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Multi-parametric MRI Characterization of Healthy Human Thigh Muscles at 3.0T - Relaxation, Magnetization Transfer, Fat/Water, and Diffusion Tensor Imaging

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Abstract

Muscle diseases commonly have clinical presentations of inflammation, fat infiltration, fibrosis, and atrophy. However, the results of existing laboratory tests and clinical presentations are not well correlated. Advanced quantitative MRI techniques may allow the assessment of myopathological changes in a sensitive and objective manner. To progress towards this goal, an array of quantitative MRI protocols was implemented for human thigh muscles, their reproducibility was assessed, and the statistical relationships among parameters were determined. These quantitative methods included fat/water imaging, multiple spin-echo T₂ imaging (with and without fat signal suppression, FS), selective inversion recovery for T₁ and quantitative magnetization transfer (qMT) imaging (with and without FS), and diffusion tensor imaging. Data were acquired at 3.0 T from nine healthy subjects. To assess the repeatability of each method, the subjects were re-imaged on an average of 35 days later. Pre-testing lifestyle restrictions were applied to standardize physiological conditions across scans. Strong between-day intra-class correlations were observed in all quantitative indices except for the macromolecular-to-free water pool size ratio (PSR) with FS, a metric derived from qMT data. Two-way analysis of variance revealed no significant between-day differences in the mean values for any parameter estimate. The repeatability was further assessed with Bland-Altman plots, and low repeatability coefficients were obtained for all parameters. Among-muscle differences in the quantitative MRI indices and

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inter-class correlations among the parameters were identified. There were inverse relationships between fractional anisotropy (FA) and the 2nd eigenvalue, the 3rd eigenvalue, and the standard deviation of first eigenvector. The FA was positively related to the PSR, while the other diffusion indices were inversely related to the PSR. These findings support the use of these T₁, T₂, fat/water, and DTI protocols for characterizing skeletal muscle using MRI. Moreover, the data support the existence of a common biophysical mechanism, water content, as a source of variation in these parameters.

Keywords

Reproducibility; reliability; skeletal muscle; relaxometry; quantitative magnetization transfer; diffusion tensor imaging; three-point Dixon imaging

Introduction

Muscle diseases are commonly characterized by inflammation, fat infiltration, fibrosis, and atrophy. Magnetic resonance imaging (MRI) has emerged as a powerful technique for non-invasively characterizing these pathophysiological changes. Contrast-based imaging methods, including longitudinal (T₁) and transverse (T₂) relaxation time constant-weighted sequences (1), short-tau-inversion-recovery imaging (2,3), magnetization transfer (MT) contrast imaging (4), and diffusion-weighted imaging (5), have all been used to identify muscle abnormalities. Although the contrast patterns in these images are sensitive to pathology, they are also sensitive to differences in hardware and image acquisition parameters, such as repetition time (TR) and echo time (TE); radiofrequency (RF) irradiation power and frequency offset (for MT imaging); and diffusion weighting (*b*-values (for diffusion imaging)). Consequently, the specificity and sensitivity of these methods to pathological changes are reduced.

The development of quantitative MRI methods, such as T₁ and T₂, MT, diffusion, and fat/water content measurements, may characterize tissue properties more objectively. T₁ and T₂ measurements have been widely used to study muscle diseases and injury (6-11). It is typically found that T₁ values are elevated in inflamed regions of muscle and decreased in fat-infiltrated regions (6-8), whereas T₂ values are elevated in both inflamed and fat-infiltrated regions (12). QMT has been developed and applied in healthy muscles (13,14), and a recent preliminary report appeared describing its implementation in muscle disease (15). Another quantitative MRI approach, diffusion tensor (DT) MRI, has been widely applied to assay the anisotropic diffusion behavior of water molecules in tissues with ordered, elongated structures. The indices derived from the diffusion tensor provide information about tissue structure and orientation at a microscopic level. The diffusion properties of muscle, especially the third eigenvalue of the diffusion tensor and degree of diffusion anisotropy, reflect muscle damage due to experimental injury (16), strain injury (17), and disease (5,18). Lastly, macroscopic fat infiltration is a common pathological component of muscle diseases. Fat and water signals can be most readily separated and quantified by exploiting the different resonance frequencies of fat and water protons (19-21).

