

## Using [1-<sup>13</sup>C]lactic acid for hyperpolarized <sup>13</sup>C cardiac studies

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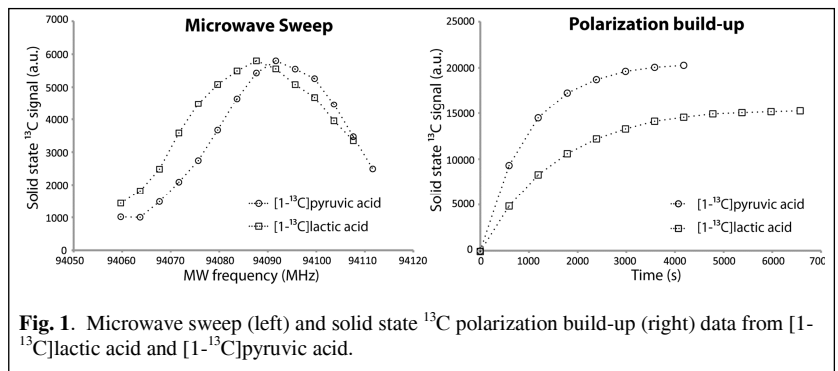
**Introduction:** MR studies using hyperpolarized [1-<sup>13</sup>C]pyruvate have been shown to allow real time assessment of PDH flux *in vivo* non-invasively (1). Although its safety has been demonstrated in a recent clinical trial of prostate cancer patients (2), it has potential inotropic effects at high doses and may not be an ideal imaging agent for patients with cardiovascular disease. Pyruvate also stimulates PDH, thus the measured apparent flux may be perturbed by the agent itself. [1-<sup>13</sup>C]lactate may be a viable alternative since its endogenous concentration is much higher than pyruvate and is administered routinely at high doses to patients in the form of lactated Ringer's solution. Hyperpolarized <sup>13</sup>C metabolic studies have been demonstrated by using sodium [1-<sup>13</sup>C]lactate with either DMSO/water or glycerol/water in the DNP sample preparations (3-4). The relatively low solubility of sodium lactate in these solvents may limit the final dose that can be achieved for studies in large animals or humans. In this study, preparation of hyperpolarized [1-<sup>13</sup>C]lactate in solution was demonstrated using neat [1-<sup>13</sup>C]lactic acid as the DNP sample. Hyperpolarized [1-<sup>13</sup>C]lactate was also compared to [1-<sup>13</sup>C]pyruvate for *in vivo* investigation of cardiac PDH flux at the same dose in the same subjects.

**Methods:** Hardware and agent: All studies were performed using a 3T MR750 scanner (GE Healthcare) and a micro-strip dual-tuned <sup>1</sup>H-<sup>13</sup>C volume coil (8cm ID) (Magvale). A HyperSense DNP polarizer (Oxford Instruments) was used to polarize the substrates. Neat [1-<sup>13</sup>C]lactic acid or neat [1-<sup>13</sup>C]pyruvic acid (99% enrichment, Isotec, Miamisburg, OH) was doped with 15mM of OX63 radical (Oxford Instruments) and 1mM of Gd chelate (Prohance®, Bracco International). The polarized samples were dissolved with a NaOH/Tris buffered solution. MRS Experiments: To estimate the polarization of hyperpolarized [1-<sup>13</sup>C]lactate generated from DNP of [1-<sup>13</sup>C]lactic acid, <sup>13</sup>C spectra were acquired from ~3ml/40mM aliquot of the hyperpolarized solution using a pulse-acquire pulse sequence just after dissolution (~15s sample transfer) and at thermal equilibrium polarization. Dynamic <sup>13</sup>C spectroscopic data were acquired from the hearts of 3 healthy rats (~350g) using a pulse-acquire pulse sequence (1.2cm axial slice through the heart, 20° nominal tip angle, TR=1s). Two experiments were performed on each animal following tail vein infusion of either hyperpolarized lactate or pyruvate (same volume and concentration: 2ml/40mM). Data acquisitions started at the start of the ~10 s infusion of the substrates.

