In Vivo Measurement of Free Creatine and Phosphocreatine Kinetics In Lower Leg Muscle.

Olusegun Adegbite[1], Prodromos Parasoglou[1], Lee Jae Seung[1], Ding Xia[1], and Ravinder R. Regatte[1]
[1]*Radiology, NYU, Langone Medical Centre, New York, New York, United States*

Audience: Those interested in muscle bioenergetics, muscle physiology and metabolism.

Purpose: Creatine kinase (CK) plays a central role in the cellular energy homeostasis. In large organs such as the brain or in skeletal muscles, wherein energy demand is high and often fluctuates over time, CK reversibly interconverts adenosine diphosphate (ADP) and phosphocreatine (PCr) to adenosine triphosphate (ATP) and creatine (Cr); $PCr + ADP + H^+ \leftrightarrow ATP + Cr$ (1). Thus, there exist correspondences between the concentrations of these endogenous metabolites. ^{31}P MRS have been used extensively to noninvasively measure the concentrations of PCr, ATP, and inorganic phosphate (P_i), while ^{1}H MRS relays information about only the total concentrations ([tCr]) of phosphorylated and unphosphorylated Cr involved in the reaction (1). Recently, free Cr participating in this energy bound reaction was measured using a novel chemical exchange saturation transfer (CEST) imaging technique, called CrCEST[1, 2]. In this study, we measured the spatial kinetics of PCr and Cr in the gastrocnemius muscle following plantar flexion exercise, consecutively by ^{31}P and CEST MRI.

Methods: Four healthy subjects (mean age ± standard deviation = 34.0 ± 10.0 years) were recruited and scanned on a 7 T MRI system (Siemens Medical Solution, Erlangen, Germany) using a dual tuned (^{31}P/^{1}H) quadrature transmit-receive kneel coil (Rapid MRI Ohio). Each subject performed a 2-min plantar flexion exercise twice, each of which was preceded by the measurement of the baseline for 2 mins and followed by the post-exercise measurement. With the first exercise, we measured PCr signal using a three-dimensional turbo spin echo (TSE)[3] ^{31}P-MR sequence with a 6-sec temporal resolution. With the second exercise, the kinetics of Cr was assessed through CrCEST MRI, using a two-dimensional GRE acquisitions (field of view = 150x150 mm^2, matrix size = 128x128, slice thickness = 5 mm) following a 500-ms long off-resonance saturations at $\pm$1.8 ppm[2] from the water signal, with a 16-sec temporal resolution for 6 mins. CrCEST effect is computed over a manually drawn region of interest covering the entire region of the gastrocnemius muscle by the following equation: $CEST(\omega) = [M_{sat}(-\omega) - M_{sat}(+\omega)]/M_{sat}(-\omega)$, where $M_{sat}(\pm\omega)$ are the water magnetizations after the off-resonance saturations at $\pm$1.8 ppm from the water signal. The CEST effect was calculated during each point of the recovery curve. ROI in the same muscle of the lower leg was drawn in the PCr images, and the data post-exercise were fitted to an exponential recovery function.

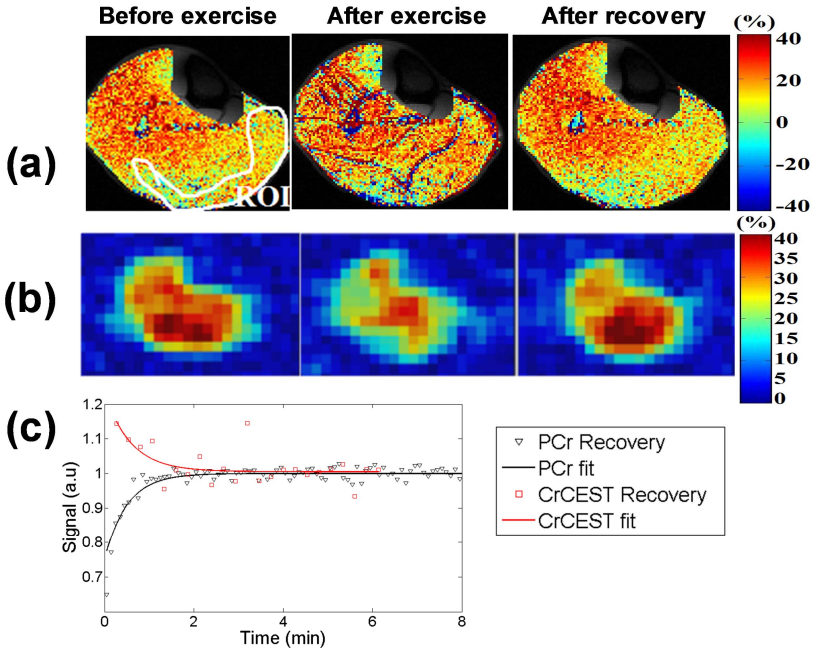

Fig.1: (a) CrCEST contrast before the plantar flexion, at the beginning and end of the recovery. (b) The corresponding PCr depletion and recovery images at the same time point. c) Combined plots of PCr and CrCEST recovery with their respective curve fits.

Result and Discussion: Fig. 1a shows the CEST at three time points; before and immediately after the plantar flexion, and after a full recovery from the exercise and the corresponding PCr recovery maps in (b). The recovery curves for PCr and CrCEST were fitted with mono-exponential curves, as depicted in the fig. 1c. The depletion of PCr at plantar flexion results in a corresponding increase in the Cr concentration as expected from Eq.1. The combined measurement of the PCr depletion and CrCEST recovery kinetics in higher temporal resolution shows the correspondence between the concentration of PCr and Cr. The time constants for all the subjects are presented in the Table 1. The percentage increase for CrCEST ranges from 13% - 45% while that of PCr is ca. 35% - 65% in all the subjects.

Conclusion: The depletion and recovery of PCr and the corresponding increase and recovery of CrCEST were monitored in lower leg muscles following the plantar flexion exercise. The time constant for the recovery of the kinetics differs in both cases, while the PCr recovers faster (shorter time constant) after the exercise; the recovery of Cr was slower. The study has the potential of characterizing CK reaction, and may in future allows the detection of abnormalities in the bioenergetics system.

Table 1. PCr depletion and corresponding Cr increase with their time constants.

Sub	ΔCr (%)	ΔPCr (%)	τ_{Cr} (s)	τ_{PCr} (S)
#1	43.78	58.81	92.99	30.93
#2	13.50	42.23	60.28	28.36
#3	14.50	35.03	34.78	23.00
#4	44.41	62.50	96.47	23.00

Acknowledgement: The study was supported by NIH grants R01 AR056260, R01 AR060238 and R01 EB016045, and was performed under the rubric of the Center for Advanced Imaging Innovation and Research (CAI^{2}R), a NIBIB Biomedical Technology Resource Center (NIH P41 EB017183).

References: [1] Kogan F. et al. JMR 2014: 40(3) 596-602. [2] Kogan F. et al. MRM 2014 71(1) 164-172. [3] Parasoglou P. et al. NMR Biomed 2013:3 348-356.