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Quantitation of *In Vivo* Metabolic Kinetics of Hyperpolarized Pyruvate in Rat Kidneys using Dynamic ^{13}C Magnetic Resonance Spectroscopic Imaging

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Abstract

With signal-to-noise ratio enhancements on the order of 10,000-fold, hyperpolarized MR spectroscopic imaging (MRSI) of metabolically active substrates allows the study of both the injected substrate and downstream metabolic products *in vivo*. Although hyperpolarized $[1-^{13}\text{C}]$ -pyruvate, in particular, has been used to demonstrate metabolic activities in various animal models, robust quantitation and metabolic modeling remain important areas of investigation. Enzyme saturation effects are routinely seen with commonly used doses of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate, however most metrics proposed to date, including metabolite ratios, time-to-peak of metabolic products, or single exchange rate constant fail to capture these saturation effects. In addition, the widely used small flip-angle excitation approach does not correctly model the inflow of fresh downstream metabolites generated proximal to the target slice, which is often a significant factor *in vivo*. In this work, we developed an efficient quantitation framework employing a spiral-based dynamic spectroscopic imaging approach. The approach overcomes the aforementioned limitations and demonstrates that the *in vivo* ^{13}C labeling of lactate and alanine after a bolus injection of $[1-^{13}\text{C}]$ -pyruvate is well approximated by saturable kinetics, which can be mathematically modeled using a Michaelis-Menten-like formulation with the resulting estimated apparent maximal reaction velocity $V_{\max}$ and apparent Michaelis constant K_M parameters being unbiased with respect to critical experimental parameters including the substrate dose, bolus shape, and duration. Although the proposed saturable model has similar mathematical formulation to the original Michaelis-Menten kinetics, it is conceptually different. In this study, we focus on the ^{13}C labeling of lactate and alanine and do not differentiate the labeling mechanism (net flux or isotopic exchange) or the respective contribution of various factors (organ perfusion rate, substrate transport kinetics, enzyme activities, and the size of the unlabeled lactate and alanine pools) to the labeling process.

Keywords

hyperpolarized ^{13}C ; *in vivo* metabolism; saturable kinetics; magnetic resonance spectroscopic imaging

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Introduction

MRSI of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate is a promising technique for mapping metabolic activities *in vivo* as demonstrated in recent animal studies [1–9]. This method uses dynamic nuclear polarization (DNP) and a rapid in-field dissolution process to produce a highly polarized metabolic contrast agent [10]. In less than one minute following bolus injection, $[1-^{13}\text{C}]$ -pyruvate and its downstream metabolic products $[1-^{13}\text{C}]$ -lactate, $[1-^{13}\text{C}]$ -alanine, and $[1-^{13}\text{C}]$ -bicarbonate can be mapped at relatively high spatial resolution, with the appearance of the ^{13}C label on the various metabolites resulting from a combination of isotopic exchange and metabolic flux [11]. Several ^{13}C NMR spectroscopic imaging techniques have proven capable of monitoring the metabolism of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate as an *in vivo* biomarker for disease diagnosis and response to therapy [1,12–18].

However, the widely used small flip-angle excitation approach in combination with a multi-site exchange model [15, 19, 20] fails to adequately account for the inflow of fresh downstream metabolites generated proximal to the target slice, often a significant factor *in vivo*. In addition, to date, most proposed metrics for such hyperpolarized ^{13}C MRS studies, including metabolite ratios, time-to-peak of metabolic products, or single exchange rate constant fail to capture the saturation effect during a single bolus injection. To address these effects, Zierhut et al. [20] recently demonstrated a non-linear relationship between *in vivo* exchange rate constants and $[1-^{13}\text{C}]$ -pyruvate dose which was mathematically well modeled using a Michaelis-Menten-like enzyme kinetics formulation parameterized by an apparent maximal reaction velocity V_{max} and an apparent Michaelis constant K_M of ^{13}C label. A modeling approach which first estimated single exchange rate constant for each of 19 dose levels (a potentially inaccurate modeling method for intermediate and large doses) was then employed to investigate the relationship between these exchange rate constants and pyruvate doses.

The primary goal of the present study is the development of a robust saturable kinetics model focusing on the ^{13}C labeling of lactate and alanine and not differentiating the labeling mechanism (net flux or isotopic exchange) or the respective contribution of various factors including organ perfusion rate, substrate transport kinetics, enzyme activities, and the size of the unlabeled lactate and alanine pools.

