Separated quantification of creatine and phosphocreatine based on a novel proton MR method combing ^{1}H-MRS and CEST MRI

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Target audience: Scientists or clinicians who are interested in cellular bioenergy and creatine kinase activity.

Introduction/Purpose: Phosphocreatine (PCr) serves as a rapidly mobilizable reserve of high-energy phosphates particularly in skeletal muscle, heart and brain[1]. The conversion of PCr to creatine (Cr) by the creatine kinase produces ATP, the body's fundamental energy currency. Thus, measurements of PCr and Cr separately may help to probe the energy deprivation in many pathologic conditions, such as muscular dysfunction, myocardia infarct and neuronal disorders[1]. However, ^{1}H-MRS measures the total creatine (tCr or PCr + Cr) due to the overlapping of PCr and Cr in ^{1}H-MRS[2]. Although ^{31}P-MRS was used to investigate PCr, creatine kinase activity and other high-energy phosphate compounds *in vivo*[3], ^{31}P-MRS suffers from rather low sensitivity (6.63% of ^{1}H-MRS), lower resolution and prolonged acquisition time. Recently, our studies demonstrated that creatine itself can be imaged based on its chemical exchange saturation transfer (CEST) MRI contrast *in vitro* and *in vivo*[4-7]. The goal of this preliminary study is to prove the concept that PCr and Cr can be individually quantified using a combined proton MR method based on CEST MRI and ^{1}H-MRS.

Methods: PCr and Cr (n=3) solutions were prepared in PBS, adjusted to pH 7.0 and placed in 5 mm NMR tubes to form two sets of imaging phantoms: 1) 10 mM Cr with various PCr concentrations ([PCr] = 0, 2.5, 5, 7.5 and 10 mM); 2) 10mM PCr with various Cr concentration ([Cr] = 0, 2.5, 5, 7.5 and 10 mM). ^{1}H-MRS and CEST z-spectrum were acquired at room temperature with a Varian 9.4-T horizontal MRI scanner. The single voxel point resolved spectroscopy sequence (PRESS) was used for ^{1}H-MRS with and without water suppression using variable pulse power and optimized relaxation delays (VAPORE), TE/TR= 13/3000 ms, 32 averages and voxel size = 2.5x2.5x6 mm^3. The tCr peak at ~3ppm was fitted as a Lorentzian function using a customized spectroscopy fitting program developed in MATLAB. The integral of the tCr peak was normalized to the reference water integral to calculate the proton concentration [H] from tCr in each solution. CEST z-spectra were collected using a custom sequence with a frequency selective rectangle saturation pulse (B_1=50 Hz, 3 s) followed by Fast Low-Angle Shot (FLASH) readout[7].

Results: Fig.1A shows in a typical ^{1}H-MRS, both peaks from Cr and PCr are shown to overlap with each other. In Fig. 1B and 1C, the calculated [H] from tCr with ^{1}H-MRS were found to be proportional to the actual [Cr] or [PCr] while with different slopes presumably due to the differences in T_1 or T_2 relaxation times. From z-spectra of PCr or Cr solutions, we found that PCr exhibits no CEST contrast (Fig. 2A). Instead, CEST contrast at 2ppm from Cr was linearly correlated to the actual [Cr] (Fig. 2B-C). As a result, [Cr] can be quantified with CEST MRI based on CEST%=k*[Cr], where k is the slope (Fig. 2C). Since [H] obtained from ^{1}H-MRS is equal to a*[PCr]+b*[Cr], where a and b are the slopes (Fig. 1B-C), PCr can then been quantified once [Cr] is known from CEST MRI.

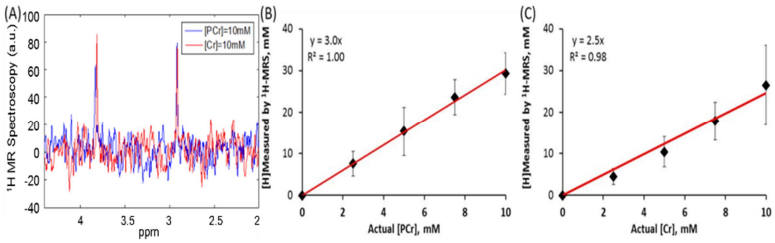

Fig. 1. (A) Representative ^{1}H-MR spectra from 10 mM PCr solution (blue) and 10 mM Cr solution (red). (B) and (C) derived from prepared PCr and Cr solutions reflects the linear dependence of measured proton concentration on PCr and Cr, respectively.

Discussion: Total creatine is typically used as an internal reference for ^{1}H-MRS under the assumption that the total creatine is relatively constant. This assumption, although it is relatively accepted in normal conditions, is not generally accurate in aging, cancer, metabolic syndrome, stroke and mental disorders[1]. Quantitative separation of PCr and Cr may provide further insights in cellular bioenergy for those pathologic conditions. This study demonstrated that PCr and Cr can be measured individually using the combined ^{1}H-MRS and CEST MRI. Future study will extend the single voxel ^{1}H-MRS to 2D or 3D ^{1}H-MRSI to generate tCr images. Together with creatine CEST maps, spatial distributions of both Cr and PCr may be quantified in a 2D or 3D fashion. This novel proton MR method is potentially a sensitive imaging tool for studying the distributions of PCr and Cr, the conversion of PCr to Cr, and the ratio of ATP/ADP, which is proportional to PCr/Cr. In addition this novel method may serve as a generic and powerful imaging biomarker for cellular bioenergy.

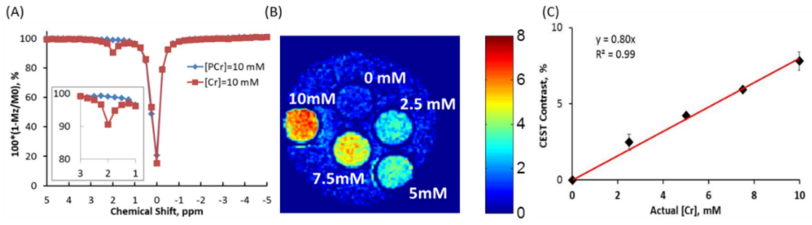

Fig. 2. (A) Z-spectrum of 10mM PCr or 10mM Cr only. (B) A representative CEST MRI contrast map of solutions with 10mM PCr and different [Cr] (in mM). CEST contrast and Cr concentration are linearly correlated (c).

References: [1] Debe J. et al. Designs for health. 2012. [2] Knight-Scott J. et al. Magn Reson Imaging. 2003;21:787-97. [3]Cadoux-Hudson TA. et al., FASEB J. 1989;3:2660-6. [4] Haris M. et al. Nature Medicine. 2014;20:209–214. [5] Haris M. et al. NMR Biomed. 2012;25:1305-9. [6] Kogan F et al. Magn Reson Med. 2014;71:164-72. [7] Cai K. et al. NMR in Biomedicine. 2014.