# Hepatic Metabolism of Hyperpolarized [1-$^{13}$C]Pyruvate in the Zucker Rat

Jian-Xiong Wang[1,2], Leila Fidelino[3], Karlos Moreno[3], A. Dean Sherry[3,4], Craig Malloy[3,5], and Matthew E Merritt[1,6]

*[1]AIRC, UT Southwestern Medical Center, Dallas, TX, United States, [2]Radiology, UT Southwestern Medical Center, Dallas, TX, United States, [3]AIRC, UT Southwestern Medical Center, TX, United States, [4]Chemistry, University of Texas at Dallas, TX, United States, [5]Internal Medicine, UT Southwestern Medical Center, TX, United States, [6]Radiology, UT Southwestern Medical Center, Dallas, United States*

## Target Audience

Investigators interested in hepatic gluconeogenesis and hyperpolarized $^{13}$C metabolic imaging.

## Purpose

Hepatic dysfunction is often characterized by abnormal energy metabolism. The Krebs cycle provides reducing equivalents needed for generation of ATP. Subsequently, ATP can be utilized to provide the energy for the first committed step of gluconeogenesis (GNG), flux through phosphoenolpyruvate carboxykinase (PEPCK). Previously, we have shown in the perfused mouse liver that PEPCK can be the source of hyperpolarized (HP) [$^{13}$C]bicarbonate when presented with HP [1-$^{13}$C]pyruvate (1). However, subsequent experiments *in vivo* showed that pyruvate dehydrogenase (PDH) is the primary source of [$^{13}$C]bicarbonate (Figure 1, top) (2) in fed and fasted rats. Zucker (fa/fa) rats are characterized by morbid obesity, insulin resistance and high glucose production. We hypothesized that the abnormal metabolism of these rats might lead to increased PEPCK flux *in vivo*, and that these animals might serve as a convenient model of human diabetes mellitus for pyruvate imaging. To test this hypothesis, Zucker rats were imaged in a 3 T GE scanner using HP [1-$^{13}$C]pyruvate.

## Experimental Methods

All experiments were carried out with protocols approved by the UTSW Animal Care and Use Committee. Animals were prepositioned inside a 6 cm rat coil (General Electric, Waukeesha, WI) in a General Electric MR 750W 3T scanner. Isoflurane gas was used for anesthesia and respiration was monitored using a small animal monitoring system from SAI (Stonybrook, NY). Samples of [1-$^{13}$C]pyruvic acid and 15 mM trityl radical were placed in a homebuilt polarizer operating at 129 GHz ESR frequency and 1.2 K. The sample was dissolved with 4 mL of phosphate buffer with a resultant pH of ~7. Five seconds prior to initiation of imaging, 3 mL of 80 mM [1-$^{13}$C]pyruvate was injected via a tail vein catheter. A slice selective spectroscopy protocol using a 15 degree pulse was applied every 3 seconds to acquire the $^{13}$C kinetic data. A $^{13}$C IDEAL sequence was used to acquire images with ~2 mm in-plane resolution. The IDEAL sequence used 6 excitations with a 7$^{o}$ flip angle for each.

## Results and Discussion

GNG in the liver is a primary target for development of diabetes drugs. While hepatic GNG can be measured using a variety of stable isotope methods, a cost effective real time *in vivo* method for assessing hepatic glucose production is not available. This driving need was the focus of the project. After injection of HP [1-$^{13}$C]pyruvate, [$^{13}$C]bicarbonate production was similar between the fed and fasted condition. This was not the case in lean animals where HP [$^{13}$C]bicarbonate was not detected in the fasted state presumably due to increased levels of circulating fatty acids. The appearance of HP-bicarbonate in fasted Zucker rats most likely reflects an increase carboxylation of [1-$^{13}$C]pyruvate followed by an enhanced flux through PEPCK (Figure 1, top). Alternatively, increased hepatic lipogenesis may be driving increased PDH flux in the liver even in the case where peripheral fatty acid stores have been liberated by fasting. The separate observation of a much stronger HP [1-$^{13}$C]lactate signal likely reflects higher levels of NADH in the fasting Zucker rat, a subject that will be investigated.