In this work, we developed an efficient spiral dynamic spectroscopic imaging approach such that the saturation effects can be quantified *during a single bolus injection* [21, 22]. In particular, following an injection of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate, the pyruvate concentration is time varying due to the nature of a bolus injection. Typically, the $[1-^{13}\text{C}]$ -pyruvate signal increases rapidly, reaches a maximum shortly after the end of injection and then decays due to T_1 relaxation, metabolic flux, isotopic exchange, and dilution into the blood and extracellular space. By exploiting this inherently time-varying pyruvate concentration in combination with an RF excitation scheme that uses all of the available magnetization within the target slice, we can obtain independent estimates of the apparent reaction velocities of ^{13}C label in each TR interval, then the respective apparent V_{max} and K_M parameters of ^{13}C label can be estimated using the entire observation time window. Although the proposed model has similar mathematical formulation to the original Michaelis-Menten kinetics, it is conceptually different. The observed dose-dependent exchange rate constants and reaction velocities of ^{13}C label reflect the collective effects of the factors including the organ perfusion rate, substrate transport kinetics, enzyme activities, and the size of unlabeled substrate pools.

Methods

Experimental Setup and Animal Model

All measurements were performed on a clinical 3 T Signa MR scanner (GE Healthcare, Waukesha, WI) equipped with self-shielded gradients (40 mT/m, 150 mT/m/ms). A custom-built dual-tuned ($^1\text{H}/^{13}\text{C}$) quadrature rat coil (inner diameter: 80 mm, length: 90 mm), operating at 127.7 MHz and 32.1 MHz, respectively, was used for both RF excitation and signal reception. Four healthy male Wistar rats (350–390 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 ^{13}C -pyruvate that was hyperpolarized via DNP. For each experimental run, the 2–4 ml of the hyperpolarized pyruvate solution were injected via a bolus at a rate of 0.2 ml/sec.

Pulse Sequence and Data Processing

The spectroscopic imaging experiments were designed to measure the total hyperpolarized [$1\text{-}^{13}\text{C}$]-pyruvate, [$1\text{-}^{13}\text{C}$]-lactate, and [$1\text{-}^{13}\text{C}$]-alanine signals within an excited slice each TR interval. The ^{13}C -bicarbonate signal was quite small in the rat kidney and thus not quantified in this study. Because about 20% of rat blood volume flows through the kidneys, the perfusion of these organs is high. Therefore, we assume the pyruvate signal at the end of each TR represents a snapshot of the newly inflowing pyruvate concentration within the slice, i.e., new hyperpolarized pyruvate completely replenishes the slice each TR interval. In addition, during each TR, a fraction of this pyruvate is metabolically converted to, or isotopically exchanged with, lactate and alanine. In contrast to pyruvate, efflux of lactate and alanine signals during each TR was assumed to be negligible. To verify the validity of these influx and efflux assumptions, a series of experiments with TR values ranging from 1.5 to 5 sec were performed and the corresponding ^{13}C -pyruvate, ^{13}C -lactate, and ^{13}C -alanine signals were recorded (see *TR selection experiment* part of *Results* section). To approximate the dynamic nature of pyruvate concentration within each TR interval, we employed the arithmetic average of pyruvate concentration at the beginning and end of each TR interval to approximate the *effective* pyruvate concentration within this TR interval.

Exploiting the fact that the pyruvate concentration is inherently time-varying following a bolus injection, independent estimation of apparent reaction velocities of lactate and alanine ^{13}C label within each TR interval can be used to plot the relationship between apparent reaction velocity of ^{13}C label and pyruvate concentration. Based on this non-linear relationship, the apparent $V_{\max}$ and K_M parameters are extracted.

Dynamic imaging data were obtained using an extension of our prior fast multi-shot spiral-based MRSI acquisition and reconstruction algorithm [23] optimized for imaging of hyperpolarized [$1\text{-}^{13}\text{C}$]-pyruvate and its downstream metabolic products. Specifically, three clustered slice-selective pulses with variable flip angles (35.3° , 45° , 90°) [24], each followed by an interleaved spiral readout trajectory, were used to repeatedly excite a slice through rat kidneys and measure the resulting spectroscopic signals from pyruvate, lactate, and alanine with the following parameters: $5 \times 5 \text{ mm}^2$ nominal in-plane resolution, 10 mm slice thickness, FOV = 8 cm, TR = 5 s, TE = 5 ms, 8.6 Hz nominal spectral resolution, and 276.24 Hz spectral bandwidth. The three flip angles were chosen to achieve equal transverse magnetization for each of the three interleaves. Initial *in vivo* experiments showed significant hyperpolarized lactate, produced outside of the target slice (likely produced in the heart) flowing into the kidney slice during the imaging experiment. To minimize this undesired lactate inflow, we added a lactate-selective saturation pulse at the beginning of each TR interval. Therefore, the lactate signal we observed at the end of each TR was almost the locally generated lactate within that TR. Data were processed as described in [23] and

