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In vivo CEST Imaging of Creatine (CrCEST) in Skeletal Muscle at 3T

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Abstract

Purpose—To characterize the chemical exchange saturation transfer (CEST) based technique to measure free creatine (Cr), a key component of muscle energy metabolism, distribution in skeletal muscle with high spatial resolution before and after exercise at 3T.

Methods—CrCEST saturation parameters were empirically optimized for 3T. CEST, T₂, magnetization transfer ratio (MTR) and ³¹P magnetic resonance spectroscopy (MRS) acquisitions of the lower leg were performed before and after mild plantar flexion exercise on a 3T whole-body MR scanner on 6 healthy volunteers.

Results—The feasibility of imaging Cr changes in skeletal muscle following plantar flexion exercise using CrCEST was demonstrated at 3T. This technique exhibited good spatial resolution and was able to differentiate differences in muscle utilization among subjects. CrCEST results were compared with ³¹P MRS results showing good agreement in the Cr and PCr recovery kinetics. A relationship of 0.45 % CrCEST_{asym}/mM Cr was observed across all subjects.

Conclusion—Demonstrated the CrCEST technique could be applied at 3T to measure dynamic changes in creatine in *in vivo* muscle. The widespread availability and clinical applicability of 3T scanners has the potential to clinically advance this method.

Keywords

CrCEST; Muscle; Creatine; Chemical Exchange; Endogenous Contrast

Introduction

Creatine (Cr) plays an essential role in the storage and transmission of phosphate-bound energy (1). During exercise, Adenosine triphosphate (ATP) is broken down into Adenosine diphosphate (ADP) and phosphate group for energy. To maintain the ATP supply, phosphocreatine (PCr) is broken down by the creatine kinase (CK) reaction leading to an increase in Cr (2).

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Thus exercise leads to a decrease in PCr and an increase in Cr

Aliphatic protons of free creatine and PCr have the same chemical shifts making it rather difficult to measure their individual contributions to the observed proton magnetic resonance spectrum (^1H MRS) at clinical field strengths (3). On the other hand, ^{31}P Magnetic Resonance Spectroscopy (MRS) is able to relay information about the concentrations of PCr, Pi, as well as muscle pH and has been used extensively to study oxidative metabolism of skeletal muscle (4). ^{31}P MRS been applied to studies of bioenergetic impairment in various muscle impairments as well as in cardiac energetics (5-7). However, ^{31}P MRS, like all spectroscopy techniques, suffers from poor spatial resolution as well as low sensitivity due the low gyromagnetic ratio of ^{31}P . Additionally, neither ^1H MRS nor ^{31}P MRS can measure free Cr.

Chemical Exchange Saturation Transfer (CEST) is a new contrast enhancement technique that enables the indirect detection of molecules with exchangeable protons and exchange-related properties (8,9). CEST makes MRI sensitive to the concentrations of endogenous metabolites and their environments (10-12). Cr exhibits a concentration dependent CEST effect between its amine ($-\text{NH}_2$) and bulk water protons (13,14). This technique allows for the monitoring of Cr concentration changes with high spatial resolution (15).

More recently, the potential of measuring the CEST effect from Cr (CrCEST) in spatial and temporal mapping of muscle Cr following exercise was demonstrated at ultra-high field (7T) (15). Furthermore, it was shown that due to differences in amine proton exchange rates, the CEST effect from Cr can be isolated from the other metabolites of the creatine kinase reaction (PCr, ATP, ADP). CrCEST also showed complementary recovery kinetics to the ^{31}P MRS PCr signal under the same exercise conditions (15). Nonetheless, ultra-high field scanners are still not widely available and they are currently limited for clinical research only. In order for rapid clinical translation, it is important to characterize the CrCEST effect at lower fields.

In this work, we demonstrate the feasibility of imaging Cr at 3T in skeletal muscle using CrCEST. Changes in CrCEST in the calf muscles of healthy human volunteers were mapped following plantar flexion exercises. Additionally, changes in PCr levels under the same exercise conditions were assessed using ^{31}P MRS. Furthermore, we also measured changes in magnetization transfer ratio (MTR) and T_2 following exercise. Finally, we showed complementary recovery kinetics of CrCEST and under the same exercise conditions. Finally, the potential advantages and disadvantages of CrCEST at 3T compared to 7T are discussed.

