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Influence of Parameter Accuracy on Pharmacokinetic Analysis of Hyperpolarized Pyruvate

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Abstract

Purpose—To explore the effects of noise and error on kinetic analyses of tumor metabolism using hyperpolarized [1-¹³C] pyruvate.

Methods—Numerical simulations were performed to systematically investigate the effects of noise, the number of unknowns, and error in kinetic parameter estimates on kinetic analysis of the apparent rate of chemical conversion from HP pyruvate to lactate (k_{PL}). A pharmacokinetic model with two physical and two chemical pools of hyperpolarized spins was utilized to generate and analyze the synthetic data.

Results—The reproducibility of k_{PL} estimates worsened quickly when peak SNR for HP pyruvate was below ~20. The accuracy of k_{PL} estimates was most sensitive to errors in high excitation angles, the vascular blood volume fraction (v_b), and the rate of pyruvate extravasation (k_{ve}), and was least sensitive to errors in the T_1 of pyruvate. When v_b and/or k_{ve} were fit as additional unknowns, the accuracy of k_{PL} estimates suffered, and when the vascular input function (VIF) of pyruvate was also fit, the reproducibility of k_{PL} estimates worsened.

Conclusion—The accuracy and precision of k_{PL} estimates improve substantially for peak SNR above ~20. Accurate estimates of perfusion parameters (combinations of v_b , k_{ve} , and the pyruvate vascular input function) and transmit calibration at high excitation angles have the greatest impact on the accuracy of kinetic analyses.

Keywords

hyperpolarized pyruvate; kinetic modeling; tumor metabolism; dynamic nuclear polarization

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Introduction

Magnetic resonance imaging (MRI), spectroscopy (MRS), and spectroscopic imaging (MRSI) of hyperpolarized (HP) substrates enables the investigation of key metabolic and biochemical pathways *in vivo* with sensitivity and spatiotemporal resolution that was previously impossible (1,2). Advances in dissolution dynamic nuclear polarization technology and instrumentation have enabled significant increases in ^{13}C MRI signal and the rapid acquisition and reconstruction of *in vivo* ^{13}C data in nearly real time. Integration of HP MRI in preclinical research is widespread, and the safety and feasibility of the approach has been proven in a recent clinical trial involving patients with prostate cancer (3). Imaging HP $[1-^{13}\text{C}]$ -pyruvate and its metabolites has tremendous potential to inform on cancer because pyruvate occupies an important branching point in metabolism that is often altered in cancer (4,5). Many tumors display high aerobic glycolysis (the “Warburg effect”) where pyruvate is converted into lactate in the presence of oxygen. Recent studies have shown the conversion of HP pyruvate into lactate is sensitive to changes in tumor metabolism due to expression of oncogenes (6), tumor suppressors (7), and a range of therapeutic strategies (8–14). Quantitative imaging biomarkers of tumor metabolism from HP MRI (15–21) could provide early feedback about response to therapy. However, methods to quantify HP MRI remain under development (16), and must be better understood before they can be utilized to guide clinical care.

Several methods have been proposed to quantify the apparent conversion rate of HP pyruvate into lactate. The HP lactate signal normalized to the total metabolically active HP signal (pyruvate plus lactate) is a simple semi-quantitative method (22,23), but this approach does not account for signal arising from within vasculature or the effects of perfusion on apparent chemical exchange. A model with two-site chemical exchange has been used to quantify the apparent rate constant (k_{PL}) for conversion of HP pyruvate into lactate (22,24,25). In addition, pharmacokinetic (PK) models that incorporate multiple spatial pools have been proposed (15,26). We recently introduced a suite of PK models that sequester the intravascular HP signal component from extravascular compartments where chemical conversion occurs to more accurately account for effects of substrate delivery on signal evolution (15). Preliminary studies demonstrated that a pharmacokinetic model comprised of two spatial and two chemical pools balances accuracy and complexity, and is sufficient to characterize signal evolution in a range of animal models and disease conditions.

Pharmacokinetic modeling and analysis of signal from HP $[1-^{13}\text{C}]$ -pyruvate and its metabolites can be used to estimate k_{PL} , the apparent rate constant for conversion of pyruvate into lactate, as a quantitative imaging biomarker for tumor metabolism. Models include a number of nuisance parameters, such as relaxation losses of HP pyruvate and lactate ($T_{1,\text{Pyr}}$ and $T_{1,\text{Lac}}$), the local excitation angle, and terms that account for microvascular function including the vascular input function (VIF) and extravasation rate (k_{ve}) of pyruvate, and the vascular blood volume fraction (v_b), which have been shown to influence estimation of k_{PL} (26–28). These parameters may be assumed as unknowns that must be derived from HP MRI data; alternatively, some parameters may be designated as constants during analysis, or estimated from complementary ^1H MRI data. The accuracy of each of these parameters may have profound effects on the accuracy of k_{PL} estimates,

however, it is not immediately clear which parameters most influence the measurement. An effective strategy to reduce the degrees of freedom in pharmacokinetic analysis of HP MRI data requires thorough understanding of the effects of bias and error in ‘nuisance’ parameters on the target parameter of interest. Given the relatively large parameter space, number of potentially confounding unknowns, and current lack of gold standard methods for validation, it is not feasible to carry out such an investigation using phantoms or animal models. In this study, we assume a range of parameter values that are consistent with our prior *in vivo* observations in order to exhaustively study through simulation the effects of noise and bias in model parameters on the quantitative analysis of tumor metabolism using HP [1-¹³C]-pyruvate.

Methods

Using simulated HP data, we measured the effects of noise and assumed values of T_1 for pyruvate and lactate, excitation angle, k_{ve} , v_b , and the VIF on k_{PL} estimates. We used a modified Bloch-McConnell simulator (29) to generate simulated HP data from physiologically relevant ranges of model parameters. The effect of noise on k_{PL} measurement was assessed for a range of k_{PL} values. The effects of errors in each model parameter on k_{PL} were individually explored using noise-free synthetic data. The effects of fitting the perfusion parameters k_{ve} , v_b and the VIF in addition to k_{PL} were also tested to examine the sensitivity of k_{PL} estimation to VIF measurements when fitting multiple unknowns. Our general approach was to use a known set of model parameters to generate synthetic data, then determine the accuracy and reproducibility of k_{PL} estimates under various conditions. All data was analyzed using Matlab (The Mathworks, Natick, MA).

