**Introduction:** Creatine (Cr) plays an essential role in the storage and transmission of phosphate-bound energy. During exercise, phosphocreatine (PCr) is depleted to maintain the adenosine triphosphate (ATP) supply leading to an increase Cr concentration. $^{31}$P Magnetic Resonance Spectroscopy (MRS) is able to relay information about the concentrations of Cr, Pi, as well as muscle pH and has been used extensively to study oxidative metabolism of skeletal muscle(1). $^{31}$P MRS has been applied to studies of bioenergetic impairment in various muscle impairments as well as in cardiac energetics. However, $^{31}$P MRS, like all spectroscopy techniques, suffers from poor spatial resolution as well as low sensitivity due to the low gyromagnetic ratio of $^{31}$P. Chemical exchange saturation transfer (CEST) is a new sensitivity enhancement mechanism that can indirectly detect metabolite content based on exchange-related properties(2). Recently it has been shown that Cr exhibits a concentration dependent CEST effect between its amine (-NH$_2$) and bulk water protons(3) and that this CEST effect can be isolated from the other metabolites of the creatine kinase reaction (PCr, ATP, ADP). This technique allows for the monitoring of Cr concentration changes with high spatial resolution. In this work, we demonstrated the potential of measuring the CEST effect from Cr (CrCEST) in spatial mapping of free creatine in exercised muscle.

**Methods:** All imaging experiments were performed on a 7T whole body scanner (Siemens Medical Systems, Erlangen, Germany) under an approved Institutional Review Board protocol. An MR compatible pneumatically controlled foot pedal was used for plantar flexion exercise with a 28 channel $^1$H knee coil utilized for proton imaging and a custom built 7 cm diameter $^{31}$P transmit/receive surface coil was used for $^{31}$P MRS. CEST, T$_2$, MTR and $^{31}$P MRS acquisitions of the calf were performed at 7T on healthy volunteers (n=8, 5 male, 3 female, ages 19-30) with various activity levels (sedentary to active). For each methodology, baseline imaging was performed for 2 minutes, followed by 2 minutes of mild plantar flexion exercise and then 8 minutes of post exercise imaging. CEST images were acquired with a 500 ms long saturation pulse consisting of a series of 100 ms Hanning windowed saturation pulses and a $B_1$ of 123 Hz (2.9 $\mu$T) followed by a FLASH readout. Water saturation shift reference (WASSR) images and $B_1$ maps were collected, as described previously (4,5), for all CEST studies before and after exercise to correct for $B_0$ and $B_1$ inhomogeneities. CrCEST asymmetry was calculated using the $B_0$ corrected signal intensity at ±1.8 ppm, the chemical shift of Cr amine proton, using the equation: $\text{CrCEST}_{\text{asym}} = [S_{-nh2} - S_{+nh2}] / S_{+nh2}$. $^{31}$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:** Plantar flexion exercise led to an increase in the CrCEST$_{\text{asym}}$ in all subjects. Figure 1 shows CrCEST$_{\text{asym}}$ maps for the same subject before and after mild plantar flexion exercise with a temporal resolution of 48 seconds. The time dependence of the mean CrCEST$_{\text{asym}}$ for each segmented muscle group is plotted in figure 2. The post exercise CrCEST$_{\text{asym}}$ map shows that the majority of CrCEST$_{\text{asym}}$ increases were localized to the muscles of the posterior compartment of the leg responsible for plantar flexion. Whereas the CEST maps showed fairly uniform CrCEST$_{\text{asym}}$ at baseline, the first post-exercise map showed a 6.7% (±1.0%) and 7.2% (±0.8%) increase in CrCEST$_{\text{asym}}$ in the medial (MG) and lateral (LG) gastrocnemius muscles, respectively, immediately following exercise. A 2.6% (±0.9%) increase was observed in the soleus, the other major muscle of the posterior compartment. The tibialis anterior is predominantly involved in dorsiflexion and as a result, less than a 1% increase in CrCEST$_{\text{asym}}$ was observed. The CrCEST$_{\text{asym}}$ in all the muscles recovered exponentially to baseline after roughly three time points or ~2 minutes, $T_2$ and MTR maps for all subjects didn’t show any significant differences post exercise (AT < 0.5 ms, AMTR < 1.0%) (data not shown).

A free induction decay (fid) was used for $^{31}$P MRS acquisition in the current study, and thus the signal was quickly decayed back to baseline. No shift or splitting of the Pi peak was observed indicating that there were negligible pH changes. There was good agreement in the observed rates at which the CrCEST$_{\text{asym}}$ from a region of interest equivalent to the $^{31}$P MRS coil excitation profile and the $^{31}$P MRS signal recovered (fig 4).

Data from all 8 volunteers showed that there was some subject variability in regards to the level and involvement of each muscle group during exercise which was able to be detected in CrCEST$_{\text{asym}}$ maps. To compare the CEST$_{\text{asym}}$ and $^{31}$P MRS results, the % decrease in $^{31}$P MRS signal from baseline at the first integrated measurement following exercise was used to determine the decrease in PCr based on a 33 mM PCr baseline concentration(6). As the total Cr remains constant, the decrease in PCr concentration should equate to an equivalent increase in Cr concentration. Thus we can compare the change in the CEST$_{\text{asym}}$ to the Cr concentration change across all subjects (fig 5). This yields a slope of 0.84 % CrCEST$_{\text{asym}}$mM Cr with an R$^2$ = 0.75.

**Conclusion:** It is feasible to use CEST imaging to measure changes in Cr concentration in muscle following plantar flexion exercise. Further, CrCEST$_{\text{asym}}$ maps showed good spatial resolution and were able to differentiate muscle recruitment during exercise. There was good agreement in the recovery kinetics of $^{31}$P MRS and CrCEST$_{\text{asym}}$ following exercise.