Materials and Methods

Subjects

All Studies were conducted under an approved Institutional Review Board protocol. Written informed consent from each volunteer was obtained after explaining the study protocol.

CEST, T_2 , MTR and ^{31}P MRS acquisitions of the lower leg were performed in separate exercise bouts at 3T on healthy volunteers ($n=6$, 3 male, 3 female, ages 19-30) with various activity levels (sedentary to active).

MRI Scans

Imaging experiments were performed using an 8-channel ^1H knee coil (In vivo, Gainesville, FL, USA) on a 3T whole body scanner (Siemens Medical Systems, Erlangen, Germany). ^{31}P MRS was performed with a rigid $^1\text{H}/^{31}\text{P}$ dual-tuned transmit/receive surface coil (RAPID Biomedical GmbH, Rimpfing, Germany) with a 7 cm diameter. Plantar flexion exercise was performed inside the scanner using an MR compatible pneumatically controlled foot pedal as described previously (15). The pressure applied to the pedal was held constant at 7.5 psi across all subjects to ensure the exercise was not strenuous but still resulted in PCr changes. CEST imaging experiments utilized a 500 ms long saturation pulse train consisting of a series of 96 ms Hanning windowed saturation pulses with a 4 ms inter-pulse delay (96% duty cycle, 100 ms pulse train), followed by a single shot RF spoiled gradient echo (GRE) readout with centric phase encoding order. The excitation bandwidth of this saturation pulse train was 10 Hz for a 500 ms saturation duration with a 1% bandwidth of 40 Hz. A chemical shift selective quad phasic fat saturation pulse with a bandwidth of 2 kHz centered at 5 ppm from water was applied prior to magnetization preparation to reduce the signal from fat in the muscle. In order to empirically optimize imaging parameters and observe the effect of varying saturation parameters, CrCEST maps were acquired at varying saturation amplitude and pulse durations. Z-spectra at varying saturation amplitudes and durations were also acquired of the lower leg muscles from -4 to 4 ppm. Water saturation shift reference (WASSR) images and B_1 maps were collected, as described previously (16,17), for all CEST studies before and after exercise to correct for B_0 and B_1 inhomogeneities. WASSR images were collected with a saturation pulse train amplitude, $B_{1\text{rms}}$ of 12.4 Hz ($0.3 \mu\text{T}$) and 200 ms duration from -0.5 ppm to 0.5 ppm with a step size of 0.05 ppm. Cr amine protons have a chemical shift of 1.8 ppm downfield from water under physiological conditions (pH 7.0 & 37°C) 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 -1.5 ppm to -2.1 ppm with a 0.3 ppm step size to calculate $\text{CrCEST}_{\text{asym}}$ and allow for adequate B_0 inhomogeneity correction (14).

In vivo Image Acquisition Parameters

For each imaging methodology (CEST, T_2 /MTR, ^{31}P MRS), baseline imaging was performed for 2 minutes, followed by 2 minutes of mild plantar flexion exercise and then 8 minutes of post exercise imaging. Lower leg CrCEST imaging was performed with imaging parameters: slice thickness = 8 mm, flip angle = 10° , TR = 5.6 ms, TE = 2.7 ms, field of view = $140 \times 140 \text{ mm}^2$, matrix size = 128×128 , saturation pulse amplitude, $B_{1\text{rms}}$ = 93 Hz ($2.25 \mu\text{T}$), saturation duration t_{sat} = 500 ms, with one saturation pulse every 8 seconds. To compute MTR maps, the same sequence with a saturation duration of 1 second was performed at 20 ppm along with a sequence without selective saturation. For T_2 maps, a T_2 spin-echo prepared FLASH sequence was used with the parameters mentioned above at echo times of 2.4, 10, 20, 30, and 40 ms (18). Finally, non-localized ^{31}P MRS was performed with the surface coil positioned on the calf using a free induction decay (fid) sequence with parameters: spectral width = 2 kHz, number of points = 512, averages = 5,

and TR = 2.4 s. In order to determine the depth of penetration of the ^{31}P surface coil, a ^{31}P fid based chemical shift imaging (CSI) sequence was used with the following parameters: FOV = $200 \times 200 \text{ mm}^2$, Slice Thickness = 25 mm, TR = 1500 ms, matrix size = 32×32 , flip angle = 20° , averages = 4.