Pharmacokinetic Model

The kinetic model utilized in this study accounts for two well-mixed physical compartments (vascular and extravascular) and two chemical pools (HP pyruvate and lactate), as illustrated in Figure 1a. Chemical conversion of HP pyruvate to lactate occurs only in the extravascular compartment and the reverse reaction is assumed to be negligible (24). It is also assumed that there is initially no HP pyruvate or lactate present in the volume of interest. Under those conditions, the equations for total HP pyruvate and lactate signal in a volume of interest are (15):

$$Py_r(t) = v_e Py_{r_{ev}}(t) + v_b Py_{r_{iv}}(t) = k_{ve} \int_0^t e^{\alpha_{Py_r}(t-\tau)} Py_{r_{iv}}(\tau) d\tau + v_b Py_{r_{iv}}(t), \quad [1]$$

$$Lac(t) = v_e Lac_{ev}(t) = \frac{k_{ve} \cdot k_{PL}}{\alpha_{Lac} - \alpha_{Py_r}} \int_0^t (e^{\alpha_{Lac}(t-\tau)} - e^{\alpha_{Py_r}(t-\tau)}) Py_{r_{iv}}(\tau) d\tau, \quad [2]$$

where

$$\alpha_{Pyr} = - \left(\frac{k_{ve}}{v_e} + k_{PL} + R_{Pyr} + \frac{1 - \cos\theta}{TR} \right) \quad [3]$$

$$\alpha_{Lac} = - \left(\frac{k_{ve}}{v_e} + R_{Lac} + \frac{1 - \cos\theta}{TR} \right) \quad [4]$$

As noted, k_{PL} describes the apparent rate of chemical conversion of HP pyruvate to lactate. $Pyr_{iv}(t)$ is the time-dependent intravascular HP pyruvate signal, or VIF. The intravascular volume fraction is given by v_b . The rate of HP spin exchange across the capillary membrane, k_{ve} , is normalized by the extravascular volume fraction, v_e , in loss terms to conserve spins transferred between volume compartments of unequal size. The excitation angle is given by θ , and TR denotes the repetition time. R_{Pyr} and R_{Lac} describe the rate of HP losses due to spin-lattice relaxation ($T_{1,Pyr}$ and $T_{1,Lac}$, respectively) and can account for additional losses other than capillary exchange and RF excitation, including conversion to chemical endpoints other than pyruvate and lactate (15).

Numerical Simulations

Simulated HP data was generated using a Bloch-McConnell simulator that is driven by a pharmacokinetic model for perfusion (29). A simple dynamic pulse-acquire sequence (20° excitation angle unless otherwise specified, 2s repetition time, 50 repetitions, 4096Hz readout bandwidth over 2048 spectral points) was used to generate complex-valued free induction decay signals. A perfectly homogeneous B_0 of 7T was assumed, with the RF excitation pulses modeled as five-lobed sinc pulses with 5kHz bandwidth centered halfway between the lactate and pyruvate resonances.

A ‘default’ set of parameter values were assumed, based on our prior observations *in vivo*, and were used to generate all synthetic HP data except when specifically varied in order to explore the impact of error over a broader range of physiologically reasonable values. The default value for $T_{1,Pyr}$ was assumed to be 43s, and $T_{1,Lac}$ was assumed to be 33s (28). The VIF shape was modeled as a gamma-variate function (29–31). The rate constant of extravasation (k_{ve}) characteristic of tumor tissue was assumed to be $0.02s^{-1}$, the vascular blood volume fraction (v_b) was 0.09 and k_{PL} was $0.1s^{-1}$. Washout of HP lactate (the first term on the right hand side of Eq. 4) was assumed to be negligibly small (32).

Fitting and Analysis

After transformation of synthetic raw data, dynamic spectra were phase corrected and the full width at half maximum (FWHM) area under pyruvate and lactate peaks were integrated to generate dynamic metabolite curves for kinetic analysis (Fig. 1c). Equations [1–4] were then fit to the data to determine estimates for unknown parameters. In all cases, k_{PL} as the parameter of primary interest was assumed to be an unknown in the fitting process. Unless otherwise indicated, the remaining pharmacokinetic parameters were assumed to be known.

Impact of Noise

Noisy synthetic HP data was generated using default parameter values and several values of k_{PL} (0.05s^{-1} , 0.1s^{-1} , 0.2s^{-1} , and 0.4s^{-1}). Complex Gaussian noise was added to the simulated free induction decays to achieve peak signal-to-noise ratios (SNR) of 10–50 (see Fig. 1). Here, peak SNR is defined as the maximum value in the dynamic spectra divided by the standard deviation of noise. For statistical analysis, fitting was repeated 100 times at each SNR with the addition of fresh Gaussian noise to otherwise noise-free synthetic data.

Impact of Error in Fixed Parameters

To test the effects of error in model parameters, k_{PL} was fit to noise-free synthetic data utilizing intentionally inaccurate values for one of the remaining model parameters at a time. The degree of inaccuracy in the model parameter of interest relates to assumed values by

$$P_e = P_t \times f_e \quad [5]$$

where P_e represents the parameter value with error, P_t is the true value used in simulation, and f_e is the error factor. Fits were performed across a range of error factors and physiologically relevant “true” values for each of the parameters listed in Table 1 (except for the VIF amplitude, for which only a single value was used).

