Measuring Michaelis-Menten Kinetics of Hyperpolarized 13C1-Pyruvate Metabolism in a Single Bolus Injection

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Introduction: With signal-to-noise ratio (SNR) enhancement on the order of 10,000-fold [1], hyperpolarized MRS of metabolically active substrates allows the study of both the injected substrate and downstream metabolic products *in vivo*. To date, most dynamic studies have employed slice-select excitation pulses with small flip angles to measure the metabolic time curves following bolus injection of the hyperpolarized substrate. There are three major limitations to this approach. First, the bolus injection function needs to be accurately modeled. Second, the curve fitting assumes a constant metabolic exchange rate throughout the observation window. Third, there is no distinction between inflow and locally produced metabolites within the slice. A recent study [2] involving multiple $^{13}C_1$-pyruvate injections at various dose levels showed decreased pyruvate-to-lactate and pyruvate-to-alanine exchange rates with increased pyruvate concentration, and such nonlinear relationships can be described by Michaelis-Menten kinetics [3] in order to extract the maximum reaction velocities V_{maxL}, V_{maxA} and the Michaelis-Menten constants K_{mL}, K_{mA}. In this work, we developed a new technique addressing the above limitations and to estimate the Michaelis-Menten parameters in a single bolus injection of hyperpolarized $^{13}C_1$-pyruvate**.**

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 (Φ = 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 2.5 ml of a 100-mM solution of $^{13}C_1$-pyruvate that was hyperpolarized via DNP (15-20% liquid-state polarization). The pyruvate solution was carefully injected at a rate of 0.25 ml/s.

A 90° slice-select pulse was used to repeatedly excite a slice through rat kidneys and measure the signals of pyruvate and its downstream products of lactate and alanine. During each TR, new pyruvate flows into the slice, part of which is then converted to lactate and alanine. By exploiting the fact that the pyruvate concentration is time-varying following a bolus injection, the use of a 90° slice-select readout allows an independent estimate of the exchange rates to be calculated each TR interval. In modeling the data, we ignored the backward reactions from lactate- or alanine-to-pyruvate. In addition, only pyruvate is assumed to flow into the slice each TR. In practice this second assumption was violated, as significant lactate, produced primarily in the heart, was also observed to flow into the targeted slice. To eliminate this undesired lactate inflow, we added a lactate-selective saturation pulse immediately after each readout. The overall pulse sequence is shown in Figure **1 (A)**. To simplify modeling, we assume that the inflow rate of pyruvate was constant within each TR interval, hence effectively modeling the pyruvate input function to the slice as a piecewise linear function. The choice of TR is critical. If TR is too small, the constant pyruvate inflow rate assumption well approximates the true inflow curve, but the SNRs of the received signals are low because there is little time for the metabolic conversion. If TR is too large, the SNRs of the pyruvate, lactate, and alanine signals are higher, but the piecewise linear model of the pyruvate bolus may be violated. Based on simulations, an optimal TR value of 5 second was used for the experiments described here. Each TR, the estimated metabolic rate constants are given by Equation **1**. According to the Michaelis-Menten enzyme kinetics, the rates of enzyme-catalyzed first-order reactions only proceed linearly with respect to substrate concentration when there is an abundance of enzyme as shown in Equation **2**. For high substrate concentrations, the reaction rates approaches a constant, limited by the available enzyme activity as shown in Equation **3**. Here, we only give the equations for lactate, as the analogous alanine equations are similar.

$$k_{PL} = \frac{1}{TR/2}\frac{M_L}{M_P + M_L + M_A} \quad \textbf{(1)} \qquad \frac{dC_L}{dt} = k_{PL}C_P \quad \textbf{(2)} \qquad \textbf{and} \qquad \frac{dC_L}{dt} = \frac{V_{max\,L}}{C_P + K_{mL}}C_P \quad \textbf{(3)}$$

where M_P, M_L, and M_A are magnetization measurements, C_P and C_L are pyruvate and lactate concentrations in the slice respectively, k_{PL} is the exchange rate from pyruvate to lactate, V_{maxL} is the maximum lactate reaction velocity, and K_{mL} is the lactate Michaelis-Menten constant.

