Investigation of substrate dose on rat cardiac metabolism in vivo using MRS of hyperpolarized [2-^{13}C]pyruvate

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Introduction

Hyperpolarized ^{13}C MRS has been used to measure changes in cardiac metabolism. Using [1-^{13}C]pyruvate allows the in vivo assessment of pyruvate dehydrogenase (PDH) flux, which converts pyruvate to acetyl-CoA releasing the ^{13}C label as $^{13}CO_2$/^{13}C-bicarbonate [1]. However, changes in PDH flux measured via bicarbonate may not reflect tricarboxylic acid (TCA) cycle activity, and using [1-^{13}C]pyruvate does not permit following the fate of acetyl-CoA into the downstream metabolic steps. Hyperpolarized [2-^{13}C]pyruvate and [1,2-^{13}C]pyruvate have been used to track the ^{13}C label into TCA cycle and measure [5-^{13}C]glutamate (in fast exchange with α-ketoglutarate in the TCA cycle) as well as into [1- ^{13}C]acetylcarnitine (ALCAR) generated from acetyl-CoA via carnitine acetyltransferase (CAT) [2,3]. Schroeder et al [2] showed changes in acetylcarnitine in response to increased cardiac workload with dobutamine (DOB), but did not observe any change in glutamate using a 0.25 mmol/kg dose of [2-^{13}C]pyruvate. Moreno et al [4] demonstrated that substrate concentration can have a substantial impact on metabolic response in cardiac studies using hyperpolarized ^{13}C pyruvate with greater pyruvate oxidation relative to fatty acids and ketones observed at high pyruvate concentrations. Thus, probing cardiac metabolism using a higher pyruvate dose may provide additional information about the ^{13}C label incorporation into different metabolic pathways and may permit the ability to observe changes in TCA cycle activity as reflected by ^{13}C labeling of glutamate. This work compares the changes in the metabolic fate of acetyl-CoA in response to increased cardiac workload with dobutamine in vivo in rat heart at two different pyruvate doses.

Methods

All measurements were performed on a GE 3T MR scanner using a custom-built ^{13}C transmit/receive surface coil (dia=28 mm) placed over the heart with rat supine. An 80-mM solution of [2-^{13}C]pyruvate, hyperpolarized using HyperSense (Oxford Instruments, UK), was injected into healthy male Wistar rats (350-400 g, n=9) at a dose of 0.8 mmol/kg (group A, n=5) or 0.2 mmol/kg (group B, n=4). Each animal received 2 injections of pyruvate. Dobutamine (0.5 mg/kg body weight) was infused i.v. over 10 min immediately before the second pyruvate injection to acutely increase cardiac workload. Dobutamine increases heart rate and myocardial oxygen demand, and is also known to increase flux through PDH and the TCA cycle in rats.

Dynamic free induction decay ^{13}C-MRS data were acquired using a hard RF pulse with a flip angle of 5°, spectral width of 10 kHz and 4096 points from the heart every 3 s starting at the same time as the pyruvate injection. Metabolite levels were measured after summing up absorption mode spectra from time 6s to 72s. To account for the upfield peak of $^{13}C_1$-lactate doublet (generated from natural abundance $^{13}C_1$-pyruvate) overlapping with glutamate, the lactate signal from the downfield peak was subtracted from glutamate.

Results and Discussion

Representative ^{13}C spectra from group A (Fig. 1) demonstrate the effect of dobutamine. In addition to increased lactate and alanine labeling, higher glutamate was also observed reflecting increased TCA cycle activity with elevated cardiac workload. Fig. 2 shows average metabolite ratios and illustrates the effect of pyruvate dose. For group A, Glu/Pyr increased from 0.0048 ± 0.0017 at baseline to 0.0072 ± 0.0009 post-dobutamine. In contrast, group B showed no significant difference with dobutamine (baseline Glu/Pyr = 0.0114 ± 0.0048, post-DOB=0.0124 ± 0.0079, p=0.6093). Both groups showed similar increases in Lac/Pyr and Ala/Pyr. The effect on acetylcarnitine was not significant for either group, in contrast to [2] which showed a decrease in acetylcarnitine with dobutamine. Metabolite time-courses are shown in Fig. 3.

