

Differentiation of Pyruvate Flux versus Exchange in Rat Liver In Vivo Using a Three-Site Exchange Model

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Introduction: Among several applicable substrates to dynamic nuclear polarization, ¹³C-labeled pyruvate (Pyr) has been most frequently used to date because of its essential role connecting multiple metabolic pathways. However, the metabolic conversions of Pyr to lactate (Lac) and alanine (Ala) via Lac dehydrogenase and Ala transaminase, respectively, are reversible reactions, making it difficult to differentiate metabolic flux from isotopic exchange, which is important to understand the metabolic fate of the injected Pyr. Several studies showed that the contribution of isotopic exchange for observation of products is important and non-negligible [1, 2]. Park, et al. [3] proposed a method to differentiate flux and exchange by co-administration of hyperpolarized [2-¹³C]Pyr and [1-¹³C]Lac, using a two-site exchange model [4], and showed an increased flux of Pyr to Lac in a rat liver after ethanol infusion. In this work, we propose an improved three-site exchange model (Fig.1) that considers conversion between Pyr, Lac, and Ala, and measure the ratio of flux and isotopic exchange among them by injecting co-polarized pairs of [2-¹³C]Pyr and [1-¹³C]Ala, and [2-¹³C]Pyr and [1-¹³C]Lac.

Method: All measurements were performed on a clinical 3-T GE MR scanner. A custom-built transmit/receive ¹³C surface coil (Ø = 28mm) was placed on top of the livers of healthy male Wistar rats



Fig. 1. Three-site exchange model

$$(1) Pyr_{RFcor,n} = \frac{Pyr_{measured,n}}{\sin \theta}$$

$$(2) TR \cdot k_{PL} = \frac{lac_n}{pyr_{RFcor,n} + lac_n + ala_n}, \quad TR \cdot k_{PA} = \frac{ala_n}{pyr_{RFcor,n} + lac_n + ala_n}$$

$$(3) lac_{acm,n} = lac_n + lac_{n-1} \cdot e^{-TR/T_1} \cdot \cos \vartheta_{n-1} + lac_{n-2} \cdot e^{-2TR/T_1} \cdot \cos \vartheta_{n-1} \cdot \cos \vartheta_{n-2} + \dots$$

$$ala_{acm,n} = ala_n + ala_{n-1} \cdot e^{-TR/T_1} \cdot \cos \vartheta_{n-1} + ala_{n-2} \cdot e^{-2TR/T_1} \cdot \cos \vartheta_{n-1} \cdot \cos \vartheta_{n-2} + \dots$$

$$(4) TR \cdot k_{AP} = \frac{pyr_n + lac_n}{ala_{RFcor,n} + pyr_n + lac_n}, \quad TR \cdot k_{PL} = \frac{lac_n}{pyr_n + lac_n}$$

$$(5) Flux_{Pyr \rightarrow Ala} = \frac{[Pyr] \cdot k_{PA} - [Ala] \cdot k_{AP}}{\min([Pyr] \cdot k_{PA}, [Ala] \cdot k_{AP})}, \quad Flux_{Pyr \rightarrow Lac} = \frac{[Pyr] \cdot k_{PL} - [Lac] \cdot k_{LP}}{\min([Pyr] \cdot k_{PL}, [Lac] \cdot k_{LP})}$$

Method: All measurements were performed on a clinical 3-T GE MR scanner. A custom-built transmit/receive ¹³C surface coil (Ø = 28mm) was placed on top of the livers of healthy male Wistar rats (459 ± 23 g, n = 4), and a birdcage ¹H coil (Ø = 70mm) was used for anatomical reference. Each animal was anesthetized with 1-3 % isoflurane in oxygen (~1.5 L/min), then administered two hyperpolarized solutions with a 3-h interval in-between: a co-polarized solution of 80-mM [2-¹³C]Pyr and 40-mM [1-¹³C]Ala with 40-mM ¹²C-Lac (injection 1), and a solution of 80-mM [2-¹³C]Pyr and 40-mM [1-¹³C]Lac with 40-mM ¹²C-Ala (injection 2). The unlabeled substrates were included in the dissolution buffers. Independent phantom experiments were performed to estimate liquid polarization levels and T₁'s of ¹³C-labeled substrates. MRS data were acquired following the injection of the hyperpolarized compounds using the dynamic free induction decay sequence with a 10° hard RF pulse (pulse width = 40 μs, spectral width = 10,000 Hz, 4096 spectral points, T_{acq} = 4 min, temporal resolution = 3 s). The obtained time-course data were fit using the following two types of three-site exchange models to estimate apparent conversion rate constants (k's) and apparent T₁'s.

Model 1 k_{PL} and k_{PA} from the injected [2-¹³C]Pyr were estimated using model 1. After correcting for RF sampling of measured [2-¹³C]Pyr curve (eq.1), produced Lac and Ala are calculated using k_{PL} and k_{PA} (eq.2). Then, accumulated Lac and Ala during pulse repetition time (TR) are calculated (eq.3) with the apparent T₁ decay and RF sampling. Finally, the best combination of the apparent T₁ and the apparent conversion rate constants, k_{PA} and k_{PL}, are estimated by comparing measured Lac and Ala with the lac_{acm} and ala_{acm}, respectively.

Model 2 k_{AP} and k_{PL} from the injected [1-¹³C]Ala, or k_{LP} and k_{PA} from the injected [1-¹³C]Lac were estimated using model 2. Procedure is similar to model 1 except eq.2. For example, when [1-¹³C]Ala is injected, eq.2 in model 1 is replaced by eq.4. From the obtained conversion rate constants and injected concentrations, the ratio of isotopic exchange and flux are calculated using eq.5.

