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Stimulated echo diffusion tensor imaging and SPAIR T2-weighted imaging in Chronic Exertional Compartment Syndrome of the lower leg muscles

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Abstract

Purpose—To evaluate the performance of diffusion tensor imaging (DTI) in the evaluation of chronic exertional compartment syndrome (CECS) as compared to T₂-weighted imaging.

Materials and Methods—Using an IRB-approved HIPAA-compliant protocol, spectral adiabatic inversion recovery (SPAIR) T₂-weighted imaging (T2w) and stimulated echo DTI were applied to 8 healthy volunteers and 14 suspected CECS patients before and after exertion. Longitudinal and transverse diffusion eigenvalues, mean diffusivity (MD), and fractional anisotropy (FA) were measured in 7 calf muscle compartments, which in patients were classified by their response on T2w: normal (<20% change), and CECS (>20% change). Mixed model analysis of variance compared subject groups and compartments in terms of response factors (post-/pre-exercise ratios) of DTI parameters.

Results—All diffusivities significantly increased ($p < 0.0001$) and FA decreased ($p = .0014$) with exercise. Longitudinal diffusion responses were significantly smaller than transversal diffusion responses ($p < 0.0001$). 19 of 98 patient compartments were classified as CECS on T2w. MD increased by $3.8 \pm 3.4\%$ (volunteer), $7.4 \pm 4.2\%$ (normal), and $9.1 \pm 7.0\%$ (CECS) with exercise.

Conclusion—DTI shows promise as an ancillary imaging method in the diagnosis and understanding of the pathophysiology in CECS. Future studies may explore its utility in predicting response to treatment.

Keywords

DTI; stimulated echo; chronic exertional compartment syndrome; skeletal muscle; exercise

INTRODUCTION

Diffusion tensor imaging (DTI) (1) provides quantitative markers of tissue microstructure and anisotropy, and as such can serve as a diagnostic probe of pathologic conditions affecting skeletal muscle fibers (2–5). Ischemia (6), inflammation, and injury (7) can result

in myofiber damage, disorganization, or deterioration and are therefore detectable via DTI metrics such as mean diffusivity (MD) and fractional anisotropy (FA). In healthy subjects, elevated muscle signal intensity on T₂-weighted imaging following exercise is a well-known phenomenon with multiple possible contributions (8–10). Quantitative relaxation imaging in muscle suggests intra/extra-cellular compartmentation determines multi-exponential T₂ behavior (though macromolecule hydration layers may also play a role), and exercise may involve exchange between these compartments. The increase of apparent diffusion with exercise is also well-established, and has been observed to correlate with T₂ increases in the same subjects (11,12), though its sensitivity to compartmentation and to temperature differ from that of relaxation. Vascular contributions to diffusion contrast are also recognized in skeletal muscle (13,14).

In chronic exertional compartment syndrome (CECS) (15–17), muscle compartments retain excess fluid following exercise leading to elevated pressure, reduced perfusion, pain, and possibly ischemic injury (18). Standard diagnosis of CECS involves intracompartmental pressure (ICP) measurement, and fasciotomy is a common surgical intervention. Both steps can be invasive and/or debilitating, and are not always successful. Therefore, noninvasive MRI markers can be very attractive alternative or supplemental diagnostic tools. Multiple factors may be involved in CECS, including vascular deficits, elevated compartmental pressure, muscular edema, myofiber dilation/separation, and membrane degradation.

In the CECS population, elevated T₂-weighted signal intensity have correlated with ICP and CECS severity (19). Turbo spin echo (TSE) T₂-weighted sequences are often used for this characterization, and uniform fat saturation is crucial to separate evaluation of all muscle compartments. Frequency selective or short time inversion recovery approaches alone can suffer from transmit field inhomogeneity or low signal-to-noise ratio, while spectral adiabatic inversion recovery (SPAIR) (20) combines both approaches for more effective performance.

Given the history of complementary diffusion and relaxation imaging approaches in muscle, DWI may also have diagnostic value in CECS. Quantitative approaches such as DTI have potential to shed light on the microscopic pathophysiology of CECS, and possibly inform upon treatment options. In this study, we tested the performance of stimulated echo DTI and SPAIR T₂-weighted MRI in calf muscles of healthy volunteers and suspected CECS patients at 3 T before and after exertion.