Data Processing

All image processing and data analysis was performed using in-house written MATLAB (Mathworks Inc., Natick, MA, version 7.5, R 2009b) scripts. B_0 maps were used to generate corrected CEST images ($\pm 1.8 \text{ p.p.m.}$) using the WASSR method (16). Similarly, B_1 maps were created from two images obtained using preparation square pulses with flip angles of 30° and 60° and the FLASH readout parameters described above for CrCEST imaging. A B_1 calibration curve for muscle was developed from calf CEST data at varying saturation amplitudes and used in conjunction with B_1 maps to correct for B_1 inhomogeneities (17) CEST contrast was computed by subtracting the normalized magnetization signal at the Cr proton frequency ($\omega = +1.8 \text{ ppm}$), from the magnetization at the corresponding reference frequency symmetrically at the opposite side of the water resonance ($-\omega$) (19)

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

Z-spectra were computed by fitting a 3rd degree polynomial to B_0 corrected water magnetization data as a function of saturation frequency offset (16,20). Similarly, asymmetry curves were calculated by applying equation [2] to each frequency acquired in the z-spectra. The entire soleus, medial and lateral gastrocnemius muscles were manually segmented from anatomical images and applied to CrCEST_{asym}, T_2 , and MTR maps in order to determine the changes in each individual muscle group before and after exercise.

Similarly, MTR maps were computed by

$$MTR = \frac{M_0 - M_{sat}}{M_0} \times 100\% \quad [3]$$

Where M_0 is the magnetization without saturation and M_{sat} is the magnetization with a saturation pulse applied at $\omega = 20 \text{ ppm}$. T_2 maps were created by fitting images acquired with various echo times to $S = S_0 e^{-TE/T_2}$ and solving for T_2 on a pixel-wise basis. ^{31}P MRS Spectra were phased and baseline corrected and then fitted using nonlinear squares methods with Lorentzian functions.

Results

In vivo Z-spectra and Parameter Optimization

Figure 1 shows the z-spectra and corresponding asymmetry curves for varying saturation amplitudes and constant 500 ms duration (Fig 1a,b) as well as varying saturation durations at a constant saturation amplitude of $B_{1rms} = 2.25 \mu\text{T}$ (Fig. 1c,d). The z-spectra and CEST asymmetry plot from the soleus muscle at baseline was broad and showed maximum CEST

assymetry at ~ 1.8 ppm, the chemical shift of Cr amine protons. Increasing B_{1rms} or the saturation duration results in a broadening of the z-spectra and assymetry curves. At lower saturation power, increases in the saturation amplitude result mainly in increases in $CEST_{asym}$ due to a higher labile proton saturation efficiency. However, when the saturation efficiency is already high, increases in B_{1rms} result in increased direct water saturation, which decrease the $CEST_{asym}$ as well as the SNR of base CEST images. Thus in order to maximize $CEST_{asym}$ and SNR, a B_{1rms} of 93 Hz (2.16 μ T) and saturation duration of 500 ms were used for the 3T CEST experiments in this study. We define the CEST effect at $\omega = 1.8$ ppm using those saturation parameters as CrCEST.

In vivo human studies

Figure 2 shows CrCEST_{asym} maps with a temporal resolution of 24 seconds generated before and after mild plantar flexion exercise at 3T for a single subject. Following plantar flexion exercise, an increase CrCEST_{asym} is observed in the posterior compartment of the leg, which then recovers to basal values. The average CEST_{asym} in each segmented muscle of posterior compartment of the lower leg, the soleus as well as the medial (MG) and lateral (LG) heads of the gastrocnemius muscles, is plotted as a function of time at baseline and following exercise in figure 3. Following exercise, an increase in CEST_{asym} of 2.0 %, 2.7 % and 4.2 % was observed in the soleus, medial and lateral gastrocnemius muscles, respectively. The CrCEST_{asym} in all the muscles was seen to recover back to baseline after roughly about 2 minutes.

