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Perfusion has no effect on the in vivo CEST effect from Cr (CrCEST) in skeletal muscle

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Abstract

Creatine, a key component of muscle energy metabolism, exhibits a chemical exchange saturation transfer effect (CEST) between its amine group and bulk water which has been exploited to spatially and temporally map creatine changes in skeletal muscle before and after exercise. Additionally, exercise leads to an increase in muscle perfusion. In this work we determined the effects of perfused blood on the CEST effects from creatine in skeletal muscle. Experiments were performed on healthy human subjects (n= 5) on whole-body Siemens 7T MRI scanner with a 28 channel RF coil. Reactive hyperemia, induced by inflation and subsequent deflation of a pressure cuff secured around the thigh was used to increase tissue perfusion while maintaining the levels of creatine kinase metabolites. CEST, arterial spin labeling (ASL), and ^{31}P MRS data were acquired at baseline and for 6 minutes post cuff deflation. Reactive hyperemia resulted in substantial increases in perfusion in human skeletal muscle of the lower leg as measured by ASL mean percent difference. However, no significant changes in the $CrCEST_{asym}$ as well as in ^{31}P MRS derived PCr levels of skeletal muscle were observed following cuff deflation. This work demonstrates that perfusion changes do not have a major confounding effect on CrCEST measurements.

Keywords

CEST; Creatine; Muscle; ASL; Perfusion

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Introduction

The creatine kinase (CK) reaction plays a vital role in muscle energetics by catalyzing the exchange of high energy phosphates from phosphocreatine (PCr) to adenosine triphosphate (ATP)[1].

$$\mathrm{Cr} + \mathrm{ATP} \leftrightarrow \mathrm{ADP} + \mathrm{PCr} + \mathrm{H}^{+} \quad [1]$$

During exercise, PCr is depleted to maintain ATP levels, resulting in an increased creatine (Cr) concentration[2].

Magnetic resonance has been a valuable tool for studying CK kinetics since the discovery of ^{31}P Magnetic Resonance Spectroscopy (MRS)[3]. ^{31}P MRS can measure high-energy phosphate compounds including PCr and ATP. Additionally, intracellular pH can be measured noninvasively from the chemical shift of the inorganic phosphate (P_i) peak[4]. This technique has been used extensively to study metabolism of skeletal and cardiac muscle metabolism[5–7]. However, like all spectroscopy techniques, ^{31}P MRS suffers from poor spatial resolution due to the low concentration of measured metabolites as well as a reduced sensitivity due to the low gyromagnetic ratio of ^{31}P.

More recently, chemical exchange saturation transfer (CEST) has been used to measure CK kinetics in vivo with high spatial resolution. In a CEST experiment, saturated magnetization from exchangeable protons on a pool of solute molecules is transferred to a much larger pool of bulk water protons. The corresponding reduction in water signal results in a solute-concentration dependent contrast in CEST water images[8–10], which can be utilized to provide contrast to the solute as well as potentially quantitative information about solute content and exchange rate[11,12]. Cr has been shown to exhibit a concentration dependent CEST effect between its amine group (-NH_2) protons and bulk water protons (CrCEST)[13]. Furthermore, the feasibility of spatially and temporally mapping the CrCEST effect following exercise has also been demonstrated in skeletal muscle at 3T and 7T[14,15].

Plantar flexion exercise has been shown to increase the CrCEST effects in exercised muscle, and the complementary recovery kinetics to the ^{31}P MRS PCr signal under the same exercise conditions[15]. Furthermore, due to differences in amine proton exchange rates, the other metabolites of the creatine kinase reaction (PCr, ATP, ADP) do not exhibit a CEST effect with the utilized pulse parameters and therefore do not confound measurements of Cr changes after exercise. However, exercise also leads to an increase in muscle perfusion, and it has recently been shown ex vivo, that blood also exhibits a CEST effect[16]. Accordingly, increases in tissue perfusion could potentially confound measurements of CrCEST increases during and after exercise.

On the other hand, a short bout of ischemia, where blood flow is occluded for several minutes and then rapidly restored, has little effect of the PCr signal, but results in a blood flow increases during reactive hyperemia when hyperemia is restored[17,18]. Thus, the purpose of this investigation was to assess whether blood-flow changes alone were confounding/

contaminating the measured CrCEST increase by measuring the CrCEST and ^{31}P MRS during reactive hyperemia.

