

Detection of altered hepatic gluconeogenesis by Nuclear Magnetic Resonance (NMR) and hyperpolarized ^{13}C pyruvate

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Introduction

Poor regulation of hepatic gluconeogenesis is a hallmark feature of obesity, insulin resistance and all forms of diabetes. Among approaches available to measure gluconeogenesis and other hepatic fluxes, MR in combination with stable isotope tracers is attractive because of its ability to detect positional enrichment patterns in individual metabolites. However, the poor sensitivity of MR compared to radioisotope or mass detection has limited its widespread application. Dynamic nuclear polarization (DNP) of NMR spins improves the sensitivity limits of MR by 10,000-fold or more and thereby allows one to consider experiments that were once impractical. Spectroscopy of hyperpolarized (HP) metabolic intermediates has been applied to a number of different tissues *ex vivo* and *in vivo* after exposure to HP ^{13}C pyruvate. However, only intermediates metabolically adjacent to pyruvate have been typically observed (lactate, alanine, bicarbonate) raising some concern about the utility of the approach in complex metabolic systems. For example, the metabolic fate of pyruvate in liver is largely anaplerotic rather than oxidative so detection of HP metabolites normally associated with mitochondrial metabolism is essential (**Figure 1A**). The purpose of this study was to determine whether detection of TCA cycle intermediates derived from HP ^{13}C pyruvate in liver is possible and whether these hyperpolarized intermediates are sensitive to hepatic gluconeogenesis.

Methods

Livers isolated from WT or liver specific PEPCK knockout mice were perfused through the portal vein in a 14T vertical bore NMR spectrometer equipped with an 18 mm BB probe tuned to ^{13}C . The perfusion media consisted of a standard Krebs-Henseleit buffer containing 1.5/0.15 mM lactate/pyruvate and 0.75 mM octanoate. DNP was used to hyperpolarize $[1-^{13}\text{C}]$ pyruvate and this solution was immediately added to the liver perfusate. Carbon-13 spectra were collected using a 66 degree pulse in 1 second increments just prior to injection and the carbonyl region was monitored for the polarization of hepatic metabolites over 60 seconds.

Results and Discussion

As anticipated, HP lactate and alanine were observed during the 60 seconds following perfusion with HP $[1-^{13}\text{C}]$ pyruvate (**Figure 1B**). Notably, four other polarized signals, presumably TCA cycle associated metabolites, were also observed in control livers during this period (**Figure 1C**). To determine whether the appearance of polarization in these intermediates is sensitive to gluconeogenesis, we performed experiments on livers from mice that lack the gluconeogenic enzyme PEPCK and thus cannot produce glucose from pyruvate. ^{13}C signals from these same HP intermediates were substantially suppressed and in most cases not observed at all in livers from PEPCK knockout mice. This observation is consistent with impaired anaplerotic flux of HP $[1-^{13}\text{C}]$ pyruvate into the TCA cycle and the absence of gluconeogenesis. Interestingly, a signal from HP bicarbonate was largely lacking in perfused livers, consistent with low hepatic PDH activity in this tissue.

Conclusions

These data demonstrate that ^{13}C spectroscopy can be used to monitor production of hyperpolarized TCA cycle intermediates derived from HP $[1-^{13}\text{C}]$ pyruvate in perfused liver. This is significant because these metabolites have not been detected in prior HP studies and it establishes that the hepatic pathways of anaplerosis and gluconeogenesis can be monitored by ^{13}C spectroscopy after administration of HP $[1-^{13}\text{C}]$ pyruvate.

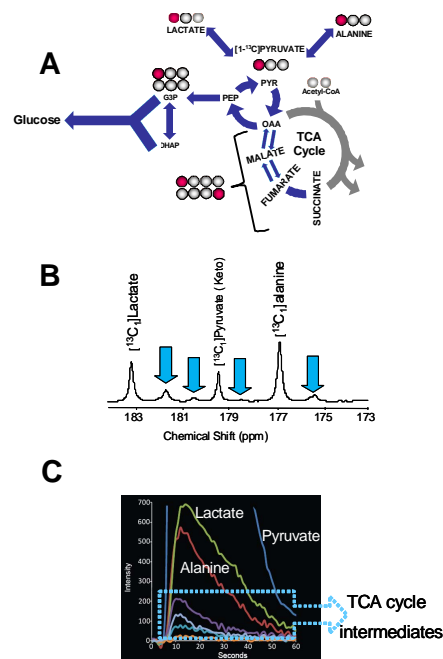


Figure 1. Gluconeogenic flux in liver can be detected by hyperpolarized ^{13}C pyruvate. **A)** $[1-^{13}\text{C}]$ pyruvate enters the TCA cycle through pyruvate carboxylase and will become labeled in C1 or C4 of dicarboxylic acid intermediates. **B)** This enrichment can be observed in the carbonyl region of ^{13}C spectra acquire from the liver after perfusion of HP ^{13}C pyruvate. **C)** The time course of polarization in these intermediates are similar to the appearance of lactate and alanine.