metabolic images were calculated by peak integration in absorption mode with integration intervals of 36 Hz for pyruvate, lactate, and alanine. Figure 1 depicts the overall spectroscopic imaging pulse sequence we developed for the study, and Figure 2 shows representative *in vivo* spectroscopic imaging results.

Mathematical Model

Our proposed approach first estimates the apparent reaction velocities of lactate and alanine ^{13}C label within each TR interval, and then extracts the apparent V_{max} and K_M parameters using nonlinear regression with respect to the corresponding hyperpolarized $[1-^{13}\text{C}]$ -pyruvate concentration.

We first assume the backward exchange rate constant k_{LP} of ^{13}C label is a fraction α of the forward exchange rate constant k_{PL} of ^{13}C label (analogous equations hold for alanine). We model the forward and backward reactions of ^{13}C label as a two-step process, i.e., a fraction of the hyperpolarized pyruvate ^{13}C label is first metabolically converted to or isotopically exchanged with lactate ^{13}C label, and then a fraction of the locally generated lactate ^{13}C label is converted back to pyruvate ^{13}C label. Denote the effective pyruvate ^{13}C label concentration as P , the intermediate lactate ^{13}C label concentration as $\hat{L}$, the final lactate ^{13}C label concentration as L , and the incremental concentration of pyruvate ^{13}C label converted back from locally generated lactate ^{13}C label as ΔP . Based on the definition of apparent exchange rate constants of ^{13}C label, the following set of four equations hold:

$$\begin{aligned} k_{PL} &= \frac{\hat{L}}{P \cdot \text{TR}} \\ k_{LP} &= \frac{\Delta P}{\hat{L} \cdot \text{TR}} \\ k_{LP} &= \alpha \cdot k_{PL} \\ \hat{L} - \Delta P &= L \end{aligned} \quad (1)$$

By solving these four equations with four unknowns k_{PL} , k_{LP} , $\hat{L}$, and ΔP , we can obtain the analytical solutions for the forward rate constant k_{PL} of lactate ^{13}C label:

$$k_{PL} = \frac{P - \sqrt{P^2 - 4\alpha P \cdot L}}{2\alpha P \cdot \text{TR}} \quad (2)$$

In [20], the backward exchange rate of ^{13}C label, here denoted by k_{LP} , was assumed to be negligible as compared to the forward exchange rate of ^{13}C label based on the high $[1-^{13}\text{C}]$ -pyruvate concentration following a bolus injection and prior work using hyperpolarized $[1-^{13}\text{C}]$ -lactate [25]. To assess the impact of ignoring the backward exchange rate of ^{13}C label, we performed a series of simulations and quantified the effects of this assumption. Based on these simulations (see *Backward Reaction Rate Constant Simulations* part of *Results* section), we verified the validity of these assumptions and showed the inclusion of the ^{13}C label backward reactions had numerically insignificant impact on the final apparent V_{max} and K_M estimates.

Ignoring the backward reaction of ^{13}C label, i.e., $\alpha \rightarrow 0$, the apparent reaction velocity of lactate ^{13}C label V_{PL} which is estimated as the product of the effective pyruvate ^{13}C label concentration P and the apparent rate constant k_{PL} is,

$$V_{PL} = k_{PL} \cdot P = \frac{L}{\text{TR}} \quad (3)$$

Repeating the aforementioned calculation for each of N TR intervals yields V_{PL}^i and P^i ($i = 1, 2 \dots N$). The relationship between the apparent reaction velocities of lactate ^{13}C label and the effective pyruvate ^{13}C label concentration is then mathematically approximated using a Michaelis-Menten-like formulation as below

$$V_{PL}^i = V_{max} \frac{P^i}{P^i + K_M} \quad i=1, 2 \dots N \quad (4)$$

where V_{max} is the apparent maximal reaction velocity of lactate ^{13}C label, and K_M is the apparent Michaelis constant of lactate ^{13}C label.