Arterial spin labeling (ASL) is widely used for non-invasive magnetic resonance imaging of perfusion[19,20]. In ASL, inflowing arterial blood is magnetically labeled proximal to the tissue of interest. This labeled blood then functions as an endogenous tracer that flows downstream to the capillary bed and exchanges with unlabeled protons[19,20]. The signal difference between images collected with and without magnetic labeling is directly proportional to tissue perfusion.

In this work, we investigated the effects of increased muscle perfusion on the CrCEST effect during reactive hyperemia following blood flow occlusion. Changes in CrCEST with increased perfusion were also compared with changes in PCr levels from ^{31}P MRS.

Methods

Subjects

All studies were conducted in healthy volunteers (n=5, 3 male, 2 female, ages 23–31) under an approved Institutional Review Board protocol. Prior to data collection, informed written consent was obtained from each volunteer after explaining the study protocol. All MR data acquisitions of the lower leg were performed in separate trials of blood flow occlusion and subsequent hyperemia.

MRI Scans

All imaging experiments were performed on a 7T whole body MRI scanner (Siemens Medical Systems, Erlangen, Germany). CEST and flow-sensitive alternating inversion recovery (FAIR) ASL imaging experiments utilized a 28-channel receive and single channel transmit ^{1}H knee coil (Quality Electrodynamics, Mayfield Village, OH, USA)[20]. ^{31}P MRS was performed with a ^{1}H/^{31}P dual-tuned transmit/receive surface coil (RAPID Biomedical GmbH, Rimpar, Germany) with a 7 cm inner diameter. Reactive hyperemia was induced with an inflatable pressure cuff (Hokanson, Bellevue, WA, USA) secured around the superior thigh and rapidly 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), FAIR ASL perfusion (reconstructed with 32s effective temporal resolution), and ^{31}P MRS data (12s effective temporal resolution) were acquired at baseline and for 6 minutes post cuff deflation. CEST, ASL, and ^{31}P MRS data was acquired in a single scan session in 3 separate bouts of reactive hyperemia with a swap of coils for ^{31}P MRS acquisition following CEST and ASL acquisition.

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 single shot RF spoiled gradient echo (GRE) readout with centric phase encoding order as previously utilized for 7T CrCEST studies[15] and imaging parameters: TR/TE = 6.1/2.9 ms; slice thickness = 10 mm; FA = 10°, matrix size = 128 × 128; FOV = 130 × 130 mm. Water saturation shift reference (WASSR) images and B_1 maps were collected, as described previously, for all CEST studies before and after cuff occlusion to correct for B_0 and B_1

inhomogeneities[21,22]. Cr amine protons have a chemical shift of 1.8 ppm downfield from water and thus CEST images were collected in a frequency shift range with respect to the water resonance of +1.5 ppm to +2.1 ppm and −1.5ppm to −2.1 ppm with a 0.3 ppm step size to calculate $CrCEST_{asym}$ and allow for adequate B_0 inhomogeneity correction.

The FAIR ASL data was acquired using a pulse sequence developed in-house with echo-planar imaging readout and imaging parameters: TR/TI/TE = 2,000/1,000/20 ms; slice thickness = 10 mm; matrix size = 128 × 128; FOV = 200 × 200 mm[20]. Non-selective and slice-selective adiabatic inversion pulses were used for the control and label conditions, respectively. Eight label and control pairs were averaged to provide an effective frame rate of one perfusion image per 32 seconds (*i.e.,* the perfusion map calculated at a given time-point is the mean result of 32 seconds of FAIR acquisition). A chemical shift selective fat saturation pulse was applied prior to image readout to reduce the signal from fat in the muscle.

Finally, non-localized ^{31}P MRS was performed using the $^{1}H/^{31}P$ dual-tuned 7-cm diameter surface coil positioned on the calf and the following parameters: spectral width = 5 kHz, number of points = 512, averages = 5, TR = 2.4 s, for an effective temporal resolution of 12 s.