The effects of bias and errors in each parameter on the accuracy of k_{PL} estimates were quantified in two ways. For each parameter tested, the assumed value associated with the smallest range of error factors that led to $\pm 10\%$ errors in k_{PL} estimates was identified as the ‘worst-case’ parameter value. Next, the slope of $k_{PL}(f_e)$ was calculated at $f_e = 1$ for that worst-case value. For kinetic parameters P_e with approximately equal and uncorrelated variance, errors in these parameters will result in errors in k_{PL} estimates that scale with this slope:

$$k_{PL}^{est} \approx k_{PL}^{true} + \sum_i \frac{\partial k_{PL}^{est}}{\partial P_{e,i}} P_{e,i} \approx k_{PL}^{true} + \sum_i \frac{\partial k_{PL}^{est}}{\partial P_{e,i}} f_{e,i} \quad [6]$$

Thus, the slope in $k_{PL}(f_e)$ for a single parameter provides an estimate of the relative propagation of errors in that parameter to errors in k_{PL} (33,34).

Impact of Error in Bolus Arrival Time

In simulation, it is possible to know the exact time at which the bolus of HP pyruvate arrives in the region-of-interest. In practice, the bolus will arrive between readouts, and its arrival time can only be bracketed to within the TR period before the first nonzero observation of HP ^{13}C signal. To investigate the impact of differences between the actual and assumed timing of bolus arrival during PK analysis, synthetic data was generated using default parameter values and a vascular input function that is exactly known except for a slight offset in time (t_{offset}) from its expected arrival at $t = t_b$. If $gvf(a, \beta, t)$ is a gamma variate

function (29,31) that is nonzero for $t > 0$, with shape terms α and β , then the HP pyruvate VIF was modeled as:

$$Pyr_{iv}(t) = gvf(\alpha, \beta, t - t_b + t_{offset}) \quad [7]$$

Synthetic data was generated with $-TR - t_{offset} - TR$ and analyzed assuming $t_{offset} = 0$.

Impact of Fitting Multiple Unknowns

The effects of estimating multiple parameters simultaneously from one HP data set were also investigated. Multi-parametric fitting of noisy data with SNR=25 was repeated 100 times to characterize the mean and standard deviation of k_{PL} estimates as a function of whether key parameters are assumed to be already known or treated as unknowns that must be determined from HP data. Noisy data was fit to determine k_{PL} in each of the eight combinations of states (known or unknown) for three parameter sets (k_{ve} , v_b , and VIF shape). For each combination of these parameters as fixed or fit parameters, the accuracy and reproducibility of k_{PL} estimates was measured. Lastly, to assess the impact of errors in VIF scaling on kinetic analysis with multiple unknowns, noisy data was analyzed with k_{ve} and v_b as fixed or unknown parameters and with error factors of 1 ± 0.1 applied to the VIF amplitude.

Results

Effects of noise on pharmacokinetic analysis

Repeated analyses of synthetic noisy data revealed that the standard deviation of k_{PL} estimates were inversely related to SNR, while mean values were relatively stable (Fig. 2). A small underestimate of the mean k_{PL} was observed for all SNRs, which was due in part to the approximation of excitation losses as averaged over the entire repetition time. The coefficient of variation of k_{PL} estimates rapidly improved up to a peak-SNR threshold of ~20, then continued to gradually improve with higher SNR (Fig. 2e).

Effects of individual model parameters on pharmacokinetic analysis

The sensitivity of k_{PL} to errors in the estimation of nuisance parameters is summarized in Figures 3–5 and Table 1. Overestimates in $T_{I,PyT}$ resulted in nearly accurate values of k_{PL} for all P_t values of $T_{I,PyT}$ (Fig. 3a). However, for underestimates of $T_{I,PyT}$ the modeled k_{PL} increased almost exponentially with decreasing error factor, with greater error observed for smaller values of $T_{I,PyT}$. The range of error factors that resulted in $\pm 10\%$ errors in k_{PL} estimates was larger for $T_{I,PyT}$ than for any of the other parameters tested, indicating that k_{PL} estimates are relatively insensitive to errors in $T_{I,PyT}$. Overestimates in $T_{I,Lac}$ had a more significant impact on the accuracy of k_{PL} fits than underestimates (Fig. 3b). $T_{I,Lac}$ overestimates resulted in proportionate underestimates of k_{PL} , while underestimates of $T_{I,Lac}$ led to overestimates of k_{PL} which were more severe for higher values of $T_{I,Lac}$.

Conversion of HP pyruvate into lactate occurs predominately within cells, and downstream of vascular delivery of pyruvate. The presence of the vascular input function for pyruvate

and descriptors of vascular function in Eqs. 1–2 indicate a clear relationship between the delivery of HP pyruvate and its subsequent conversion to lactate. As expected, errors in estimation of pyruvate delivery affect quantification of its chemical conversion. Inaccuracies in the extravasation rate constant, k_{ve} , resulted in errors in k_{PL} estimates (Fig. 4a). Low values of k_{ve} led to underestimation of extravascular pyruvate that must be compensated by high estimates of k_{PL} , to ensure sufficient lactate is generated. Excessively high values of k_{ve} , conversely, led to underestimation of k_{PL} . Estimates of k_{PL} were also sensitive to errors in the vascular volume fraction, v_b (Fig. 4b). Errors in k_{PL} estimates were greater for inaccurately high than for inaccurately low values of v_b used in fitting HP data. Errors in k_{PL} estimates were greater for higher values of both k_{ve} and v_b .

Mis-estimation of the VIF amplitude and timing also led to errors in the kinetic analysis (Fig. 5). When an inaccurately high value of VIF amplitude is used for fitting, the HP pyruvate in both physical compartments is overestimated at all times. This necessitates an increase in k_{PL} as a means to attenuate extravascular pyruvate and thus the overall pyruvate signal. The reverse is true for VIF amplitude underestimates. When the VIF used for PK analysis is of the correct amplitude but assumed to lag in time after the true bolus arrives ($t_{offset} > 0$), elevated k_{PL} values match the observed lactate signal with artificially low extravascular pyruvate early in the dynamic timecourse, while also enhancing attenuation of artificially high pyruvate after the VIF peak. Conversely, k_{PL} will be under-estimated when the VIF used for PK analysis leads the bolus arrival ($t_{offset} < 0$) to reconcile observed lactate with artificially high pyruvate as the pyruvate signal rises, and preserve pyruvate signal as it is falling. These errors can easily be minimized by determining the offset that is most consistent with dynamic HP data as part of the PK analysis algorithm.

