Introduction: 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 (-NH2) 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 asymmetry in exercised muscle, consistent with 31P 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 31P MRS acquisitions of the calf were performed at 7T on a healthy volunteer with a reactive hyperemia protocol. A 28 channel 1H knee coil was utilized for proton imaging and a dual tuned 13P/1H transmit/receive surface coil was used for 31P 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 31P 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 T1ms of 123 Hz (2.9 μT) followed by a FLASH readout (TR/TE=6.1/2.9; slice thickness = 10 mm; FA=10°, 128 x 128 matrix; FOV = 130 x 130 mm). Water saturation reference (WASSR) images and B1 maps were collected for all CEST studies before and after exercise to correct for B1 and Binhomogeneity[5,6]. CrCEST asymmetry was calculated using the B1-corrected signal intensity at ±1.8 ppm, the chemical shift of Cr amine protons, using the equation: CrCEST asym = |S -S +|/S. 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 x 128 matrix; 200 x 200 mm FOV)[7]. Motion correction was performed using SPMS (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. 31P MRS spectra were acquired using an unlocalized free induction decay (fid) sequence. 31P MRS Spectra were phased and baseline corrected and fitted using nonlinear square 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, CrCEST asym maps of the muscles of the lower leg in the same slice do not show any significant differences (∆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, 31P MRS was performed to demonstrate that the Cr concentration 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 31P 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) peak indicating that there were negligible changes in muscle pH which could also confound CrCEST measurements.

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


Figure 1: CrCEST asym (Top) & ASL ΔM% mean (Bottom) Maps before and after reactive hyperemia

Figure 2: 31P MRS PCr signal of reactive hyperemia