Contribution of Tissue Perfusion to the CEST Effect from Creatine in Skeletal Muscle

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Introduction: Creatine kinase (CK) reaction plays a vital role in the muscle energetics by catalyzing the exchange of high energy phosphates from phosphocreatine(PCr) to adenosine triphosphate (ATP) [1]. During exercise, PCr is depleted to maintain ATP levels, resulting in an increased Cr concentration. Chemical exchange saturation transfer (CEST) can indirectly detect metabolite content based on exchange-related properties[2] and recently it has been shown that Cr exhibits a concentration dependent CEST effect between its amine (-NH_2) protons and bulk water protons (CrCEST)[3]. The feasibility of spatially and temporally mapping the CrCEST effect following exercise has also been demonstrated [4]. Plantar flexion exercise increases $CrCEST_{asym}$ in exercised muscle, consistent with ^{31}P MRS data. Additionally, exercise leads to an increase in muscle perfusion. It has also been hypothesized that blood also exhibits a CEST effect. If true, this would confound measurements of Cr CEST increases post exercise. In this work we investigated the effects of increased perfusion on the CrCEST effect by comparing CrCEST to muscle blood flow measured by ASL during cuff inflation and subsequent hyperemia.

Methods: All imaging experiments were performed on a 7T whole body scanner (Siemens Medical Systems, Erlangen, Germany) under an approved Institutional Review Board protocol. CEST, arterial spin labeling (ASL) and ^{31}P MRS acquisitions of the calf were performed at 7T on a healthy volunteer with a reactive hyperemia protocol. A 28 channel ^{1}H knee coil was utilized for proton imaging and a dual tuned $^{31}P/^{1}H$ transmit/receive surface coil was used for ^{31}P MRS. Reactive hyperemia was induced with a cuff (Hokanson) secured around the superior thigh inflated to >200 mmHg. The protocol included 2 minutes of rest, followed by 3 minutes of blood flow occlusion induced by cuff inflation, and then cuff deflation. CEST MRI (30s temporal resolution), ASL Perfusion, and ^{31}P MRS data (12s temporal resolution) were acquired continuously through rest, cuff inflation and for 6 minutes post cuff deflation.

CEST images were acquired with a 500-ms saturation pulse consisting of a series of 100-ms Hanning windowed saturation pulses and a B_{1rms} of 123 Hz (2.9 μT) followed by a FLASH readout (TR/TE=6.1/2.9; slice thickness = 10 mm; FA=10°, 128 × 128 matrix; FOV = 130 × 130 mm). Water saturation shift reference (WASSR) images and B_1 maps were collected for all CEST studies before and after exercise to correct for B_0 and B_1 inhomogeneities[5,6]. CrCEST asymmetry was calculated using the B_0-corrected signal intensity at ±1.8 ppm, the chemical shift of Cr amine protons, using the equation: $CEST_{asym} = [(S_{-\Delta\omega} - S_{+\Delta\omega})/S_{-\Delta\omega}]$. The arterial spin labeling sequence used flow-sensitive alternating inversion recovery (FAIR) technique with echo-planar imaging readout (TR/TE=2,000/20; slice thickness = 10 mm; 128 × 128 matrix; 200 × 200 mm FOV)[7].Motion correction was performed using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK), and ASL analysis was performed using MATLAB (MathWorks, Natick, MA). Mean percent signal change maps were generated by calculating the average signal difference between pairwise control and label images, and dividing by the mean of all the controls for 12 label/control pairs.^{31}P MRS spectra were acquired using an unlocalized free induction decay (fid) sequence. ^{31}P MRS Spectra were phased and baseline corrected and fitted using nonlinear squares methods with Gaussian functions.

