Metabolism of Hyperpolarized [1-^{13}C]pyruvate to Plasma Glucose in the Rat Liver

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Target Audience: Clinicians and investigators using hyperpolarized ^{13}C for monitoring metabolism.

Purpose: Metabolism of hyperpolarized (HP) [1-^{13}C]pyruvate in the liver generates HP [^{13}C] bicarbonate. The appearance of HP [^{13}C] bicarbonate has been interpreted as evidence of flux through alternative pathways. One possibility is pyruvate carboxylation followed by randomization in the fumarate / succinate pool and subsequent decarboxylation of carbon 4 in oxaloacetate by phosphoenolpyruvate carboxykinase (PEPCK), the key enzyme regulating gluconeogenesis (1). The second possibility is flux through pyruvate dehydrogenase, a pathway not directly linked to gluconeogenesis (2). HP technology is being developed to image flux in gluconeogenic pathways, a capability that would open new applications in clinical research. To achieve this goal it is important to understand the sources of HP [^{13}C] bicarbonate in the liver, but analysis of pyruvate metabolism is difficult because of possible metabolism through both pathways simultaneously. The analysis of ^{13}C labeling patterns in plasma glucose during metabolism of ^{13}C-labeled octanoate, pyruvate, propionate or other substrates is a powerful and essentially non-invasive tool for measuring flux through these pathways in animals and humans. However it is not known whether the HP [1-^{13}C]pyruvate significantly labels the plasma glucose pool. This study was designed to determine if the injected HP [1-^{13}C]pyruvate is converted to plasma glucose, providing unequivocal evidence for flux through PEPCK under the conditions of the HP experiment, and to assess whether the ^{13}C labeling pattern in plasma glucose is sensitive to the injected HP [1-^{13}C]pyruvate.

Methods: Sprague-Dawley rats (280-350 g) were studied under a protocol approved by the local Animal Care Committee. Two groups were examined: 1) Fasted, infused intravenously for > 30 min with [3-^{13}C]pyruvate (4.8 mmol/kg/hr) and [U-^{13}C]octanoate (0.9 mmol/kg/hr); 2) Fed, infused for > 30 min with saline. [1-^{13}C]Pyruvate was hyperpolarized with an Oxford HyperSense DNP polarizer using the trityl radical. After a transfer delay of ~ 30 sec, hyperpolarized [1-^{13}C]pyruvate (2.5 mL, 80 mM) was injected *via* jugular vein over a period of 60 sec. ^{13}C NMR spectra were collected with a 15 cm birdcage volume coil (transmit/receive for 1H, transmit only for ^{13}C), a 4 cm ^{13}C surface receiving coil using a 20mm single, axial slice on rat liver, 12 cm FOV, 20 deg pulse, TR = 2 s, 5 kHz bandwidth and 120 acquisitions for a total time of 4 min on a GE 3T 750W. Blood plasma was collected and the livers were excised and freeze-clamped. Blood glucose was purified and converted to monoacetone glucose (MAG). Metabolites from livers were extracted by perchloric acid and the acid-soluble fraction was isolated. The MAG and the liver extract were studied by conventional ^{1}H decoupled ^{13}C NMR at 14.1 T. If [1-^{13}C]pyruvate contributes to gluconeogenesis, then excess ^{13}C should appear in plasma glucose carbons 3 and 4. Excess ^{13}C enrichment was measured relative to an internal standard.

