Metabolism of Hyperpolarized [1-^{13}C]Pyruvate in Isolated Perfused Mouse Livers – A Comparison of Fed and Fasted States

B. M. Pullinger[1], S. J. Kadlecek[1], H. Chen[2], Q. Chu[2], N. N. Kuzma[1], and R. R. Rizi[1]

[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, United States

INTRODUCTION: Much current research in hyperpolarized ^{13}C NMR is focused around the Langendorff perfused heart model,[1] owing to the control, specificity, and high SNR achievable with a perfused organ at high field. Similarly to the heart, a liver can be isolated, perfused, and kept viable for the duration of NMR study, and is of considerable physiological interest due to its pivotal role in regulation of energetic metabolism. It has been established for some time that the hepatic processes of lipogenesis (LG) and gluconeogenesis (GNG) hold a reciprocal relationship – one being upregulated while the other is downregulated – that depends upon whether the liver is in a fed or fasted state. LG requires acetyl-CoA, which in a fed state comes predominantly from the oxidative decarboxylation of pyruvate by pyruvate dehydrogenase (PDH). In a gluconeogenic state, pyruvate may be diverted through pyruvate carboxylase (PC) to oxaloacetate, which can in turn leave the TCA cycle when it is converted to phosphoenolpyruvate in the first step of GNG. In insulin resistance (precursor to Type II diabetes), the reciprocal relationship of GNG and LG is violated, and both processes are simultaneously active[2]. Consequently, the molecular fate of pyruvate – conversion by PDH or PC – serves as an interesting point of study, since the mechanism by which insulin regulates these enzymes is not yet fully understood.[3]

METHODS: _Animal Handling_: Male C57BL6 mice (8-10 wks) were used for all experiments. In the fed group (n=4), the mice were fasted overnight and re-fed standard chow _ad libitum_ for 2-3 hrs prior to the experiment. The livers were perfused via portal vein with a modified Krebs-Henseleit buffer containing 15 mM glucose, 100 nM insulin, and either 3% or 0.5% w/v BSA (n=2 each). In the fasted group (n=2), the animals were not re-fed, and the perfusion media contained 5.5 mM glucose and no insulin. ^{31}P spectra were taken 10-15 min before and after pyruvate injection to assess viability.

Hyperpolarization and ^{13}C NMR: [1-^{13}C]pyruvic acid was polarized to ~30% in a HyperSense DNP polarizer. This sample was dissolved in ~4 mL of a Tris-buffered isotonic solution, and the resulting 75 mM sodium pyruvate solution was diluted in 25 mL of oxygenated perfusion medium (without BSA). The flow of recirculating perfusion medium was temporarily stopped, and 10 mL of the hyperpolarized sodium pyruvate solution (9 mM, pH 7.4) was injected at a rate of 7.0 mL/min. ^{13}C NMR acquisition began before the arrival of injected pyruvate. Spectroscopy was performed with a 20-mm Varian ^{1}H/X probe in a 9.4-T vertical bore magnet, and nominal 90° pulses were acquired every 4 sec. A phantom experiment was performed according to the above protocol in the absence of a liver to determine which resonances belonged to impurities in the hyperpolarized pyruvate. Arrayed spectra were processed with a custom fitting routine written in MatLab.

RESULTS: _^{31}P NMR:_ Spectra indicated stable bioenergetics for over 3.5 hours of perfusion, and no significant perturbation in ATP:ADP ratio was observed after the injection. Fed livers exhibited a large 3-phosphoglycerate peak (Figure 1), which
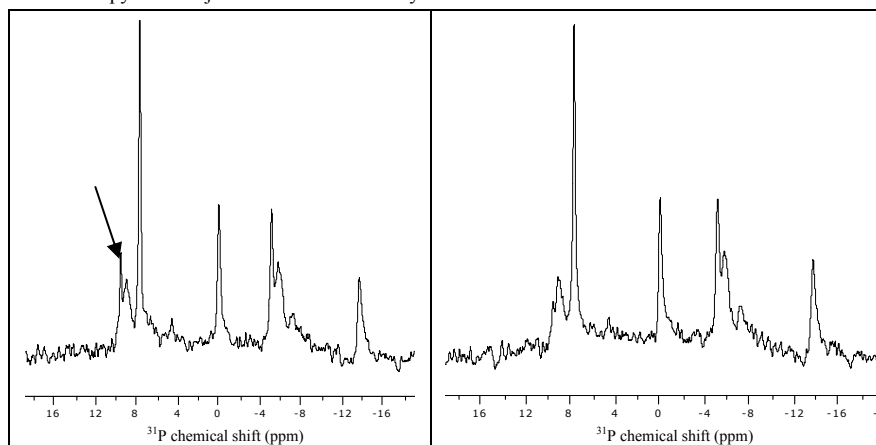
decreased greatly after injection, most likely due to inhibition of glycolysis by the introduction of the large pyruvate bolus. Additionally, there were no detectable differences for livers perfused with 0.5% and 3% BSA. _^{13}C NMR:_ Lactate and alanine were observed with high SNR, and despite low PDH activity, the evolution of bicarbonate was quantifiable. Additional metabolites were observed at 10.7, 9.5, 7.5, and 3.3 ppm downfield of [1-^{13}C]pyruvate. The latter two peaks were too small to accurately quantify, and could not be unambiguously assigned. The first two peaks were assigned to [1-^{13}C]- and [4-^{13}C]malate, respectively. Because conversion of [1-^{13}C]pyruvate by PDH truncates the label, the combination of these peaks was taken to represent PC flux. The label at both positions indicates that the label traveled as far as fumarate. Perfusion can differ from one liver to another, and it is impossible to differentiate free pyruvate in the NMR tube from intracellular pyruvate. Therefore, metabolism was quantified by comparison between metabolites, and the time courses of individual metabolites were parameterized by calculating the area under the curve (AUC). The ratio of AUC for the malate peaks to bicarbonate peak (MAL-1 + MAL-4 / HCO$_3^-$) did not

