Using CEST to Detect Glycogen-depleting Exercise-Induced Changes In Vivo

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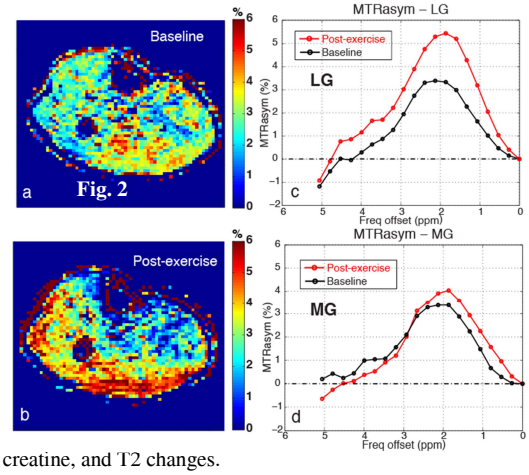
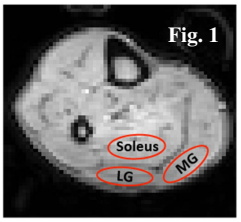
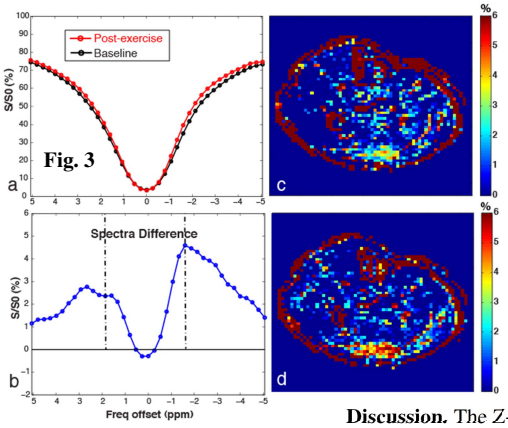
Target audience: Researchers and clinicians interested in applying the CEST technique to detect energy metabolism of musculoskeletal system.

Purpose: Magnetic resonance spectroscopy (MRS) has been applied to follow energy metabolism of the musculoskeletal system at baseline, during exercise and recovery. For example, ^{31}P MRS can provide valuable information in particular on muscle high-energy phosphates[1]. Natural abundance ^{13}C MRS has been applied to detect glycogen synthesis and glycogenolysis via the ^{13}C-C1 of glycogen[2]. However, MRS suffers from low sensitivity and concomitant poor spatial resolution for imaging. Chemical exchange saturation transfer (CEST) is a new MRI method that can indirectly detect cellular substances such as metabolites, glycogen, proteins and peptides through exchangeable protons[3]. Recently, the creatine CEST (CrCEST) effect based on its amine group has been suggested for spatial and temporal mapping of creatine changes in skeletal muscle at 7T[4]; the feasibility of detecting the CEST effect from hydroxyl proton (–OH) groups of glycogen (glycoCEST) was also demonstrated previously in vitro as well as in vivo in animal studies of the liver at 4.7T[5]. However, due to broad resonances (> 500Hz), the CEST effects of OH groups and NH_2 groups overlap in the exchangeable proton signal range at positive frequencies with respect to water and are difficult to separate. However, glycogen may also have a nuclear Overhauser enhancement (NOE) effect in vivo at negative frequency, depending on its chain mobility. In 1998 Gore *et al.* described a glycogen-depleting exercise protocol[6], and by using ^{13}C and ^{31}P they reported a 43% decrease of glycogen level but no significant phosphate (PCr and P_i) changes in the gastrocnemius after the exercise. In this study, we applied a similar exercise protocol in calf muscles of healthy human volunteers at 3T, and compared CEST imaging pre- and post- exercise. We were able to detect exercise-induced CEST-MRI changes, which could in principle include glycogen, creatine, and T2 changes.





Methods: Calf imaging experiments were performed on a 3T Philips Achieva system (Philips Healthcare, Best, The Netherlands) using a body coil for RF transmission and an 8-channel ^{1}H knee coil for reception. Parallel transmission (pTX) was used to alternate the saturation pulses in order to have long saturation[7]. Five healthy volunteers (3 male, 2 female) were recruited to do a 12-min foot toe-raise. During the exercise, each subject performed toe-pulls for 1 min using 5 therapeutic bands (REP band, Magister Corp, Chattanooga TN) with a combined force of 70lbs at 150% length, followed by rest for 1 min, and repeated 6 times. Such on/off exercise protocol was reported to deplete gastrocnemius glycogen levels by ~40% while avoiding anaerobic fatigue[6]. Baseline water saturation shift reference (WASSR)[8] and CEST images were collected followed by this 12-min exercise protocol. Post-exercise, CEST and WASSR images were acquired 1-min after stopping the exercise protocol. In CEST experiments, Z spectra were collected from -5 to 5 ppm with a frequency step size of 34Hz (~1/4 ppm) using a ten-block RF saturation pulse (100-ms duration per block and 2.25-μT amplitude) with 400-μs interpulse delay, followed by a single-shot RF-spoiled gradient echo readout with centric phase encoding order (flip angle=15°, TR_{GRE}/TE = 4.0/1.91ms, FOV=140x140cm^2, matrix size=64x64, slice thickness = 8mm, SENSE factor=2. WASSR images for B0 inhomogeneity correction of the CEST spectra were acquired from -2 to 2ppm with a step size of 14 Hz, and by applying six 100-ms sinc-Gaussian pulses with B_{1rms} of 0.22-μT and 400-μs interpulse delay using the same imaging parameters as CEST. *Data Processing:* All data processing was performed using in-house MATLAB (The Mathworks, Natick MA) programs. CEST MTR asymmetry (MTR_{asym}) maps were also generated at the creatine amine proton frequency offset ($\Delta\omega$ = +1.8ppm): $MTR_{asym}(+1.8ppm) = [S(-\Delta\omega) - S(+\Delta\omega)]/S_0$.