These quantitative techniques therefore offer great potential for characterizing muscle damage by disease or injury at the microscopic and macroscopic levels at a greater specificity and sensitivity than qualitative, contrast-based methods. Because muscle diseases exhibit heterogeneous pathology, with respect to both the spatial presentation of the disease and the presence or absence of individual pathological components, a multi-parametric MRI approach may be required. Multi-parametric MRI may improve the ability to resolve individual pathological components; improve the understanding of the biophysical basis for the variations in single MRI parameters; and allow better data interpretation by accounting for potential confounding variables.

It is particularly interesting to implement quantitative MRI methods specifically for thigh muscle imaging, because the proximal muscles are early sites of muscle involvement in many muscle disorders. However, quantitative MRI studies in muscles are challenged by short T_2 values and static (B_0) and RF (B_1^+) field inhomogeneities. For these methods to be appropriately applied in longitudinal studies of therapeutic response and disease progression, it is important to develop means for overcoming these issues, measure the normal values for different muscles, establish the repeatability of the fitted parameters, and understand how the parameters relate to each other under conditions of healthy muscle. We have implemented a multiple spin-echo sequence for T_2 measurement, a selective inversion recovery (SIR) sequence for T_1 and qMT imaging (22), a three-point Dixon imaging sequence for fat/water imaging (21), and a DT-MRI sequence for *in vivo* healthy human thigh muscles. Using these sequences, we determined quantitative MRI indices at 3.0 T, compared the indices among the major thigh muscles, characterized and interpreted the relationships among the parameters, and assessed the repeatability of these methods.

Materials and Methods

Subjects

The study was approved by the local Institutional Review Board. Nine subjects (five female) with no known history of cardiovascular, metabolic, neurological, orthopedic, and muscle disorders participated in this study. None of the subjects engaged in a regular exercise program, as defined in Ref. (23). Their average age (mean and standard deviation, SD) was 26 ± 6 yr, with height 173 ± 13 cm and mass 69 ± 20 kg (BMI 22.6 ± 3.7). Written informed consent was obtained from each subject. To test the repeatability, the subjects were imaged on two occasions (on average, 35 days apart). Before each scan, the subjects adhered to 24-hour restrictions against moderate and heavy exercise, alcohol and drug use, and over-the-counter medications. They also refrained from caffeine use for at least 6 hours before each MRI scan. The subjects were instructed not to perform moderate or heavy exercise between the two MRI scans. To ensure consistent subject position in the two scans, the positioning details were recorded at the first scan and repeated in the second scan.

MRI Data Acquisition

MRI data were obtained on a 3.0-T Philips Achieva MR imager/spectrometer (Philips Medical System, Best, The Netherlands). A two-channel body coil was used for signal excitation and a six-channel sensitivity encoding (SENSE) cardiac coil array was used for

signal reception. All images had a field of view (FOV) of $256 \times 256 \text{ mm}^2$ and slice thickness of 7 mm. Four bovine serum albumin (BSA) (Sigma-Aldrich, St Louis, MO, USA) samples with weight percentages of 5%, 10%, 15%, and 20% served as standard references. The samples were fully cross-linked with 1% glutaraldehyde (Sigma-Aldrich). The samples were attached to the lateral side of the imaged thigh while imaging, as illustrated in Figure 1.

Anatomical Images—High-resolution anatomical images were acquired using a T_1 -weighted sequence with a turbo-spin-echo (TSE) readout, with sequence parameters $TR/TE = 530/6.2 \text{ ms}$, matrix size = 340×340 with 512×512 reconstruction, excitation flip angle (α) = 90° , refocusing pulse flip angle = 110° , SENSE factor = 1.4, TSE factor = 6, 11 slices, and number of excitations (N_{EX}) = 1. The acquisition time was ~ 2 minutes.