Kinetic analyses are also sensitive to errors in RF transmit calibration (Fig. 6). Over-estimation of the actual excitation angle leads to higher modeled attenuation of extravascular spin pools, which necessitates an increase in k_{PL} to maintain extravascular lactate levels. The reverse is true for inaccurate under-estimation of the excitation angle. This effect is more pronounced at higher excitation angles.

Table 1 summarizes the worst-case conditions for each of these parameters, except for the VIF offset which was analyzed with an absolute offset rather than a multiplicative error factor. In the pulse-acquire sequence examined here, with $TR=2s$, errors in estimation of the HP pyruvate bolus arrival only yielded 10% error in k_{PL} when the simulation incorporated a VIF that led the true bolus by approximately $1.2s = 0.6 \times TR$. The worst-case error range for the remaining parameters was identified with the “true” assumed parameter value (P_t) that resulted in the greatest slope in $k_{PL}(f_e=1)$ and the narrowest range of error factors that remained within $\pm 10\%$ of the “true” assumed k_{PL} ($0.1 s^{-1}$). Among these parameters, $T_{1,PyT}$ exhibited the lowest slope and greatest range of error factors ($=f_{e,high}-f_{e,low}=2.44$), suggesting that errors in the estimation of $T_{1,PyT}$ had the smallest overall effect on k_{PL} estimation. The VIF amplitude resulted in the next largest range ($=2.05$), followed by $T_{1,Lac}$ ($=0.56$). The parameters that had the greatest effect on kinetic analyses were k_{ve} ($=0.48$), v_b ($=0.38$), and excitation angle ($=0.17$ for $\theta=30^\circ$). Errors due to excitation calibration depended heavily on the target excitation angle. When an excitation angle of $\theta=10^\circ$ was used, v_b and k_{ve} had the greatest impact on errors in k_{PL} estimates.

Effects of the number of unknown parameters on pharmacokinetic analysis

Figure 7 shows the mean and standard deviation of k_{PL} estimates derived from synthetic data with an SNR of 25 using various combinations of fitting parameters. If the overall shape of VIF is known but scaled by an unknown factor such that its absolute amplitude is unknown, k_{PL} estimates were most accurate when both k_{ve} and v_b were known. The accuracy of k_{PL} estimates was slightly worse when v_b was unknown, and progressively worse when k_{ve} , or both k_{ve} and v_b , were unknown. Reproducibility was markedly worse when the VIF shape and amplitude, and either k_{ve} or v_b , were not known.

The effects of small errors in estimation of the VIF amplitude were least substantial when k_{ve} and v_b were known (Fig. 7b). k_{PL} estimates obtained when v_b was an additional unknown were most sensitive to errors in VIF amplitude. When k_{ve} was unknown, k_{PL} estimates were also sensitive to VIF amplitude errors. Accuracy was more substantially affected than reproducibility when both k_{ve} and v_b were fit as unknowns.

Discussion

Our goal in PK modeling of HP pyruvate is to measure k_{PL} as a quantitative imaging biomarker for aerobic glycolysis. The unique metabolic information that is made accessible through dynamic imaging of HP pyruvate and lactate is encapsulated by k_{PL} ; other unknowns in Equations 1–4 can be treated as nuisance parameters that are not necessarily of interest, but must be effectively managed. We have explored the effects of error and bias in model parameters to help identify the conditions under which accurate and reproducible k_{PL} measurements can be expected. These results indicate that the SNR of the underlying data, the number of unknown parameters, and the accuracy of RF calibration and externally fixed model parameters are all critical considerations for accurate and reproducible quantification of k_{PL} .

The range of error in individual model parameters that led to a 10% error in k_{PL} , as summarized in Table 1, shows that the excitation angles above 15° had the tightest range of error factors (13–15% for the default 20° excitation), and the highest k_{PL}/f_c , indicating that errors in excitation calibration propagate to k_{PL} measurements more substantially than other parameters. Accurate calibration of excitation pulses and characterization of B_1 uniformity over the volume of interest are critical for accurate and reproducible PK analyses. The vascular blood volume fraction (v_b) and the extravasation rate constant for HP pyruvate (k_{ve}) had the second and third highest impact on k_{PL} measurements, followed by $T_{1,Lac}$, scaling of the HP pyruvate vascular input function, and underestimation of $T_{1,PyT}$.

One approach to minimizing the number of unknowns in these equations, and thus enhance the reproducibility of PK analysis, is to estimate as many as possible from 1H MRI. If the vascular blood volume fraction and/or extravasation rate for HP pyruvate can be accurately estimated from 1H arterial spin labeling, dynamic contrast-enhanced MRI, diffusion- or susceptibility-weighted MRI, for example, then the accuracy and reproducibility of PK analyses can be high (Fig. 7a). In these analyses, 23–25% errors in the estimation of k_{ve} and 18–20% errors in the estimation of v_b led to 10% error in k_{PL} measurements (Table 1, Fig. 4). If these values are not known, the reproducibility of PK analyses can still be acceptable,

albeit with additional bias, as long as the shape of the pyruvate vascular input function is known or dynamically measured in nearby vessels that reasonably approximate delivery to tissues of interest. Differences in the timing and amplitude of the pyruvate VIF can be determined during PK analysis with relatively lower impact on reproducibility because the VIF must be consistent with the total observed HP pyruvate and lactate signals described in Equations 1–4 above. If none of these parameters are known or measured *a priori*, the VIF and vascular parameters can still be derived from HP MRI data as part of PK analysis, with some sacrifice in accuracy and reproducibility (Fig. 7).

