^{31}P MRS and Creatine CEST: A Method to Monitor Creatine Kinase Metabolism in a Perfused Heart Model

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Purpose: The activity of creatine kinase (CK) plays a crucial role in cardiac muscle bioenergetics[1]. Phosphocreatine (PCr), a substrate of CK, is utilized as an energy reserve; during periods of metabolic stress, muscle cells utilize CK to replenish ATP while converting the pool of PCr to creatine (Cr), which accumulates in myocytes. While the cell immediately disposes of ATP, Cr accumulates until the energy demands on the cell are returned to normal. ^{31}P MR spectroscopy has been able to measure high energy phosphate metabolites in the heart as well as the intracellular pH of cardiac muscle[2,3]. However, ^{31}P spectroscopy lacks spatial resolution and cannot measure the depleted Cr pool. Creatine Chemical Exchange Saturation Transfer (CrCEST) has recently been demonstrated as a technique that can provide high sensitivity and high resolution contrast based specifically on Cr concentration[4]. Herein, we demonstrate that CrCEST combined with ^{31}P spectroscopy can be potentially used as a tool to monitor the energy economy of living *ex vivo* cardiac tissue under metabolic stress.

Materials and Methods: All animal procedures are approved by IACUC of the University of Pennsylvania. Male Wistar rats (300 g, purchased from Charles River) was anesthetized and injected with 2000 U heparin. The heart was quickly removed and immersed in ice cold cardioplegia solution. The aorta was cannulated and the heart was perfused retrograde in Langendorff flow mode. To allow simultaneous MR studies, perfusion took place in a 20 mm NMR sample tube, which was placed inside a 400 MHz vertical bore (89 mm ID) Oxford magnet interfaced with DirectDrive console (Agilent, Palo Alto, CA). MR spectra and images were acquired using a 20-mm ID broadband liquids probe doubly tuned to ^{1}H/^{31}P.

The perfusion media made of modified Krebs-Henseleit (KH) buffer containing 10 mM glucose was warmed and aerated with a mixture of 95:5 % O_2:CO_2 to maintain pH of 7.4 ± 0.1. The heart was stabilized for 15 min and heart rate (HR) and left ventricle (LV) pressure were measured by a balloon catheter introduced into the LV. The heart was then arrested by increasing potassium chloride concentration in the KH buffer to 20 mM; the HR was reduced to zero within 3 min; following this, the balloon catheter was removed from LV. Non-flow ischemia was then induced by turning off perfusion pump. CrCEST and ^{31}P-NMR data were then acquired sequentially.

Localized shimming was performed using a customized Varian shim interface, and the full width at half maximum of the water peak for all CEST acquisitions was maintained at less than 55 Hz. All CEST experiments utilized a 500 ms saturation pulse train consisting of four 125 ms square pulses of 2.85 μT peak B_1 power each. These pulses were then followed by a conventional spoiled gradient-echo readout, with a TR of 8s. Frequency offsets saturated and imaged during each CEST acquisition varied linearly from 1.5 to 2.1 ppm and -1.5 to -2.1 ppm with respect to water in 0.1 ppm steps. Water saturation shift referencing (WASSR) data[5] was acquired for B_0 mapping at the beginning and end of imaging, and was collected from 0 to 0.5 and -0.5 to 0 ppm in steps of 0.1 ppm.

Results and Discussion: Due to the action of creatine kinase, low levels of PCr in a living cardiac tissue sample should correlate with relatively high concentrations of Cr. CrCEST (Fig. 1 A, B, C) and ^{31}P spectra (D, E, F) acquired sequentially show this correlation clearly. PCr/γATP ratios and associated average CrCEST asymmetry percents are plotted in Fig. 2. It is important to note that the CrCEST asymmetry maps represent only a single axial slice out of the whole sample volume over which the ^{31}P MRS was collected. However, these preliminary observations - consistent with both theory and results from previous *in vivo* studies[4,6] - are an important first step towards the realization of a number of *ex vivo* cardiac stress experiments. Work is in progress towards utilizing ^{31}P MRS and creatine CEST to probe *ex vivo* models of ischemia, drug interaction with heart tissue metabolism, and metabolic response to tachycardia. In addition, we expect that this combined approach will provide a valuable vehicle to study *ex vivo* cardiac muscle energetics on models that more closely approximate the human heart, as well as a foundation on which to build *in vivo* studies of human heart metabolism using CrCEST.

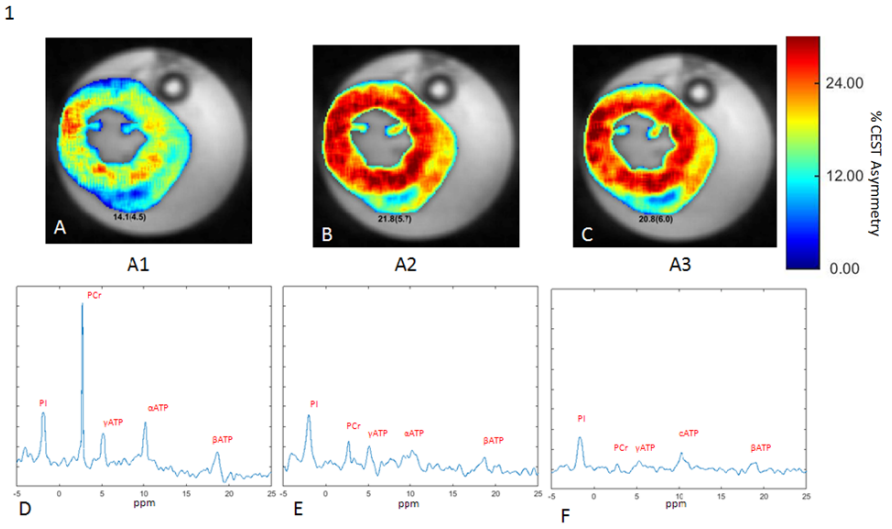

Fig. 1. B_0-corrected CrCEST maps and time-associated ^{31}P spectra from various acquisitions. Reductions in PCr correlate with increased average creatine CEST asymmetry, as measured at 1.8 ppm offset from the water resonance.

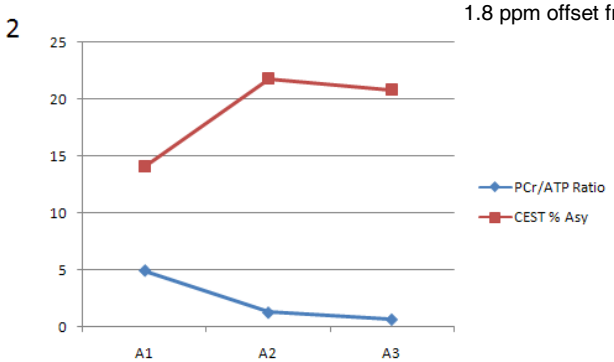

Fig 2. PCr/γATP ratio versus percent creatine CEST asymmetry for acquisitions 1-3 (A1-A3).

References: **1.** Chance, B. et al. *J BiolChem* 1955;217:409-427 **2.** Bendahan, D et al *Cell Molec. Life Sci* 2004;61:1001-1015 **3.** Neubauer, S et al. *Circulation* 1992;86:1810-1818 **4.** Kogan, F et al. *Mag Res In Med* 2014;71:164-172 **5.** Kim, M. et al *Magn Reson Med* 61:1441-1450 **6.** Kogan, F et al. *J Magn Reson Imaging* 2014;40(3):596-602

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