Alternating Acquisition for Quantification of Pyruvate Metabolism in Hyperpolarized 13C Studies

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Introduction Observation of pyruvate-lactate conversion through hyperpolarized ^{13}C spectroscopy and spectroscopic imaging provides valuable insight in cellular metabolism. Previous studies were focused on quantification and modeling of lactate dehydrogenase-catalyzed flux of ^{13}C label exchange between injected pyruvate and lactate *in vivo*[1]. To increase the SNR of the product and prolong the hyperpolarization of the substrate for accurate observation of dynamic kinetics, methods such as dual or multi-band RF excitation or simultaneous spectro-spatial excitation for in-vivo ^{13}C imaging have been developed[2-4].However, in *in vitro* experiment that may be limited by cell number count, product SNR can be typically lower compared to in vivo environment, making accurate measurement and quantification of pyruvate-lactate conversion difficult. To overcome this, an alternating single voxel spectroscopic acquisition scheme which uses a narrow-band RF pulse for selective excitation of pyruvate and lactate between every TR is proposed. In addition, a modified version of the two-site exchange model was formulated to calculate the exchange and relaxation rate constants from this acquisition method.

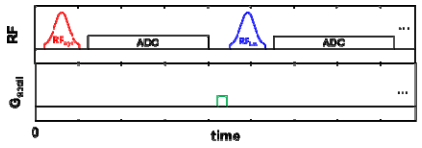

Figure 1: Pulse sequence used for alternate acquisition of pyruvate and lactate. The RF transmit frequency is switched every TR between pyruvate and lactate.

Methods A narrow-band RF was designed using finite impulse response (FIR) filter design method. The required bandwidth of the RF pulse for selective excitation was determined from ^{13}C spectrums obtained from previous in-vitro studies conducted in 3T environment. Typical resonance peaks of pyruvate and its metabolic products showed approximate linewidth of 15Hz at FWHM, which required the RF passband to be wider than 15Hz. Also, proximity of lactate and pyruvate-hydrate peaks ($\Delta f \cong 120$Hz) prevented accurate measurement and quantification of converted lactate due to pyruvate-hydrate baseline. Therefore the RF stopband was designed to be less than 100Hz to ensure selective excitation. The FWHM of the designed RF pulse resulted in $\cong 80$Hz with duration of 20.0ms.

The narrow-band RF excitation pulse was implemented into a free-induction decay spectroscopy sequence. For selective excitation, the transmit frequency of the RF excitation pulse was alternated between pyruvate and lactate at every TR. Also, to compensate for relatively low SNR of lactate product compared to the injected pyruvate, flip angles were also alternated between 1° for pyruvate and 10° for lactate as shown in Fig 2.

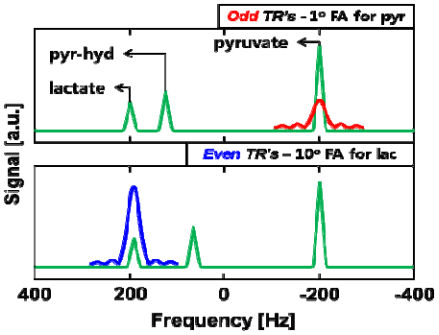

Figure 2: Excitation scheme illustrating the relative chemical shifts of metabolites in ^{13}C experiment (green) along with alternating excitation RF pulses with different transmit frequencies. 1°flip RF pulse for pyruvate is shown in red, with 10° flip RF pulse for lactate shown in blue.

For *in vitro* demonstration [1-^{13}C] pyruvic acid doped with 15mM Trityl radical and 1.5M Dotarem was polarized using HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK). After dissolution into aqueous state, 2.0mL of the hyperpolarized substrate was injected into the test tube filled with 4.0mL of LDH (15U) and 60uL of NADH (500mM) solution over 9s, and conversion from hyperpolarized pyruvate to lactate was monitored. TR of 750ms was used with 512 acquisitions (256 acquisitions each for pyruvate and lactate) resulting in 6.4 minutes of scan time. The procedure was repeated with a constant flip angle scheme using a hard excitation pulse with 5° flip angle for comparison. All experiments were performed on a GE MR750 3T clinical MRI system (GE Healthcare, Waukesha, WI, USA) equipped with a broadband amplifier. A custom built ^{13}C-tuned solenoid coil was used for transmission and reception.