B_1^+ mapping— B_1^+ field maps were acquired using an actual flip angle method (24), with $\alpha = 60^\circ$, delays of $TR_1/TR_2 = 30/130 \text{ ms}$, $TE = 2.2 \text{ ms}$, 11 slices, and matrix size of 64×64 with 128×128 reconstruction. The acquisition time was ~ 1 minute.

Fat/water Imaging—The fat/water imaging data were acquired with a 3D three-echo gradient-echo sequence with $TR = 75 \text{ ms}$, $TE = 1.34 \text{ ms}$, echo spacing (ΔTE) = 1.53 ms , matrix = 128×128 with 256×256 reconstruction, SENSE factor = 1.3, $\alpha = 22^\circ$, 11 slices, and $N_{EX} = 1$. The acquisition time was ~ 9 seconds.

Multiple spin-echo T_2 imaging—Multiple spin-echo data were acquired with a single-slice sequence and $90(x)$ - $180(y)$ - $90(x)$ composite refocusing pulses (25). The imaging parameters were $TR = 3000 \text{ ms}$, $TE = 10, 20, 30 \dots 320 \text{ ms}$, $\Delta TE = 10 \text{ ms}$, matrix = 128×128 , SENSE factor = 1.3, and $N_{EX} = 2$. A crusher gradient scheme proposed by Poon and Henkelman (26) was used to destroy stimulated echoes from imperfect refocusing pulses. Multi-echo data with fat suppression (FS) were acquired using a spectrally selective adiabatic inversion recovery pulse, applied to the $\sim 3.5 \text{ ppm}$ lipid resonance with an inversion recovery time (TI) of 202 ms, and an additional saturation pulse (90° , 18ms, 100Hz offset, Gaussian shape, bandwidth = 45 Hz) on the olefinic proton resonance ($\sim 0.75 \text{ ppm}$). The other instrumental settings (receiver gain, transmitter attenuation, etc.) and sequence parameters (timing, geometry, etc.) were identical to the non-FS image. The acquisition time for each scan was ~ 11 minutes.

SIR for T_1 and qMT imaging—The SIR data were acquired with a 1-ms 180° block inversion pulse, TSE readout with 16 inversion recovery time (TI) values (15 logarithmically spaced between 10 ms and 2 s and one at 10 s) (27), pre-delay (TD) = 2.5 s, matrix 128×128 , SENSE factor = 2, TSE factor = 12, $\Delta TE = 5.9 \text{ ms}$ (and thus effective $TE = 38 \text{ ms}$), 1 slice, and $N_{EX} = 2$. Twenty-four additional refocusing pulses were added to the end of the TSE pulse train to ensure full saturation of both pools (22). SIR-qMT data with FS using the gradient-reversal off-resonance suppression technique (28) were acquired with identical instrumental, contrast, and geometric settings. The acquisition time for each scan was ~ 7 minutes.

DT-MR imaging—The DT-MRI data were acquired with $TR/TE = 4000/48 \text{ ms}$, matrix = 128×64 , with 128×128 reconstruction, SENSE factor = 1.5, 11 slices, b -value = 450

$\text{s}\cdot\text{mm}^{-2}$ in 15 diffusion-weighted directions (and one $b = 0$ image), single-shot spin-echo echo-planar-imaging (EPI) readout, and $N_{\text{EX}} = 6$. Fat signal suppression used, in part, a gradient-reversal off-resonance suppression technique that suppressed signals from spins >138 Hz off-resonance from the water proton peak. Also, we used a saturation pulse on the olefinic proton resonance as described above. To reduce TE and geometric distortion during the EPI readout, partial k-space sampling along the phase encoding direction was adopted with k-space coverage of 80%. The acquisition time was approximately 7 minutes.

The total acquisition time of this protocol was ~ 75 minutes. This included ~ 46 minutes for quantitative imaging, plus additional time for subject positioning, scout images, slice prescription, and preparation steps for each sequence.

MRI Data Analysis

Parameter Fitting—All analyses were performed in Matlab 2011a (The MathWorks Inc, Natick MA) using scripts written in-house. All data were processed on a pixel-wise basis and the quantitative indices were determined by calculating the mean and SD within each specified region-of-interest (ROI).

