# Dynamic Interleaved Imaging of Hyperpolarized Metabolites for Lactate Dehydrogenase Kinetics
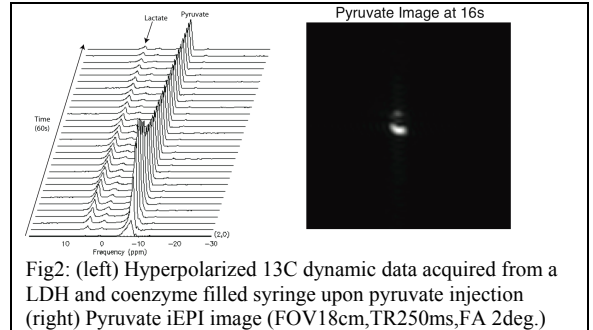
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**INTRODUCTION** Dynamic Nuclear Polarization (DNP) of metabolically active 13C-labelled substrates has been reported as a method of generating MR images of in vivo cellular metabolism [1] and hence has the potential to characterize aggressive cancers that exhibit heightened energy metabolism non-invasively[2]. In a phenomenon known as the Warbrug effect, aggressively proliferating tumor cells preferentially up-regulate lactate-dehydrogenase (LDH) enzyme to consume glucose at a high rate and release lactate in glycolysis despite the presence of adequate oxygen [3,4]. It has been demonstrated that quantitative pyruvate-lactate exchange rates can be obtained by modeling the temporally resolved hyperpolarized dynamic 13C MR spectroscopic data [5] and it may be feasible to create an in vivo map of enzymatic rates if 13C images of the various metabolites can be acquired dynamically over time. In this abstract, we describe the use of a metabolite specific rapid imaging pulse sequence for acquisition of spatially and temporally resolved hyperpolarized pyruvate and lactate images in vitro and in vivo. The dynamic imaging data are fitted to a kinetic two-pool model and compared to results from dynamic MR spectroscopic data.

Fig 1: Interleaved pyruvate and lactate imaging scheme
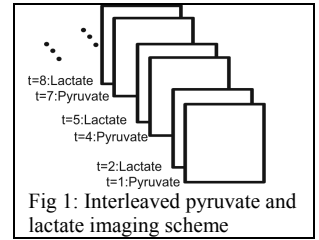
METHODS *In-vitro* studies: A syringe containing 3mL of 40mM unlabeled sodium lactate, 20mM NAD+, 10mM NADH, and 530 activity units LDH-5 (Sigma, L1254) was prepared immediately before the experiments. Samples of [1-13C] pyruvic acid (Isotec,Miamisburg,OH) and 15 mM trityl radical were hyperpolarized at 1.4K with a Hypersense DNP hyperpolarizer (Oxford Instruments, Tubney Woods, UK). It was rapidly dissolved with NaOH/Tris/EDTA buffer solution to a concentration of 80mM, pH7.5, and a bolus of 1.5mL was injected into the enzyme mixture inside the collection tube at the MRI scanner over 10s. *In-vivo* studies: 2.5mL of the 80mM solution was injected into the tail vein of an adult Sprague-Dawley rat over 10s. All experiments were performed on a GE MR750 3T scanner with a dual-tuned transmit-receive birdcage coil. The dynamic MR spectroscopic data was acquired with a double spin echo pulse sequence without spatial localization [6] (TR=2s, TE=35ms, flip angle=2.5deg (*in-vitro*); 10deg (*in-vivo*)). The dynamic lactate and pyruvate images were acquired with a spectral-spatial (sp-sp) selective RF pulse (Fig. 1) implemented with a flyback echo-planar imaging sequence [7] with interleaved pyruvate (2deg flip angle) and lactate (10deg flip angle) excitations and four-shot EPI acquisitions (Fig 1.); a 2D axial image of pyruvate and lactate with 18 cm FOV, TR 60ms, 32x32 matrix, 2cm thick slice were acquired every 3s. Pyruvate and lactate dynamic data was extracted from an ROI on the reconstructed images after correcting for tip-angle differences. Data analysis was performed in SAGE (GE Healthcare, Waukesha, WI) and Matlab (The Mathworks, Inc., Natick, MA).