This work demonstrates in vivo measurement of changes in cardiac metabolism using MRS of hyperpolarized [2-^{13}C]pyruvate, and illustrates the importance of pyruvate dose. No change in glutamate was detected in the low dose group, similar to results in [2], whereas the high dose group showed increased glutamate with dobutamine. Using a high dose of pyruvate may provide an improved ability to observe changes in glutamate, and thus probe TCA cycle activity, with metabolic perturbations in cardiovascular diseases.

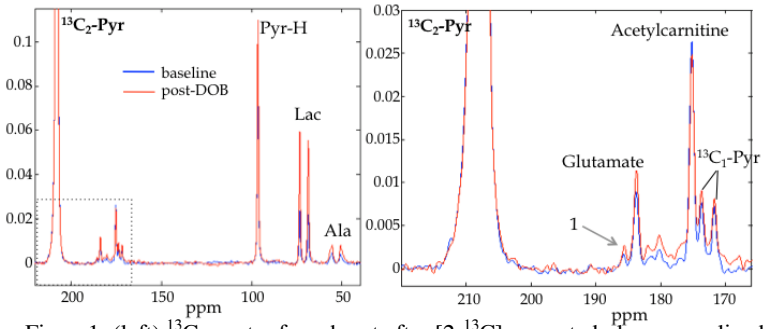

Figure1: (left) ^{13}C spectra from heart after [2-^{13}C]pyruvate bolus, normalized to pyruvate signal, for a rat in group A. Lactate and alanine increased with dobutamine (DOB). (right) The zoomed-in spectra show the increase in glutamate. Peak 1 is the downfield peak of natural abundance $^{13}C_1$-lactate doublet with the upfield peak overlapping with glutamate.

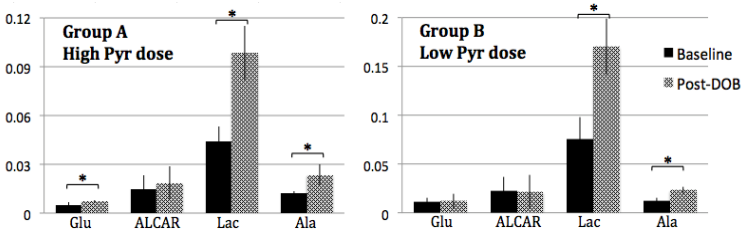

Figure2: Metabolite-to-pyruvate ratios at baseline and after dobutamine (DOB). Glutamate increased for group A, but not group B. Lactate and alanine signals increased similarly for both groups. *paired t-test p<0.026

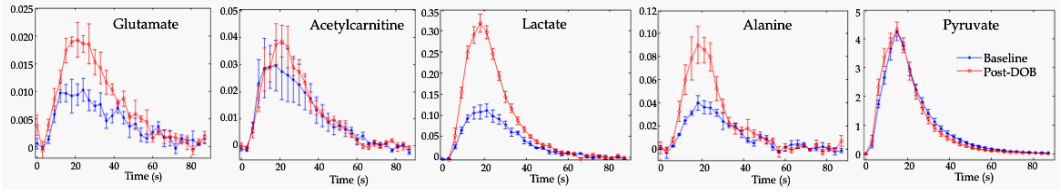

Figure3: Average metabolite time-courses from group A rats show increased glutamate, lactate and alanine signals with dobutamine.

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References: [1] Golman et al, MRM 2008, p1005 [2] Schroeder et al, Circ Cardiovasc Imaging 2012,p201 [3] Chen at al, NMR Biomed 2011, p305 [4] Moreno at al, Am J Physiol Heart Circ 2010,p H1556