Fat/water imaging data: The three-point Dixon imaging data were processed with the algorithm described in (21), assuming a nine-peak model with fixed ratios among the peaks (29). The analytical solutions of water and fat signals were obtained for each voxel. Then the true solution was identified by a multiseed three-dimensional region-growing scheme with a dynamic path. Confident regions were solved before unconfident regions. With TE of 1.53 ms, phase unwrapping was not required. T_1 -weighting effects were corrected by assuming T_1 values of 1.4 s and 0.3s for water and fat, respectively. The calculated fat fractions (f_{fat}) are presented.

Multiple spin-echo data: Preliminary analyses using non-negative least squares methods (12) did not reveal multi-exponential transverse relaxation behavior under these experimental conditions. Therefore, the data were fitted to a mono-exponential decay model with a noise baseline to determine T_2 and $T_2(\text{FS})$.

SIR data: To determine T_1 and $T_1(\text{FS})$, the SIR data were fitted to a mono-exponential model with reduced TD (30). To determine qMT parameters, the SIR data were fitted to a bi-exponential model, as described in (30). Five parameters were determined: the longitudinal relaxation rate of the free water pool (R_{1f}), PSR, fast exchange rate (k_{mf}), inversion ratio of the free water pool (S_f), and the equilibrium magnetization (M_0), where “f” and “m” denote the free water and macromolecular proton pools, respectively. SIR data with FS were processed with the same model. Only R_1 , $R_1(\text{FS})$, PSR and PSR(FS) are presented and discussed in detail; k_{mf} and $k_{mf}(\text{FS})$ are not presented due to the high SNR required for accurate parameter characterization and the insensitivity of this parameter to pathophysiological changes (31).

DT-MRI data: All diffusion-weighted images were first registered to the $b = 0$ image using an affine transformation algorithm written by local image analysis core. Then the data were fitted to a tensor model (32) using multivariate linear regression. The diffusion tensor was

diagonalized to determine the eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and corresponding eigenvectors. The mean diffusivity (ADC) and FA were then calculated (32). Of the diffusion indices, λ_3 , ADC, and FA are of greatest interest because of their correlation to muscle damage (16); thus λ_1 and λ_2 are not discussed in detail. To characterize the normal variability in the estimated fiber orientation within each muscle, the standard deviation of the first eigenvectors were calculated. Firstly, the mean signal within each ROI was fitted to the tensor model and the first eigenvector was used as a reference vector. Then for each voxel, the angle between its first eigenvector and the reference vector was calculated. Finally, the standard deviation of all angle values within the ROI was determined, noted as $SD(v_1)$.

ROI definition

Eight muscles were studied, including the quadriceps (rectus femoris (RF), vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM)), hamstrings (biceps femoris (BF), semitendinosus (ST), semimembranosus (SM)), and adductor magnus/longus (AD). The sartorius, gracilis, and adductor brevis muscles were also identified in the images, but were not analyzed because of their relatively smaller dimensions and/or their location outside of the region of highest image quality. ROIs were initially drawn on the anatomical images. The ROIs were then applied to the quantitative model parameter maps. As necessary, the alignment of ROIs was adjusted to avoid muscle boundaries. Each ROI was visually examined and pixels were removed to avoid partial volume artifacts, non-contractile tissue, and flow artifacts.

Statistical Analyses

Repeatability and reliability analyses—Repeatability was assessed using the intra-class correlation coefficient (ICC); Bland-Altman analysis including determination of the repeatability coefficient (RC) (33); and comparison of between-day mean values (see the statistical comparisons below). An ICC of 1.0 indicates a perfect correlation between the two measurements. The RC was defined as $RC = 1.96 \cdot SD_{diff}$, where SD_{diff} is the standard deviation of the differences between the two scans, and represents the maximum difference between scans that is likely due to measurement error. Therefore, a low RC is desirable.

Statistical comparisons—Statistical comparisons were made for each variable using a two-factor analysis of variance (ANOVA), with main effects for Muscle and Day and considering $p < 0.05$ to be statistically significant. When significant main effects were observed, multiple comparisons were made with Tukey's honestly significant difference (HSD) test.