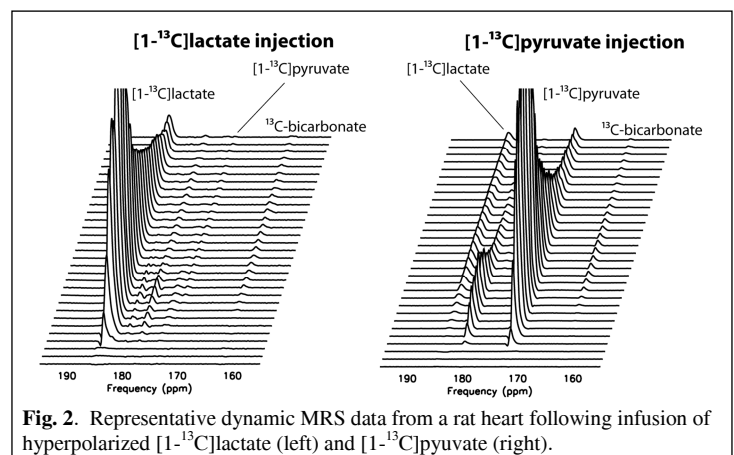
**Results and Discussion:** Microwave sweep and representative dynamic polarization build-up data from the two substrates are shown in Fig. 1. Slightly lower (4 MHz) optimal MW frequency and approximately 0.25 fold lower equilibrium solid state <sup>13</sup>C polarization were observed for [1-<sup>13</sup>C]lactic acid as compared to [1-<sup>13</sup>C]pyruvic acid (build-up plots were normalized by total <sup>13</sup>C nuclei in the sample). Polarization of hyperpolarized [1-<sup>13</sup>C]lactate measured in solution was 17.0% (n=3, stdev.=3.7 %); and similar to the observation in solid state, the polarization of [1-<sup>13</sup>C]lactate in solution was ~0.3 fold lower than the polarization of [1-<sup>13</sup>C]pyruvate in solution (23.1%, n=3, stdev.=0.9%). Representative dynamic MRS data from a rat heart following infusion of hyperpolarized [1-<sup>13</sup>C]lactate and [1-<sup>13</sup>C]pyruvate are shown in Fig. 2. Conversion of [1-<sup>13</sup>C]lactate to [1-<sup>13</sup>C]pyruvate and <sup>13</sup>C bicarbonate was observed in experiments in which hyperpolarized [1-<sup>13</sup>C]lactate was injected. Unknown resonances (~178 – 181 ppm) associated with the hyperpolarized substrate solution were also observed in the data and were likely due to lactyllactic acid and lactide formed in the neat lactic acid sample (5). Average peak (temporal maximum in the dynamic data) bicarbonate over peak substrate ratio was 0.014 (stdev.=0.004) in experiments where hyperpolarized lactate was injected, which was slightly lower than the peak bicarbonate over peak substrate ratio of 0.019 (stdev.=0.005) in experiments where pyruvate was injected. Although this result indicated that more product of PDH flux could be observed when using pyruvate as the substrate at this dose (~0.2 mmol/kg), possibly due to PDH stimulation by the injected pyruvate, the potential loss in sensitivity using hyperpolarized [1-<sup>13</sup>C]lactate is marginal, especially if purity and polarization of [1-<sup>13</sup>C]lactic acid can be improved.

**Conclusions:** This study demonstrated that using neat [1-<sup>13</sup>C]lactic acid as the DNP sample is a viable alternative to [1-<sup>13</sup>C]pyruvic acid for cardiac hyperpolarized <sup>13</sup>C MR studies. Hyperpolarized [1-<sup>13</sup>C]lactate may provide non-invasive assessment of cardiac PDH flux with minimal perturbation of the enzymes involved in this metabolic pathway and a superior safety profile in cardiac patients

**Reference:** 1. Schroeder, MA et al. PNAS.,2008;105(33):12051–12056. 2. Nelson, SJ et al. Proc ISMRM., 2012;274. 3. Chen, AP et al. MRI., 2008;26(6):721-6. 4. Mayer, D et al. NMR Biomed., 2012;25(10):1119-24. 5. Watson, PD et al. Ind. Eng. Chem.1940, 32 (3), pp 399–401.



**Fig. 1.** Microwave sweep (left) and solid state <sup>13</sup>C polarization build-up (right) data from [1-<sup>13</sup>C]lactic acid and [1-<sup>13</sup>C]pyruvic acid.



**Fig. 2.** Representative dynamic MRS data from a rat heart following infusion of hyperpolarized [1-<sup>13</sup>C]lactate (left) and [1-<sup>13</sup>C]pyruvate (right).