MATERIALS AND METHODS

In this HIPPA-compliant study approved by the local institutional review board (IRB), fourteen patients (8 F, age 25±8 years, range 15 to 40 years; 6 M, age 31±10 years, range 18 to 44 years) with clinical suspicion of CECS and eight healthy volunteers (2 F, age 29±1 years, range 28 to 29 years; 6 M, age 27±3 years, range 21 to 29 years) provided written informed consent and underwent MR imaging of the legs including SPAIR T₂-weighted imaging and diffusion tensor imaging (DTI) protocols. SPAIR T₂-weighted (T2w) imaging and DTI results were obtained both at rest and after at least 10 minutes of treadmill exertion. Specifically, the subjects came off the scanner table and jogged on a commercial exercise treadmill (Reebok S 9.80, Model#RBTL69608.0), at their own pace and resistance level, for 10 minutes or, in the case of patients, until pain onset, up to maximum of 30 minutes. Subjects were then quickly repositioned on the scanner bed for the post-exercise scan. The distribution of time intervals for the subjects enrolled in this study are given in the Results section. Images were collected in either a Siemens TIM Trio 3 T (6 patients) or wide-bore Siemens Verio 3 T (8 volunteers, 8 patients) scanner, determined by scanner availability.

T2w and DTI scans were carried out in that order for all subjects and exercise status; further timing details for the full subject cohort are given in the Results section. Bilateral axial T2w images were collected with a 2D turbo spin echo (TSE) sequence with SPAIR fat suppression (TR/TE = 5380/62 ms, $204 \times 256 \times 50$ matrix, $1.6 \times 1.3 \times 3$ mm resolution, 2 averages, acquisition time 5:55) and a combination of anterior body matrix coil and posterior spine array elements using 5–6 elements total. Axial DTI used a fat-saturated stimulated echo diffusion sequence (Figure 1) (21) with echo-planar imaging (EPI) readout and mixing time TM (TR/TE/TM = (10700–12400)/(31–42)/1000 ms, $64 \times 64 \times 10$ matrix, $3 \times 3 \times 5$ mm resolution, 6 directions, $b = 0$, 500 s/mm², 3 averages, acquisition time 5:07) and a unilateral multi-channel knee coil (4 channels for Tim Trio and 8 channels for Verio). DTI scans in the Tim Trio system used frequency-selective fat suppression, while those in the Verio system used SPAIR fat suppression. In a subset of the entire study cohort (7 patients and 8 volunteers), an additional axial DTI scan was acquired with shorter mixing time (TM = 10 ms, giving a diffusion time of $T_d = TM + TE/2 = 30$ ms) before and after exercise. The b-value of 500 s/mm² was used for consistency with the literature (22–25). The stimulated echo diffusion preparation used monopolar gradients in the first and third intervals of the sequence, followed by an echo-planar imaging (EPI) readout (Figure 1) (21,26), to enable long diffusion times and correspondingly large diffusion lengths for amplified microstructural sensitivity. Spoilers were included in the mixing time interval and, for the $b=0$ acquisition, in the 1st and 3rd intervals. Since our protocol employed a fixed b-value of 500 s/mm² at a long diffusion time (~1000 ms), the diffusion gradient moment on one axis (59 mT/m ms) was close enough to those of unbalanced spoiler (17 mT/m ms) and slice-selective imaging gradients (8 mT/m ms) that the full diffusion encoding matrix (“b-matrix”) was required in the diffusion tensor inversion to obtain quantitative accuracy (27–29). The vendor sequence software on the Verio platform calculated the full b-matrix for both the nominally unweighted (b_0) and diffusion-weighted images, according to standard formalism (27,30). Specifically, if the diffusion-weighted signal attenuation is written as

$$\frac{S}{S_0} = \exp \left(- \sum_{i,j=x,y,z} b_{ij} D_{ij} \right) \quad [1]$$

where D_{ij} is the diffusion tensor and b_{ij} is the diffusion encoding or b-matrix, then the latter is related to the gradient waveforms according to

$$b_{ij} = \int_0^{TE} k_i(t) k_j(t) dt \quad [2]$$

$$k_i(t) = \int_0^t g_{eff,i}(t') dt'$$

where g_{eff} is the effective applied diffusion gradient waveform, including all diffusion and imaging gradients and magnetization inversions from RF pulses.

Phantom validation

In order to validate the analysis procedure, DTI scans with the same imaging protocol described above were acquired in the Verio 3 T scanner with several mixing times T_m (30 ms, 500 ms, 1000 ms) in a water phantom having isotropic, time-independent diffusion. The calculated b-matrices were employed in the DTI estimation of the water data. The two main perturbations to the applied diffusion gradients were (1) a spoiler gradient applied on all three gradient axes in the b_0 image, and (2) the unbalanced slice gradient influence over the course of the diffusion time. For 6 patient scans in the Tim Trio system, internal b-matrix

calculations were not available and were thus empirically generated by modification of those from identical protocols on the Verio platform. A spoiler magnitude of 0.73 times that in the Verio system was found to correctly reveal time-independent, isotropic behavior in water phantom acquisitions using the patient scans' protocols. The resulting b-matrices were then used to analyze the Tim Trio patient data.