Fig2: (left) Hyperpolarized 13C dynamic data acquired from a LDH and coenzyme filled syringe upon pyruvate injection (right) Pyruvate iEPI image (FOV18cm,TR250ms,FA 2deg.)

RESULTS A dynamically stacked MRS spectral plot shown in Fig. 2a illustrates the LDH-5 catalyzed transfer of 13C-labelled carbon from the hyperpolarized pyruvate to lactate. The signal for each metabolite and time point was computed by integrating the corresponding spectral line. An example of interleaved pyruvate images acquired with the sp-sp pulse and flyback-EPI is shown in Fig. 2b. The time courses of the metabolites from the *in vitro* and *in vivo* dynamic images are compared to the MRS data in Fig 3 and 7 respectively. Representative *in vivo* dynamic images of pyruvate and lactate, and the corresponding proton anatomical image from a slice through the kidneys are shown in Fig 4-5. The dynamic imaging and MRS data was fitted to the modified Bloch equations for a two-compartment kinetic model: $dP/dt = -(k_{0P} + k_{LP}) P + R_A$, $dL/dt = k_{LP} P - k_{0L} L$ where P, L denote the peak integrals of z-magnetizations of pyruvate and lactate, respectively; t is time; and R_A is the constant rate of appearance. The peak integrals were least-squares fitted to these equations using the Levenberg-Marquardt method to obtain R_A, pyruvate to lactate conversion rate constant k_{LP}, and the apparent spin lattice relaxation rates, k_{0P}, k_{0L}. The metabolite kinetics of the right kidney is shown in Fig. 6 and the results are summarized in Table 1.

Fig3: Pyruvate-to-lactate conversion time course of *in vitro* enzyme mixture with dynamic imaging and MRS acquisitions.
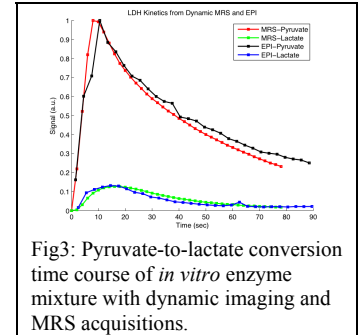
DISCUSSION and CONCLUSIONS In this study, a spectral-spatial RF pulse and a rapid flyback echo planar encoding technique were used to acquire hyperpolarized 13C images of specific metabolites (pyruvate and lactate) at high spatial and temporal resolution. In an *in vitro* model system, dynamic data from a series of periodically acquired pyruvate and lactate image reveals comparable conversion time course to that obtained with non-localized dynamic MR spectroscopy. This approach was also tested *in vivo* in a normal rat to demonstrate the feasibility of mapping first order enzymatic conversion rates non-invasively with hyperpolarized 13C MR imaging. This imaging approach could be useful for creating spatially resolved kinetics maps for investigating heterogeneous tumours and comparing tissue types.

Table1	Axial slice	Axial slice	Right kidney	Left kidney
Method	Dynamic MRS	iEPI	iEPI	iEPI
k_{LP} (1/s)	0.009±0.005	0.010±0.003	0.014±0.007	0.017±0.006
k_{0P} (1/s)	0.11±0.01	0.114±0.006	0.12±0.01	0.15±0.01
k_{0L} (1/s)	0.06±0.05	0.05±0.02	0.06±0.04	0.07±0.03

REFERENCES:[1] Golman et al. Proc. Natl. Acad. Sci. USA 103 (2006) 11270-11275. [2] Golamn K et al, Cancer Res 66 (2006) 10855- 10860 [3] Vander Heiden et al., Science 324 (2009) 1029-1033 [4] Feron Rad. And Onc. 92 (2009) 329-333; [5] Day et al. Nature Medicine 13 (11) (2007) 1382-1387,[6] Cunningham, JMR 2008,193:146; [7] Cunningham et al., JMR 193 (2008) 139-146;

Fig4: T2 FSE axial image of a rat and the prescribed ROI of the kidneys

Fig5: Interleaved EPI images of pyruvate at 19s (left) and lactate at 20s (right) after the start of the hyperpolarized [1-13C]pyruvate injection.

Fig6: Changes in z-magnetization dynamics in the right kidney ROI fitted to a two-compartment kinetic model

Fig7: Z-magnetization dynamics in the entire 2cm slice through kidneys with dynamic imaging and MRS acquisitions