This simulation examines quantitative analysis of data where the VIF is known or accurately estimated with less than TR=2s uncertainty in the timing of its arrival. This analysis cannot be directly applied in cases where data acquisition starts significantly after injection and nothing quantitative is known about the VIF or distribution of HP agents in the interim. In such circumstances, initial conditions for extravascular HP pyruvate (P_0) and lactate (L_0) are nonzero and must be considered:

$$Pyr_{ev}(t) = P_0 e^{\alpha_{Pyr} t} + \frac{k_{ve}}{v_e} \int_0^t e^{\alpha_{Pyr}(t-\tau)} Pyr_{iv}(\tau) d\tau \quad [8]$$

$$Lac_{ev}(t) = L_0 e^{\alpha_{Lac} t} + \frac{k_{PL} P_{ev,0}}{\alpha_{Lac} - \alpha_{Pyr}} [e^{\alpha_{Lac} t} - e^{\alpha_{Pyr} t}] + \frac{k_{ve} \cdot k_{PL}}{v_e (\alpha_{Lac} - \alpha_{Pyr})} \int_0^t (e^{\alpha_{Lac}(t-\tau)} - e^{\alpha_{Pyr}(t-\tau)}) Pyr_{iv}(\tau) d\tau$$

[9]

The first term on the right hand side of these equations accounts for extravascular HP agents that are present when data acquisition begins ($t=0$). The second term on the right hand side of Equation 9 describes conversion of extravascular HP pyruvate to lactate, and the rightmost terms in both equations describe fresh infusion and conversion of HP pyruvate from vasculature. The relative importance of initial conditions and loss terms is clearly higher here than in Equations 1–2, and would likely change the priority and influence of parameters examined under the conditions described in this study. Starting data acquisition significantly after the bolus arrives risks missing critical dynamic information contained in the initial rise of the HP lactate signal, when k_{PL} has the greatest influence on signal evolution.

Simulations undertaken in this work were performed using a range of parameter values that are consistent with prior observations. To minimize the likelihood that a specific set of assumptions could lead to conclusions that are only valid over a narrow range of conditions, error factors were applied to parameter values (P_i) that were allowed to vary over a range of physiologically relevant values. The VIF was modeled as a gamma-variate function, which is often used to describe dispersion of a bolus as it transits a series of compartments (30,31). The specific shape of the VIF used in this work was derived from bolus injections carried out in preclinical experiments. Injection profiles that differ substantially from the VIF that

we have used could alter the relative importance and propagation of errors from assumed parameter values. Different acquisition schemes that sample HP signal pools much more slowly or quickly, or with significantly different excitation pulse amplitudes, could also change the relative impact of bias and error in assumed parameter values. The framework described here can easily be modified to characterize effects of noise, bias, and error under different experimental conditions. We anticipate that accuracy and reproducibility will broadly be enhanced by understanding the dynamics of vascular delivery and by rationally minimizing the number of unknowns that must be derived from HP MRI data.

A peak HP pyruvate SNR above ~20 leads to improved reproducibility and lower coefficient of variation in k_{PL} values for the experimental conditions considered in this work. In preclinical dynamic MRS measurements, the peak SNR of HP pyruvate is often observed to be several hundred. SNR is affected by coil sensitivity, polarization level, and tumor characteristics, and must be balanced against target image resolution during protocol optimization.

This work is focused on quantification of k_{PL} , the apparent rate constant for chemical conversion of HP pyruvate into lactate and imaging biomarker for tumor metabolism. In vivo, HP pyruvate is also converted into alanine, or acetyl coenzyme A where the ^{13}C label is lost to $^{13}\text{CO}_2$ (which is in rapid equilibrium with bicarbonate, whose resonance is close enough to HP [1- ^{13}C]-pyruvate that it is readily observed with current spectroscopic imaging sequences). This framework can easily be extended to accommodate and evaluate quantification of conversion rates to these additional chemical endpoints. Rate constants for chemical conversion to alanine (k_{PA} , for example) and bicarbonate (k_{PB}) would be added to the pyruvate attenuation term (Eq. 3), and new equations that are similar to Eq. 2 would be needed for each endpoint, along with attenuation terms similar to Eq. 4. The present analysis remains applicable for *in vivo* studies where these reactions occur but quantification of conversion to alanine or bicarbonate is not desired, since chemical conversion to those endpoints simply accelerates the attenuation of HP pyruvate via modestly higher values for R_{PyT} and lower apparent $T_{1,PyT}$.

Evaluation of the effects of multiple model parameters, noise, and bias in intact biological systems would be extremely challenging owing to complex and confounding biology, the dynamic nature of the HP signal, lack of robust controls, and prohibitive costs associated with repeated sampling of the parameter space. Exhaustive analysis would be nearly impossible to perform experimentally, and therefore simulated data was used to derive important guidance on the design of imaging protocols and analysis strategies for ongoing studies. These results are based on simulation of a simple pulse-acquire sequence acting on a uniform volume of interest. Perfect intrinsic localization to a homogeneous voxel permits consideration of these results within the context of an idealized imaging sequence. Partial volume averaging at the boundary between tumor and adjacent normal tissue will lead to a weighted average that is biased towards tumor tissue due to higher vascular blood volume fraction, rate of extravasation, and metabolic activity (data not shown); analysis of these effects are ongoing and must be considered alongside the spatial resolution and point-spread function of dynamic spectroscopic imaging strategies. This work shows the importance of prior information and substrate delivery on kinetic analysis of tumor metabolism, and further

studies are needed to establish methods by which the perfusion characteristics of ^{13}C substrates can be derived from ^1H MRI.

Although semi-quantitative analysis may suit a range of HP MRI applications, quantitative analyses will be preferred in many cases. The feasibility of k_{PL} as a quantitative imaging biomarker for tumor metabolism is clear. Optimized imaging strategies that maximize the accuracy and reproducibility of these measurements require rigorous evaluation of its complexities to guide the design of new approaches to data acquisition and analysis. Such strategies will help realize the tremendous potential for HP MRI to provide new insight into disease and response to therapy.