Relationships among quantitative MRI parameters—For each MRI parameter, the mean value for scans 1 and 2 was calculated. Then, the strengths of the relationships among selected parameters were determined by calculating the Pearson correlation. For this analysis, relaxation behavior was expressed as relaxation rate constants ($R_N \equiv 1/T_N$) rather than time constants, in order to characterize more appropriately the expected linear behavior of certain parameters with relaxation rate constants, but not with time constants.

Results

Supplementary Table 1 lists the relaxation, qMT, and diffusion parameters for the BSA samples obtained during a phantom-only experiment. As shown in Supplementary Table 2, R_{1f} , R_2 , and PSR were strongly positively correlated with weight percentage and each other (minimum r^2 , 0.899), while the ADC was strongly negatively correlated with weight percentage and the other parameters (minimum r^2 , 0.947). No significant between-scan differences were observed in mean values for these indices (details not shown).

Representative T_1 -weighted images from two scans of a female subject are shown in Figure 1a and b. No noticeable between-day differences in the positioning of any subject occurred. The mean, SD, RC, and ICC of the quantitative indices of the eight muscles analyzed in this work are summarized in Table 1 and Table 2.

Fat/water Imaging Data

Example data and repeatability characteristics—After completing scans of five subjects, two changes were made to the fat/water imaging and analysis protocol to achieve consistent quality. The changes included (a) use of monopolar readout of the multiple gradient echoes and (b) preservation of the original image domain phase information without any undesired post-processing by the MR scanner's reconstruction software. The final four subjects were scanned with the finalized fat/water imaging protocol, and fat/water analysis results are presented for only those four subjects. Figure 2a shows a representative f_{fat} map. The ICC for f_{fat} was 0.814; the Bland-Altman plot is shown in Figure 2b. The descriptive statistics and RC values for each muscle are listed in Tables 1 and 2. The mean values did not differ between days ($p = 0.24$).

Among-muscle differences—There was a significant Muscle main effect ($p < 0.001$), but the Muscle $\times$ Day interaction was not significant ($p = 0.96$). There were significantly different f_{fat} values among several of the muscle pairs; details are given in Table 1.

Multiple Spin-echo T_2 Data

Example data and repeatability characteristics—Example multi-echo data fitting curves of a pixel in the VL muscle are shown in Figure 3a. Figure 3b shows a representative B_1^+ map from the same subject, with areas of low B_1^+ noted in the lateral region of the VL ($\sim 75\%$ of the nominal flip angle) and SM, AD, and gracilis (as low as $\sim 65\%$ of the nominal flip angle). Figure 3c shows a representative T_2 map from this subject. The T_2 (FS) maps (not shown) were spatially more uniform than the T_2 maps. Bland-Altman plots are shown in Figure 3d; the ICC's for T_2 and T_2 (FS) were ICC = 0.860 and 0.854, respectively. The descriptive statistics and RC values for each muscle are listed in Table 1. Neither the T_2 nor the T_2 (FS) Day main effects were significant ($p = 0.14$ and 0.34 , respectively).

Among-muscle differences—Table 1 presents the descriptive statistics for T_2 and T_2 (FS). For T_2 and T_2 (FS), there were significant Muscle main effects, with Tukey's HSD results also given in Table 1. For neither T_2 nor T_2 (FS) was the Muscle $\times$ Day interaction significant ($p = 1.00$).

SIR T_1 and qMT data

Example data and repeatability characteristics—As revealed by position shifts of the thigh or ringing artifacts, the SIR data in four subjects were severely confounded by motion. The following discussion pertains only to those five datasets in which such artifacts were not observed. Example SIR data-fitting curves of a pixel in the VL muscle are shown in Figure 4a. Figure 4b shows a representative T_1 map; Figure 4c shows an example PSR map. The corresponding FS data had similar patterns and are not shown. The Bland-Altman plots for T_1 and T_1 (FS) values are shown in Figure 4d. The corresponding data for PSR and PSR(FS) are shown in Figure 4e. The descriptive statistics and RC values for these data are listed in Table 1. The main effects for Day were not significant for T_1 , T_1 (FS), PSR, and PSR(FS) (minimum p value = 0.157).