Human subject analysis

Increased T2w signal on post exercise images was evaluated as a percent increase on ROI values. Small regions of interest (ROIs) (average size 10 mm²) were independently drawn using vendor software by two radiologists blinded to clinical diagnosis (xx, xx) with 14 and 25 years of experience within the following muscle compartments: anterior tibialis (AT), extensor digitorum longus (EDL), posterior tibialis (PT), peroneus longus (PL), soleus (SOL), gastrocnemius lateralis (GL) and gastrocnemius medialis (GM). T2w signal intensity was recorded for each slice and muscle compartment for both legs in the bilateral T2w scans before and after exercise. Results for all slices were averaged to produce representative values for each muscle compartment and leg. Any discrepancies between reader evaluations were resolved by consensus. Relative changes in intensity were evaluated as a percentage of the baseline T2w signal intensity. A 20% or greater T2w change for a particular muscle compartment was defined as positive for CECS, an approximate threshold consistent with prior studies (17,19,31–33).

DTI data were processed offline (Igor Pro, Wavemetrics, Portland, USA) to generate maps of MD, FA, and diffusion eigenvalues (λ_1 , λ_2 , λ_3), including full b-matrices as described above. Regions of interest (ROI) were manually segmented on axial b₀ images, separately for pre-and post-exercise scans, enclosing entire muscle compartments for the same areas sampled in the T2w analysis above (see Figure 2 for example DTI ROI placement). For each muscle compartment, mean DTI metrics were evaluated in each slice ROI and the set of values for all slices were averaged as a representative value. Signal-to-noise ratio (SNR) of DWI at b = 0 and b = 500 s/mm² were separately evaluated for each subject, exercise condition, and muscle compartment by dividing the mean signal intensity in compartment ROI by the standard deviation of signal intensity in a region outside the leg, and averaging results from different slices, directions, and compartments in a given subject. For the 15 subjects with both short and long time diffusion data, physiological variance was quantified separately for each slice, compartment, diffusion direction, and diffusion time via a normalized standard deviation, i.e. standard deviation/mean value, of signal intensity across the acquired averages, and then averaging results from directions and compartments in a given subject.

Statistical analysis

For each subject the exercise response factor for each parameter within each muscle compartment was computed as the ratio of the post-exercise value divided by the pre-exercise value. Exact Wilcoxon tests were used to assess whether the parameters within each muscle compartment exhibited a pre- to post-exercise change by testing the null hypothesis that the response factor ratio was one. Mixed model analysis of variance was used to compare muscle compartments and response categories in terms of the response factors and baseline levels of each parameter. To test consistency, the mixed model analysis was also used to compare subjects scanned in the Tim Trio 3 T and those scanned in the Verio 3T scanner in terms of DTI metrics in each muscle compartment at baseline. To account for the lack of statistical independence among measures from the same subject (e.g., subjects contributed for data for each of the muscle compartments being compared), the correlation structure was modeled by assuming responses to be symmetrically correlated when acquired from the same subject and independent when derived from different subjects. All tests were

conducted at the two-sided 5% comparison-wise significance level using SAS 9.3 (SAS Institute, Cary, NC).

To compare results acquired at different diffusion times, mixed model analysis of variance was used to compare volunteers to the combined group of CECS and Normal patients in terms of the response factor ratios and the pre- and post-exercise levels of each parameter. For each parameter, the analysis was stratified by diffusion time (short, 30 ms vs. long, 1020 ms) and the ratio, the pre-exercise level and the post-exercise level were analyzed separately. In each case, the relevant data from all muscle compartments were pooled into a single overall analysis and the model included subject group and muscle compartment as fixed classification factors. A standardized difference for each parameter was computed as the absolute value of the mean difference between groups divided by the standard error of the mean difference. To account for statistical dependencies among measures from the same subject, the correlation structure was modeled by assuming responses to be symmetrically correlated when acquired from the same subject and independent when derived from different subjects. The error variance was allowed to differ across comparison groups to remove the unnecessary assumption of variance homogeneity. Since the mixed model analyses used data from all muscles combined, the sample size was considered sufficiently large for asymptotic justification of the underlying normality assumption. All tests were conducted at the two-sided 5% comparison-wise significance level using SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

Water phantom

Water phantom DTI metrics (mean $\pm$ interslice standard deviation for ROIs in 10 slices) as a function of mixing time with and without b-matrix correction are shown in Table 1. Without correction, the water mean diffusivity shows artificial reduction with diffusion time and fractional anisotropy artificially increases. With the correct b-matrices, the mean diffusivity is time-independent within the precision of the measurement, and the FA is at a minimal noise level (0.1) for all three diffusion times. The tertiary eigenvalue λ_3 shows slightly larger discrepancy at 1000 ms (7.5%) but this is consistent with numerical eigenvalue repulsion effects driven by lower signal to noise ratio (34) and would not affect relative changes in λ_3 with exercise.