Data Processing

All image processing and data analysis was performed in MATLAB (Mathworks Inc., Natick, MA, version 7.5, R 2009b). B_0 maps were used to generate corrected CEST images (± 1.8 p.p.m.) using the WASSR method. Similarly, B_1 maps were created from two images obtained using preparation square pulses with flip angles of 30° and 60°. The B_1 calibration curve for muscle at varying saturation amplitudes developed for previous CrCEST muscle studies was used in conjunction with B_1 maps to correct for B_1 inhomogeneities[15]. CrCEST asymmetry ($CrCEST_{asym}$) was calculated using the B_0-corrected signal intensity at ±1.8 ppm,[23]

$$CEST_{asym} = \frac{M_{sat}(-\Delta\omega) - M_{sat}(+\Delta\omega)}{M_{sat}(-\Delta\omega)} \quad [2]$$

For ASL data, motion correction was performed using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK), and ASL analysis was performed using MATLAB. 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 8 label/control pairs. ^{31}P MR Spectra were phased and baseline corrected and fitted using nonlinear squares methods with Gaussian functions.

Statistical analyses were done using Stata Release 14.1 (StataCorp LP, College Station, TX) and R 3.1.2 (www.r-project.org). One-sided exact Wilcoxon tests were used to compare measures of $CrCEST_{asym}$, ASL mean percent difference, and relative PCR peak area between time points. Further, exact Kruskal-Wallis tests stratified by subject were used to test whether each measure differed over time.

Results

Figure 1 shows $CrCEST_{asym}$ maps with a temporal resolution of 30 seconds at baseline and after cuff deflation following 3 minutes of blood flow occlusion induced by cuff inflation. Similarly, Figure 2 shows ASL mean percent difference maps for 8 label control pairs with a total temporal resolution of 32 seconds generated at baseline and following cuff deflation. While ASL percent difference maps exhibited a large increase in perfusion during reactive hyperemia caused by cuff deflation which returns to baseline levels in a couple of minutes, $CEST_{asym}$ maps of the muscles of the lower leg in the same slice did not show any significant differences ($\Delta CrCEST_{asym} < 1.0\%$) throughout the reactive hyperemia experiment. Thus, under the saturation parameters utilized for CrCEST experiments in skeletal muscle at 7T, the CEST effect from perfusion seems negligible as there was no change in $CEST_{asym}$ following cuff release.

To further validate the CrCEST results, ^{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 a decrease in PCr concentration would equate to an equivalent increase in Cr concentration. For short durations of occlusion, PCr and thus Cr levels were relatively unaffected. This is seen in Figure 3a which shows a stacked plot of every ^{31}P MRS spectra acquired with a 12 second temporal resolution at baseline, during cuff inflation and following cuff release. This is further exhibited the plot of the normalized PCr peak integral from ^{31}P MR spectra as a function of time in Figure 3b. During reactive hyperemia following cuff deflation, a less than 3% change in the area of the PCr peak (<1 mM Δ[PCr]) was observed[24]. 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 4 shows $CrCEST_{asym}$, ASL mean percent difference, and relative PCr peak integral values at baseline, immediately following cuff deflation and 5 min post cuff deflation for each of the 5 subjects. For $CrCEST_{asym}$ and ASL mean percent difference measurements, a central region of interest between the gastrocnemius and soleus muscles away from any major vessels was used. Error bars represent the standard deviations of measurements in the region of interest. As expected, a significant increase in perfusion was observed between baseline and cuff deflation ($p=0.003$) and decrease between cuff deflation and 5 minutes post cuff deflation ($p=0.005$) across all subjects as measured by ASL mean percent difference. However, differences in $CrCEST_{asym}$ and PCr peak area observed across subjects immediately after cuff release compared to those made at baseline and 5 minutes after cuff release (Baseline vs. Peak Hyperemia and Peak Hyperemia vs. Recovery) were not significant (for $CrCEST_{asym}$ $p=0.28$ and $p=0.41$, respectively, and for PCr peak area $p=0.16$ and $p=0.10$, respectively). Additionally, no significant differences were observed between measurements of ASL mean percent difference, $CrCEST_{asym}$ or PCr at baseline and 5 minutes after cuff release (Baseline vs Recovery) ($p=0.81$, $p=0.63$, $p=.60$, respectively). Further, exact Kruskal-Wallis tests stratified by subject showed that ASL measurements of perfusion changed significantly over time ($P=0.008$) while $CrCEST_{asym}$ and PCr peak measurement did not ($p= 0.97$ and $p= 0.15$, respectively).

