Observing Gluconeogenesis in Real-Time in the Zucker Rat Using Hyperpolarized [2-^{13}C]Dihydroxyacetone

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Target Audience

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

Purpose

Recently we have shown that hyperpolarized (HP) [2-^{13}C]dihydroxyacetone (DHA) can readily distinguish between glycogenolytic and gluconeogenic states in the perfused mouse liver (1). DHA has been used for nearly 90 years to assay liver function using an oral tolerance test and is safe given in 50 g quantitites for humans (2). Immediately after entry into the cytosol, DHA is phosphoylated to dihydroxyacetone phophate and rapidly exchanges into the triose phosphate pool. Subsequently, the hyperpolarized species transits multiple sites of enzymatic regulation, though only pyruvate kinase exerts control over the kinetics on the time scale of the HP experiment. To assess the suitability of HP DHA for the evaluation of gluconeogenesis (GNG) *in vivo*, its metabolism was monitored in a Zucker rat model of obesity and insulin resistance using a GE 3T MR scanner.

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 [2-^{13}C]dihydroxyacetone in a water/glycerol matrix and 15 mM trityl radical were placed in a homebuilt polarizer operating at 129 GHz ESR frequency and 1.2 K. The sample was polarized for ~1.5 hours and dissolved with 4 mL of phosphate buffer with a resultant pH of ~7. Five seconds prior to acquisition, 3 mL of 100 mM [2-^{13}C]DHA was injected via a tail vein catheter. A slice selective spectroscopy protocol using a 25 degree pulse was applied every 3 seconds to acquire the ^{13}C kinetic data.

Results and Discussion

The precursors of hepatic GNG are normally glycerol and phosphoenolpyruvate (PEP) generated from oxaloacetate, a Krebs cycle intermediate. Spectra acquired after injection of HP DHA show rapid labeling of both upstream and downstream metabolites including PEP and glucose. As was the case in the perfused liver, PEP and glucose appear concurrently, indicating that the multiple reaction steps between glyceraldehde-3-phosphate, glucose, and PEP are all fast *in vivo* as well as in the perfused liver. Previously, these pathways could only be probed simultaneously with separate isotopically labeled compounds. This experimental necessity left questions about differential uptake of the tracers and the impact it might have on the flux measurements open. The method proposed here has no such complication, proving in detail simultaneous, bidirectional flux in the liver. Future imaging experiments should be able to address differential uptake of DHA in the various lobules of the liver and its possible impact on its fate. We cannot exclude the possibility that multiple pools may exist within the cell or that uptake and utilization of DHA may depend upon the distance of the cell from the vasculature. Future experiments will also probe the effects of hormones on DHA metabolism.

Conclusions

Increased hepatic GNG is a hallmark of insulin resistance and the onset of diabetes. Glucose production can be readily visualized in the Zucker rat liver using HP [2-^{13}C]DHA. The lack of chemical shift dispersion at 3 T suggests that experiments at higher field strengths will yield more biological information, though simple metrics like the ratio of PEP to glucose might yield substantial information about the relative rates of glycolysis, glycogenolysis, and GNG.

References

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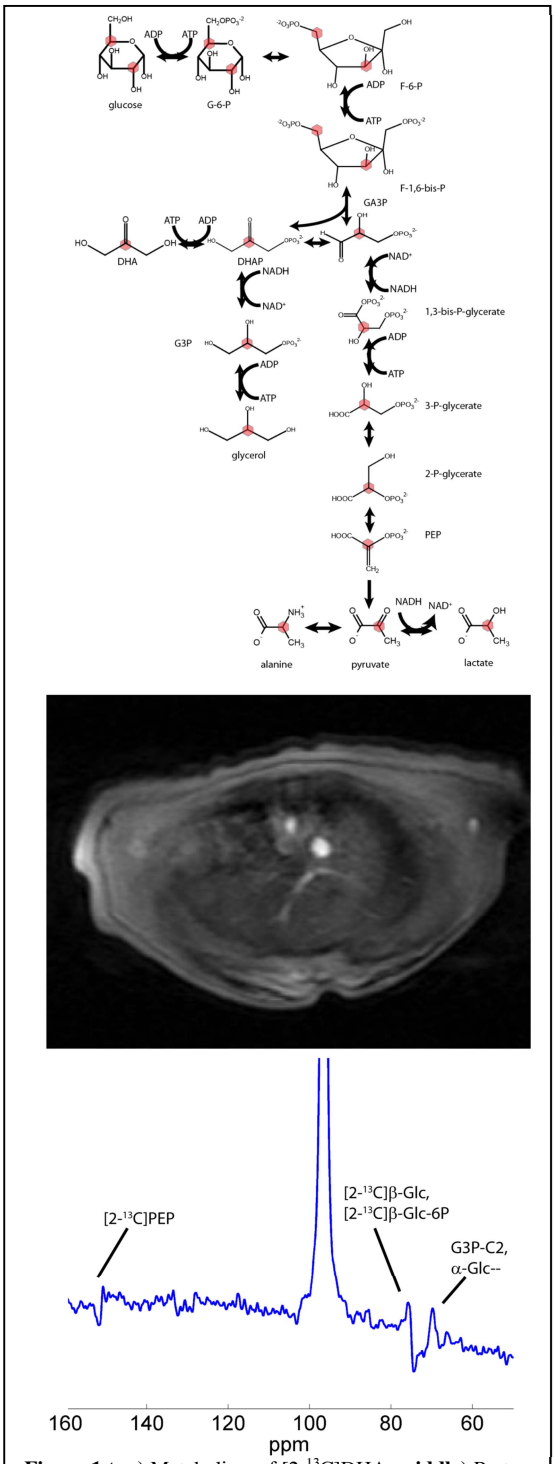

Figure 1 top) Metabolism of [2-^{13}C]DHA. **middle)** Proton planning image through the rat liver. **bottom)** Spectrum showing detection of upstream and downstream metabolites of DHA *in vivo*. Many metabolites overlap in the ^{13}C spectrum at 3 T. Nonetheless, products of DHA phosphorylation (glycerol-3-phosphate, G3P), glycolysis (phosphoenolpyruvate, PEP), and gluconeogenesis (glucose-6-phosphate and glucose), all appear.