Rapid Sequential Injections of Hyperpolarized [1-^{13}C]Pyruvate *In Vivo* Using a Sub-Kelvin, Multi-Sample DNP Polarizer

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Introduction: Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the measurement of ^{13}C metabolism *in vivo* with 5 orders of magnitude higher SNR compared to thermal equilibrium [1]. Traditionally, consecutive injections of a hyperpolarized compound in an animal have been separated temporally, with the practical minimum time between injections determined by the polarization build-up time, which is on the order of an hour for [1-^{13}C]pyruvate [1]. This has precluded the monitoring of metabolic changes occurring on a faster time scale. The feasibility and effects of greatly reducing the time separation between injections has not been investigated previously. In this study, using a recently developed GE SpinLab^{TM} sub-Kelvin, dynamic nuclear polarizer with the capability of simultaneously polarizing up to 4 samples under sterile conditions [2], we performed initial *in vivo* investigations of dynamic and chemical shift imaging of [1-^{13}C]pyruvate in normal rats with 5 minute injection intervals.

Methods: Normal male Sprague-Dawley rats were used. All studies were conducted using a GE 3T scanner with a custom ^{1}H/^{13}C rat coil [3]. 80 μL samples of [1-^{13}C]pyruvate were loaded into and polarized with the SpinLab instrument [2] funded by NIH S10RR029570. Each rat received 3 injections (timed 5 minutes apart) of 2.5 mL of 100 mM hyperpolarized pyruvate injected over 12 sec. Slab-localized (from top of liver to bladder) dynamic data were collected (starting upon injection) with a double spin-echo sequence [4] with 5 degree excitation, TE = 35ms, and TR = 3 sec. For the chemical shift imaging, the double-spin echo sequence was modified to use a single 180 degree adiabatic refocusing pulse, converting it to a single spin-echo sequence. The imaging parameters were: 8x8x16 x/y/z imaging matrix, 1 cm^3 isotropic resolution, TE = 120 ms, TR = 195 ms, 12.5 sec imaging time, and 25 sec delay between start of injection and start of imaging. The other parameters were the same as those described in [5].

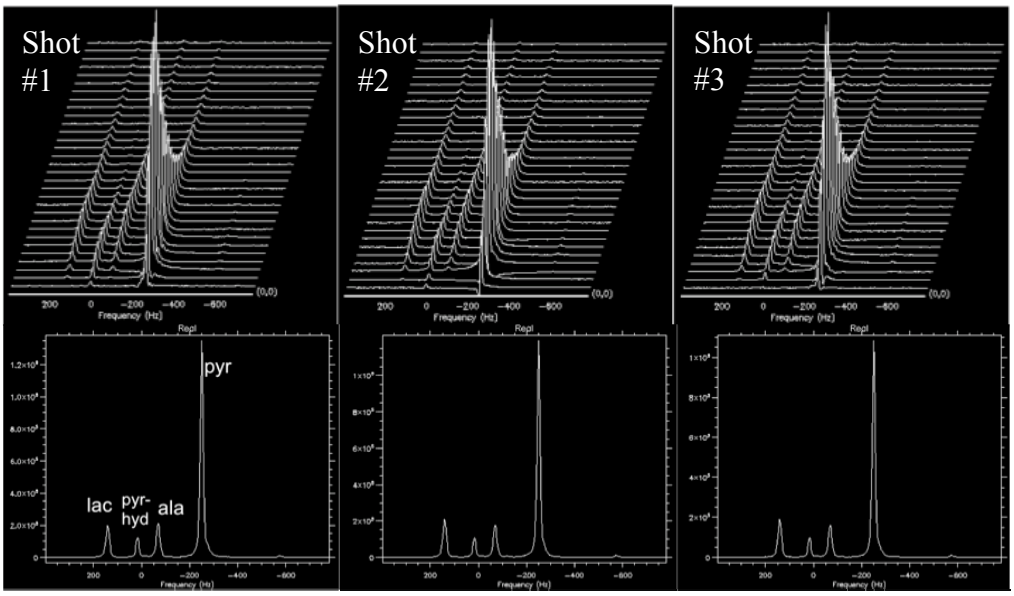

Figure 1: Slab-localized rat dynamic curves for three consecutive injections of hyperpolarized [1-^{13}C]pyruvate spaced 5 min apart along with the corresponding time-summed spectra. The peaks from left to right, as labeled in the lower left, are lactate, pyruvate-hydrate, alanine, and pyruvate. The ratios of the peaks to each other remained relatively constant across the injections with no significant variations detected.

Table 1: Summary of voxel-by-voxel statistics from the 3D-CSI injections. The lac/tCar, ala/tCar, and pyr/tCar area ratios for each voxel were divided by the corresponding value from the same voxel in the first injection of the experiment. The means and std are shown below.

	Shot #1	Shot #2	Shot #3
Lac/tCar Exp #1	1.00 ± 0.00	1.08 ± 0.17	1.20 ± 0.17
Ala/tCar Exp #1	1.00 ± 0.00	0.86 ± 0.17	0.90 ± 0.19
Pyr/tCar Exp #1	1.00 ± 0.00	1.03 ± 0.07	0.99 ± 0.06
Lac/tCar Exp #2	1.00 ± 0.00	1.03 ± 0.12	1.08 ± 0.15
Ala/tCar Exp #2	1.00 ± 0.00	0.87 ± 0.15	0.83 ± 0.15
Pyr/tCar Exp #2	1.00 ± 0.00	1.04 ± 0.07	1.04 ± 0.08

Results: Figure 1 shows the dynamic curves and corresponding summed spectra for the slab-localized data. The mean/std of the liquid state polarizations (at time of dissolution) and pHs were 33.5% ± 4.2% / 7.76 ± 0.12. The peak ratios remained relatively constant across the 3 injections with no significant variations observed. There was perhaps a slight increase in lactate and slight decrease in alanine from the first injection. Table 1, summarizing the results from the 3D chemical shift imaging experiments, shows a similar trend. The lac/tCar, ala/tCar, and pyr/tCar ratios for each voxel of each injection were first computed. Then, on a voxel-by-voxel basis, the lac/tCar, ala/tCar, and pyr/tCar ratios were divided the lac/tCar, ala/tCar, and pyr/tCar ratios respectively from the first injection. The columns in Table1 show the mean/std of these comparisons. Of course, for the column corresponding to the first injection, the values are all unity. Table 1 indicates that lac/tCar increased slightly (but not significantly) from the first injection, ala/tCar decreased slightly, and pyr/tCar remained constant.

Discussion: We demonstrated for the first time the ability to do rapid sequential *in vivo* injections of a hyperpolarized compound using the SpinLab polarizer. In this initial study, the time separation between injections was 5 minutes, but that could be decreased to ~1 minute with the current system. The data appear consistent across shots, suggesting that rat physiology stabilizes quickly and that each injection minimally perturbs the normal rat's metabolism as detected by HP-pyruvate. The initial data gathered serves as a baseline for future studies. For example, the rapid multiple injection capability would allow drug dynamics to be studied on the timescale of drug action in the body as well as monitoring reperfusion following ischemia with higher temporal resolution.

References: [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158 [2] Ardenkjaer-Larsen et al. NMR Biomed (2011) 24:927 [3] Hu et al. MRI (2011) 29:1035 [4] Cunningham et al. JMR (2007) 187:357 [5] Hu et al. Mol Imaging Biol (2009) 11:399

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