Human subjects

The average durations of relevant exercise intervals in our subject cohort were as follows. The time interval from final pre-exercise to first post-exercise scan was 22.8 ± 8.4 minutes (26.0 ± 8.5 minutes for patients and 17.1 ± 4.7 minutes for volunteers). The start times of the post-exercise T2w and long diffusion time DTI scans following subject repositioning were 4.8 ± 6.7 minutes (pts: 2.8 ± 2.2 minutes; volunteers: 8.9 ± 10.7 minutes) and 12.4 ± 6.7 minutes (pts: 14.7 ± 6.6 minutes; volunteers: 12.3 ± 3.2 minutes), respectively. Similarly, start times of short diffusion time DTI scans in a subset of subjects (8 volunteers and 7 patients) were 3.9 ± 1.9 minutes (pts: 4.0 ± 2.5 minutes; volunteers: 3.8 ± 1.2 minutes) following repositioning. 6/14 patients underwent 2 separate exertion periods before T2w and DTI scans to ensure activation effects had not subsided, one of which had their T2w and DTI scans performed in different scanners (Verio and Tim Trio, respectively). 2 patients had their T2w and DTI scans performed in the same scanner on different days. Given these timing data, it is to be appreciated that the observed MR diffusion biomarker changes with exercise were sampled in a long term steady state period following initial response to exercise, and that timing heterogeneity may play some role.

Average SNR values were as follows for the 15 subjects with both short and long diffusion time data. At $b=0$ the average SNR before/after exercise was $84.7 \pm 18.5 / 85.9 \pm 14.1$ at short times and $43.9 \pm 8.4 / 45.2 \pm 9.2$ at long times, while at $b=500 \text{ s/mm}^2$ average SNR was $51.3 \pm 10.8 / 49.3 \pm 8.1$ at short times and $35.6 \pm 7.4 / 36.1 \pm 7.5$ at long times. Similarly, the normalized standard deviation of all DWI before/after exercise for $b=0 \text{ s/mm}^2$ was $0.136 \pm 0.025 / 0.139 \pm 0.015$ at short diffusion time and $0.148 \pm 0.028 / 0.154 \pm 0.019$ at long diffusion times, and for $b=500 \text{ s/mm}^2$, $0.156 \pm 0.023 / 0.161 \pm 0.019$ at short diffusion time and $0.166 \pm 0.026 / 0.173 \pm 0.019$ at long diffusion times. At long times, the 6 subjects scanned in the Trio 3 T and 4-channel knee coil showed an average SNR before/after exercise of $36.0 \pm 16.1 / 39.9 \pm 16.1$ for $b=0$ and $28.2 \pm 7.7 / 30.4 \pm 8.2$ for $b=500 \text{ s/mm}^2$, while the 14 subjects scanned in the Verio 3 T and 8-channel knee coil showed an average SNR of $43.9 \pm 8.5 / 45.3 \pm 9.2$ for $b=0$ and $35.6 \pm 7.5 / 36.1 \pm 7.5$ for $b=500 \text{ s/mm}^2$. Note that these SNR values refer to individual DWI and the final SNR including all 3 averages was thus higher by $\sqrt{3} = 1.73$.

SPAIR T2w and DTI (MD, FA) results from a healthy volunteer and a CECS patient are illustrated in Figure 3. In the volunteer, T2w signal intensity and MD increase diffusely in all muscle compartments. In the CECS patient, T2w images show focally elevated signal intensity in the lateral and medial gastrocnemius muscles following exercise. Correspondingly, MD values increase, and FA values decrease, more strongly in the same GM and GL compartments.

Nine of 14 patients showed elevated T2w signal intensity both subjectively and based on ROI values in one or more muscle compartments after exercise. A total of 19 muscle compartments (see Table 2) were identified as CECS positive based on T2w. DTI metrics at baseline as a function of skeletal muscle compartment and subject group are shown in Table 2, and those with significant ($p < 0.05$) changes with exercise are highlighted. Figures 4a and 4b show the MD distribution for all subject groups and muscle compartments, respectively, before and after exercise. Regarding magnet consistency, the average ratio of baseline values from the two magnet types (Trio/Verio) for all compartments and parameters was 0.98 ± 0.06 (average deviation $5.0 \pm 4.2\%$), and that of response factors was 1.02 ± 0.03 (average deviation $3.1 \pm 2.1\%$).