The exercise protocol was repeated to measure any T_2 and MT changes that could potentially confound the CEST effect. Figure 4 shows MTR (Fig 4a) and T_2 (Fig 4b) maps at baseline and following plantar flexion exercise with a temporal resolution of 28 seconds. As shown in the figure, changes in T_2 or MTR ($T_2 < 1.0$ ms, $MTR < 1.0\%$) observed following exercise were not appreciable ($T_2 < 1.0$ ms, $MTR < 1.0\%$).

In order to validate the observed increase in creatine, ^{31}P MRS was performed to observe changes in PCr. The concentrations of PCr and Cr are tightly coupled in the creatine kinase reaction. Figure 5a shows every alternate ^{31}P MRS spectra acquired 2 minutes before and 8 minutes after exercise with a temporal resolution was 12 seconds. Following exercise, there is a decrease in the PCr peak area, which recovers back to the pre-exercise levels in about 2 minutes. The size and area of the three ATP peaks remained constant ($\pm 3\%$ variation) before and after this exercise. The P_i peak is seen to increase after exercise and then quickly decay back to baseline levels. No shift or splitting of the P_i peak was observed indicating that there were negligible intracellular pH changes ($\text{pH} = 7.02 \pm 0.04$). Since a free induction decay (fid) was used to acquire ^{31}P MR spectra, the signal was unlocalized. A separate chemical shift imaging (CSI) sequence was used to determine the depth of penetration of the surface coil and showed that the majority of the fid signal ($\sim 85\%$) from the surface coil is obtained from a depth less than 1.8 cm from the coil and thus the majority of the ^{31}P MRS signal comes from the gastrocnemius muscle. Finally, when comparing the CrCEST_{asym} from the area of the gastrocnemius muscles equal to that of the ^{31}P surface coil excitation to the relative PCr peak area integrated to a 24 second temporal resolution, a very good agreement was observed in the rate and shape of recovery (fig 5b).

Exercise data from all 6 volunteers is shown in figure 6. Figure 6a shows the ^{31}P MRS PCr peak integral as a function of time. The mean $\text{CrCEST}_{\text{asym}}$ corresponding to the ^{31}P MR signal is plotted as a function of time for each subject in figure 6b. Some subject variability was observed in regards to the level and involvement of each muscle group. However, good agreement is seen between the two figures in the rate and shape at which they recover back to baseline following exercise for each subject. To determine the dependence of the change in $\text{CEST}_{\text{asym}}$ on the change in creatine concentration, the % decrease in the PCr signal following exercise was used to determine the decrease in PCr based on a 33 mM PCr baseline concentration (21). Due to the coupling of PCr and Cr in the CK reaction, the decrease in PCr concentration should equate to an equivalent increase in Cr concentration. Thus comparing the average change in the $\text{CEST}_{\text{asym}}$ corresponding to the ^{31}P MR signal to the calculated change in Cr concentration across all subjects produces a slope of 0.45 % $\text{CrCEST}_{\text{asym}}/\text{mM Cr}$ with an R^2 value 0.65 (Fig. 7).

Discussion

The results of this work demonstrate the feasibility of using the CrCEST technique at 3T to measure creatine with high spatial resolution in muscle following exercise.

There are several challenges to translating the CrCEST technique from 7T to 3T. The chemical shift (ω) between Cr amine protons and free water protons is directly proportional to the static magnetic field (B_0) and thus is decreased at 3T (~ 540 Hz at 7T vs. ~ 225 Hz at 3T). The decrease in chemical shift at 3T results in the frequency selective saturation pulse being applied closer to the water resonance, leading to an increase in direct water saturation. This greatly decreases the signal-to-noise ratio (SNR) of base CEST images. To address the decrease in SNR to some extent, a larger slice thickness was used in this study. Another consequence of increased direct water saturation is that the B_1 that gives the optimal $\text{CrCEST}_{\text{asym}}$ is lower at 3T than at 7T. This decreases the labile proton saturation efficiency, which similarly decreases $\text{CrCEST}_{\text{asym}}$. However, the optimal exchange rate of Cr amine protons lends itself to CEST imaging at lower fields. With empirically optimized parameters, the dependence of the change in $\text{CEST}_{\text{asym}}$ on the change in creatine concentration following exercise at 3T (0.45 % $\text{CrCEST}_{\text{asym}}/\text{mM Cr}$) is approximately half of that observed at 7T (0.84 % $\text{CrCEST}_{\text{asym}}/\text{mM Cr}$)(15).