Results and Discussion: Figure 1 shows $CrCEST_{asym}$ and ASL mean percent difference maps before and during cuff inflation and reactive hyperemia after cuff deflation. ASL percent difference maps show a small decrease during the time blood flow is restricted followed by a large increase in perfusion during reactive hyperemia which returns to baseline levels over time. On the other hand, $CEST_{asym}$ maps of the muscles of the lower leg in the same slice do not show any significant differences ($\Delta CrCEST_{asym}$< 1.0%) throughout the reactive hyperemia experiment. Thus, under these saturation parameters, the CEST effect from perfusion is negligible as there was no significant change in $CEST_{asym}$ following cuff release. Cuff inflation resulted in notable changes in the signal from blood in the large vessels, as expected. The signal decreased considerably during cuff inflation, followed by a significant increase immediately following release of the cuff and then recovery to baseline values. To validate the CrCEST technique, ^{31}P MRS was performed to demonstrate that the Cr concentration also did not change during reactive hyperemia. The concentrations of Cr and PCr are tightly coupled due to the activity of creatine kinase and thus the decrease in PCr concentration should equate to an equivalent increase in Cr concentration. For short durations of occlusion, it has been shown that PCr and thus Cr levels are unaffected. This was confirmed by ^{31}P MRS data, which showed that there was no change in the area of PCr peak integral during blood flow obstruction or reactive hyperemia (fig 2). In addition, there was no shift in the inorganic phosphate (P_i) peak indicating that there were negligible changes in muscle pH which could also confound CrCEST measurements.

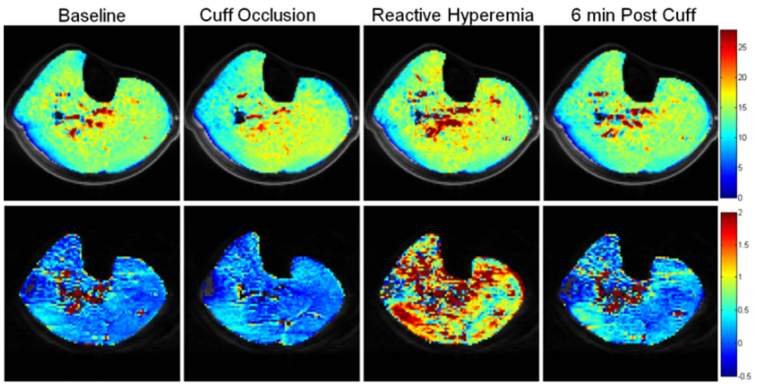

Figure 1: $CrCEST_{asym}$(Top) & ASL ΔM% mean (Bottom) Maps before and after reactive hyperemia

Another possible confound to the measured $CEST_{asym}$ is the effect of the potential increase in water content due to increased perfusion. This increases the signal intensity in muscles with increased perfusion in base CEST images. However, this signal enhancement is equivalent in +Δω and -Δω frequency saturation images and for small increases in signal intensity this will be subtracted out in the asymmetry analysis. In this study, less than a 5% increase in signal enhancement was observed with reactive hyperemia which did not affect the calculated $CEST_{asym}$ (fig 1).

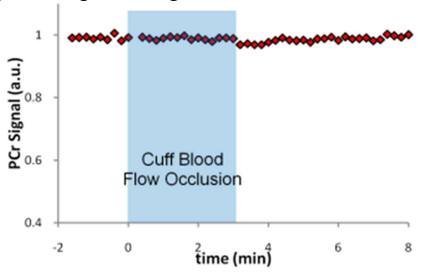

Figure 2: ^{31}P MRS PCr signal of reactive hyperemia

Plantar flexion exercise leads to an increase in both $CEST_{asym}$ and tissue perfusion in exercised muscle. The observed increase in tissue perfusion with reactive hyperemia in this study is expected to be larger than in exercise studies. Thus, as the observed effects of increased perfusion on the CESTasym were negligible, changes in tissue perfusion with exercise likely do not contribute appreciably to the observed CrCEST effects. This is relevant for faster exchanging labile protons such as amine protons which require a larger saturation amplitude such as the one used in this work. Further studies need to be conducted on the effect of perfusion on slower exchanging spins such as amide protons where a smaller saturation amplitude and longer duration are generally utilized.

Conclusion: Reactive hyperemia showed that the effect from perfused blood on the CEST effect from Cr is negligible.

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