Discussion

In this study, reactive hyperemia was used to demonstrate that the impact of perfused blood on muscle CrCEST asymmetry are negligible. The observed increase in tissue perfusion with reactive hyperemia is expected to be larger than or equal to the perfusion response in exercise studies[25]. Therefore, although exercise leads to an increase in both $CEST_{asym}$ and tissue perfusion in exercised muscle, any perfusion effects with exercise likely do not appreciably confound observed CrCEST effects. These findings are 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.

Other potential confounders to the CrCEST effect in mild plantar flexion exercise including changes of cellular pH, water content, and other metabolites have been previously reported at 7T[15]. Muscle pH is not expected to change for short durations of occlusion, as used in this study, and this is supported by ^{31}P MRS findings, which showed negligible differences in the shape or position of the P_i peak. Increases in muscle signal intensity reflecting elevated water content due to increased perfusion may be observed in base CEST images. However, for small changes in hydration, this signal enhancement is largely equivalent in $+\Delta\omega$ and $-\Delta\omega$ frequency saturation images and will be subtracted out in the asymmetry analysis[9].

Cuff inflation and deflation did result in notable changes in the signal from blood in the large vessels. Retrograde flow in the large vessels of the lower leg could lead to differences in saturation of labile protons and bulk water, which would result in inaccuracies of CEST asymmetry analysis. Furthermore, inhomogeneities from flow effects could confound the measurement, thus areas near these large vessels were avoided in our analysis. The large vessels supplying muscle in the leg are located centrally, not within the major muscles of the lower leg, therefore avoidance of regions surrounding the feeding vessels is not expected to be a limitation of this investigation.

It should be noted that the ^{31}P signal was localized only by the position of the ^{31}P surface coil and as a result was not obtained from the identical region as CEST data but rather from part of the gastrocnemius and soleus muscles from a large area of the calf, similar to previous studies[15]. Thus, comparisons made between $CrCEST_{asym}$ and ^{31}P MRS are rough estimates. Nonetheless, changes in both the $CrCEST_{asym}$ and ^{31}P MRS PCr signal were not significant between the baseline and reactive hyperemia periods. It should also be noted that measurements of perfusion with ASL mean percent difference were calculated with a 32 second effective temporal resolution for comparison with the temporal resolution used for acquisition of $CrCEST_{asym}$ maps. This likely underestimated the peak perfusion during reactive hyperemia. However, as we are not interested in the physiologic response of blood flow but rather whether blood flow changes impact $CrCEST_{asym}$ measurement, we felt it was more appropriate to use similar temporal resolution for both measurements. Measurements of perfusion were used primarily to show that cessation of induced ischemia resulted in an expected increase in tissue perfusion. Lastly, CEST, ASL and ^{31}P MRS data were acquired in separate cuff occlusion trials and subtle differences in perfusion and muscle

changes between trials could also have occurred. Nevertheless, any such variances are expected to be small[26], as occlusion was relatively short and subjects were healthy volunteers.

In summary, this work demonstrated that measurements of $CrCEST_{asym}$ at 7T in skeletal muscle following induced ischemia are not confounded by perfusion effects, suggesting that blood flow alone does not contribute to $CrCEST_{asym}$ changes following exercise. The CrCEST approach has the potential to provide key information about primary disorders of muscle metabolism as well as the secondary complications of muscle metabolism associated with other pathologies.

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Abbreviations

ATP	Adenosine triphosphate
ASL	Arterial Spin Labeling
CEST	Chemical Exchange Saturation Transfer
CK	Creatine Kinase
Cr	Creatine
CrCEST	Chemical Exchange Saturation Transfer of Creatine
FAIR	Flow-sensitive Alternating Inversion Recovery ASL
P_i	Inorganic Phosphate
PCr	Phosphocreatine
WASSR	Water Saturation Shift Reference

