at baseline and after DCA. Relative to baseline, glutamate increased by approximately 1.3 ± 0.2 ($p = 0.036$), acetyl-carnitine by 1.6 ± 0.3 ($p = 0.035$) and acetoacetate by 4.1 ± 0.6 ($p = 0.016$).

References: [1] Atherton et al NMR Biomed 2011,p201 [2] Schroeder et al, FASEB J 2009,p2529 [3] Chen et al, NMR Biomed 2011; DOI 10.1002/nbm.1749

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