Quantitation of In-Vivo Metabolic Kinetics of Pyruvate using Hyperpolarized 13C MRSI

T. Xu[1], D. Mayer[2,3], M. Gu[2], Y-F. Yen[4], S. Josan[2,3], E. Johansson[5], J. Tropp[6], R. Hurd[4], and D. Spielman[1,2]

[1]Department of Electrical Engineering, Stanford University, Stanford, CA, United States, [2]Department of Radiology, Stanford University, Stanford, CA, United States, [3]Neuroscience Program, SRI International, Menlo Park, CA, United States, [4]Global Applied Sciences Laboratory, GE Healthcare, Menlo Park, CA, United States, [5]Medical Diagnostics R&D, GE Healthcare, Oslo, Norway, [6]Global Applied Sciences Laboratory, GE Healthcare, Fremont, CA, United States

Introduction: With signal-to-noise ratio enhancement on the order of 10,000-fold [1], hyperpolarized MRSI of metabolically active substrates allows the study of both the injected substrate and downstream metabolic products *in vivo*. Although hyperpolarized $^{13}C_1$-pyruvate (Pyr), in particular, has been used to demonstrate metabolic activity in various animal models, robust quantitation remains an important area of investigation. Enzyme saturation effects are routinely seen with commonly used doses of hyperpolarized $^{13}C_1$-Pyr [2], however most metrics proposed to date, including metabolite ratios, time-to-peak of metabolic products, or an estimated single rate constant fail to capture these saturation effects. In addition, the widely used small flip-angle excitation approach doesn't model the inflow of fresh spins correctly, which is a significant factor *in vivo*. In this work, we developed a quantitative 90°-excitation dynamic spectroscopic imaging approach which can overcome the aforementioned limitations, and demonstrated that the *in-vivo* conversion of Pyr to its downstream metabolic products of lactate (Lac) and alanine (Ala) is well approximated by Michaelis-Menten kinetics with the resulting estimated apparent V_{max} and K_m parameters being unbiased with respect to critical experimental parameters including the substrate dose and bolus shape.

Method: All measurements were performed on a GE 3 T MR scanner equipped with self-shielded gradients (40 mT/m, 150 mT/m/ms). A custom-built dual-tuned ($^1H/^{13}C$) quadrature rat coil (∅ = 80 mm) was used for both RF excitation and signal reception. Healthy male Wistar rats (350-450 g body weight) were anesthetized with 1-3% isoflurane in oxygen (~1.5 L/min). The rats were injected in a tail vein with the 80-mM solution of Pyr that was hyperpolarized via DNP (15-20% liquid-state polarization). The Pyr solution was carefully injected at a rate of 0.2 mL/s.

We have extended our prior fast multi-shot spiral-based MRSI acquisition and reconstruction algorithm [3] optimized for imaging of hyperpolarized $^{13}C_1$-Pyr and its downstream metabolic products. Three clustered slice-select pulses with variable flip angles (35.3°, 45°, 90°) [4] were used to repeatedly excite a slice through rat kidneys and measure the signals of Pyr and its downstream products of Lac and Ala. Within each 5 s TR, fresh $^{13}C_1$-Pyr replenishes the slice. A fraction of the Pyr is metabolically converted to Lac and Ala, which are assumed to stay in kidney cells within this TR interval. Thus, the Pyr signal at the end of each 5 s TR is a "snapshot" of Pyr in the slice while Lac and Ala signals are an "integral" of Lac and Ala in the slice accumulated during the prior 5 s. By exploiting the fact that the Pyr concentration is time-varying following a bolus injection, the use of a 90° slice-select pulse allows an independent estimate of the reaction velocity to be calculated each TR interval. In modeling the data, we ignored the backward reactions from Lac to Pyr because simulations show the inclusion of the backward reactions has numerically insignificant impact on the estimated apparent V_{max} and K_m. Significant $^{13}C_1$-Lac, produced primarily in the heart, was observed to flow into the targeted slice. To eliminate this undesired Lac inflow, we added a Lac-selective saturation pulse at the beginning of each TR [5]. The overall pulse sequence is shown in Fig. **1A**. We use an external 8-M ^{13}C-enriched urea phantom to quantify the *in-vivo* metabolite concentrations. To simplify modeling, we assume that the *in-vivo* concentration of $^{13}C_1$-Pyr is constant within each TR interval and the Lac is continuously generated. During a given TR interval, the average Lac reaction velocity is given by $V_{\mathrm{Pyr}\to\mathrm{Lac}} = \frac{[^{13}\mathrm{C}_1\text{-Lac}]}{TR} = V_{\max}\frac{[^{13}\mathrm{C}_1\text{-Pyr}]}{[^{13}\mathrm{C}_1\text{-Pyr}]+K_m}$ where $[^{13}C_1$-Lac] and $[^{13}C_1$-Pyr] are the *in-vivo* Lac and Pyr concentrations, V_{max} and K_m are the apparent maximum Lac reaction velocity and the apparent Lac Michaelis constant. Similar equations hold for Ala production.