Table 3 shows exercise response factor results of all DTI indices as a function of group classification. MD and eigenvalues λ_1 , λ_2 , λ_3 increased with exercise in all muscle compartments by an average of 4.1% for VOL groups, 8.1% for NML groups, and 9.7% for CECS groups, while FA decreased by an average of 1.3%, 2.8%, and 3.4%, respectively. Significant differences ($p < 0.05$) were found between the response factors of all diffusivities (MD, λ_1 , λ_2 , λ_3) for VOL groups vs. either NML or CECS groups. Significantly lower response factors for λ_1 were found than for λ_2 and λ_3 in the entire study group ($p < 0.0001$) and in NML ($p < 0.0001$) groups, and lower λ_1 response factors than for λ_2 in VOL ($p = 0.0166$). At the individual muscle compartment level, most diffusivity parameters and muscle compartments showed significant ($p < 0.05$) changes. Peripheral compartments (AT, EDL, GL, GM) show larger changes than do deeper compartments (PT, SOL). Significant responses ranged from as low as $2.6 \pm 2.3\%$ (for λ_1 in AT in VOL) to as high as $13.4 \pm 8.9\%$ (for λ_3 in GM in NML). Figures 5a and 5b show the distribution of the MD response factors among subject groups and muscle compartments, respectively. MD exercise response factors in CECS patients in AT, GL, and GM compartments were the highest: $8.6 \pm 6.3\%$, $8.0 \pm 9.4\%$, and $13.9 \pm 7.6\%$, respectively.

Table 4 and Figure 6 show the results comparing the DTI data acquired at two different diffusion times (30 ms and 1020 ms) in a subset of 8 volunteers and 7 patients. All diffusivities are observed to be lower, and fractional anisotropies higher, at longer diffusion

times than smaller times. Also, nearly all parameters, and even more so their response factors, show greater differentiation between volunteers and suspected CECS patients at longer diffusion times, as manifested in larger and more significant standardized difference values. This is particularly evident in the response factor standardized difference for radial diffusivity being 5 times as high for long as for short diffusion time, as compared to the axial diffusivity $D_{||}$, which shows comparable response factor standardized differences for the two diffusion times.

DISCUSSION

Diffusion contrast in skeletal muscle has been explored extensively, and models have been proposed relating DTI parameters to microstructural features (myofiber size, aspect ratio, sarcoplasmic reticulum) (3,24,25,35,36). Most studies employ diffusion times on the order of 40 ms and diffusion lengths of 15 μm . Since the typical fiber size of 50 μm significantly exceeds this probing lengthscale, contrast is suboptimal. Longer diffusion times can improve diffusion contrast for skeletal muscle quantitative imaging. Stimulated echo DTI has shown signal-to-noise ratio (SNR) benefits by adjusting transverse and longitudinal relaxation weighting (26) as well as structural quantification via time-dependent restricted diffusion in *ex vivo* muscle tissue (37). The present work uses long diffusion times for *in vivo* human skeletal muscle for higher microstructural contrast, in particular for DTI of the CECS pathology.

The regional variation in diffusivities from different muscle compartments is consistent with reports finding lower diffusivities in anterior (AT) than posterior (GM, GL) compartments (3,23,38). The anisotropy ($FA \sim 0.4\text{--}0.5$) observed in our long diffusion time (~ 1000 ms) protocol is larger than that reported from shorter diffusion times, due to more restricted diffusion. Globally, all diffusion parameters except FA show significant increase with exercise in all subjects. This response was also anisotropic, with transverse diffusivities ($D_{\perp}$, $D_{\perp 2}$, $D_{\perp 3}$) increasing more than longitudinal ($D_{||}$) diffusivities. Furthermore, the exercise response factors of these parameters are significantly higher in CECS patients. These trends mostly hold true at the individual muscle compartment level, though smaller sample size limits statistical power. The most affected compartments are the peripheral ones (AT, GM, GL), consistent with the activation pattern of the treadmill exercise.

The comparison of DTI results at short and long diffusion times in Table 4 and Figure 6 is also informative. The reduced diffusivities, particularly in the radial direction, at longer diffusion times is well known as a signature of restricted diffusion, with reductions comparable to *ex-vivo* muscle diffusion observations (37). This higher degree of restriction should provide higher sensitivity to structural modifications, consistent with the larger volunteer/patient group differentiation with standardized difference quantification.

The observed changes following exertion may have multiple contributions. Intra- and extracellular compartmentation contribute to multi-component T_2 relaxation (9,10). T_2 increases with exercise may involve fluid exchange to the extracellular space or changes in macromolecule hydration layers. Elevated temperature would increase the water diffusion rate isotropically, and *in vitro* experiments suggest it plays a larger role in diffusion than relaxation contrast (39). Several studies have shown diffusion metrics to be sensitive to exercise (40,41), cooling regimens (42,43), and training (44), affected in part by perfusion-induced temperature redistribution. However, in a hindered environment heating alone without a structural modification would not change (or if anything increase) apparent anisotropy, in contrast to our observations. Increased SNR (such as the 3% increase in average SNR for the whole subject group) from higher fluid content could increase apparent anisotropy at sufficiently low SNR; however, our dataset does not appear to lie in this