Absolute Quantitation

For our experiments, we included an external 8-M ^{13}C -enriched urea phantom to quantify the *in vivo* metabolite concentrations. To obtain absolute quantitation, both percentage polarization and T_1 relaxation must be taken into account. The basic idea is to use the proportionality between the readout signal and the *in vivo* concentration as shown below:

$$\begin{aligned} S_P &= \beta \cdot \text{pol}_P(\%) \cdot P \\ S_U &= \beta \cdot \text{pol}_U(\%) \cdot U \end{aligned} \quad (5)$$

where S_P and S_U are the average signal intensity (A.U.) per pixel within the region of interest, $\text{pol}_P(\%)$ and $\text{pol}_U(\%)$ are pyruvate and urea percentage polarization, P is the unknown pyruvate concentration at the readout time, U is the known urea concentration of 8M. Analogous equations hold for lactate and alanine.

Metabolite signal intensity S_P and urea phantom signal S_U are calculated from spectral peak integration without polarization correction. The polarization correction is considered in the estimation of pyruvate percentage polarization discussed below. A representative metabolite signal curve is shown in Figure 3(c).

Pyruvate percentage polarization at dissolution $\text{pol}_P^{\text{diss}}(\%)$ is estimated from the solid-state polarization value and the amount of the pyruvic acid (PA) placed in the polarizer. Starting from $\text{pol}_P^{\text{diss}}(\%)$ after dissolution, the pyruvate percentage polarization $\text{pol}_P(\%)$ first experiences an *in vitro* T_1 (approximately 60 sec in our study) decay before injection and then an *in vivo* T_1 (approximately 40 sec in our study) decay after injection until the readout time.

The relationship between the pyruvate percentage polarization at dissolution $\text{pol}_P^{\text{diss}}(\%)$ and the pyruvate acid amount is determined for several samples of polarized and dissolved PA mix using the ratio of the polarized signal to its thermal equilibrium signal. Then, the relationship is extrapolated back to the time of dissolution using an estimate of pyruvate T_1 and correlated with the solid state signal level. The empirical formula for the pyruvate percentage polarization at dissolution in our experiment setup is found to be

$$\text{pol}_P^{\text{diss}}(\%) = \frac{SS}{4.17 \times PA} \quad (6)$$

where SS is the solid state polarization value (A.U.), PA is the amount (umol) of the pyruvic acid placed in the polarizer and 4.17 is the regressed constant multiplier determined from our measurements.

Urea percentage polarization pol_U (%) was estimated from the thermal polarization definition as shown below:

$$\text{pol}_U(\%) = \frac{1 - \exp(-h\nu/KT)}{1 + \exp(-h\nu/KT)} \quad (7)$$

where h is the Plank constant, ν is the urea resonant frequency (approximately 32.1 MHZ at 3T), K is the Boltzmann constant, T is the absolute temperature of urea.

After estimating pyruvate signal S_P , urea signal S_U , pyruvate percentage polarization pol_P (%), and urea percentage polarization pol_U (%), we can calculate the value of pyruvate concentration P at each readout time according to Equation 5.

Results

To verify the selected pulse sequence and metabolic modeling equations, a series of *in vivo* experiments were conducted on normal adult male Wistar rats. Specifically, dynamic spectroscopic imaging data were acquired from a 10-mm thick slice through the center of the right kidney as shown in Figure 3. Pyruvate, lactate, and alanine peak integrals were computed from Region of Interests (ROIs) in the right kidney, carefully chosen to avoid major renal blood vessels. Pyruvate signal was typically 10 times larger than lactate and alanine as shown in Figure 3(c).

TR Selection Experiment

The selection of TR is critical for these experiments. An overly short TR does not allow appreciable accumulation of ^{13}C label on the downstream products of lactate and alanine and also invalidates the assumed complete replenishment of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate between measurements. In contrast, a too long TR would result in undesirable T_1 decay for hyperpolarized metabolite signals and invalidate the assumption that products come from the imaging slice.