Among-muscle differences—For T_1 , there was a significant main effect for Muscle ($p = 0.005$; see Table 1 for Tukey's HSD results); however, for T_1 (FS), the main effect for Muscle was not significant ($p = 0.605$). For PSR and PSR(FS), there were significant main effects for Muscle ($p < 0.001$ in both cases). None of the Muscle $\times$ Day interactions were significant (minimum p-value, 0.970).

DT-MRI Data

Example data and repeatability characteristics—Figures 5a, 5b, and 5c show representative λ_3 , ADC, and FA maps; the corresponding Bland-Altman plots are shown in Figures 5d, 5e, and 5f. Regarding the repeatability, there was no significant main effect for Day for λ_3 ($p = 0.89$, ICC = 0.786), ADC ($p = 0.61$, ICC = 0.870), and FA ($p = 0.23$, ICC = 0.866). The RC values are given in Table 2. The $SD(\mathbf{v}_1)$ values of individual muscles and their RC values are listed in Table 2.

Among-muscle differences—For λ_3 , ADC, and FA, there was a significant main effect for Muscle ($p < 0.001$). The *post hoc* test results for the Muscle main effects are listed in Table 2. The Muscle $\times$ Day interactions were not significant for any of these variables ($p = 0.98$). In general, lower mean values of λ_3 and ADC, and higher mean values for FA, were observed in the hamstring muscles than in other muscles.

Relationships among Parameters

Table 3 shows the correlation coefficients among all measured parameters. Predictably, there were significant correlations among most of the relaxation parameters and between the PSR and relaxation rates. In addition, there were correlations among several of the indices of the diffusion tensor. While FA and λ_1 were not significantly related (Figure 6a), there were inverse relationships between FA and λ_2 (Figure 6b); λ_3 (Figure 6c); and $SD(\mathbf{v}_1)$ (Figure 6d). All of these indices were inversely related to the PSR (Figure 7). In general, the diffusion coefficients were inversely related to the relaxation rates (illustrative examples in Figure 8).

Discussion

We have implemented a quantitative, multi-parametric MRI protocol for thigh muscle imaging at 3.0 T. Quantitative indices, including T_2 , $T_2(\text{FS})$, T_1 , $T_1(\text{FS})$, PSR, PSR(FS), f_{fat} , λ_{1-3} , ADC, and FA, were determined in eight thigh muscles. The analyses indicate that for most of these indices, a high repeatability exists. The data also reveal differences in some of the quantitative indices among thigh muscles, as well as the relationships among these indices. These inter-muscle differences can be used to suggest that a common biophysical mechanism, water content, causes these parameters to vary among healthy skeletal muscles. We note that these conclusions are drawn from a sample that was healthy and relatively restricted in age and muscle fat content, and so merit further examination in other populations.

Reproducibility and Inter-muscle Variability of the Parameters

Fat/Water Imaging—Considerable evidence supports using f_{fat} as a biomarker. Fat replacement of necrotic muscle fibers is a typical pathological component of muscle diseases. There is a higher correlation between lower extremity functional scores and the proportion of fat within various thigh muscles than between functional scores and manual or dynamometric measures of lower extremity strength (34). Close correlations have been reported between MRI-observed fibro-adipose replacement and histologic abnormalities (35) and between fibro-adipose tissue replacement and performance on functional measures (36,37). Muscle fat content predicts the loss of ambulation in patients with Duchenne muscular dystrophy (38).

Because this aspect of the disease process is often framed in terms of fat replacement, rather than muscle loss, we presented the fat/water imaging data as fat fractions. The f_{fat} data reported here ranged between 2-6% (Figure 2 and Table 1). Despite this low dynamic range of values, the f_{fat} data had high ICC and low absolute RC values. Because the mean f_{fat} values were low, the relative RC values were quite high (up to ~76%). But when expressed relative to the water signal fractions, the relative RC values were ~2%, which are similar to values previously reported for muscle (39) and subcutaneous adipose tissue (40). Because complete muscle replacement by fat can occur, the dynamic range of values for f_{fat} that can be encountered is nearly 20-fold. Collectively, these data indicate that the fat/water imaging protocol implemented here can repeatably measure f_{fat} in this range of values. The reported increases in the proton T_1 of the methylene peak with increasing fat fractions (41) and the existence of B_1 inhomogeneity indicate that under conditions of high fat content, a longer TR and/or incorporating a flip angle map and measured T_1 values into the data analysis may reduce T_1 bias.