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References

1. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of $> 10,000$ times in liquid-state NMR. *Proc Natl Acad Sci USA*. 2003; 100(18):10158–10163. [PubMed: 12930897]
2. Golman K, in 't Zandt R, Thaning M. Real-time metabolic imaging. *Proc Natl Acad Sci U S A*. 2006; 103(30):11270–11275. [PubMed: 16837573]
3. Nelson SJ, Kurhanewicz J, Vigneron DB, et al. Metabolic imaging of patients with prostate cancer using hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Sci Transl Med*. 2013; 5(198):198ra108.
4. Kurhanewicz J, Vigneron DB, Brindle K, et al. Analysis of Cancer Metabolism by Imaging Hyperpolarized Nuclei: Prospects for Translation to Clinical Research. *Neoplasia*. 2011; 13(2):81–97. [PubMed: 21403835]
5. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009; 324(5930):1029–1033. [PubMed: 19460998]
6. Venkatarayanan A, Raulji P, Norton W, et al. IAPP-driven metabolic reprogramming induces regression of p53-deficient tumours in vivo. *Nature*. 2015; 517(7536):626–630. [PubMed: 25409149]
7. Phan L, Chou PC, Velazquez-Torres G, et al. The cell cycle regulator 14-3-3sigma opposes and reverses cancer metabolic reprogramming. *Nature Comm*. 2015; 6:7530.
8. Chaumeil MM, Ozawa T, Park I, Scott K, James CD, Nelson SJ, Ronen SM. Hyperpolarized ^{13}C MR spectroscopic imaging can be used to monitor Everolimus treatment in vivo in an orthotopic rodent model of glioblastoma. *NeuroImage*. 2012; 59(1):193–201. [PubMed: 21807103]
9. Park I, Bok R, Ozawa T, Phillips JJ, James CD, Vigneron DB, Ronen SM, Nelson SJ. Detection of early response to temozolomide treatment in brain tumors using hyperpolarized ^{13}C MR metabolic imaging. *J Magn Reson Imaging*. 2011; 33(6):1284–1290. [PubMed: 21590996]
10. Radoul M, Chaumeil MM, Eriksson P, Wang AS, Phillips JJ, Ronen SM. MR Studies of Glioblastoma Models Treated with Dual PI3K/mTOR Inhibitor and Temozolomide: Metabolic Changes Are Associated with Enhanced Survival. *Mol Cancer Ther*. 2016; 15(5):1113–1122. [PubMed: 26883274]
11. Sandulache VC, Chen Y, Lee J, et al. Evaluation of hyperpolarized $[1-^{13}\text{C}]$ -pyruvate by magnetic resonance to detect ionizing radiation effects in real time. *PLoS One*. 2014; 9(1):e87031. [PubMed: 24475215]

12. Sandulache VC, Skinner HD, Wang Y, Chen Y, Dodge CT, Ow TJ, Bankson JA, Myers JN, Lai SY. Glycolytic inhibition alters anaplastic thyroid carcinoma tumor metabolism and improves response to conventional chemotherapy and radiation. *Mol Cancer Ther.* 2012; 11(6):1373–1380. [PubMed: 22572813]
13. Ward CS, Venkatesh HS, Chaumeil MM, et al. Noninvasive detection of target modulation following phosphatidylinositol 3-kinase inhibition using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Cancer Res.* 2010; 70(4):1296–1305. [PubMed: 20145128]
14. Witney TH, Kettunen MI, Hu DE, Gallagher FA, Bohndiek SE, Napolitano R, Brindle KM. Detecting treatment response in a model of human breast adenocarcinoma using hyperpolarised $[1-^{13}\text{C}]$ pyruvate and $[1,4-^{13}\text{C}_2]$ fumarate. *Br J Cancer.* 2010; 103(9):1400–1406. [PubMed: 20924379]
15. Bankson JA, Walker CM, Ramirez MS, et al. Kinetic Modeling and Constrained Reconstruction of Hyperpolarized $[1-^{13}\text{C}]$ -Pyruvate Offers Improved Metabolic Imaging of Tumors. *Cancer Res.* 2015; 75(22):4708–4717. [PubMed: 26420214]
16. Daniels CJ, McLean MA, Schulte RF, et al. A comparison of quantitative methods for clinical imaging with hyperpolarized ^{13}C -pyruvate. *NMR Biomed.* 2016; 29(4):387–399. [PubMed: 27414749]
17. Brindle KM, Bohndiek SE, Gallagher FA, Kettunen MI. Tumor imaging using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Magn Reson Med.* 2011; 66(2):505–519. [PubMed: 21661043]
18. Zierhut ML, Yen YF, Chen AP, Bok R, Albers MJ, Zhang V, Tropp J, Park I, Vigneron DB, Kurhanewicz J, Hurd RE, Nelson SJ. Kinetic modeling of hyperpolarized ^{13}C -pyruvate metabolism in normal rats and TRAMP mice. *J Magn Reson.* 2010; 202(1):85–92. [PubMed: 19884027]
19. Pages G, Kuchel PW. Mathematical Modeling and Data Analysis of NMR Experiments using Hyperpolarized (^{13}C) Metabolites. *Magn Reson Insights.* 2013; 6:13–21. [PubMed: 25114541]
20. Witney TH, Kettunen MI, Brindle KM. Kinetic modeling of hyperpolarized ^{13}C label exchange between pyruvate and lactate in tumor cells. *J Biol Chem.* 2011; 286(28):24572–24580. [PubMed: 21596745]
21. Bahrami N, Swisher CL, Von Morze C, Vigneron DB, Larson PE. Kinetic and perfusion modeling of hyperpolarized ^{13}C pyruvate and urea in cancer with arbitrary RF flip angles. *Quant Imaging Med Surg.* 2014; 4(1):24–32. [PubMed: 24649432]
22. Day SE, Kettunen MI, Gallagher FA, Hu D-E, Lerche M, Wolber J, Golman K, Ardenkjaer-Larsen J, Brindle KM. Detecting tumor response to treatment using hyperpolarized ^{13}C magnetic resonance imaging and spectroscopy. *Nat Med.* 2007; 13(11):1382–1387. [PubMed: 17965722]
23. Li LZ, Kadlecsek S, Xu HN, Daye D, Pullinger B, Profka H, Chodosh L, Rizi R. Ratiometric analysis in hyperpolarized NMR (I): test of the two-site exchange model and the quantification of reaction rate constants. *NMR Biomed.* 2013; 26(10):1308–1320. [PubMed: 23625472]
24. Harris T, Eliyahu G, Frydman L, Degani H. Kinetics of hyperpolarized ^{13}C -pyruvate transport and metabolism in living human breast cancer cells. *Proc Natl Acad Sci USA.* 2009; 106(43):18131–18136. [PubMed: 19826085]
25. Khagai O, Schulte RF, Janich MA, et al. Apparent rate constant mapping using hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *NMR in Biomedicine.* 2014; 27(10):1256–1265. [PubMed: 25156807]
26. Harrison C, Yang C, Jindal A, DeBerardinis RJ, Hooshyar MA, Merritt M, Dean Sherry A, Malloy CR. Comparison of kinetic models for analysis of pyruvate-to-lactate exchange by hyperpolarized ^{13}C NMR. *NMR Biomed.* 2012; 25(11):1286–1294. [PubMed: 22451442]
27. Hill DK, Orton MR, Mariotti E, et al. Model free approach to kinetic analysis of real-time hyperpolarized ^{13}C magnetic resonance spectroscopy data. *PLoS One.* 2013; 8(9):e71996. [PubMed: 24023724]
28. Kazan SM, Reynolds S, Kennerley A, Wholey E, Bluff JE, Berwick J, Cunningham VJ, Paley MN, Tozer GM. Kinetic modeling of hyperpolarized (^{13}C) pyruvate metabolism in tumors using a measured arterial input function. *Magn Reson Med.* 2013; 70(4):943–953. [PubMed: 23169010]
29. Walker CM, Chen Y, Lai SY, Bankson JA. A novel perfused Bloch–McConnell simulator for analyzing the accuracy of dynamic hyperpolarized MRS. *Medical Physics.* 2016; 43(2):854–864. [PubMed: 26843246]