Figure 4 shows the measured metabolite signals from the same ROI in a rat kidney acquired with different TR values (5 sec, 3 sec, and 1.5 sec respectively). Figure 4(a) indicates that pyruvate signal is largely unchanged when TR value changes from 5 sec to 3 sec while lactate and alanine signals decrease in proportion to the TR reduction. When TR is reduced to 1.5 sec, the assumption of the complete replenishment of the fresh hyperpolarized $[1-^{13}\text{C}]$ -pyruvate between measurements breaks down (as shown by decreased pyruvate signal) and lactate and alanine signals decrease further. Hence, for subsequent studies, we chose 5 sec as a reasonable compromise among lactate and alanine ^{13}C labeling process, the pyruvate replenishment, and T_1 decay.

Backward Reaction Rate Constant Simulations

To assess the validity of ignoring the backward exchange rate, we performed a series of simulations and quantified the change of the estimated apparent V_{max} and K_M parameters.

In particular, focusing on the flow of the ^{13}C -labeled fraction in organs with large preexisting lactate pools, ^{12}C lactate backward reaction dominates the backward reaction so little ^{13}C lactate label will go back. The worst case is where there is no or low preexisting

unlabeled lactate pool. Our simulation results showed the numerical effect of ignoring the backward exchange rate of ^{13}C -labeled lactate on the estimated parameters in the worst case (i.e., no preexisting lactate pool) is small. As shown in Figure 5, $V_{\max}$ is insensitive to the inclusion of the backward reaction. Even if α , the ratio between backward and forward rate constant of ^{13}C label, equals 1, $V_{\max}$ only has about 10 – 15% change for both lactate and alanine. Note in practice, the value of α is typically smaller than 1, approximately 0.1 for lactate for most tissues. K_M experiences relatively larger change because it appears in the denominator of Equation 4.

Metabolic Modeling

Using the pulse sequence, mathematical modeling, and absolute quantitation procedures described in *Methods*, experimental results demonstrate that a Michaelis-Menten-like formulation for saturable enzyme kinetics provides a good model for fitting the observed relationship between the reaction velocities and the effective pyruvate concentration as shown in Figure 6. Table 1 shows that the regression results of apparent $V_{\max}$ and K_M are robust to ROIs encompassing varying kidney volumes.

We also repeated our experiments on four rats to study the intra- and inter-subject variability of the estimated apparent $V_{\max}$ and K_M parameters. For the intra-subject variability study, 2.3 ml, 2.7 ml, and 2.7 ml of 80 mM pyruvate were injected to a single rat respectively. The different volumes were due to variations in the manually injected pyruvate bolus. For the inter-subject variability study, 3.25 ml, 2.8 ml, 3 ml, and 2.7 ml of 80 mM pyruvate were injected to four rats respectively. As we can see from Table 2, the inter-subject variability is approximately twice that of the intra-subject variability.

One attractive feature of this mathematical formulation is the estimated apparent $V_{\max}$ and K_M of ^{13}C label are unbiased with respect to the critical experimental parameters including the substrate dose, bolus shape, and duration. To investigate this robustness, we performed a series of bolus injections using different pyruvate dosages (2 ml, 3 ml, 4 ml respectively). As shown in Figure 7(a) and (b), the proposed pulse sequence and data analysis algorithm yielded consistent values for the apparent $V_{\max}$ and K_M . Although the estimated apparent $V_{\max}$ and K_M were unbiased with respect to the dosage, the most accurate estimates (small variance) were obtained with medium to large pyruvate dosages that ensured saturated enzyme kinetics. Table 3 shows the estimated apparent $V_{\max}$ and K_M values of ^{13}C label under three different dosage conditions. Low-dose injections (2 ml in our study) gave inaccurate parameter estimates since the effective pyruvate concentration remained in the linear region.

Discussion

In vivo results clearly demonstrate the proposed dynamic spectroscopic imaging approach overcomes limitations of widely used small flip-angle approaches. The described technique explicitly exploits the inflow of fresh hyperpolarized $[1-^{13}\text{C}]\text{-pyruvate}$, suppresses undesired contributions from $[1-^{13}\text{C}]\text{-lactate}$ generated proximal to the target slice, and estimates the apparent $V_{\max}$ and K_M parameters of ^{13}C label in a single bolus injection, thus obtaining quantitative and robust metabolic information.

The estimated apparent $V_{\max}$ and K_M parameters of ^{13}C label are unbiased with respect to the critical experiment parameters including the substrate dose, bolus shape, and duration, with high-dose injections yielding the most accurate parameter estimates. If the substrate dose versus reaction velocity is in the linear region, a single exchange rate constant is likely a good model whereas if the substrate dose is in the saturation region, our proposed approach outperforms a model based on a single exchange rate constant. This technique

could be potentially applied to tumor detection, treatment monitoring, identification of cardiovascular pathologies, and the study of other metabolic disorders.

