In Vivo Carbon-13 Dynamic MRS and MRSI of Rat Liver with Hyperpolarized ^{13}C-1-Pyruvate

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Introduction: Development of methods to achieve high polarization of nuclear spins in liquid state (greater than 50,000-fold enhancement relative to thermal equilibrium) [1] has enabled the direct monitoring of ^{13}C metabolites in vivo, allowing for rapid assessment of tissue metabolism. Non-localized dynamic spectra from normal rats have been published [2,3]. The goal of this study was to obtain hyperpolarized ^{13}C dynamic spectra localized to the liver to measure characteristics such as time to peak for the various metabolites. Additionally, we conducted initial comparisons between non-fasted and fasted rats. We observed differing alanine metabolism in the liver, which agrees with prior biochemical studies showing elevated levels of alanine aminotransferase (ALT) during fasting in rats [4]. This study demonstrates that hyperpolarized ^{13}C MR can detect significant changes in liver metabolic states in vivo and may be useful in studying liver disease.

Methods: ^{13}C-1-pyruvate was hyperpolarized using an Oxford Instruments HyperSense™ system (~18% polarization) and was injected through a rat tail vein catheter over a 12s duration. Injections of ~3ml of ~80mM hyperpolarized pyruvate were given to all rats. Non-fasted rats were allowed to feed freely while fasted rats had their food supply removed ~24 hours before data acquisition. All experiments were performed on a GE 3T system using a custom dual-tuned ^{1}H/^{13}C RF coil. A slice selective (15mm slab select centered on the liver) RF pulse with 5º flip angle was applied every 3s starting with the injection. The collected data, processed using MATLAB, were apodized with a 10 Hz Lorentzian filter before Fourier transformation, and the dynamic data points were taken from magnitude spectra. 3D acquisitions were performed using a double-spinecho sequence [5] with variable flip angle, centric phase encoding order, TE = 140 ms, TR = 215 ms (total acquisition time of 14 s), FOV = 8x8cm, and 1cc resolution.

Results: Figure 1 shows an axial T2-weighted image corresponding to a typical slice from which liver dynamic data were taken. Figure 2 shows both a dynamic plot from non-fasted liver (representative plot, with similar results seen for repeat cases) and a previously unreported/unpublished dynamic plot centered at the level of the kidney. Representative liver/kidney voxels from a 3D acquisition are also shown in Figure 2. The purpose of the comparison in Figure 2 is to establish that liver/kidney metabolism and dynamics are indeed different. These differences are important for future liver studies, e.g. time to peak for metabolites is crucial for determining an imaging time window for voxel localized studies that will maximize SNR. Figure 3 shows a liver dynamic plot from a fasted rat. Note that the pyruvate line in each dynamic plot has been scaled down by a factor of 4 for easier viewing, and data points for the first 90 seconds after injection are shown. Figure 4 shows a comparison of the peak lactate-to-alanine ratio from the liver data collected in this study (n = 5 for both groups, P < .01 using a Mann-Whitney Rank-Sum test).

Discussion: As shown in Figure 2, one noticeable difference between liver and kidney dynamic plots is the delayed peak/plateau time for lactate and alanine, meaning the best imaging window for liver hyperpolarized ^{13}C MRSI studies is different than that for kidney. A clear difference between the non-fasted and fasted dynamic plots is the lactate to alanine ratio. In non-fasted liver dynamic spectra, the lactate and alanine levels were similar. For the fasted rat liver dynamic spectra, lactate signal intensity was much higher than that of alanine. Such a change in the lactate to alanine ratio could be caused by a decrease in alanine due to heightened ALT levels. During fasting, ALT levels in rats have been shown to increase, promoting the use of alanine as a gluconeogenic substrate and its conversion to pyruvate for eventual glucose generation [4]. ALT levels are particularly elevated in rats after 2 days of fasting [4]. The dynamic liver data prompted us to acquire 3D localized MRSI data to compare 1cc liver voxels in fasted/non-fasted rats (Figure 5), which showed a decrease in alanine relative to lactate and pyruvate. In the limited number of non-fasted and fasted 3D MRSI acquisitions we performed, we noticed consistently lower alanine area to total carbon area ratios in fasted rat liver, while lactate area to total carbon area ratios showed no significant differences. Note that alanine area is defined as the area under the alanine peak for liver voxels with little partial voluming. Similarly, lactate area applies to the area under the lactate peaks, and total carbon refers to the sum of alanine, lactate, pyruvate, and pyr-H_2O areas.