There are also advantages to CrCEST imaging at 3T. At 3T, muscle T_2 relaxation times are longer ($T_2(3\text{T}) = 29.3\text{ms}$, $T_2(7\text{T}) = 23.0\text{ms}$ (22)) which allows us to regain some of the SNR lost due to higher direct water saturation due to irradiation closer to water. Similarly, T_1 relaxation times are shorter at lower fields. As the repetition time between saturation pulses (Shot TR) is directly correlated to the T_1 , this allows for increased temporal resolution. This is significant for translation of this method to studies of muscle energetics, which have a time course on the order of 30 seconds to a few minutes. In this study, the TR was minimized to optimize the temporal resolution while maintaining adequate SNR. There is potential for further improving the temporal resolution by decreasing the resolution. Additionally, methods to reduce CEST scan times while maintaining adequate B_0 correction will also increase the temporal resolution and further advance the clinical applicability of

this technique. Finally, the widespread availability and clinical applicability of 3T scanners will allow this method to be advanced and translated into clinical applications more rapidly.

The potential confounders to the CrCEST effect in this model of mild plantar flexion exercise are thoroughly discussed in muscle CrCEST experiments done at 7T (15). Mild plantar flexion exercise is not expected to result in a decrease in muscle pH. This is supported by ^{31}P MRS data in this study, which showed no shift or splitting of the P_i peak. Similarly, as no pH changes were observed, we assume that there is no glycogen contribution to the observed CrCEST changes in the mild exercise employed in this study (23). Additionally, MTR maps showed negligible changes before and after exercise, and thus we do not expect any MT contamination to the measured increase in $\text{CrCEST}_{\text{asym}}$ following exercise. Finally, it was shown that the other CK metabolites (ATP, ADP, and PCr) are not expected to contribute to the CrCEST effect (14). This is mainly due to the exchange rate of their amine group protons which are about an order of magnitude lower than that of Cr and thus do not show CEST enhancement with parameters that optimize $\text{CrCEST}_{\text{asym}}$. Since there were negligible changes in the pH, T_2 , or MTR of exercised muscle as well as negligible CEST effects from other creatine kinase reaction metabolites, it can be concluded that the observed changes in $\text{CrCEST}_{\text{asym}}$ in this model of plantar flexion exercise are predominantly due to changes in muscle Cr concentration.

It is also necessary to point out several potential limitations of the study. In this study, the ^{31}P signal was unlocalized and as a result was not obtained from same slice as CEST data but rather from part of the gastrocnemius muscles from a large area of the calf. Thus, as the spectral data is obtained from a larger volume of the leg, comparisons made between $\text{CrCEST}_{\text{asym}}$ and ^{31}P MRS are estimates. It should also be noted that multiple exercise bouts were used to acquire CEST, T_2 , MTR, and ^{31}P MRS data. This could result in differences in muscle changes between exercise bouts. Nevertheless, the variances are expected to be small, as exercise was mild and subjects were healthy volunteers.

In summary, this work has demonstrated the feasibility of in vivo CrCEST imaging on routine clinical scanners (3T) to measure changes in Cr concentration in muscle following plantar flexion exercise. Similar to ultra-high fields, at 3T there was good agreement between the recovery kinetics of ^{31}P MRS and $\text{CrCEST}_{\text{asym}}$ following exercise. The widespread availability and clinical applicability of 3T scanners will allow for more rapid translation to clinical applications for the diagnosis and treatment of muscle disorders as well as to enhance research into conditions such as heart and renal failure, and other secondary complications of metabolic disorders.