For the rat kidney, a well perfused organ, we exploited the rapid inflow of fresh hyperpolarized [1-¹³C]-pyruvate during each TR interval. The validity of this high inflow assumption and subsequent rapid metabolic conversion and isotopic exchange depends on the key physiological parameters such as the organ perfusion rate, substrate transport kinetics, enzyme activities, and pool sizes of unlabeled substrates. For other organs and tissues, as well as substrates other than pyruvate, such modeling assumptions will need to be verified and modified where appropriate. In particular, a set of acquisitions with varying TR intervals, as depicted in Figure 4, would likely be required to choose the optimum acquisition parameters. In future work, we will extend our current single-slice approach to the multi-slice version to provide volumetric apparent V_{max} and K_M parameters of ¹³C label in a single bolus injection.

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List of Abbreviations

MRSI	Magnetic Resonance Spectroscopic Imaging
DNP	Dynamic Nuclear Polarization
ROI	Region of Interest
PA	Pyruvic Acid
A.U	Arbitrary Unit

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Figure 1.

Three-shot spiral-based pulse sequence diagram. At the beginning of each TR, the lactate-selective saturation pulse eliminates all hyperpolarized lactate signal existing within the rat body, allowing fresh hyperpolarized $[1-^{13}\text{C}]$ -pyruvate subsequently to flow into the target slice (kidney in our study) and get metabolically converted to or isotopically exchanged with lactate and alanine. Finally, the three-shot spiral sequence excites and reads out pyruvate and locally generated lactate and alanine signals.

Figure 2.

Metabolite maps Fourier-interpolated by a factor of 2 with (a)–(c) and without (d) the lactate-selective saturation pulse. Scanning starts with the beginning of the pyruvate injection and time t is the post-injection time. Strong lactate inflow from the renal aorta is observed (d), and the lactate-selective saturation pulse effectively suppresses this undesired lactate inflow (c). Independent scaling factors are used in (a)–(c) for better visualization whereas (c) and (d) use the same scaling factor for fair comparison. The pyruvate scaling factor (i.e. maximal pyruvate signal intensity) is 1.12×10^{12} A.U., the lactate scaling factor is 5.68×10^{10} A.U., and the alanine scaling factor is 2.79×10^{10} A.U.. Metabolite signals are not corrected for polarization.

Figure 3.

(a) The target slice is positioned through the center of the right kidney. (b) A Region of Interest (ROI) from the right kidney is selected. The ROI is carefully chosen to avoid major renal blood vessels. (c) The metabolite signal from the ROI within the right kidney. Pyruvate signal is typically 10 times larger than lactate and alanine. Metabolite signals are not corrected for polarization.

Figure 4.

Average (a) $[1-^{13}\text{C}]$ -pyruvate, (b) $[1-^{13}\text{C}]$ -lactate, (c) $[1-^{13}\text{C}]$ -alanine signals (A.U.) from the same ROI in a rat kidney using different TR values (5 sec, 3 sec, and 1.5 sec respectively). In these experiments, 2.8 ml hyperpolarized $[1-^{13}\text{C}]$ -pyruvate (80 mM) were injected. Pyruvate signal was largely unchanged when TR value changed from 5 sec to 3 sec whereas lactate and alanine signals decreased in proportion to the TR reduction. When TR was reduced to 1.5 sec, the assumption of the complete replenishment of the fresh $[1-^{13}\text{C}]$ -pyruvate between measurements broke down (as shown by decreased pyruvate signal), lactate and alanine signals further decreased.

Figure 5.

Relative change of (a) the estimated apparent V_{max} of lactate ^{13}C label, (b) the estimated apparent K_M of lactate ^{13}C label, (c) the estimated apparent V_{max} of alanine ^{13}C label, and (d) the estimated apparent K_M of alanine ^{13}C label when the ratio between the ^{13}C label backward and forward rate constant α changes from 0 to 1. The apparent V_{max} is insensitive to the inclusion of the backward reaction while the apparent K_M experiences relatively larger change because it appears in the denominator of Equation 4 and is more prone to the regression input variations. In these simulations, we assume there is no unlabeled lactate and alanine pools, thus giving the upper bounds for estimation error.

Figure 6.