There were few inter-muscle variations in f_{fat} , with just the VL and hamstring muscles having higher fat contents than other muscles. The limited number of inter-muscle variations in this parameter, and the spatial pattern of the variations that did exist, can be used to inform the interpretation of the inter-muscle variations in the other parameters.

T_2 Measurements— T_2 is frequently used for MRI characterization of muscle function and disease. The T_2 values determined in this work generally agree well with reported

values for 3.0T (42,43) but are lower than others (44). For T_2 and $T_2(\text{FS})$, the RC values ranged from 2.7-5.2% of the corresponding mean values (Table 1) and high ICC values were observed. The high reproducibility observed is consistent with other reports (45). From these data, and considering the 50-100% elevation of T_2 in muscle diseases (6) and the up to $\sim 30\%$ increase in T_2 that occurs during and following exercise (46), we conclude that T_2 can serve as a biomarker with high sensitivity to pathophysiological changes and excellent measurement properties.

However, close attention should be paid to B_1^+ and B_0 inhomogeneities when there is a significant fat component. The bandwidth of the composite pulse used in this work was not wide enough to refocus completely the fat component (which has a ~ 450 Hz chemical shift from water at 3.0T); this is seen in the subcutaneous fat region of Figure 3. The B_1^+ field varied between 130% and 70% (Figure 3b), and B_0 varied by ± 20 Hz (not shown). Collectively, these data indicate that the composite pulse used here is robust to B_1^+ field inhomogeneities; but, as illustrated by the absence of smoothness to the non-FS signal decay data in Figure 3a, the data are susceptible to the additional fat component. Therefore, for fat-infiltrated muscles, the presence of the increased fat component may require that a strategy such as a broader-bandwidth refocusing pulse, RF shimming using multiple transmit coils, dielectric padding, or an advanced data analysis model (such as the extended phase graph algorithm (47,48)) be used.

T_1 and qMT Imaging Using SIR—Because the T_1 of inflamed muscle is elevated and the T_1 of fat is as much as 3-fold lower than healthy muscle, T_1 measurements or T_1 -based contrast can distinguish among healthy, inflamed, and fat-infiltrated muscles. Across the eight thigh muscles studied, T_1 ranged from 1.33 and 1.37 s; these values are slightly lower than (43) or similar to (44) previously reported values. With fat suppression, $T_1(\text{FS})$ values were slightly longer than T_1 values. Both the T_1 and $T_1(\text{FS})$ data had high ICC's (Figure 4) and RC values much smaller than the expected differences due to disease (Table 1). Note that in this work, the TR was reduced with a short TD, as presented in (22,30). Alternatively, if only T_1 is of interest, a smaller number of TI values and varied TD may be used (30). There were only a small number of inter-muscle differences in T_1 , which were eliminated by using FS.

The PSR values across all muscles generally agree with previously reported values obtained using a pulsed saturation method (14). For the full cohort of subjects, a relatively poor repeatability was obtained in PSR and PSR(FS) values. However, when the data displaying significant motion artifacts were eliminated, the repeatability substantially improved. This improvement, along with the high quality of the phantom data, suggests that motion artifacts were a major complication for this method. The normal motion sensitivity of MRI data was probably exacerbated in this case by using a multi-shot TSE readout and large number of separate inversion time images. An alternative approach may be to replace the TSE readout with a turbo-field-echo (TFE) readout (49), which is also feasible for 3D imaging. Unexpectedly, the repeatability of PSR(FS) did not improve when the motion-corrupted data were excluded. Although a definitive explanation for these data is lacking, it may be that the subject's motion altered the B_0 distribution, reducing the efficacy of the fat suppression routines.

