

# In Vivo Measurement of Normal Rat Intracellular Pyruvate and Lactate Levels after Injection of Hyperpolarized [1-<sup>13</sup>C]Alanine

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**Introduction:** Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the measurement of <sup>13</sup>C metabolism *in vivo* at very high SNR [1]. The most commonly used *in vivo* agent thus far has been [1-<sup>13</sup>C]pyruvate. In preclinical studies, not only is its uptake detected but also its intracellular enzymatic conversion to metabolic products including [1-<sup>13</sup>C]lactate. However, the ratio of <sup>13</sup>C-lactate/<sup>13</sup>C-pyruvate measured in this data does not accurately reflect cellular values since much of the [1-<sup>13</sup>C]pyruvate is extracellular depending on timing, vascular properties and extracellular space and monocarboxylate transporter activity. In order to measure the relative levels of intracellular pyruvate and lactate, we hyperpolarized [1-<sup>13</sup>C]alanine and monitored the *in vivo* conversion to [1-<sup>13</sup>C]pyruvate and [1-<sup>13</sup>C]lactate. With the assumption that normal cells exhibit little or no leakage of ALT and LDH, then hyperpolarized alanine must be transported into cells in order for conversion to hyperpolarized pyruvate and then lactate to take place, and thus, the detected levels of pyruvate and lactate would reflect intracellular ratios.

**Methods:** Normal male Sprague-Dawley rats were used. All studies were performed on a GE 3T scanner with a custom <sup>1</sup>H/<sup>13</sup>C rat coil. Non-localized dynamic <sup>13</sup>C spectroscopic data (30 degree flip, TE = 35ms, start of acquisition 35 seconds after injection, 3 seconds between time points) were acquired after injection of ~2.5mL of ~60mM hyperpolarized [1-<sup>13</sup>C]alanine (with 0.3 mM Dotarem® gadolinium, 5-15% polarization at t = 0 sec). A 10 Hz gaussian apodization filter was used in the data processing.

**Results/Discussion:** Figure 1 shows a non-localized phased spectrum from a pulse and acquire acquisition with 16 averages. The lactate peak is very prominent, and farthest to the right, on the shoulder of the alanine peak, is the pyruvate peak. The ratio of lactate area to pyruvate area (with alanine shoulder area subtracted off) was 7.26. (Note: To improve the linewidth, subsequent acquisitions used a spin-echo scheme [2].) In Figure 1, 1.25 ppm (40 Hz) to the left of pyruvate is a natural abundance lipid peak. To obtain clearer confirmation of this natural abundance lipid peak, we performed a separate experiment in which we injected hyperpolarized [1-<sup>13</sup>C]pyruvate. Figure 2 shows a non-localized spin-echo acquisition in which increased averaging resulted in the increase of the lipid peak but not the hyperpolarized metabolite peaks, suggesting that the increasing peak is a natural abundance component. Furthermore, with the hyperpolarized [1-<sup>13</sup>C]pyruvate injection, the distance in ppm between the lipid and pyruvate peaks was confirmed to be the distance between the two rightmost peaks in Figure 1. Figure 3 shows another data set obtained after injecting hyperpolarized [1-<sup>13</sup>C]alanine, this time using the spin-echo sequence. The lactate area to pyruvate area ratio in this acquisition was 8.37.

**Conclusion:** In this study, a method involving the injection of [1-<sup>13</sup>C]alanine was developed to investigate the relative levels of *in vivo* intracellular hyperpolarized lactate and pyruvate.

**References:** [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158  
[2] Cunningham et al. JMR (2007) 187:357

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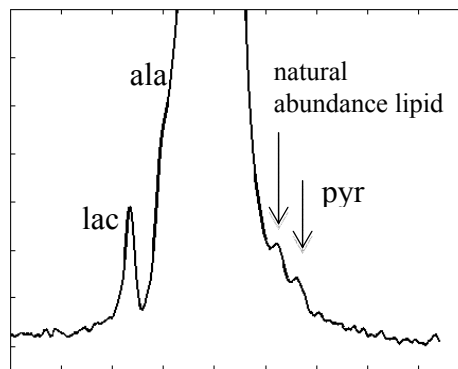


Figure 1: Non-localized pulse and acquire spectrum with 16 averages (polarization at t=0 was ~15%). The peaks from left to right are [1-<sup>13</sup>C]lactate, [1-<sup>13</sup>C]alanine, natural abundance lipid, and [1-<sup>13</sup>C]pyruvate. The distance between lactate and pyruvate is 12.15 ppm, and the distance between the lipid and pyruvate is 1.25 ppm (40 Hz at 3T). The ratio of lactate area to pyruvate area (with alanine baseline subtracted off) in this acquisition was 7.26.

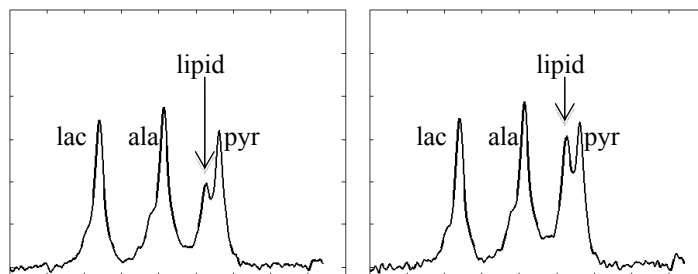


Figure 2: Non-localized spectra when [1-<sup>13</sup>C]pyruvate was injected. The peaks from left to right are once again [1-<sup>13</sup>C]lactate, [1-<sup>13</sup>C]alanine, natural abundance lipid, and [1-<sup>13</sup>C]pyruvate. Note that pyruvate is relatively low because the first 10 dynamic time points, in which pyruvate is very high, have been excluded. The left shows a spectrum with 91 averages, and the right shows the same spectrum with another 91 averages included. The hyperpolarized metabolite peaks do not increase, but the lipid peak does, suggesting that it is a natural abundance component.

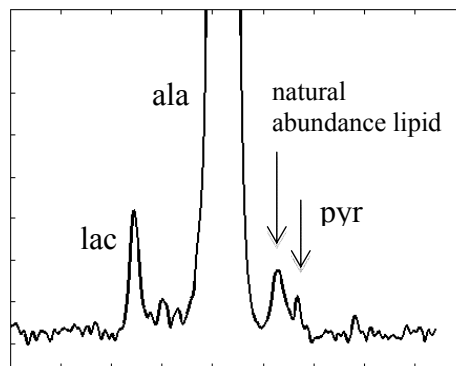


Figure 3: Non-localized spin-echo spectrum with 10 averages (polarization at t=0 was ~5%). The ratio of lactate area to pyruvate area in this data was 8.37.