regime. Thus, some myofiber structural modification is likely, and may be exaggerated in CECS; the longer probing lengthscale of the present study was likely key to emphasizing this anisotropy. There is also substantial precedent for structural diffusion contrast mechanisms in skeletal muscle. Passive muscle shortening or stretching are known to affect diffusion anisotropically (23,45,46). Microscopic dilation would parallel the macroscopic engorgement of skeletal muscle compartments following exertion. Functional changes such as myofiber permeability may also be modified under the hypoxic conditions of CECS. Physiological suspicion and histological evidence (33) also exists for interfiber fluid accumulation or muscular edema in CECS. Differentiating between these mechanisms (dilation, permeability, edema) may require higher order acquisitions and modeling. Inversion recovery preparation to suppress free fluid may help clarify the role of edema. Further experiments and modeling of the muscle DTI metrics in CECS, particularly as a function of diffusion time (37) may shed further light on the CECS pathophysiology and its detection.

This study had a number of limitations. The total number of subjects (22) was small and the volunteer cohort was not number, gender, or age-matched. The SPAIR T₂-weighted sequence and the stimulated echo DTI sequence provide additional information but at some cost in scan time (5 minutes per sequence). The subject classification (VOL, NML, CECS), though supported by prior T₂-weighted imaging studies (15–17,19), was an approximate tiered labeling of a continuous spin relaxation contrast; parametric mapping of the quantitative T₂ relaxation time might provide a useful correlate to quantitative DTI metrics (9,39). In a related sense, our analysis used T₂w and DTI information in conjunction to understand the differences between volunteers and degrees of CECS-affected muscle compartments; future studies might explore the comparative utility of quantitative T₂ and quantitative DTI examinations taken individually. ROIs were drawn separately by different investigators on T₂w and DTI images. While the RF sensitivity profile determined by flexible coil array placement for the bilateral T₂w imaging was similar for pre- and post-exercise scans, no reference normalization was performed and thus T₂w response factor quantification may have been affected by variations in coil placement. Similarly, different knee coils and fat suppression methods were employed in the Tim Trio and Verio systems, which may have limited the accuracy of SNR quantification. While SNR was found to be sufficient in all cases, the 8-channel knee coil and Verio system showed 20% higher sensitivity than the 4-channel knee coil and Tim Trio system. The DTI acquisitions did not use cardiac gating, and thus could have been subject to pulsation-induced signal variation near leg vessels(26). However, we also note that the normalized standard deviation, reflecting physiological noise, is quantitatively consistent with studies (26) and is at most 10% larger at long compared to short diffusion times; thus we expect pulsation not to have played an outsized role in our results. Some variability existed in the timing of the exercise procedure, including (1) the running duration required to induce symptoms in patients, (2) the interval between exercise cessation and MRI scanning due to patient setup/compliance, (3) the interval from scan initiation to the time of the DTI scan, during which activation effects may have lessened. The need to evaluate both T₂ and DTI contrast soon after exertion necessitated two exertion periods for some subjects, in between which complete baseline status may not have been achieved. These sources of variability may have diluted our results. Finally, we only evaluated MRI biomarker changes after exertion, though measurement sooner after or even during exertion would also be informative (11,17). While these issues may have limited sensitivity, the general findings indicating the potential of DTI biomarkers of CECS pathophysiology remain clear.

In conclusion, we have performed T₂-weighted imaging and stimulated echo DTI at 3T in the leg muscles of healthy volunteers and suspected CECS patients before and after exercise. T₂w signal intensities increased following exercise, with >20% change defining CECS

affected compartments. Mean, transverse, and longitudinal diffusivities all increased significantly following exercise, with greater changes in transverse than longitudinal diffusion, particularly in patients affected by CECS. Further studies with advanced acquisition and modeling may help improve noninvasive diagnosis, monitoring, and treatment selection in the setting of CECS.

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Figure 1. Diagram of monopolar stimulated echo diffusion tensor imaging pulse sequence with echo-planar imaging readout. Spoiler gradients shown in black are applied only for the $b=0$ acquisition.

Figure 2.
Example region of interest (ROI) placement on axial unweighted (b0) DW image.

Figure 3. T2w-MRI and DTI in (a) a healthy volunteer and (b) a CECS patient right calf muscle before (pre) and after (post) treadmill exercise.

Figure 4.

Mean diffusivity box-plot distributions before and after exercise as a function of (a) subject group (VOL=volunteer, NML = normal appearing patient group, CECS = apparent CECS muscle group) and (b) muscle group location (abbreviations in text).

Figure 5. Exercise response factor distribution for mean diffusivity as a function of (a) subject group and (b) muscle compartment.

Figure 6. Standardized difference between volunteer and patient groups of exercise response factors for each DTI parameter at short or long diffusion times. * indicates significant ($p < 0.05$) differentiation of volunteer and patient groups.

