Investigation of cardiac malate-aspartate shuttle at high workload using hyperpolarized [1,2-13C2]pyruvate

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Introduction: At high cardiac workload, the increase in carbohydrate oxidation plays an important role to meet the increased energy demand (1). It has been shown in hearts with adrenergic stimulation that transport of cytosolic reducing equivalent (NADH) produced by glycolysis into mitochondria via malate-aspartate shuttle is limited (2). This was observed as a lower isotopic enrichment of the cytosolic glutamate (resulting from mitochondrial efflux of -ketoglutarate that is part of the shuttle) pool in ^{13}C NMR of tissue extract from canine hearts perfused with [2-^{13}C]acetate and dobutamine. With the development of the DNP-dissolution technique, it is possible to monitor the production of -ketoglutarate-derived glutamate *in vivo* non-invasively using hyperpolarized [2-^{13}C]pyruvate (3). It has also been shown that with hyperpolarized [1,2-$^{13}C_2$]pyruvate, it may be feasible to investigate changes in glutamate production independent from modulations in pyruvate dehydrogenase complex in the same experiment (4). In this study, the potential of using hyperpolarized [1,2-$^{13}C_2$]pyruvate to monitor the limited transport of -ketoglutarate across mitochondrial membrane during high cardiac workload is examined.

Methods: Hardware and agent: All studies were performed using a 3T GE MR750 scanner (GE Healthcare) with a ^{13}C transmit volume coil and a dual-tuned 1H/^{13}C receive-only surface coil (Rapid Biomedical). A HyperSense DNP polarizer (Oxford Instruments) was used to polarize the substrate. Neat [1,2-$^{13}C_2$] pyruvic acid (Isotec) doped with 15mM of OX63 radical and 1mM Gd chelate (Prohance®, Bracco International) was polarized and dissolved with a NaOH/Tris buffered solution. *In vivo* MRS Experiments: Cardiac gated, dynamic ^{13}C MRS data was acquired from 3 normal pigs (~25 kg) with and without dobutamine (Sandoz Canada) stimulation in each animal, using a pulse-acquire pulse sequence (10° tip angle, TR=3-6R/R, ~2 s). For the experiments under high workload, the animals were infused with 40 g/kg/min dose of dobutamine for ~30 min prior to hyperpolarized ^{13}C substrate infusion. For all experiments, data acquisition started at the beginning of the ~15 s infusion of a solution of 15 ml/83 mM of pre-polarized [1,2-$^{13}C_2$] pyruvate. ^{1}H cine images were also acquired in the short-axis orientation through the heart to assess cardiac function at rest and high workload (at 20 min post dobutamine infusion).

Results and Discussion: A 2.2- and a 2.1-fold increase in ejection fraction and cardiac output respectively were observed with dobutamine stimulation using cine ^{1}H cardiac MR imaging. Representative ^{13}C spectra from a pig heart at rest and with dobutamine stimulation are shown in Fig.1 and signal amplitudes of all metabolites (relative to pyruvate) appeared to have increased at higher cardiac workload. The apparent changes in cardiac metabolism of ^{13}C pyruvate from the three pig studies are summarized in Fig. 2 (left) as metabolite to substrate ratios. Significant increases in both ^{13}C bicarbonate and [1-^{13}C]lactate were found at higher workload (Student's T-test, $P < 0.01$). Although an increase in [5-^{13}C]glutamate was also observed, this increase was smaller compared to the increase in ^{13}C bicarbonate, as indicated in the significantly lower glutamate / bicarbonate ratio at high workload (Fig 2., right). This observation agrees with the prior study using ^{13}C isotopomer analysis of cardiac tissue (2). If hyperpolarized [2-^{13}C]pyruvate (3) was used in this study, an apparent increase in -ketoglutarate to glutamate flux at high workload may have been interpreted from the result. Also, [1-^{13}C]pyruvate would demonstrate only an increase in PDC flux with stimulation (5). With hyperpolarized ^{13}C pyruvate labeled at both the C1 and C2 positions, changes in ^{13}C glutamate derived from mitochondrial -ketoglutarate could be assessed relative to the changes in PDC flux at different workload in this study.

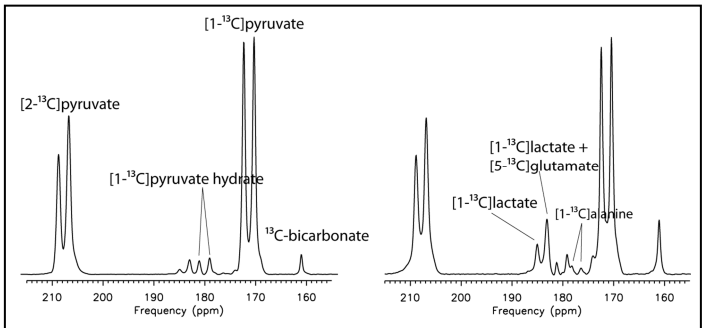

Fig 1. Representative hyperpolarized ^{13}C spectra from a pig heart in vivo without (left) and with (right) dobutamine stimulation.

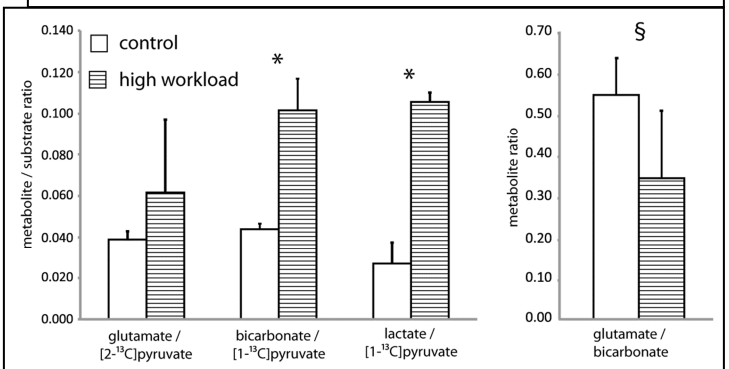

Fig 2. [5-^{13}C]glutamate, 13C bicarbonate and [1-^{13}C] lactate to substrate ratios (left) and [5-^{13}C]glutamate to ^{13}C bicarbonate ratios in ^{13}C cardiac studies at rest and under dobutamine stimulation (* $P < 0.01$); § $P < 0.05$).

Conclusions: This study demonstrated that changes in -ketoglutarate derived glutamate could be monitored non-invasively and independently from PDC flux using hyperpolarized [1,2-$^{13}C_2$]pyruvate at different cardiac workloads. A reduction in the glutamate to bicarbonate ratio may indicate a limited transport of reducing equivalent across mitochondria membrane via malate-aspartate shuttle at high cardiac workload.

References: 1. Goodwin, GW et al. *Am J Physiol Heart Circ Physiol.* 1998, 274: H1239 –H1247. 2. O'Donnell, MF et al. *Am J Physiol Heart Circ Physiol.* 2004, 286: H2237–H2242. 3. Schroeder, MA et al. *FASEB J.* 2009, 8:2529-38. 4. Chen, AP et al. *NMR Biomed.* 2012, 2:305-11. 5. Menichettia, L et al. *Contrast Media Mol Imaging.* 2012, 7:85–94.