Metabolic Imaging of the Perfused Rat Heart Using Hyperpolarized [1-13C]Pyruvate

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Introduction : The recent development of liquid state Dynamic Nuclear Polarization (DNP) techniques has dramatically increased the signal available from ^{13}C MRS experiments and has opened up new possibilities for metabolic imaging of the heart [1]. Oxidative decarboxylation of DNP hyperpolarized [1-^{13}C]pyruvate, mediated by the pyruvate dehydrogenase (PDH) complex, is a critical reaction that produces acetyl-CoA for ATP synthesis and also produces the byproducts NADH and [1-^{13}C]carbon dioxide ($^{13}CO_2$). $^{13}CO_2$ is in pH-dependent equilibrium with bicarbonate ($H^{13}CO_3^-$) and observation of $H^{13}CO_3^-$ production has been shown to be an effective biomarker of real-time, *in vivo* PDH flux [2]. Further, reduction of hyperpolarized [1-^{13}C]pyruvate to [1-^{13}C]lactate and transamination to [1-^{13}C]alanine can be monitored and it has been shown that [1-^{13}C]lactate accumulation is a metabolic indicator of cardiac ischaemia [3]. Thus, the capability to visualize these metabolites *in vivo* allows us to probe metabolic processes that are critical to energy production and the physiological condition of the heart [1]. However, to fully understand the changes observed *in vivo* it is useful to be able to study a model system where the exact parameters of the system can be carefully controlled. The perfused rat heart has been used as such a model system for many years, but the limitations of a small heart size and the resulting requirement for high spatial resolution have so far prevented metabolic imaging of the perfused heart with hyperpolarized [1-^{13}C]pyruvate. In this study, we aimed to demonstrate the feasibility of mapping the spatial distribution of hyperpolarized pyruvate, lactate, alanine and bicarbonate in the perfused rat heart via the implementation of a rapid, high spatial resolution, chemical shift imaging (CSI) approach.

Materials and Methods

***Isolated Heart Perfusion*:** Rat hearts were rapidly excised from male Wistar rats (~350 g) and perfused in the recirculating retrograde Langendorff mode [3] and then placed in the centre of an 11.7 T, vertical bore MRI scanner (Bruker-Biospin, Germany). ***Pyruvate Polarization and Dissolution*:** Approximately 40 mg of [1-^{13}C]pyruvic acid (Sigma, UK), doped with 15 mM trityl radical and a trace amount of Dotarem (Guerbet, France), was hyperpolarized in a HyperSense polarizer (Oxford Instruments, UK). The sample was subsequently dissolved in a pressurized and heated sodium hydroxide solution, containing 100 mg/litre EDTA, to yield a solution of 80 mM hyperpolarized sodium [1-^{13}C]pyruvate with 30% polarization at physiological temperature and pH. The dissolved solution was then mixed with oxygenated Krebs-Henseleit perfusion buffer, diluted to ~5mM and delivered to the isolated heart via the coronary arteries. ***1D Spectroscopy*:** Immediately before delivery of the hyperpolarized [1-^{13}C]pyruvate to the heart, a 1D pulse-acquire spectroscopy sequence was initiated (TR = 1 s, flip angle (FA) = 30°, hard pulse duration = 60 µs, sweep width (SW) = 100 ppm, acquired points (SP) = 2048, acquisition centered on the pyruvate resonance). 120 transients were collected to monitor the arrival and decay kinetics of the infused pyruvate and its metabolic derivatives, lactate, alanine and bicarbonate. MR spectra were analyzed with the AMARES algorithm within the jMRUI software package [4]. ***Chemical Shift Imaging*:** A 2D gradient-echo CSI pulse sequence was implemented with acquisition begun 18 s after the start of infusion following identification of an optimal imaging window in the 1D spectroscopy time course. A single CSI image was acquired axially through the middle of the heart over a time-course of 64 s with TR = 250 ms, TE = 0.85 ms, FA = 25°, SW = 40 ppm, SP = 512, FOV = 24 x 24 mm, matrix = 16 x 16, slice thickness = 5 mm. Post-processing with Matlab included zero-filling the data to 128 x 128, 2D Fourier Transformation and automatic detection of the resonance of each metabolite to generate metabolic maps of pyruvate, lactate, alanine and bicarbonate. A reference proton image was acquired with a FLASH imaging sequence (matrix = 128 x 128, TR = 100 ms, TE = 1.28 ms and slice thickness = 1 mm).