In summary, we have demonstrated for the first time the ability to use hyperpolarized ^{13}C MR to observe significant changes in liver metabolic state due to fasting.

References: [1] Ardenkjaer-Larsen et al, Proc Natl Acad Sci USA, 100:10158-10163(2003). [2] Kohler et al, Magn Reson Med 58:65-69(2007). [3] Golman et al, Proc Natl Acad Sci USA, 103:11270-11275(2006) [4] Rosen et al, J Bio Chem, 234:476-480(1959) [5] Cunningham et al, J Magn Reson 187:357-362(2007)

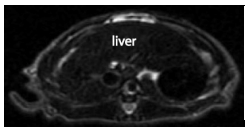

Figure 1. Axial T2-weighted image corresponding to a typical slice from which liver dynamic data were taken.

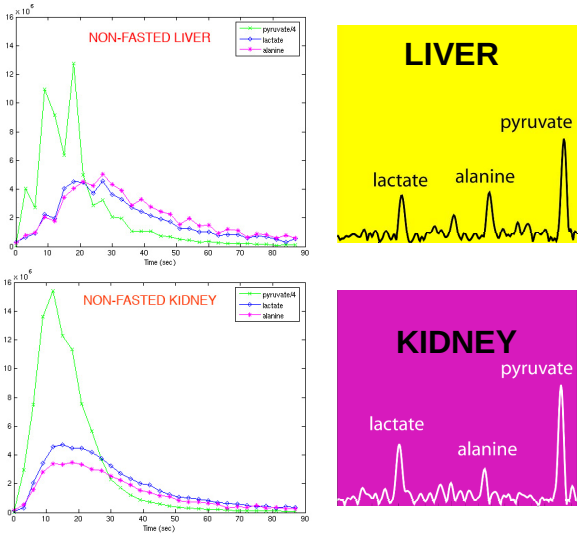

Figure 2. Top: dynamic spectra from non-fasted rat liver and a single liver voxel from a 3D study. Bottom: Comparison with non-fasted kidney.

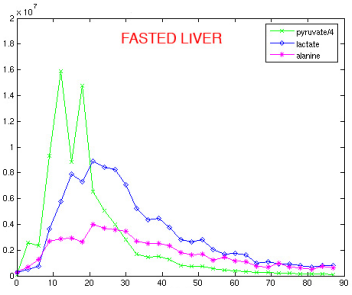

Figure 3. Dynamic spectra of liver from a rat fasted for ~24 hours. Note that lactate is significantly higher than alanine. Compare with top left of Figure 2.

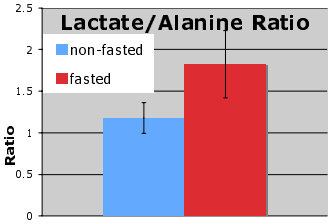

Figure 4. Comparison of peak lactate to peak alanine ratios.

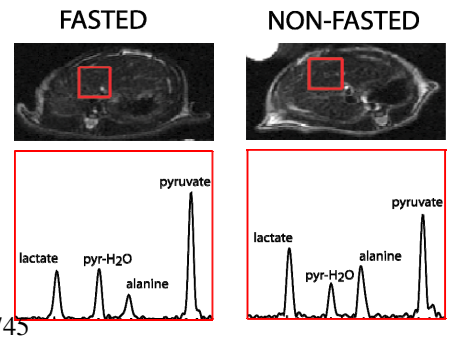

Figure 5. Comparison of single voxel spectra from 3D localized liver acquisitions. The fasted liver voxel showed decreased alanine relative to lactate, as was observed in the dynamic data.