30. Davenport R. The derivation of the gamma-variate relationship for tracer dilution curves. *J Nuc Med.* 1983; 24(10):945–948.
31. Madsen MT. A simplified formulation of the gamma variate function. *Phys Med Biol.* 1992; 37(7): 1597–1600.
32. Kettunen MI, Kennedy BW, Hu DE, Brindle KM. Spin echo measurements of the extravasation and tumor cell uptake of hyperpolarized [1-13C] lactate and [1-13C]-pyruvate. *Magn Reson Med.* 2013; 70(5):1200–9. [PubMed: 23280500]
33. Cheng HL. Investigation and optimization of parameter accuracy in dynamic contrast-enhanced MRI. *J Magn Reson Imaging.* 2008; 28(3):736–743. [PubMed: 18777534]
34. Luypaert R, Sourbron S, Makkat S, de Mey J. Error estimation for perfusion parameters obtained using the two-compartment exchange model in dynamic contrast-enhanced MRI: a simulation study. *Phys Med Biol.* 2010; 55(21):6431–6441. [PubMed: 20952813]

FIG. 1. The pharmacokinetic model and representative dynamic spectra and metabolite curves
 (a) The PK model is comprised of two chemical pools (HP pyruvate, HP lactate) in two spatial compartments (intravascular, with volume fraction v_b , and extravascular, with complimentary volume fraction v_e). k_{ve} is the rate constant for exchange between compartments, while k_{PL} and k_{LP} are the apparent rate constants for conversion of HP pyruvate to lactate and HP lactate to pyruvate, respectively. All rate constants have units (s^{-1}). This PK model was integrated into a Bloch-McConnell simulator that was used to generate raw synthetic data. (b) The Fourier transform of synthetic noisy data (SNR=30) yields dynamic spectra. (c) The real-valued full-width at half maximum area of absorption peaks associated with pyruvate and lactate were calculated to yield dynamic data for individual metabolites (symbols), which were then analyzed using the pharmacokinetic model for HP signal evolution (lines).

FIG. 2. Reproducibility of k_{PL} estimates improves with higher SNR

Synthetic data was generated assuming $k_{ve} = 0.02s^{-1}$, $v_b = 0.09$, $T_{l,Pyv} = 43s$, $T_{l,Lac} = 33s$, a gamma-variate shaped vascular input function, and (a) $k_{PL} = 0.05s^{-1}$, (b) $k_{PL} = 0.1s^{-1}$, (c) $k_{PL} = 0.2s^{-1}$, and (d) $k_{PL} = 0.4s^{-1}$. Noise was added to achieve the target SNR at the maximum of the dynamic HP pyruvate signal. The mean and standard deviation for k_{PL} estimates exhibit improved reproducibility with increasing SNR. (e) The coefficients of variation are markedly worse for peak-SNR levels below ~ 20 .

FIG. 3. Effects of errors in longitudinal relaxation rates on pharmacokinetic analysis

Synthetic data was generated assuming $k_{PL} = 0.1\text{s}^{-1}$, $k_{ve} = 0.02\text{s}^{-1}$, $v_b = 0.09$, and (a) a range of values for T_I of HP pyruvate ($T_{I,PyT} = 20\text{s}$ to 60s), with $T_{I,Lac} = 33\text{s}$; or (b) $T_{I,PyT} = 43\text{s}$ with a range of values for T_I of HP lactate ($T_{I,Lac} = 10\text{s}$ to 40s). Synthetic data was then analyzed assuming all parameters except k_{PL} were known, but with error factors applied to assumed values for (a) $T_{I,PyT}$ and (b) $T_{I,Lac}$. Results indicate that overestimation of $T_{I,PyT}$ has little impact on the accuracy of PK analyses, while overestimation of $T_{I,Lac}$ causes underestimation of k_{PL} that is independent of the underlying value. Underestimation of $T_{I,PyT}$ or $T_{I,Lac}$ causes an overestimation of k_{PL} that depends on the underlying value. Errors in k_{PL} of $\pm 10\%$ are highlighted with black vertical dotted lines. Horizontal red lines indicate the expected value for k_{PL} with no error ($f_e = 1$).

