Kinetic Modeling of Hyperpolaized [1-13C]Pyruvate Metabolism in Blood

K. K-C. Leung[1,2], W. W. Lam[1], A. P. Chen[3], and C. H. Cunningham[1,2]

[1]Imaging Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, [2]Dept. of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, [3]GE Healthcare, Toronto, Ontario, Canada

INTRODUCTION Dynamic Nuclear Polarization (DNP) of metabolically active 13C-labelled substances has been reported as a method of generating MR images of *in vivo* cellular metabolism [1] and hence has the potential to detect aggressive cancers that exhibit heightened metabolism [2]. By injecting active, hyperpolarized 13C metabolites such as pyruvate, it is possible to visualize their transformation to lactate and other products that have distinctive MRI frequencies. However, in addition to the metabolites that are transformed locally, there are also metabolic products generated in blood and other tissues that enter the tissue of interest via the systemic circulation, complicating analysis of local metabolism. Using hyperpolarized [1-13C] pyruvate and MR spectroscopy, this study attempts to quantify pyruvate-lactate conversion in *ex vivo* whole blood which might be useful in providing an estimation of blood metabolic contribution to regions of interest in *in vivo* studies.

METHODS Five milliliters of whole blood samples were taken from live Sprague-Dawley rats (n=2) and a pig and stored in lithium heparin coated blood collection tubes (BD vacutainer) immediately before the experiments. Samples of [1-13C] pyruvic acid and 15 mM trityl radical were hyperpolarized at 1.4K with a DNP hyperpolarizer (Oxford Instruments, Tubney Woods, UK). The sample was rapidly dissolved with NaOH/Tris/EDTA buffer solution to a concentration of 80mM, and a bolus of 1.9mL was injected into the blood inside the collection tube at the MRI scanner. Prior to the injection, the vacuum in the collection tube was restored with a 5mL syringe to facilitate the injection. This procedure was tested with dye and water to ensure adequate mixing. The injection took 2-3 seconds while data acquisition began immediately. The experiments were performed on a GE Excite 3T MRI scanner with a dual-tuned transmit-receive rat birdcage coil. An adiabatic double spin echo pulse sequence (TR=1s, TE=35ms, flip angle=10deg) [3] was used in this study. Data analysis was performed in SAGE (GE Healthcare, Waukesha, WI) and Matlab (The Mathworks, Inc., Natick, MA)

RESULTS [1-13C] pyruvic acid was transferred from the injected hyperpolarized pyruvate to lactate in the whole blood as expected. The z-magnetization peak integrals can be fit to the modified Bloch equations for a two compartmental linear kinetic model (figure1):

$$dP/dt = -(k_{LP} + k_{0P})P + R_A, \quad dL/dt = k_{LP}P - k_{0L}L$$

Where P, L denote the peak integrals of z-magnetizations of pyruvate and lactate, respectively; t is time; R_A is the constant rate of appearance, The peak integrals were SNR weighted, least squares fitted to these equations to obtain R_A, the rate constants k_{LP}, and the apparent spin lattice relaxation rates, k_{0P}, and k_{0L}. The metabolites' spectra and the two compartmental model fit of the metabolic kinetics are shown in figures 2 and 3, respectively. The time to peak for pyruvate and lactate are 3±1s, and 16 ±3s, respectively. Knowing the amount of [1-13C]pyruvate injected and using the parameters from the model, pyruvate-lactate substrate flux can be estimated (see Table 1).
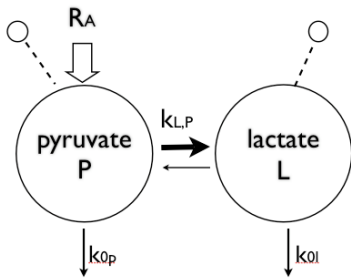

Figure 1: A two-compartment model for pyruvate metabolism and exchange with lactate in ex-vivo rat whole blood. Conversion from lactate to pyruvate is assumed to be negligible to large rate of appearance of pyruvate (Ra).
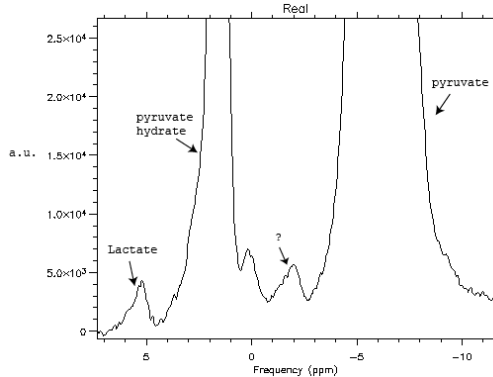

Figure 2: Spectrum of pyruvate and lactate 20 seconds after addition of 1.9mL bolus [1-13C]pyruvate to 5ml of rat whole blood

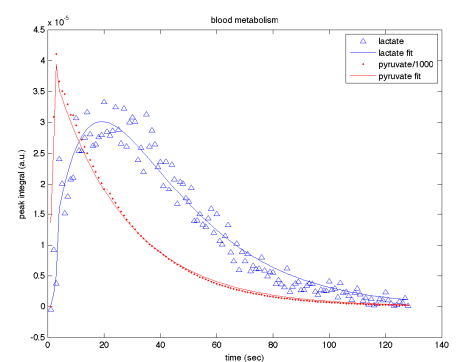Figure 3: Changes in z-magnetization peak integrals pyruvate and lactate, fit to two compartment kinetic model with addition of bolus [1-13C]pyruvate to rat whole blood in 3 seconds

Table 1.

	k_{0L} (s^{-1})	k_{0P} (s^{-1})	k_{LP} (s^{-1})	$R^2(L)$	$R^2(P)$	Pyr->Lac flux (mmol/ml/min)
Rat1	0.0631	0.0385	0.000097	0.9556	0.9811	1.28E-04
Rat2	0.1029	0.0396	0.000173	0.9663	0.9464	2.29E-04
Pig	0.218±0.056	0.036±0.003	9.2e-05± 3.4e-05	0.91±0.02	0.97±0.03	(1.21±0.5)E-04

DISCUSSIONS The bolus of 1.9mL [1-13C]pyruvate in 5mL of fresh rat whole blood is chosen to be comparable to doses used in in-vivo rat metabolic study. Given the large pyruvate bolus, it is assumed that lactate dehydrogenase predominantly converts pyruvate to lactate with negligible backward conversion. Compared to an *in vivo* study with adult Sprague-Dawley rats, the pyruvate-to-lactate conversion in *ex vivo* rat blood is approximately 1% of that in a rat's kidneys (0.015 ± 0.002)[3]. It is likely alanine is also produced by the red blood cells. However, its kinetics is not investigated due to suspected contamination near alanine's spectrum. Comparing Romijn's isotopic tracers studiy [4], the pyruvate-lactate conversion flux obtained here is 3 orders of magnitude lower. The difference might be due to the higher than normal physiological pyruvate concentration used in this study and different metabolic rates among species. Nonetheless, the variability of metabolic rates among subjects in this study is consistent with Romijn's result. In conclusion, we have demonstrated the feasibility of quantifying pyruvate metabolism in *ex vivo* blood and that metabolism in blood is very small compared to rat kidney tissues.

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] Cunningham et al. JMR 2007; 187(2):357-362 [4]K,Leung Using Spectral-Spatial Saturation RF pulse to Remove Blood Signal in Hyperpolarized 13C Metabolic Studies, ISMRM2009 abstract to be submitted. [4] Romijn et al., American Phy.Soc. 29(3) 1994 E334-E340