Figure 1. ^{31}P spectra before (left panel) and after (right panel) pyruvate injection into a fed perfused liver. The 3-phosphoglycerate peak is denoted by the arrow.
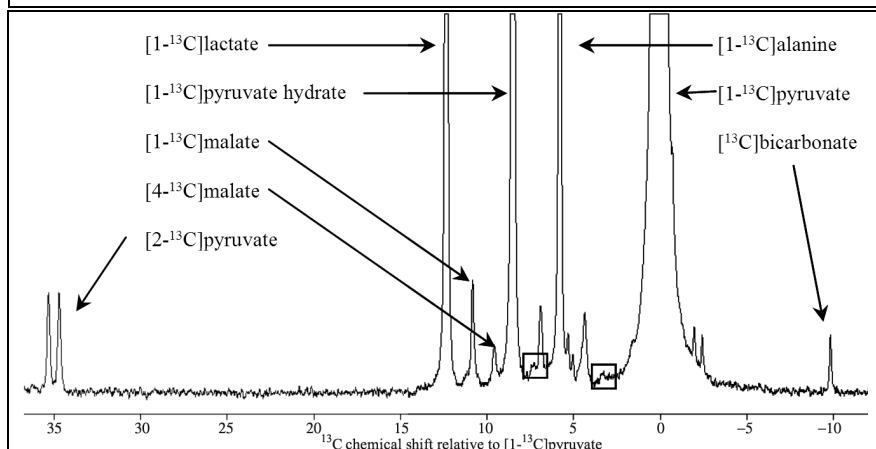

Figure 2. Representative ^{13}C spectrum after pyruvate injection, averaged over 35 individual scans. Unidentified metabolites are boxed, while the remaining peaks are identified as impurities.

differ significantly between the two groups, nor did the ratios of bicarbonate AUC to lactate or alanine (HCO$_3^-$/LAC or HCO$_3^-$/ALA). However, the ratio of the combined malate AUCs to that of lactate (MAL-1 + MAL-4 / LAC) showed an increase from 0.17 ± 0.03 in the fed livers to 0.30 ± 0.06 in the fasted livers, which corresponds to an expected increase in PC activity to support GNG. Fed and fasted states are known to have similar redox ratios,[4] so lactate production upon the introduction of excess pyruvate should be similar for both states. We therefore believe that comparison to lactate provides a helpful normalization.

CONCLUSION: We have shown here the feasibility of using hyperpolarized spectroscopy to simultaneously probe the metabolism of [1-^{13}C]pyruvate by PDH and PC in the perfused mouse liver. This bifurcation is an important point of regulation for GNG and LG, two processes deeply implicated in the pathophysiology of diabetes. In our comparison of fed and fasted livers, we saw evidence of increased PC flux in fasted livers, which is in accordance with upregulated GNG in the fasted state. This demands much more study, but the suggestion of quantifiable differences in the flux is promising for this yet unreported route of hyperpolarized pyruvate metabolism.

REFERENCES: [1] Schroeder et al., _Cardiovasc. Res._ **86** 82-91 (2010); [2] Shimomura et al., _Mol Cell_ **6** 77-86 (2000); [3] Laker and Mayes, _Biochim. Biophys. Acta_ **794** 427-430 (1984); [4] Söling et al., _Biochim. Biophys. Acta_ **115** 1-14 (1966).