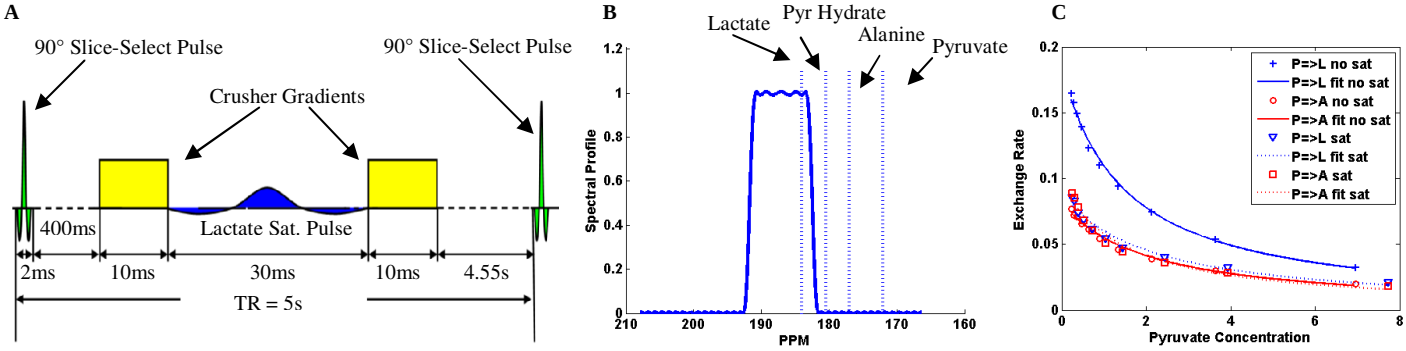

Figure 1: (A) Pulse sequence diagram. (B) Spectral profile of the lactate-selective saturation pulse. (C) Representative exchange rate and pyruvate concentration illustrating Michaelis-Menten kinetics as well as model fits. P represents Pyruvate, L represents Lactate, A represents Alanine.

Results: Figure **1 (A)** shows the pulse sequence diagram used in our experiment. Figure **1 (B)** shows the spectral profile of the lactate-selective saturation pulse we designed in our experiment. Importantly, this saturation pulse does not suppress the pyruvate-hydrate peak; any saturation of pyruvate-hydrate would result in undesired decrease in pyruvate via chemical exchange. Figure **1 (C)** demonstrates that Michaelis-Menten kinetics accurately model the relationship between metabolic rates and pyruvate concentration. The plot also demonstrates a large decrease in the artificially-enhanced lactate exchange rates with the saturation pulse as expected, while alanine exchange rates are unchanged. As anticipated, for these bolus injection experiments, where the injected pyruvate concentration is well above the normal in vivo metabolic steady-state value, low rate constants were observed when pyruvate concentration is highest (usually 15 second after bolus injection), with the rate constants increasing as the pyruvate concentration decreases over time.

Discussion: In vivo data clearly demonstrated the measurement of Michaelis-Menten enzyme kinetics for hyperpolarized $^{13}C_1$-pyruvate using a single bolus injection. We are working on incorporating outflow into the model to relax the current assumption that ignores this effect. In future work, we need to design a dual passband spectral saturation pulse to eliminate both lactate and alanine, without affecting pyruvate hydrate peaks occurring close to alanine. In addition, we are investigating how to extend this approach to a multi-slice approach. This technique is useful for obtaining quantitative metabolic kinetic information, which can be potentially applied to tumor detection, treatment monitoring, identification of cardiovascular pathologies, and the study of metabolic disorders.

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] Berg, J., et al. *Biochemistry* 2006; W.H. Freeman

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