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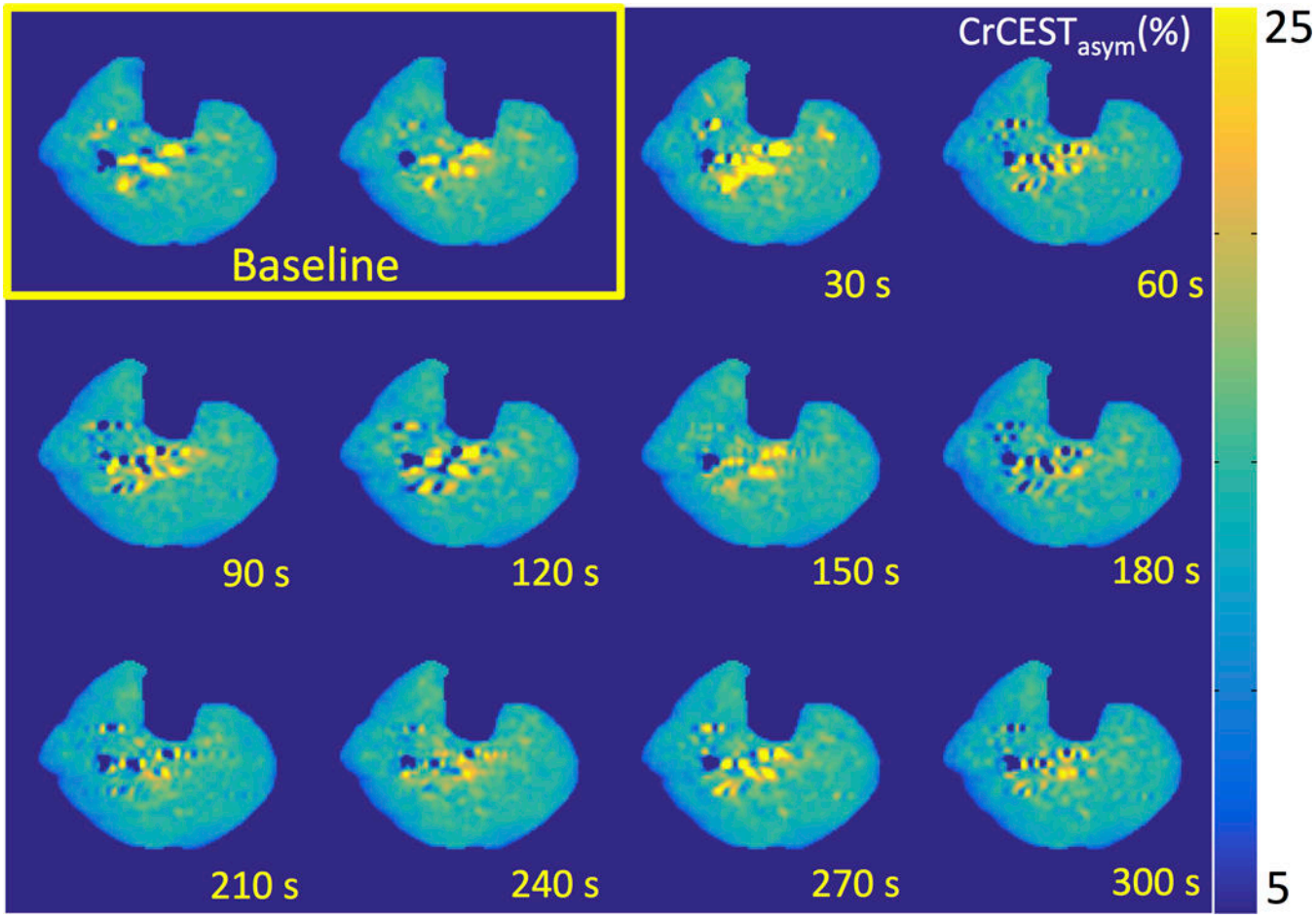

Figure 1.

$CrCEST_{asym}$ maps of a healthy human leg at baseline and every 30 seconds during recovery following 3 minutes of blood flow occlusion induced by cuff inflation (total time post cuff release labeled). Color bar represents $CrCEST_{asym}$ in percent.