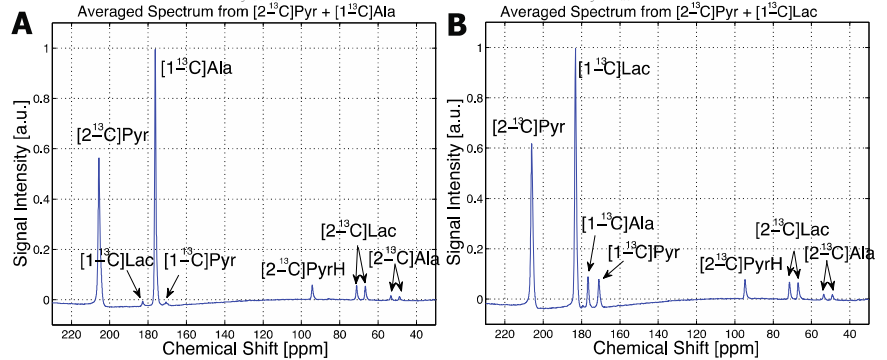


Fig. 2. Time-averaged (0-90s) spectra from (A) [2-¹³C]Pyr + [1-¹³C]Ala, and (B) [2-¹³C]Pyr + [1-¹³C]Lac

Results: Polarization levels were 28.3 ± 0.6 % (mean ± se) for [2-¹³C]Pyr (n = 5), 30.7 ± 1.7 % for [1-¹³C]Ala (n = 3), and 27.8 ± 2.6 % for [1-¹³C]Lac (n = 3). T₁'s in solution were estimated as 47.1 ± 1.7 s for [2-¹³C]Pyr, 38.2 ± 1.9 s for [1-¹³C]Ala, and 41.2 ± 2.1 s for [1-¹³C]Lac. Representative *in vivo* spectra of injections 1 & 2 acquired from the rat liver are shown in Fig.2. Representative kinetic curves from injected Pyr, Ala, and Lac are shown in Fig. 3.

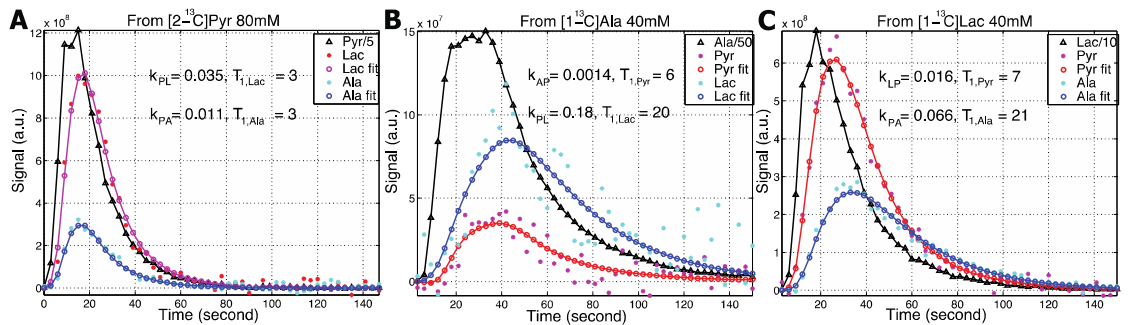


Fig. 3. Representative time-curves measured from injected (A) [2-¹³C]Pyr, (B) [1-¹³C]Ala, and (C) [1-¹³C]Lac, and their fitting curves estimated by 3-site exchange models.

The apparent conversion rate constants from [2-¹³C]Pyr were k_{PL,2} = 0.032 ± 0.002 s⁻¹, k_{PA,2} = 0.01 ± 0.0006 s⁻¹ (n = 7). Similarly, k_{AP,1} = 0.0012 ± 0.0001 s⁻¹ and k_{PL,1} = 0.18 ± 0.02 s⁻¹ (n = 4) were obtained from [1-¹³C]Ala, and k_{LP,1} = 0.015 ± 0.0006 s⁻¹ and k_{PA,1} = 0.06 ± 0.004 s⁻¹ (n = 3) from [1-¹³C]Lac were estimated. Flux-to-Exchange ratio was 14.8 ± 0.8 between Pyr and Ala, and 3.3 ± 0.1 between Pyr and Lac.

Discussion & Conclusion: Simultaneous measurements of forward and reverse apparent conversion rates are demonstrated by injecting co-polarized Pyr + Lac and Pyr + Ala. This allows flux and isotopic exchange to be differentiated, and flux-to-exchange ratios in rat liver could be estimated without knowledge of the absolute *in vivo* concentrations. When 80-mM Pyr, 40-mM Lac, and 40-mM Ala were co-administered, the ratio of flux to exchange from Pyr to Ala was much higher than for Pyr to Lac. Considering the non-negligible intrinsic Lac pool size [5], flux-to-exchange rate between Pyr and Lac could even be underestimated. Moreover, the proposed three-site exchange model compensates over- or under-estimated apparent conversion rates by introducing three interacting compartments.

References: [1] Kettunen MI, et al, *MRM* 2010;63(4):872, [2] Witney TH, et al, *J Biol Chem.* 2011;286(28):24572, [3] Park JM, et al, *ISMRM.* 2012;4298, [4] Josan S, et al, *ISMRM.* 2011;3528, [5] Phypers B, et al, doi:10.1093/bjaceaccp/mkl018

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