

The Fate of Hyperpolarized [1-¹³C]Pyruvate During Substrate Competition Reveals Increased Bicarbonate as a Potential Biomarker for Decreased Fatty Acid Oxidation

Karlos X Moreno¹, A Dean Sherry¹, Craig R Malloy¹, and Matthew E Merritt¹

¹Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, United States

INTRODUCTION.

Development of diagnostic imaging methods for hepatic dysfunction and diseases such as diabetes or cancer is important for providing early detection and care for patients. Magnetic resonance imaging of hyperpolarized (HP) ¹³C is a metabolic imaging technique ideal for this application. The liver is characterized by very complex biochemistry. For instance, the appearance of HP H¹³CO₃⁻ and ¹³CO₂ from the administration of HP [1-¹³C]pyruvate could be the result of flux through one or more of three enzyme-catalyzed reactions: pyruvate dehydrogenase (PDH), phosphoenolpyruvate carboxykinase (PEPCK), or isocitrate dehydrogenase. Previous studies indicated that HP ¹³CO₂ was derived predominantly from PEPCK in the liver. Though some diseases, such as cancer, are caused by dysfunction at the gene level, may actually manifest through a metabolic biomarker, possibly by selective substrate utilization. Here we describe the influence of available substrates on relative flux through PEPCK and PDH in the liver.

METHODS.

Livers from C57bl6 mice were used under standard conditions. All protocols were approved by the UTSW IACUC. Livers were perfused for 30 min using one of 3 different bolus conditions or a steady-state condition. All pyruvate and lactate solutions for perfusions contained [3-¹³C]pyruvate and [3-¹³C]lactate. [1-¹³C]Pyruvate was hyperpolarized with an Oxford HyperSense DNP polarizer using the trityl radical. The HP [1-¹³C]pyruvate was injected at a 4 mM concentration over a period of 90 sec. The ¹³C NMR was carried out at 9.4 T using an Agilent VNMRS console. Conventional ¹³C NMR on tissue extracts were carried out at 14.1 T using an Agilent VNMRS console.

RESULTS.

A representative spectrum from an injection of HP [1-¹³C]pyruvate into an isolated perfused liver is shown in Figure 1a. As in typical HP experiments, HP [1-¹³C]lactate and [1-¹³C]alanine are observed. Four-carbon tricarboxylic acid cycle intermediates, [1-¹³C]aspartate, [1-¹³C]fumarate, [4-¹³C]aspartate, [4-¹³C]malate and [1-¹³C]malate, were also observed in all conditions studied as was HP ¹³CO₂ and H¹³CO₃⁻. Changing available substrates for liver metabolism modulates the appearance of HP ¹³CO₂ and H¹³CO₃⁻ (Figure 1b). A 3-fold increase in HP ¹³CO₂ and H¹³CO₃⁻ appearance was observed when no octanoate was present. Previous results have established that most of the HP ¹³CO₂ and H¹³CO₃⁻ observed would arise from PEPCK flux when octanoate (a freely diffusible medium chain fatty acid) is available. To confirm the increased HP ¹³CO₂ and H¹³CO₃⁻ appearance was from PEPCK flux, glutamate C4 resonances were analyzed from conventional ¹³C spectra of the liver tissue extracts (Figure 1c). Absent any octanoate, HP ¹³CO₂ and H¹³CO₃⁻ appearance was the result of PDH flux. Thus, showing substrate availability can affect pyruvate oxidation significantly. The clinical importance of these results show that possible hepatic fatty acid oxidation deficiency could be detected by increased bicarbonate production.

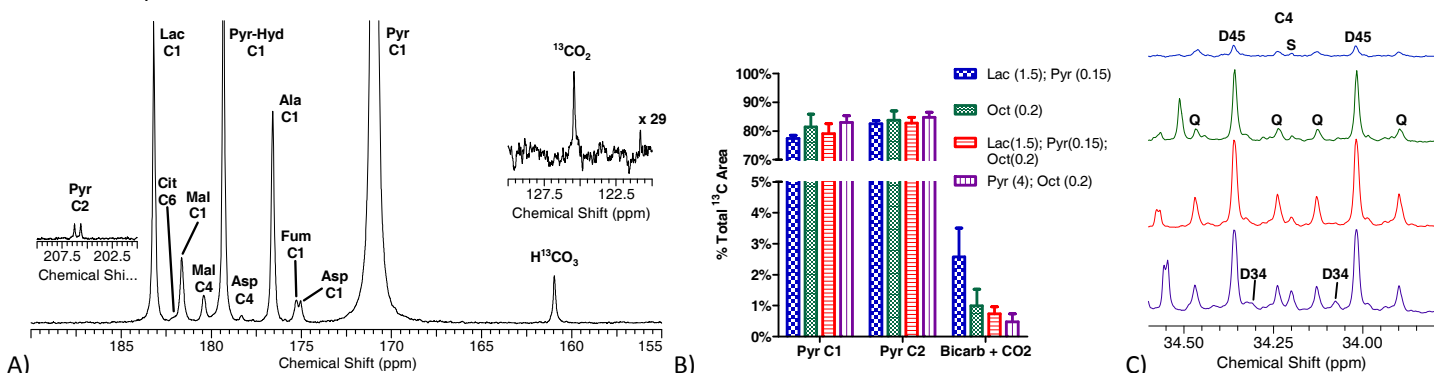


Figure 1. Effects of Substrate Availability in Hepatic Pyruvate Metabolism. A) Representative DNP ¹³C Spectrum (Sum of 50 scans). B) Changes observed with Changes in Substrate Availability. C) Glutamate C4 Resonances of Tissue Extracts. Top to Bottom: Lac (1.5):Pyr (0.5); Oct (0.2); Lac (1.5):Pyr (0.15); Oct (0.2); Pyr (4):Oct (0.2); (S = Singlet, D34 = Doublet due to C3-4 coupling, D45 due to C4-5 coupling, Q due to C3-4 and C4-5 coupling).