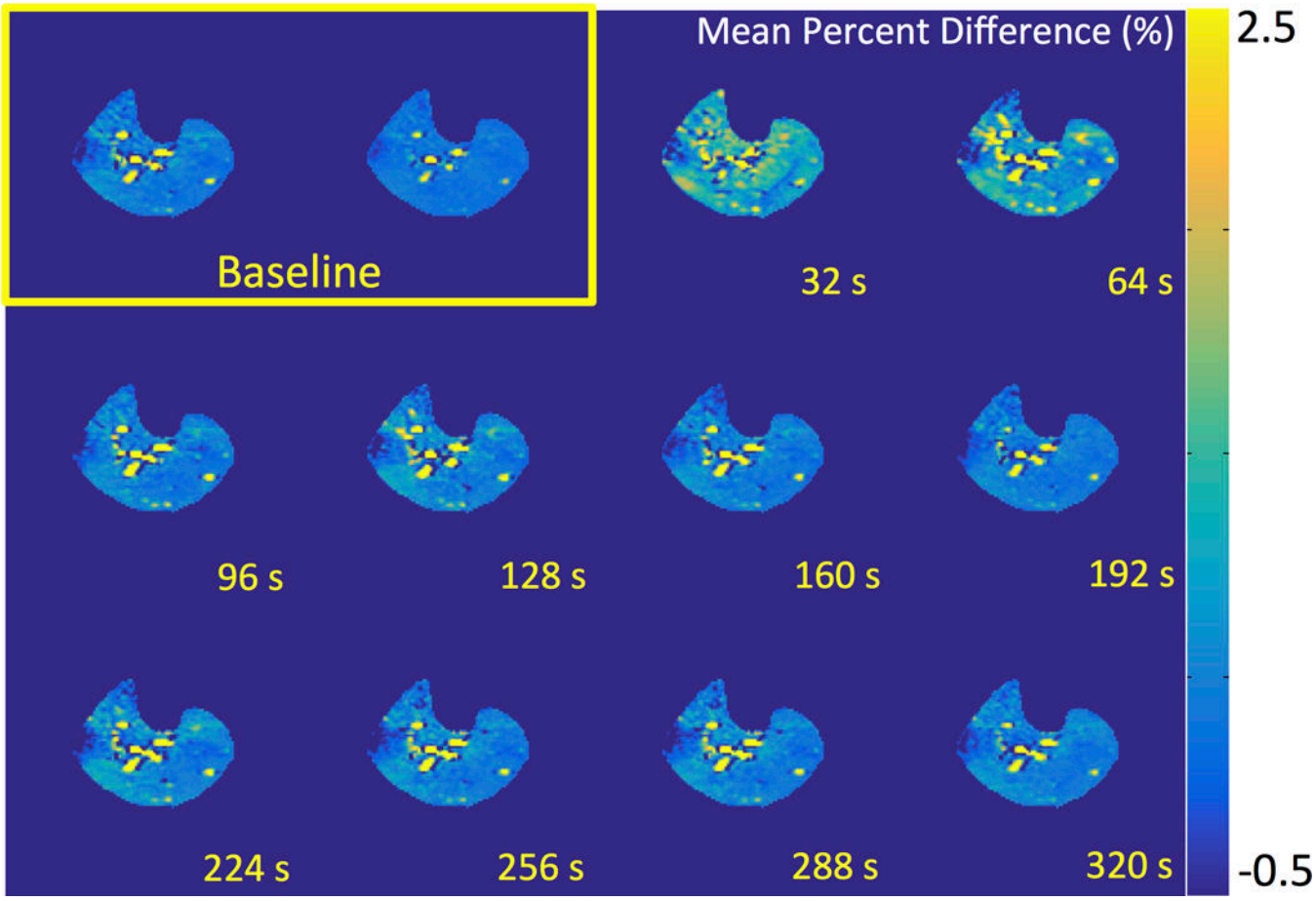

Figure 2.

ASL mean percent difference maps for 8 label/control pairs with a total temporal resolution of 32 seconds generated at baseline and recovery after 3 minutes of blood flow occlusion induced by cuff inflation (total time post cuff release labeled). Color bar represents mean percent difference.

