Quantitative Measurement of Pyruvate-Lactate Exchange Rate in Both Directions Using Exchange-Linked Dissolution Agents (ELDA) in Hyperpolarized 13C Metabolic Imaging

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INTRODUCTION The [1-^{13}C]lactate signal, as observed in hyperpolarized [1-^{13}C]pyruvate metabolic imaging, appears to be a good marker for disease progression and response to therapy. However, under the large [1-^{13}C]pyruvate bolus conditions used in these studies, the formation of [1-^{13}C]lactate is often saturated (1). The rate and extent of the [1-^{13}C]lactate produced under saturated conditions, may be limited, under some conditions, by the steady-state lactate pool size (e.g. NADH availability from the conversion of unlabeled lactate to pyruvate). If lactate pool size limits the production of hyperpolarized lactate signal, the method reduces to just an indirect measure of steady-state lactate. Such a measurement may have clinical value, but the use of ELDA (2) to overcome the limit, or as shown here, to quantify exchange rates, adds a significant level of specificity.

THEORY Ignoring any contribution from transport, the rate of conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate in dissolution-DNP metabolic imaging can be described as:

$$\boldsymbol{Vpl = Vflux + Vexchange}$$

where under typical pyruvate bolus conditions, the rate of flux depends on the concentration of labeled pyruvate [Pyr], and the rate of exchange may be limited by the steady state concentration of lactate [Lac$_0$] so that:

$$\boldsymbol{Vpl_0 = Kflux[Pyr] + Kexchange[Lac_0]}$$

If additional lactate [Lac$_\Delta$] is added to the system, the rate of [1-^{13}C]lactate production becomes:

$$\boldsymbol{Vpl_\Delta = Kflux[Pyr] + Kexchange'([Lac_0] + [Lac_\Delta])}$$

Any increase in [1-^{13}C]lactate production (steady state pool size limiting condition) can then be described as:

$$\boldsymbol{Vpl_\Delta - Vpl_0 = Kexchange'[Lac_\Delta] + (Kexchange' - Kexchange)[Lac_0]}$$

And under conditions where the increase in rate is linear with increasing concentration of the unlabeled lactate added to the system, the exchange rate constant can be assumed to be unchanged, and the increase simplifies to:

$$\boldsymbol{Vpl_\Delta - Vpl_0 = Kexchange[Lac_\Delta]}$$

Under these conditions, the increased [1-^{13}C]pyruvate to [1-^{13}C]lactate production should match the [1-^{13}C]lactate to [1-^{13}C]pyruvate conversion in an experiment in which the hyperpolarized label has been switched from pyruvate to lactate in the bolus. Thus we would expect that $[Lac_\Delta]$ would remain rate limiting for exchange and:

$$\boldsymbol{Vlp_\Delta = Kexchange[Lac_\Delta]}$$

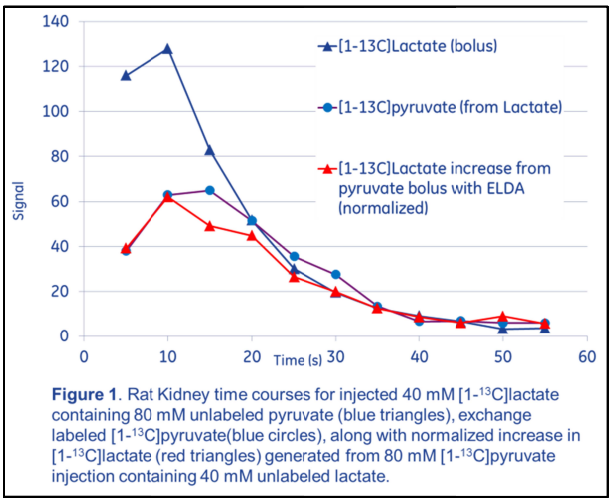

Figure 1. Rat Kidney time courses for injected 40 mM [1-^{13}C]lactate containing 80 mM unlabeled pyruvate (blue triangles), exchange labeled [1-^{13}C]pyruvate(blue circles), along with normalized increase in [1-^{13}C]lactate (red triangles) generated from 80 mM [1-^{13}C]pyruvate injection containing 40 mM unlabeled lactate.

METHODS 3 male Wistar rats were studied under conditions previously described (1). The linearity of the ELDA response (increase in the conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate) was confirmed in rat kidney for injections of 80 mM hyperpolarized [1-^{13}C]pyruvate containing 0,20,40 and 80 mM unlabeled sodium lactate. In two rats, hyperpolarized 80 mM [1-^{13}C]pyruvate, with and without 40 mM unlabeled lactate, were compared with hyperpolarized 40 mM [1-^{13}C]lactate, with and without 80 mM unlabeled pyruvate. All experiments were performed on a 3T Signa™ (GE Healthcare, Waukesha, WI), using a dual-tuned (1H/13C) quadrature rodent coil. Dynamic imaging was performed every 5 s starting at 5 s from start of tail vein injection using 3D-spCSI with a nominal isotropic resolution of 5 mm. Each imaging volume was sampled with 36 5.6-deg excitations. (Tacq=4.5s). K*exchange* in the reverse direction, was determined directly from the conversion of injected hyperpolarized [1-^{13}C]lactate to [1-^{13}C]pyruvate . The measurement of K*exchange* in the forward direction was made by fitting the difference in [1-^{13}C]lactate produced by hyperpolarized [1-^{13}C]pyruvate injections (*with* minus *without* unlabeled lactate), relative to the lactate bolus. Since the lactate in the bolus is unlabeled, signal from the [1-^{13}C]lactate (with unlabeled pyruvate in the bolus) injection was used in its place. The difference in [1-13C]lactate detected in the forward direction was corrected for the difference in the lactate and pyruvate polarization levels.

RESULTS Linear increases in [1-^{13}C]pyruvate to [1-^{13}C]lactate conversion were observed in rat kidney based on data from the addition of 20mM, 40mM, and 80mM unlabeled lactate to the 80mM hyperpolarized [1-^{13}C]pyruvate bolus. Figure 1 shows the product dynamics for one of the two rats in which K*exchange* was measured, relative to the 40mM ^{13}C lactate input. K*exchange* forward was 0.016 s^{-1} and K*exchange* in the reverse direction was 0.018 s^{-1}. In a second animal studied, K*exchange* forward was 0.018 s^{-1} and K*exchange* reverse was 0.016 s^{-1}. Within the error of the measurements, a consistent K*exchange*, determined for forward and reverse directions, confirms the prediction from theory.

CONCLUSION Under appropriate conditions, absolute values of K*exchange* can be determined using the ELDA approach. Further, the addition of metabolite quantitation, could lead to a direct measure of [NADH] availability in these studies.

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1. Zierhut et al. 2010; J Magn Reson 202:85 2. Hurd et al, ISMRM 2011 Proc. Intl. Soc. Mag. Reson. Med. 19 (2011) 654