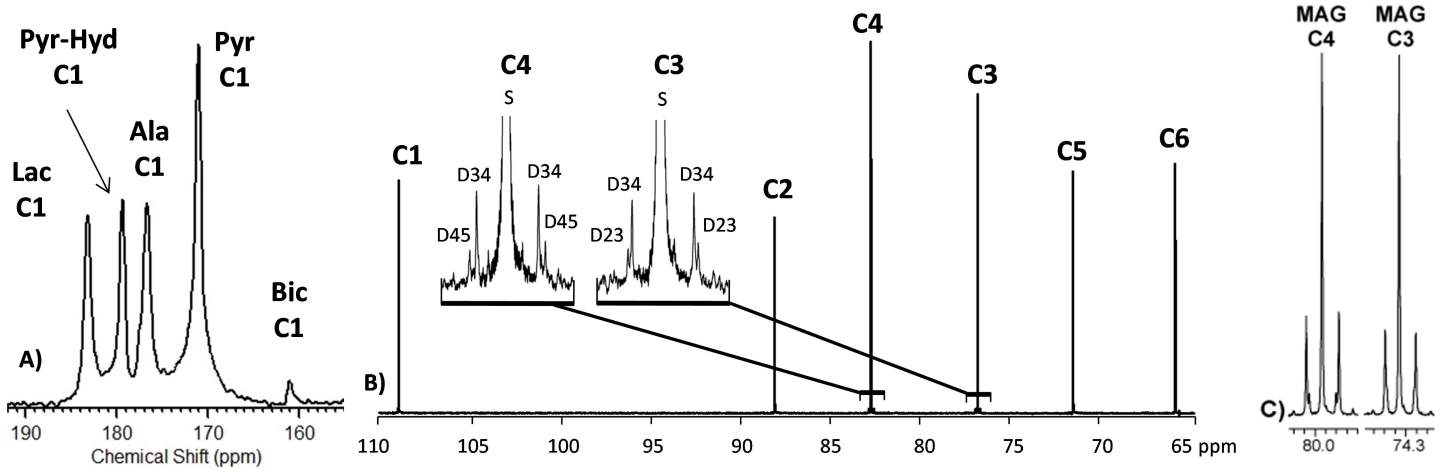

Figure 1. A) Representative ^{13}C spectrum (sum of 60 scans) from the liver of a fed rat after i.v. injection of HP-[1-^{13}C]pyruvate. B) ^{1}H decoupled ^{13}C NMR spectrum of MAG from plasma glucose of a fed rat. Excess labeling in position 3 and 4 arises from the injected HP-[1-^{13}C]pyruvate. C) ^{1}H decoupled ^{13}C NMR spectrum of MAG carbon 3 and carbon 4 from plasma of a rat infused with [3-^{13}C]pyruvate and [U-^{13}C]octanoate. These multiplets arise from metabolism of infused [3-^{13}C]pyruvate and [U-^{13}C]octanoate prior to and during the HP experiment. Abbreviations: D23, D34 and D45 refer to doublets due to ^{13}C-^{13}C J coupling.

Results and Discussion: Figure 1A shows ^{13}C spectra collected from the liver of a fed rat after injection of HP-[1-^{13}C]pyruvate. Resonances from [1-^{13}C]pyruvate, [^{13}C]bicarbonate, [1-^{13}C]alanine, [1-^{13}C]lactate and [1-^{13}C]pyruvate-hydrate were easily detected. In the animals infused with saline in addition to HP-[1-^{13}C]pyruvate, MAG spectra (Figure 1B) showed a small excess ^{13}C in carbons 3 and 4 (0.23% ± 0.15% (s.d.); 3 animals, 6 enrichment measurements). In addition, the observation that spin-spin coupling can be detected in both resonances (labeled D34) indicates that at least some of the glucose was derived from two molecules of [1-^{13}C]pyruvate. Both observations demonstrate that at least some of the original HP-[1-^{13}C]pyruvate was converted to plasma glucose via PEPCK. In fasted animals infused with [U-^{13}C]octanoate and [3-^{13}C]pyruvate, conditions designed to maximize gluconeogenesis from pyruvate, extensive ^{13}C-^{13}C spin-spin coupling was observed in the freeze-clamped livers (data not shown), demonstrating oxidation of both pyruvate and octanoate in the citric acid cycle (the ratio of octanoate:pyruvate oxidation was ~ 3:1). Plasma glucose isolated from these same animals (Figure 1C) was highly enriched (>10%) with ^{13}C demonstrating that gluconeogenesis was indeed active under these conditions.

Conclusions: Some HP-[1-^{13}C]pyruvate acts as a carbon source for gluconeogenesis in rat liver. This indicates that pyruvate carboxylation and subsequent decarboxylation of OAA via PEPCK is highly active in the intact liver and consistent with the appearance of HP-bicarbonate in the in vivo ^{13}C NMR spectrum (Figure 1A). These observations do not exclude a contribution from flux through PDH. The level of ^{13}C enrichment detected in plasma glucose in these animals after injection of a single bolus of HP-[1-^{13}C]pyruvate is low but consistent with the large amount of glucose already present in the animal prior to injection of HP-[1-^{13}C]pyruvate. This study demonstrates that a combination of hyperpolarized metabolic imaging plus conventional ^{13}C NMR of plasma glucose is a powerful approach to examine hepatic metabolism *in vivo*. Labeling of plasma glucose from the injected [1-^{13}C]pyruvate could be even higher under other metabolic conditions where gluconeogenesis is thought to be enhanced (i.e., diabetes) so this direct pathway should be considered when performing a more complete ^{13}C isotopomer analysis of plasma glucose (3).

References: 1) Merritt et al. PNAS 2011; 108: 19084-19089. 2) Lee et al. Hepatology 2013; 57: 515-524. 3) Jones et al. FEBS Lett. 1997; 412: 131-7.