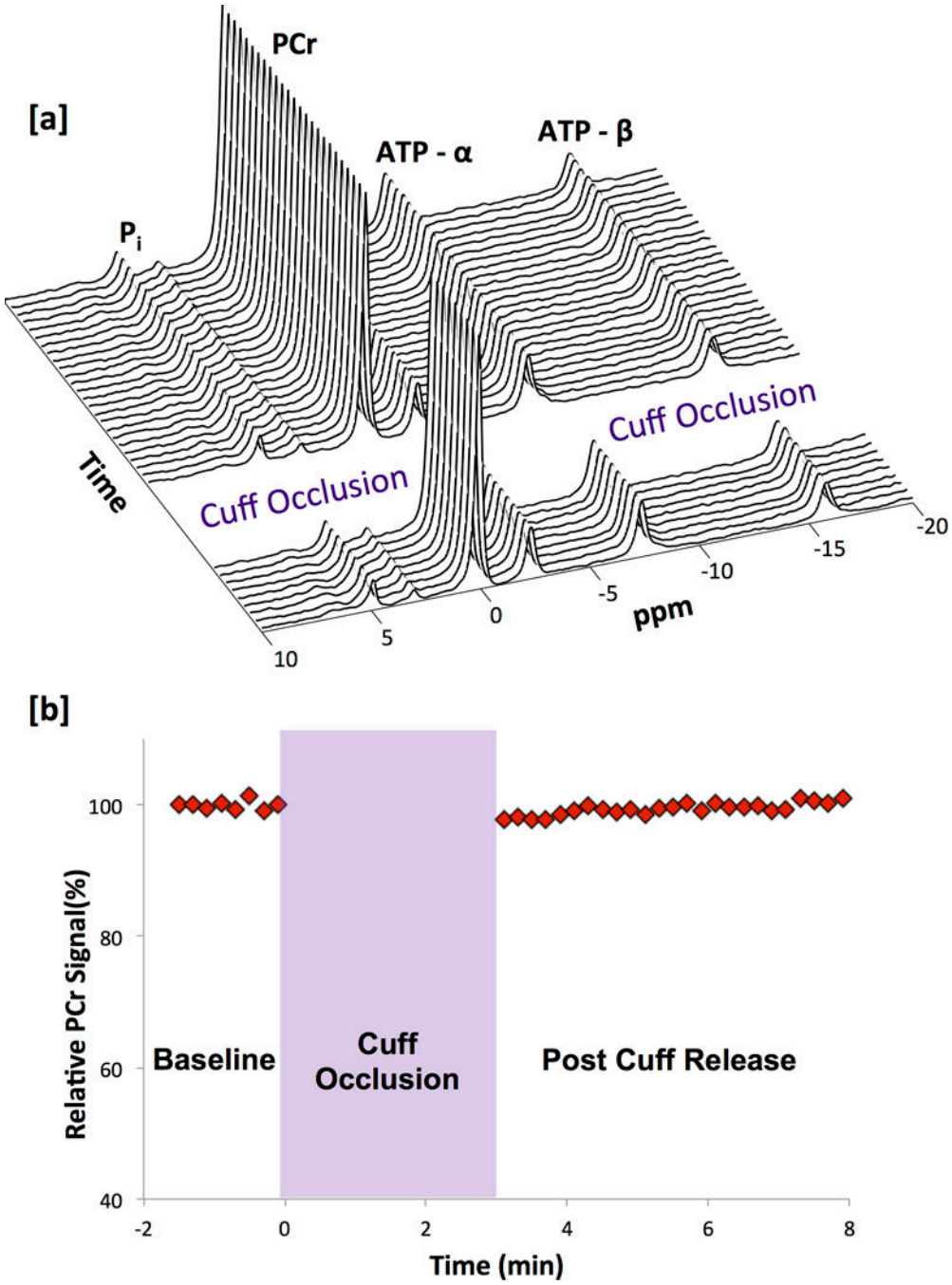

Figure 3.

(a) Stacked plot of every ^{31}P MRS spectra acquired with a 12 second temporal resolution at baseline, during cuff inflation and following cuff release. **(b)** ^{31}P MRS PCr signal integral as a function of time from ^{31}P MRS spectra acquired with a temporal resolution of 12 seconds.