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References

1. Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation .3. The steady state. *J Biol Chem.* 1955; 217(1):409–427. [PubMed: 13271404]

2. Davies RE. A molecular theory of muscle contraction - calcium-dependent contractions with hydrogen bond formation plus atp-dependent extensions of part of myosin-actin cross-bridges. *Nature*. 1963; 199(489):1068–1074. [PubMed: 14066941]
3. Bottomley PA, Lee YH, Weiss RG. Total creatine in muscle: Imaging and quantification with proton MR spectroscopy. *Radiology*. 1997; 204(2):403–410. [PubMed: 9240527]
4. Hoult DI, Busby SJW, Gadian DG, Radda GK, Richards RE, Seeley PJ. Observation of tissue metabolites using P-31 nuclear magnetic-resonance. *Nature*. 1974; 252(5481):285–287. [PubMed: 4431445]
5. Argov Z, Lofberg M, Arnold DL. Insights into muscle diseases gained by phosphorus magnetic resonance spectroscopy. *Muscle Nerve*. 2000; 23(9):1316–1334. [PubMed: 10951434]
6. Tarnopolsky MA, Parise G. Direct measurement of high-energy phosphate compounds in patients with neuromuscular disease. *Muscle Nerve*. 1999; 22(9):1228–1233. [PubMed: 10454718]
7. Yabe T, Mitsunami K, Inubushi T, Kinoshita M. Quantitative measurements of cardiac phosphorus metabolites in coronary-artery disease by P-31 magnetic-resonance spectroscopy. *Circulation*. 1995; 92(1):15–23. [PubMed: 7788910]
8. Guivel-Scharen V, Sinnwell T, Wolff S, Balaban R. Detection of proton chemical exchange between metabolites and water in biological tissues. *J Magn Reson*. 1998; 133:36–45. [PubMed: 9654466]
9. Dagher A, Aletras A, Choyke P, Balaban R. Imaging of urea using chemical exchange-dependent saturation transfer at 1.5 T. *J Magn Reson*. 2000; 12:745–748.
10. Ward K, Aletras A, Balaban R. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). *J Magn Reson*. 2000; 143:79–87. [PubMed: 10698648]
11. Zhou J, Wilson D, Sun P, Klaus J, van Zijl P. Quantitative description of proton exchange processes between water and endogenous and exogenous agents for WEX, CEST, and APT experiments. *Magn Reson Med*. 2004; 51:945–952. [PubMed: 15122676]
12. Kogan F, Hariharan H, Reddy R. Chemical Exchange Saturation Transfer (CEST) Imaging: Description of Technique and Potential Clinical Applications. *Current Radiology Reports*. 2013:1–13.
13. Sun P, Benner T, Kumar A, Sorensen A. Investigation of optimizing and translating pH-sensitive pulsed-chemical exchange saturation transfer (CEST) imaging to a 3T clinical scanner. *Magn Reson Med*. 2008; 60(4):834–841. [PubMed: 18816867]
14. Haris M, Nanga RPR, Singh A, Cai K, Kogan F, Hariharan H, Reddy R. Exchange rates of creatine kinase metabolites: feasibility of imaging creatine by chemical exchange saturation transfer MRI. *NMR Biomed*. 2012; 25:1305–1309. [PubMed: 22431193]
15. Kogan F, Haris M, Singh A, Cai K, Debrosse C, Nanga RPR, Hariharan H, Reddy R. Method for high-resolution imaging of creatine in vivo using chemical exchange saturation transfer. *Magn Reson Med*. 2013; 1002/mrm.24641
16. Kim M, Gillen J, Landman B, Zhou J, van Zijl P. Water Saturation Shift Referencing (WASSR) for Chemical Exchange Saturation Transfer (CEST) Experiments. *Magn Reson Med*. 2009; 61:1441–1450. [PubMed: 19358232]
17. Singh A, Cai K, Haris M, Hariharan H, Reddy R. On B1 inhomogeneity correction of in vivo human brain glutamate chemical exchange saturation transfer contrast at 7T. *Magn Reson Med*. 2012:n/a–n/a.
18. Parrish T, Hu X. A new T2 preparation technique for ultrafast gradient-echo sequence. *Magn Reson Med*. 1994; 32:652–657. [PubMed: 7808267]
19. Liu G, Gilad AA, Bulte JWM, van Zijl PCM, McMahon MT. High-throughput screening of chemical exchange saturation transfer MR contrast agents. *Contrast Media Mol Imaging*. 2010; 5(3):162–170. [PubMed: 20586030]
20. Zhou J, Lal B, Wilson DA, Larterra J, van Zijl PC. Amide proton transfer (APT) contrast for imaging of brain tumors. *Magn Reson Med*. 2003; 50:1120–1126. [PubMed: 14648559]
21. Kemp GJ, Meyerspeer M, Moser E. Absolute quantification of phosphorus metabolite concentrations in human muscle in vivo by P-31 MRS: a quantitative review. *NMR Biomed*. 2007; 20(6):555–565. [PubMed: 17628042]