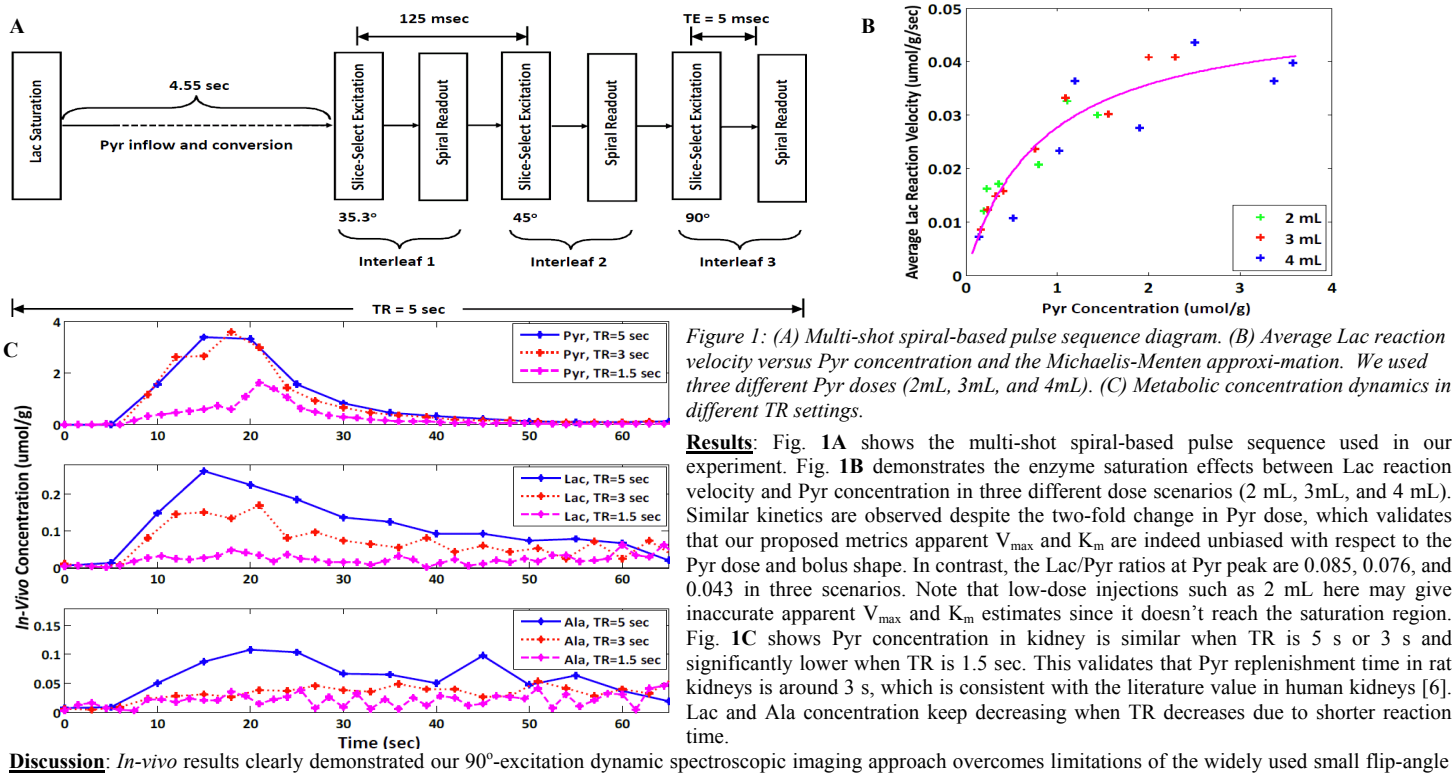

Figure 1: (A) Multi-shot spiral-based pulse sequence diagram. (B) Average Lac reaction velocity versus Pyr concentration and the Michaelis-Menten approxi-mation. We used three different Pyr doses (2mL, 3mL, and 4mL). (C) Metabolic concentration dynamics in different TR settings.

Results: Fig. **1A** shows the multi-shot spiral-based pulse sequence used in our experiment. Fig. **1B** demonstrates the enzyme saturation effects between Lac reaction velocity and Pyr concentration in three different dose scenarios (2 mL, 3mL, and 4 mL). Similar kinetics are observed despite the two-fold change in Pyr dose, which validates that our proposed metrics apparent V_{max} and K_m are indeed unbiased with respect to the Pyr dose and bolus shape. In contrast, the Lac/Pyr ratios at Pyr peak are 0.085, 0.076, and 0.043 in three scenarios. Note that low-dose injections such as 2 mL here may give inaccurate apparent V_{max} and K_m estimates since it doesn't reach the saturation region. Fig. **1C** shows Pyr concentration in kidney is similar when TR is 5 s or 3 s and significantly lower when TR is 1.5 sec. This validates that Pyr replenishment time in rat kidneys is around 3 s, which is consistent with the literature value in human kidneys [6]. Lac and Ala concentration keep decreasing when TR decreases due to shorter reaction time.

Discussion: *In-vivo* results clearly demonstrated our 90°-excitation dynamic spectroscopic imaging approach overcomes limitations of the widely used small flip-angle approach. The proposed approach explicitly exploits Lac inflow effect and the resulting estimated apparent V_{max} and K_m parameters can capture the enzyme saturation effect and are unbiased with respect to substrate dose and bolus shape. Low-dose injections may give inaccurate apparent V_{max} and K_m estimates, so it is best to use medium or large doses to get into the saturation region. This technique is useful for obtaining quantitative and robust metabolic kinetic information, which could be potentially applied to tumor detection, treatment monitoring, identification of cardiovascular pathologies, and the study of metabolic disorders. Currently, we are working to extend this approach to a multi-slice version.

References: [1] Ardenkjaer-Larsen, J.H., et al, *Proc. of the National Academy of Sciences* 2003; 100(18):10158-10163. [2] Zierhut, M.L., et al, *Proc. ISMRM* 2008, 891 [3] Mayer, D., et al, *MRM* 2009; 62:557. [4] Zhao.L, et al., *JMR* 1996. [5] Xu. T. et al, *Proc. ISMRM* 2009, 6115. [6] Roberts, D., et al., *Radiology* 1995; 196: 281-286

Acknowledgements: **NIH grants**: RR09784, AA05965, AA13521-INIA, EB009070, **Lucas Foundation, GE Healthcare**