NMR Detection of $^{13}CO_2$ and [^{13}C]Bicarbonate is Sensitive to the Duration of Reperfusion after Brief Myocardial Ischemia

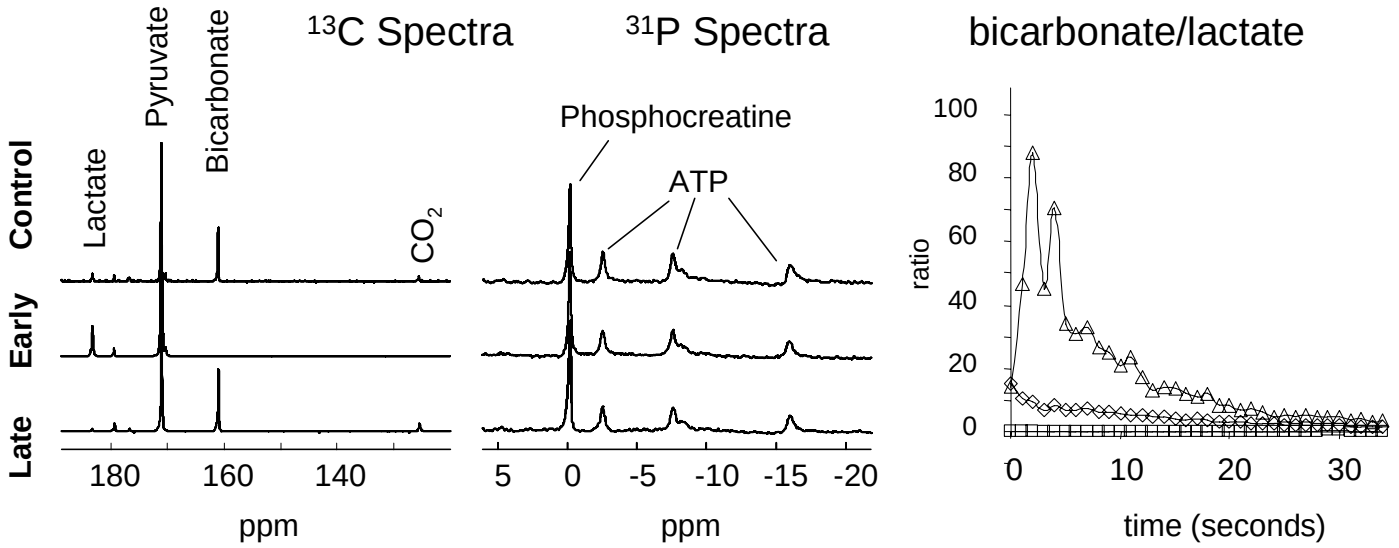
M. Merritt[1], C. Harrison[1], C. Storey[1], A. D. Sherry[1,2], and C. Malloy[1,3]

[1]Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, United States, [2]University of Texas at Dallas, Richardson, TX, United States, [3]VA North Texas Healthcare System, Dallas, TX, United States

Introduction: Increased oxidation of carbohydrates compared to fatty acids significantly improves outcomes after myocardial ischemia and heart attacks. Since oxidation of carbohydrates is largely controlled by pyruvate dehydrogenase (PDH), there is strong interest in altering flux through this enzyme by metabolic and pharmacologic means. Golman and colleagues introduced hyperpolarized (HP) [1-^{13}C]pyruvate as a metabolic imaging agent in a porcine model of myocardial infarction (1). Two hours after coronary occlusion, ^{13}C imaging following HP-[1-^{13}C]-pyruvate infusion showed a defect in the bicarbonate image consistent with impaired PDH flux. Here, PDH activity early and late after ischemia was assessed.

Methods: Rat hearts were perfused in a Langendorff mode using Krebs-Henseleit bicarbonate buffer bubbled with a 95/5 mixture of O_2/CO_2 and 2 mM pyruvate. Preparation of the HP[1-^{13}C]-pyruvate was accomplished using an Oxford Hypersense (2,3). Three different groups of hearts were studied by ^{31}P and ^{13}C NMR. Hearts in a control normoxic group were studied after administration of HP [1-^{13}C]pyruvate. Hearts in a second group were made ischemic for 10 minutes followed by reperfusion and simultaneous administration of HP[1-^{13}C]-pyruvate ("early"). A third group of hearts were made ischemic for 10 minutes, reperfused with pyruvate, and then switched to 2 mM HP [1-^{13}C]pyruvate after 20 minutes of reperfusion ("late"). ^{13}C NMR spectra were collected at 14.1 T using a 66 degree pulses with a 1 second acquisition time and no interpulse delay.

Results: During ischemia, [phosphocreatine] and developed pressure decreased dramatically. Typical ^{13}C spectra in control, early reperfusion and late reperfusion are shown below. Each spectrum is the sum of about 120 scans acquired over 2 minutes. Early after reperfusion the appearance of $^{13}CO_2$ and [^{13}C]bicarbonate was abolished in contrast to control and late reperfusion, and lactate was increased compared to control or late after reperfusion. ^{31}P NMR spectra acquired over 3 minutes for each time point were typical of isolated hearts. Notably, phosphocreatine and ATP were nearly normal in spectra gathered over the first 3 minutes of reperfusion. The ratio of bicarbonate to lactate in the ^{13}C spectra was essentially 0 early after reperfusion and significantly less than in control hearts or late after reperfusion.



Left panel. ^{13}C NMR spectra from hearts without ischemia, early after reperfusion and late after reperfusion. Bicarbonate and CO_2 were not detected early after reperfusion. **Middle panel.** ^{31}P NMR spectra acquired at the same time points (separate hearts) showing typical energetics of ischemia and very rapid recovery during the first 3 min of reperfusion. **Right panel.** The ^{13}C bicarbonate / ^{13}C lactate ratio. Triangles, late after ischemia; diamonds, control hearts; squares, early after ischemia.

Conclusion: Using hyperpolarized [1-^{13}C]pyruvate, PDH is active in normoxic hearts and recovers function within 20 minutes after transient ischemia. However, immediately after reperfusion flux through PDH is abolished consistent with inhibition by the reducing environment of ischemic tissue. Interventions designed to improve recovery after ischemia by increasing flux through PDH should focus on the very early period of reperfusion.

References: 1) Golman and Petersson. Acad Radiol. 2006; 13: 932-42. 2) Golman et al. Proc Natl Acad Sci U S A. 2006; 103: 11270-5. 3) Ardenkjaer-Larsen et al. Proc Natl Acad Sci U S A. 2003; 100: 10158-63.