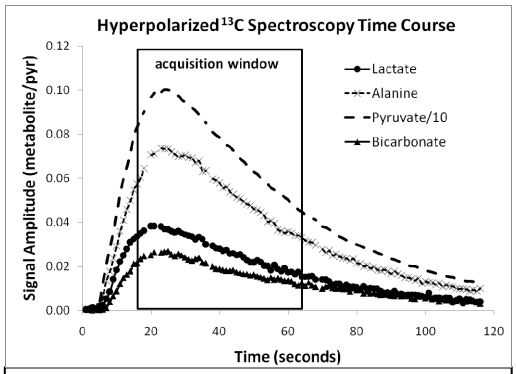

Figure 1: Metabolic time course of the infused [1-^{13}C]pyruvate and its derivatives [1-^{13}C]lactate, [1-^{13}C]alanine and $H^{13}CO_3^-$. The imaging window is overlaid on the plot.

Results & Discussion : The time course in Figure 1 illustrates the temporal evolution of hyperpolarized lactate, alanine and bicarbonate following infusion of pyruvate. All metabolites reached a peak amplitude at approximately 20 s after the start of infusion. Therefore subsequent imaging was chosen to begin at t = 18 s so that the centre of k-space coincided with the peak amplitudes of the metabolites. The imaging window is shown boxed in Figure 1. Figure 2 shows the axial proton image along with the metabolite maps for pyruvate, lactate, alanine and bicarbonate. The left and right ventricles are highlighted on the metabolite maps by the dotted lines. The peak pyruvate signal is seen just outside the heart at the location of perfusion buffer drainage. All other metabolites are localized within the contracting left ventricular tissue.

Conclusion : Hyperpolarized ^{13}C chemical shift imaging can allow 2D visualization of pyruvate metabolism in the isolated perfused heart. Localized metabolic maps, such as the bicarbonate map, provides real-time spatial information on energy production in the myocardium. A spatial resolution, before zero-filling, of approximately 11 µl permits localized detection of metabolism throughout the left ventricle. Future work will include reducing the TR and extending the imaging window to allow for the acquisition of multiple images or 3D acquisitions.

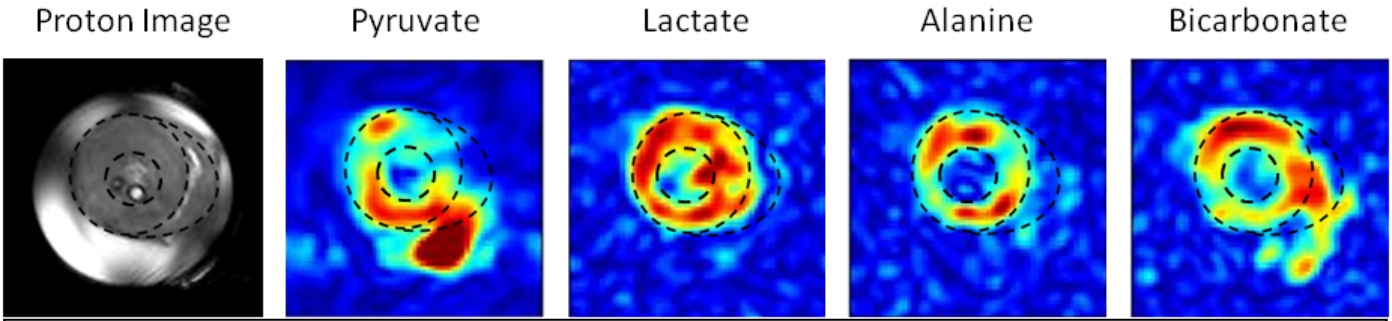

Figure 2: Metabolic images of [1-^{13}C]pyruvate, [1-^{13}C]lactate, [1-^{13}C]alanine and $H^{13}CO_3^-$ in the perfused heart along with a reference proton image. The dotted lines indicate the borders of the left and right ventricles.

References : [1] Golman *et al*, MRM 59:1005-13 (2008), [2] Schroeder *et al* PNAS 105(33), 12501-12056 (2008). [3] Schroder *et al* FASEB 23 (2009) [4] Naressi *et al* Comput Biol Med 31:269-86 (2001).

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