22. Jordan CD, Saranathan M, Bangerter NK, Hargreaves BA, Gold GE. Musculoskeletal MRI at 3.0T and 7.0T: A comparison of relaxation times and image contrast. *Eur J Radiol.* 2013; 82:734–739. [PubMed: 22172536]
23. Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR, Whipp BJ. Dynamics of intramuscular P-31-MRS P-i peak splitting and the slow components of PCr and O-2 uptake during exercise. *J Appl Physiol.* 2002; 93(6):2059–2069. [PubMed: 12391122]

Figure 1.

The data (crosses) fitted z-spectra (solid lines) and corresponding asymmetry plots for the soleus muscle at baseline for **(a,b)** varying saturation amplitudes [$B_{1rms} = 0.72 \mu T, 1.44 \mu T, 2.16 \mu T, 2.87 \mu T$, and $3.59 \mu T$] and constant 500 ms duration as well as **(c,d)** varying saturation durations [$t_{sat} = 250ms, 500ms, 1000 ms$ and $1500 ms$] at a saturation amplitude of $B_{1rms} = 2.15 \mu T$. $CrCEST_{asym}$ at $\omega = 1.8$ ppm is indicated with a dotted line.

Figure 2.

CrCEST_{asymp} maps of a human calf muscle before and every 24 seconds after (in order by number) 2 minutes of mild plantar flexion exercise. Color bar represents CrCEST_{asymp} in percent. The segmented anatomical image is displayed in the bottom left.

Figure 3. Plot of the average CrCEST_{asym} as function of time in 3 different muscles of the calf [Soleus, Medial Gastrocnemius (MG) and Lateral Gastrocnemius (LG)] segmented from anatomical images (Top Right). Error bars represent the standard deviation in the CrCEST_{asym} in each region.

Figure 4.

(a) MTR and (b) T_2 maps before and every 28 seconds after (in order by number) the 2 minutes of mild plantar flexion exercise.

Figure 5.

(a) Stacked plot of every alternate ^{31}P MRS spectra acquired 2 minutes before and 8 minutes after exercise with a 12 second temporal resolution. (b) Comparison between the $\text{CrCEST}_{\text{asym}}$ from the area of the gastrocnemius muscles equal to that of the ^{31}P surface coil excitation and ^{31}P MRS PCr signal as a function of time at an equal temporal resolution of 24 seconds.

Figure 6. CrCEST_{asy} and ^{31}P MRS data across subjects. **(a)** ^{31}P MRS PCr peak integral as a function of time. **(b)** Mean CrCEST_{asy} corresponding to the ^{31}P MR Signal is plotted as a function of time for each subject. For each subject, good agreement is seen between the two figures in the rate and shape at which they recover back to baseline following exercise

Figure 7.

Plot of the average change in the $\text{CEST}_{\text{asym}}$ corresponding to the ^{31}P MR Signal as a function of the calculated change in Cr concentration across all subjects. The % decrease in the PCr signal following exercise was used to determine the decrease in PCr based on a 33 mM PCr baseline concentration (19). As the total Cr remains constant, the decrease in PCr concentration was equated to an equivalent increase in Cr concentration. This produces a slope of 0.45 % $\text{CrCEST}_{\text{asym}}/\text{mM Cr}$ with an R^2 value 0.65 with the y-intercept set to zero.