Representative saturatable kinetics between reaction velocities of ^{13}C label and effective pyruvate concentration and the best-fit curves. The relationship can be mathematically approximated as shown in Equation 4. Although the saturatable model has similar formulation to the original Michaelis-Menten kinetics, it is conceptually different. In this experiment, 2.7 ml hyperpolarized $[1-^{13}\text{C}]$ -pyruvate (80 mM) were injected. When choosing ROI, we interpolate metabolite maps to 256×256 so that they match the size of overlay proton images. A ROI with 303 post-interpolation pixels in rat right kidney was chosen. The estimated apparent V_{max} and K_M values are listed in Table 1.

Figure 7.

The saturable kinetics between reaction velocities of ^{13}C label and effective pyruvate concentrations with different 80-mM pyruvate dosages (2 ml, 3 ml, and 4 ml respectively) for (a) pyruvate to lactate and (b) pyruvate to alanine. The dashed curve was fitted based on all three sets of data. An ROI with 560 post-interpolation pixels was chosen in the rat right kidney. The proposed pulse sequence and data analysis algorithm yielded consistent values of the apparent V_{max} and K_M parameters as shown in Table 3.

Table 1

Regression results for three ROIs with different sizes (39, 170, and 303 post-interpolation pixels respectively) within the rat right kidney in the experiment described in Figure 6. Regression results for the apparent V_{max} and K_M of ^{13}C label are insensitive to ROI size. SD represents the fitting standard deviations.

Reaction Type	ROI Size (pixels)	$V_{max} \pm SD$ (mM/sec)	$K_M \pm SD$ (mM)
Pyruvate to Lactate	39	0.047 ± 0.016	0.675 ± 0.692
	170	0.047 ± 0.016	0.718 ± 0.705
	303	0.046 ± 0.015	0.687 ± 0.676
Pyruvate to Alanine	39	0.013 ± 0.003	0.723 ± 0.481
	170	0.012 ± 0.002	0.706 ± 0.418
	303	0.012 ± 0.002	0.692 ± 0.385

Table 2

Intra- and inter-subject variability of the estimated apparent V_{max} and K_M parameters. For the intra-subject variability study, we repeated the 80 mM pyruvate injections three times (2.3 ml, 2.7 ml, and 2.7 ml respectively) with a single rat. The different volumes were due to variations in the manually injected pyruvate bolus. For the inter-subject variability study, we performed the 80 mM pyruvate injections (3.25 ml, 2.8 ml, 3 ml, and 2.7 ml respectively) with four rats separately. The variability in this table was defined as the ratio of the standard deviation to the mean value. The inter-subject variability is approximately twice that of intra-subject variability.

Variability Type	Reaction Type	V_{max} Variability	K_M Variability
Intra-Subject Variability ($n = 3$)	Pyruvate to Lactate	9.82%	15.89%
	Pyruvate to Alanine	17.01%	36.37%
Inter-Subject Variability ($n = 4$)	Pyruvate to Lactate	32.30%	39.23%
	Pyruvate to Alanine	87.04%	60.60%

Table 3

Estimated apparent V_{max} and K_M values of ^{13}C label under three different 80-mM pyruvate dosages (2 ml, 3 ml, and 4 ml respectively). An ROI with 560 post-interpolation pixels was chosen in rat right kidney. The proposed pulse sequence and data analysis algorithm yielded consistent values of the apparent V_{max} and K_M parameters of ^{13}C label. Note although the regressed parameters V_{max} and K_M were unbiased with respect to dosage, the accuracy of V_{max} and K_M depended on the dosage. Low-dose injections (2 ml in our study) gave inaccurate parameter estimates since the effective pyruvate concentration remained in the linear region. To get a reliable estimates of apparent V_{max} and K_M of ^{13}C label, we needed at least intermediate dosage (3 ml and 4 ml in our study) to achieve saturated kinetics. SD represents the fitting standard deviations.

Reaction Type	Injected Pyruvate Volume (ml)	$V_{max} \pm SD$ (mM/sec)	$K_M \pm SD$ (mM)
Pyruvate to Lactate	2	0.044 ± 0.017	0.527 ± 0.130
	3	0.068 ± 0.009	1.175 ± 0.345
	4	0.070 ± 0.005	1.591 ± 0.102
Pyruvate to Alanine	2	0.008 ± 0.003	0.435 ± 0.403
	3	0.011 ± 0.003	1.246 ± 0.905
	4	0.015 ± 0.002	1.241 ± 0.325