Table 1

Water phantom stimulated echo DTI results

Mean values $\pm$ interslice standard deviation of DTI metrics are shown as a function of mixing time T_m without (Uncorr) and with (Corr) full b-matrix processing correction.

Mixing Time T_m (ms)	$1 (\mu m^2/ms)$		$2 (\mu m^2/ms)$		$3 (\mu m^2/ms)$		MD ($\mu m^2/ms$)		FA	
	Uncorr	Corr	Uncorr	Corr	Uncorr	Corr	Uncorr	Corr	Uncorr	Corr
30	2.108 $\pm$ 0.035	2.203 $\pm$ 0.047	1.973 $\pm$ 0.030	1.992 $\pm$ 0.038	1.898 $\pm$ 0.027	1.858 $\pm$ 0.029	1.993 $\pm$ 0.024	2.018 $\pm$ 0.024	0.054 $\pm$ 0.009	0.088 $\pm$ 0.014
500	1.838 $\pm$ 0.056	2.169 $\pm$ 0.070	1.590 $\pm$ 0.051	1.944 $\pm$ 0.061	1.470 $\pm$ 0.054	1.805 $\pm$ 0.075	1.633 $\pm$ 0.042	1.973 $\pm$ 0.051	0.116 $\pm$ 0.021	0.095 $\pm$ 0.025
1000	1.589 $\pm$ 0.068	2.162 $\pm$ 0.107	1.238 $\pm$ 0.071	1.910 $\pm$ 0.096	1.071 $\pm$ 0.079	1.718 $\pm$ 0.115	1.299 $\pm$ 0.053	1.930 $\pm$ 0.092	0.203 $\pm$ 0.045	0.118 $\pm$ 0.030

DTI indices by muscle and subject group

Table 2

Mean± standard deviation over subjects in each section of baseline DTI indices in healthy volunteers and suspected CECS patients as a function of muscle compartment and subject designation (VOL=volunteers; NML=patients with <20% T2w change; CECS=patients w/ >20% T2w change). Number of cases (N) found in each category is given at left. Diffusivities are given in $\mu\text{m}^2/\text{ms}$; FA is unitless.)

Compartment	N	Subject group	1	2	3	FA	MD
AT	8	VOL	1.859±0.055	0.892±0.061	0.658±0.064	0.512±0.033	1.136±0.052
	10	NML	1.828±0.100	0.851±0.042	0.623±0.045	0.526±0.044	1.101±0.028
	4	CECS	1.851±0.079	0.862±0.041	0.656±0.036	0.517±0.015	1.123±0.044
EDL	8	VOL	1.868±0.061	0.942±0.077	0.659±0.062	0.502±0.034	1.156±0.054
	14	NML	1.829±0.091	0.904±0.050	0.615±0.044	0.517±0.036	1.116±0.026
PT	8	VOL	1.947 ± 0.094	0.997±0.088	0.664±0.061	0.502±0.037	1.203±0.068
	13	NML	1.984 ± 0.131	0.989±0.124	0.626±0.069	0.528±0.051	1.199±0.077
	1	CECS	1.97	0.99	0.64	0.52	1.20
PL	8	VOL	1.903±0.085	1.040±0.082	0.785±0.074	0.443±0.044	1.243±0.063
	13	NML	1.889±0.077	0.929±0.086	0.658±0.072	0.510±0.039	1.159±0.064
	1	CECS	1.82	0.99	0.64	0.48	1.15
SOL	8	VOL	1.946±0.083	1.053±0.078	0.745±0.061	0.465±0.042	1.248±0.050
	11	NML	2.001±0.071	0.938±0.067	0.619±0.060	0.545±0.033	1.186±0.048
	3	CECS	1.952±0.052	0.952±0.054	0.659±0.107	0.518±0.053	1.188±0.037
GL	8	VOL	1.870±0.102	1.012±0.101	0.797±0.087	0.432±0.050	1.227±0.081
	8	NML	1.834±0.088	0.938±0.078	0.709±0.051	0.469±0.042	1.160±0.033
	6	CECS	1.809±0.061	1.076±0.097	0.785±0.096	0.409±0.063	1.223±0.060
GM	8	VOL	1.967±0.067	1.002±0.083	0.721±0.072	0.489±0.043	1.230±0.053
	10	NML	1.967±0.075	0.924±0.080	0.697±0.074	0.515±0.053	1.196±0.039
	4	CECS	1.884±0.076	0.925±0.103	0.732±0.085	0.483±0.067	1.181±0.057

Groups with significant response from baseline ($p<0.05$) are shown in boldface. Compartment abbreviations: AT = anterior tibialis, EDL = extensor digitorum longus, PT = posterior tibialis, PL = peroneus longus, SOL = soleus, GL = gastrocnemius lateralis, GM = gastrocnemius medialis.