## Conclusions

Generation of [$^{13}$C]bicarbonate from HP [1-$^{13}$C]pyruvate is not altered by fasting in Zucker rats suggesting that GNG from the TCA cycle continues in both the fed and fasted states. Future experiments using [2,3-$^{13}$C]pyruvate as a stable isotope tracer will be used to identify relative flux through PC and PDH in the Zucker rat. In this commonly accepted animal model of type II diabetes, the appearance of [$^{13}$C]bicarbonate may indeed reflect liver GNG.

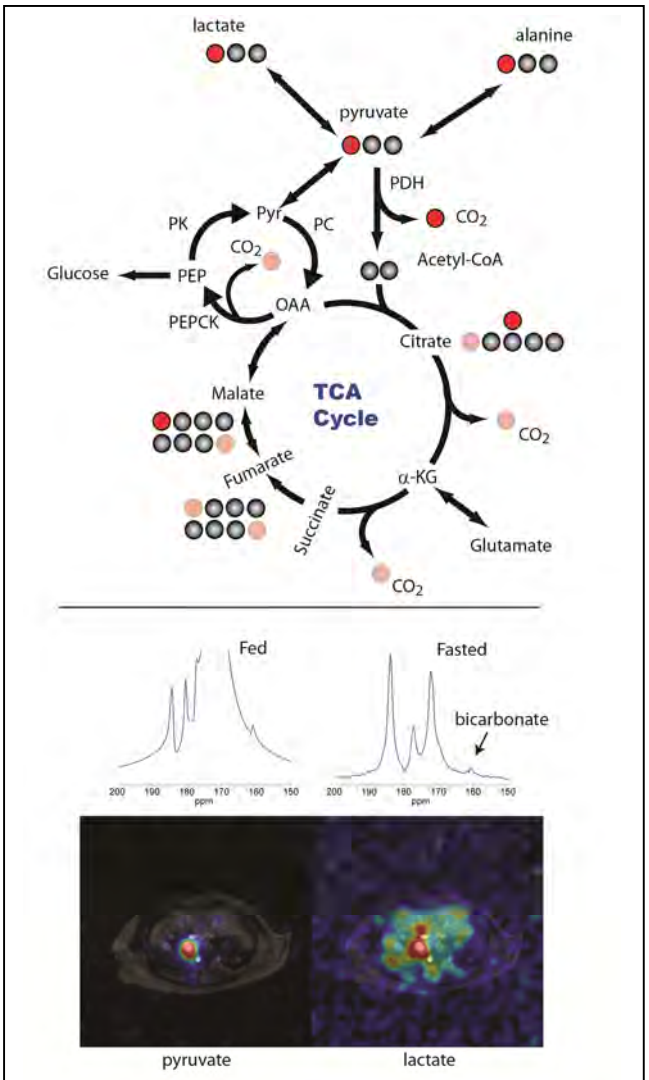


**Figure 1 top)** [1-$^{13}$C]pyruvate can produce [$^{13}$C]bicarbonate by multiple pathways in the liver. **middle)** Summed spectra from fed and fasted Zucker rat liver after injection of a bolus of HP [1-$^{13}$C]pyruvate. These data were acquired using a slice selective spectroscopy protocol. Note that while bicarbonate production is not altered by fasting, the much higher lactate signal in livers of fasted Zucker rats reflects a dramatic change in metabolic state in this animal model. **bottom)** Carbon-13 IDEAL images (axial slice through liver) of pyruvate and lactate in the fed animal.

## References

1. Merritt ME, Harrison C, Sherry AD, Malloy CR, Burgess SC. PNAS. 2011;108(47):19084-9. doi: 10.1073/pnas.1111247108; PubMed Central PMCID: PMCPMCID: PMC3223470.

2. Moreno KX, Jin ES, Wang J-X, Sherry AD, Merritt ME, Malloy CR, editors. Proc Intl Soc Mag Reson Med; 2014; Milan.