Results & Discussion: A representative anatomical image of the lower leg is shown in Fig. 1. Regions are shown for three different muscle groups: soleus, medial gastrocnemius (MG) and lateral gastrocnemius (LG). In 3 of the 5 subjects, the glycogen-depleting exercise led to an increase in the MTR_{asym} value at 1.8ppm; the other 2 subjects showed negligible change. The $MTR_{asym}(+1.8ppm)$ maps overlaid on the anatomical image at baseline and 1-min after the end of the exercise from one subject are illustrated in Fig2a, b, respectively, showing regional increases predominantly in the gastrocnemius muscles after the exercise. Average increase in $MTR_{asym}(+1.8ppm)$ from the 3 subjects were 0.6% (±0.1%) and 1.5% (±0.3%) in the medial (MG) and lateral (LG) gastrocnemius muscles, respectively, as can be seen in the pre- and post-exercise MTR_{asym} plot from the LG and MG muscles in Figs. 2c and d, resp. The MTR_{asym} spectra were broad and showed maximum CEST contrast at ~1.8ppm. Fig. 3 compares pre- and post-exercise Z-spectra (Fig. 3a) from lateral gastrocnemius from one subject, who showed large spectral differences (Fig. 3b) at both 1.8ppm and -1.8ppm, images of which are displayed in Figs. 3c and 3d, resp. Importantly, a general narrowing of the Z-spectrum can also be seen. The pre- and post- Z-spectra from soleus (not shown), on the other hand, were closely overlapping, indicating little change after exercise. The averaged (n = 5) integrated area in the difference spectra from 1 to 4ppm were 22.5% (±10.3%), 18.9% (±9.3%), and 6.5% (±2.7%) in MG, LG and soleus muscles, respectively, and 23.0% (±8.0%), 25.7% (±10.7%), 10.3% (±5.6%) from -4 to -1ppm from water.

Discussion. The Z-spectra of several volunteers performing this particular exercise regime show Z-spectral narrowing and spectral increases both at positive and negative frequencies with respect to the water resonance. Spectral narrowing of the saturation lineshape can be due to changes in B1, T1 or T2 and since B1 is constant and T1 changes not expected, we attribute narrowing to lengthening of muscle T2, in agreement with results in the literature for this exercise.[6] Signal increases in the post-pre difference spectrum at higher frequency can be due either to Cr or glycogen depletion, while such increases at lower frequency can be due only to NOE reductions. Of course Cr would be expected to increase during most exercises, leading to more saturation. This was not found in the current exercise regime, in line with expectations[6]. In the present data the exact size of the changes is hard to judge, since the effects of T2 narrowing will have to be deconvolved. These are symmetric with respect to water and thus expected to be visible only in the difference spectra and not in the MTR_{asym}. Our initial conclusion from the data is that T2 increases and glycogen is depleted, in line with the literature[6]. However, ^{13}C MRS to monitor glycogen breakdown and ^{31}P MRS to check lack of changes in phosphocreatine levels during this exercise regime in our study are needed for ultimate confirmation.

Conclusion: We applied CEST to detect changes induced by a previously described glycogen-depleting exercise. MTR_{asym} maps showed localized changes in the muscle with good spatial resolution. The difference between Z-spectra before and after exercise could reflect combined changes in water T2, creatine and glycogen levels.

Reference: (1) Hoult et al. Nature 1974;252:285 (2) Cline et al. N Engl J Med 1999;341:240 (3) van Zijl & Yadav MRM 2011;65:927 (4) Kogan et al. MRM 2013, Early View on-line; (5) van Zijl et al. PNAS 2007;104:4359 (6) Price & Gore. J Applied Physiol. 1998;84:1178 (7) Keupp et al ISMRM 19:710 (8) Kim et al. MRM 2009;61:1441. Grant support: RO1 EB015032, P41 EB015909