FIG. 4. Effects of errors in individual vascular parameters on pharmacokinetic analysis

Synthetic data was generated assuming $k_{PL} = 0.1 s^{-1}$, $T_{l,PyT} = 43s$, and $T_{l,Lac} = 33s$, and (a) a range of values for the rate of pyruvate extravasation ($k_{ve} = 0.005 s^{-1}$ to $0.04 s^{-1}$), with $v_b = 0.09$; or (b) $k_{ve} = 0.02 s^{-1}$, with a range of values for the vascular blood volume fraction ($v_b = 0.02$ to 0.1). Synthetic data was then analyzed assuming all parameters except k_{PL} were known, but with error factors applied to assumed values for (a) k_{ve} and (b) v_b . The vascular blood volume fraction and pyruvate extravasation rate have the greatest impact on accuracy, and show the most limited range of error factors that lead to errors in k_{PL} of $\pm 10\%$ (black vertical dotted lines). Horizontal red lines indicate the expected value for k_{PL} with no error ($f_e = 1$).

FIG. 5. Effects of errors in the amplitude and timing of the vascular input function on pharmacokinetic analysis

Synthetic data was generated assuming $k_{PL} = 0.1 \text{ s}^{-1}$, $k_{ve} = 0.02 \text{ s}^{-1}$, $v_b = 0.09$, $T_{I,PyT} = 43 \text{ s}$, and $T_{I,Lac} = 33 \text{ s}$, with the HP pyruvate vascular input function modeled as a gamma variate function (29,31) that arrives (a) at the expected bolus arrival time, t_b ; or (b) shifted in time by an offset of up to $\pm TR$ for arrival at $t_b' = t_b - t_{offset}$. Synthetic data was then analyzed assuming all that the VIF shape is known or accurately measured but with (a) an error factor applied to the VIF amplitude, or (b) an offset in time between true and assumed bolus arrival, with a positive offset indicating that the bolus arrived earlier than assumed in PK analysis. Incorrect scaling of the VIF will affect PK analysis, but to a lesser extent than vascular parameters (Fig 4; Table 1). Slight offsets in estimation of the bolus arrival will also impart some error in PK analysis (green curve), but that additional error can largely be mitigated by allowing a limited offset to be estimated and corrected during PK analysis (blue curve).

FIG. 6. Effects of errors in excitation angle on pharmacokinetic analysis

Synthetic data was generated assuming $k_{PL} = 0.1\text{s}^{-1}$, $k_{ve} = 0.02\text{s}^{-1}$, $v_b = 0.09$, $T_{l,pyr} = 43\text{s}$, $T_{l,Lac} = 33\text{s}$ and a range of values for the excitation angle ($\theta = 5^\circ$ to 30°). Synthetic data was then analyzed assuming all parameters except k_{PL} were known, but with an error factor applied to the excitation angle, simulating effects of poor transmit calibration or homogeneity. Errors in excitation angle can have significant impact on PK analysis of HP pyruvate, particularly when higher excitation angles are employed.

FIG. 7. Effects of prior information and number of unknowns on pharmacokinetic analysis
 Synthetic data was generated assuming $k_{PL} = 0.1 \text{ s}^{-1}$, $k_{ve} = 0.02 \text{ s}^{-1}$, $v_b = 0.09$, $T_{I,PyT} = 43 \text{ s}$, and $T_{I,Lac} = 33 \text{ s}$. Noise was added to achieve SNR=25 at the maximum of the dynamic HP pyruvate signal. Synthetic data was then analyzed assuming parameters marked with “+” were known, while k_{PL} and parameters marked with “-” were unknown and fit as free parameters during PK analysis of HP data. (a) Independent estimates of pyruvate perfusion parameters improve reproducibility of k_{PL} estimates. The standard deviations of k_{PL} estimates obtained with more than two unknowns among v_b , k_{ve} , and VIF shape are significantly larger than when fitting fewer than two of these parameters to HP data. (b) Error factors applied to VIF amplitude have a greater effect on the mean of k_{PL} estimates with v_b or k_{ve} unknown.

Table 1

Default values and ranges of parameters tested in each simulation, with summary of error factors resulting in $\pm 10\%$ errors in k_{PL} estimates (under worst-case conditions) for model parameters examined in Figures 3–5.

Model parameter	Default value	Range of P_i values	Worst case**	f_e at $\pm 10\%$ error		$\left. \frac{\partial k_{PL}^{est}}{\partial f_e} \right _{f_e=1}$
				Range		
T_1 for pyruvate ($T_{1,Py}$) *	43s	20s – 60s	20s	0.56 – 3	2.44	−0.013
T_1 for lactate ($T_{1,Lac}$) *	33s	10s – 40s	40s	0.70 – 1.26	0.56	−0.031
Vascular blood volume fraction (v_b)	0.09	0.02 – 0.1	0.1	0.82 – 1.20	0.38	0.050
Rate constant of extravasation (k_{ve})	0.02s ^{−1}	0.005s ^{−1} – 0.04s ^{−1}	0.04s ^{−1}	0.77 – 1.25	0.48	−0.044
Vascular input function (VIF) amplitude	1	***	***	0.53 – 2.58	2.05	0.014
Flip angle	20°	5° – 30°	5°	N/A	N/A	0.006
			10°	0.55 – 1.42	0.87	0.023
			15°	0.80 – 1.22	0.42	0.046
			20°	0.87 – 1.15	0.28	0.071
			30°	0.93 – 1.10	0.17	0.110

* The upper bound of T_1 values represent the maximum value used for any combination of a true value and error factor.

** All tested excitation angles are shown to illustrate excitation angle dependence.

*** Only the default value was used.