Table 3
DTI parameter exercise response factors

For each parameter and subject group, p-values for statistical comparison with zero response (p vs. 1.0), with the volunteer (VOL) group (p vs. VOL), and between different eigenvalues (p. vs. λ_2 , etc.) are shown. Significant differences (p<0.05) are highlighted in boldface.

Parameter	All (N=154)	VOL (N=56)	NML (N=79)	CECS (N=19)
λ_1	1.051 $\pm$ 0.040	1.031 $\pm$ 0.054	1.058 $\pm$ 0.039	1.077 $\pm$ 0.046
p vs. 1.0	<0.0001	0.0009	<0.0001	0.0022
p vs. VOL	-	-	<0.0001	<0.0001
p vs. λ_2	<0.0001	0.0166	<0.0001	0.2293
p vs. λ_3	<0.0001	0.0653	<0.0001	0.0546
λ_2	1.077 $\pm$ 0.069	1.046 $\pm$ 0.050	1.094 $\pm$ 0.069	1.096 $\pm$ 0.086
p vs. 1.0	<0.0001	0.0141	<0.0001	0.0193
p vs. VOL	-	-	<0.0001	0.0201
p vs. λ_3	0.5604	0.9820	0.8615	0.3133
λ_3	1.081 $\pm$ 0.088	1.047 $\pm$ 0.067	1.096 $\pm$ 0.083	1.122 $\pm$ 0.123
p vs. 1.0	<0.0001	0.0283	<0.0001	0.0134
p vs. VOL	-	-	0.0002	0.0119
MD	1.063 $\pm$ 0.038	1.038 $\pm$ 0.034	1.074 $\pm$ 0.042	1.091 $\pm$ 0.070
p vs. 1.0	<0.0001	0.0070	<0.0001	0.0061
p vs. VOL	-	-	<0.0001	0.0017
FA	0.977 $\pm$ 0.029	0.987 $\pm$ 0.054	0.972 $\pm$ 0.057	0.966 $\pm$ 0.083
p vs. 1.0	0.0014	0.2344	0.0159	0.2191
p vs. VOL	-	-	0.1214	0.3084

Table 4
Standardized difference values for diffusion metrics and response factors at short and long diffusion times

The mean and standard deviation (SD) of the post-exercise level, pre-exercise level and response factor ratio of each measure within each subject group stratified by diffusion time. FA is unitless and diffusivities are given in $\mu\text{m}^2/\text{ms}$. The standardized difference (STD Diff) and p value are also given for the comparison of volunteers to patients in terms of the mean level of the response factor ratio, the pre-exercise value and the post-exercise value of each measure, stratified by diffusion time.

Measure		Short Diffusion Time						Long Diffusion Time					
		Patient			Volunteer			Patient			Volunteer		
		Mean	SD		Mean	SD		Mean	SD		Mean	SD	
FA	status												
	Post	0.329	0.051		0.301	0.046	3.84	0.507	0.043		0.471	0.047	4.18
	Pre	0.325	0.058		0.303	0.044	2.68	0.527	0.042		0.478	0.048	6.19
1	Ratio	1.019	0.079		0.997	0.082	1.34	0.962	0.042		0.987	0.054	2.67
	Post	2.283	0.111		2.282	0.104	0.08	2.020	0.081		1.967	0.097	3.40
	Pre	2.117	0.107		2.158	0.128	2.09	1.922	0.089		1.909	0.086	0.84
2	Ratio	1.079	0.032		1.059	0.038	2.89	1.052	0.039		1.031	0.032	3.04
	Post	1.537	0.092		1.592	0.096	3.53	0.988	0.084		1.035	0.088	2.85
	Pre	1.426	0.087		1.500	0.100	4.85	0.900	0.073		0.991	0.094	5.88
3	Ratio	1.078	0.036		1.062	0.031	2.35	1.098	0.062		1.046	0.050	4.44
	Post	1.175	0.099		1.251	0.095	4.57	0.714	0.080		0.750	0.084	2.55
	Pre	1.105	0.119		1.178	0.092	3.84	0.645	0.056		0.718	0.086	6.38
rad	Ratio	1.074	0.140		1.064	0.046	0.49	1.107	0.072		1.047	0.067	4.29
	Post	1.356	0.080		1.421	0.087	4.65	0.851	0.074		0.880	0.095	1.81
	Pre	1.265	0.080		1.339	0.088	5.36	0.773	0.056		0.855	0.085	6.57
MD	Ratio	1.073	0.056		1.062	0.033	1.09	1.102	0.063		1.031	0.078	5.00
	Post	1.665	0.052		1.708	0.068	3.88	1.241	0.058		1.251	0.067	0.93
	Pre	1.549	0.054		1.612	0.083	5.22	1.156	0.043		1.206	0.071	5.19
	Ratio	1.076	0.038		1.061	0.028	2.16	1.074	0.046		1.038	0.034	4.42
	Post												
	Pre												