For quantitative analysis, the dynamic curves (signal-over-time) were drawn from odd and even TR's. Then the dynamic data was used to calculate the rate constants associated with the conversion. A 1st-order approximation of the two-site exchange model was used, and unmeasured data $L_z[2n-1]$, $P_z[2n]$ and constants k_P, k_L, ρ were iteratively estimated.

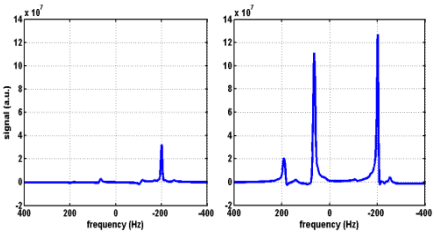

Figure 3: Real spectrums obtained with the proposed acquisition scheme. Odd TR spectrum with 1° flip angle for pyruvate (256 avg) is shown on the left, and even TR spectrum with 10° flip angle for lactate (256 avg) is shown on the right.

Results and Discussion As shown in Fig 4, the SNR of lactate increased nearly two fold (due to 10° flip) as intended compared to the constants flip angle (5°) scheme. Also, the hyperpolarized magnetization was elongated using a 1°flip angle. To avoid transient-state conversion of pyruvate to lactate in the analysis with the two-site exchange model, data fitting was limited to 100 time points after the lactate's peak signal, and the T_1 (ρ^{-1}) of pyruvate and lactate were assumed to be equal. With the constant flip angle scheme, the proposed fitting method showed T_1 of 72.7632s with $k_P = 0.0014$ and $k_L = 0.0176$. With alternate acquisition data, T_1 of 69.2214s with $k_P = 0.0024$ and $k_L = 0.0162$ was obtained. The small difference in the estimated T_1 values could be due to the minor fluctuations in the pyruvate signal (shown in Fig 4, right) possibly caused by unintended excitation from side-lobe of the 10° flip pulses used in the even TR. The RF pulse used for the excitation of lactate showed elevated sidelobes (Fig 3) and suppression of signals from pyruvate-hydrate and pyruvate was not perfect. Signals from these resonances can be further decreased by considering using various types of windows during the RF design.

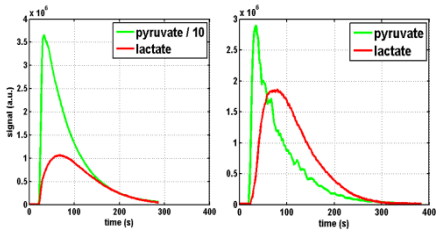

Figure 4: Dynamic curves of data obtained with constant 5° hard-pulse scheme (left) and the proposed method (right). Hyperpolarized magnetization of pyruvate is elongated and SNR of the product lactate is increased with the proposed method.

Conclusion A method for accurate quantification of hyperpolarized pyruvate and the product lactate focused at *in vitro* cell experiment is described. By using a narrow-band RF excitation pulse with alternating acquisition at every TR, metabolic product can be selectively excited with a higher flip angle for increased SNR while the hyperpolarized magnetization of the substrate can be minimally perturbed with a low flip angle. Baseline signals from neighboring resonances can be effectively suppressed to accurately quantify the metabolism kinetics. Furthermore, with the modified version of the two-site exchange model, calculation of the rate constants associated with the conversion is also possible.

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References [1] Day *et al.* Nature Medicine 13: 1382–1387, 2007. [2] Larson *et al.* JMR 194: 121-127, 2008. [3] Lau *et al.* NMR Biomed. 24: 988-996, 2011. [4] Schulte *et al.* MRM *in press*, 2012.