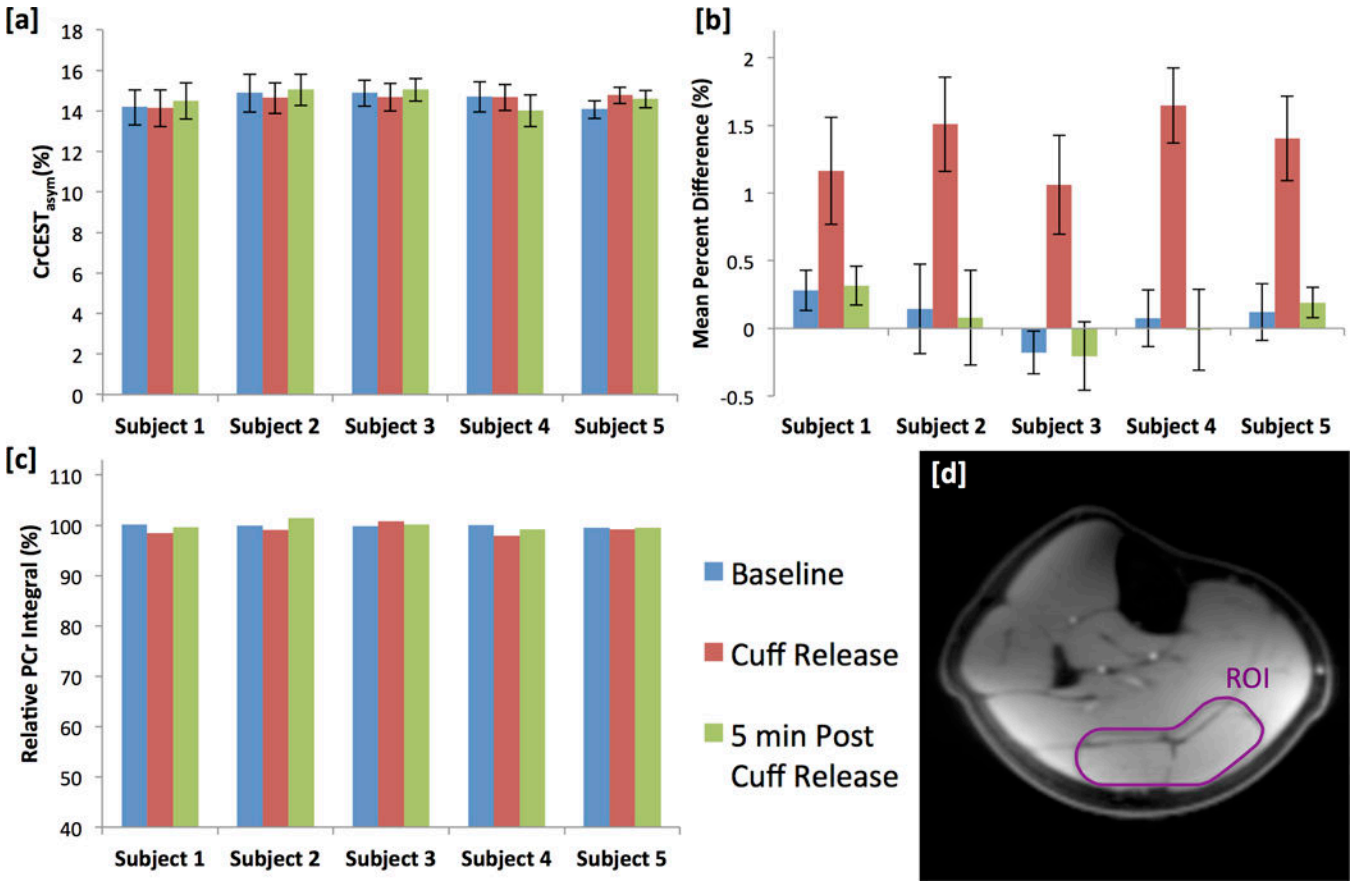

Figure 4.

(a) CrCEST$_{asym}$, **(b)** ASL mean percent difference, and **(c)** relative PCr peak integral values at baseline, immediately following cuff deflation and 5 min post cuff deflation for each of the 5 subjects. (**d**) Anatomical image of the lower leg. CrCEST$_{asym}$ and ASL mean percent difference measurements were calculated as an average from values in a region of interest (ROI) [outlined in white] for the gastrocnemius and soleus muscles chosen to correspond to the localization of the ^{31}P surface coil which was away from any major vessels. Error bars represent the standard deviation in the CrCEST$_{asym}$ and ASL mean percent difference in the ROI.