Other potential errors include SNR limitations, the effect of the (albeit small) fat component, and B_1^+ inhomogeneities. The effective TE of 38 ms is longer than the T_2 of muscle. To increase signal, the linear k-space sampling order used here could, in principle, have been replaced by a centric or asymmetric ordering; but we found these orderings of the k-space data introduced unacceptable blurring artifacts. Another potential error is from the fat component in the muscles. Previous work indicated that for PSR values present in muscle, a fat component greater than ~8-10% introduces bias and variability in qMT parameter estimations. These effects were likely very small in the present study of healthy, young people with low f_{fat} values; but this could become problematic in patient studies. Regarding B_1^+ inhomogeneity, the parameter S_f accounts for inversion flip angles of $<180^\circ$; and for the B_1^+ inhomogeneity levels we observed, we did not see systematic PSR variations. However, in the pilot studies for this project, we did observe PSR variations in areas where the B_1^+ fell to ~50% of the nominal value. A similar dependence of the MTR on B_1 was reported by Morrow *et al.* (44).

The PSR values varied among muscles, with the SM and ST muscles tending to have higher values. A possible explanation for these findings is that the hamstring muscles tend to have high Type I fiber contents (50). Compared to Type II fibers, Type I fibers are smaller in diameter and have higher relative proportions of hydroxyproline (51), an amino acid that almost uniquely exists in collagen. Either or both of these properties could increase the proportion of spins in the solid-like proton pool. For example, the smaller fiber diameter would lead to a greater spatial frequency of membranes and therefore of the exchangeable protons in the head groups of the membrane lipids. Also, because hydroxyl groups are an effective conduit for spin exchange (52), the higher content of hydroxyproline in predominantly Type I muscles could also increase the PSR. Data from Sobol *et al.* (53) suggest that lipid-water exchange is a relatively unimportant mechanism in the relaxation behavior of muscle water; thus the hydroxyproline explanation is favored. We caution that this statement is speculative.

DT-MRI data—In muscle diseases and injuries, the diffusion tensor eigenvalues can increase by up to 20% (5,16,17,54,55). In addition, DT-MRI muscle fiber tracking has been used for the quantitative assessment of muscle architecture (56-59). The SNR of the $b = 0$ images was ~60, which is high enough to achieve confident estimates of DT-MRI indices (60,61). The ADC was linearly related to BSA percentage (Table S-2). As shown by low RC and high ICC values (Table 2), there was excellent repeatability for the DT-MRI data. The relative RC's were higher for FA, the most likely explanation for which is error propagation through the use of the λ_1 , λ_2 , λ_3 , and ADC estimates in the calculation of FA. These data reflect improvements over our previous study of a single muscle group (62), and are comparable to those of Froehling *et al.* (63) and Sinha (64). Collectively, these data suggest that the DT-MRI eigenvalues and ADC have sufficient repeatability to detect the variations that are expected due to damage or disease.

We also observed a large number of among-muscle differences in diffusion parameters, especially λ_2 , λ_3 , and FA (Figure 5 and Table 2). For several reasons, we argue that these differences are not the result of image artifacts or noise. First, they are consistent with previous reports in the literature: Budzik *et al.* (65) and Kermarrec *et al.* (66) also observed

higher FA values in the hamstring muscle group than in other thigh muscles. Second, the among-muscle differences that we observed in λ_1 , λ_2 , λ_3 , and FA were not spatially correlated to the B_1^+ inhomogeneities or spatial patterns in f_{fat} , T_1 , and T_2 . Finally, bias in DT-MRI parameters can be caused by spatially varying noise levels. If so, then one would anticipate a positive correlation between $\text{SD}(\mathbf{v}_1)$ and FA (which both tend to increase with noise; (60,61)); however, a negative correlation was observed (Table 3). Thus, we consider it to be most likely that the DT-MRI parameter variations are related to some aspect of these muscle groups' microstructure. Recent work by Scheel *et al.* suggests that higher FA and lower λ_2 and λ_3 values result from a smaller fiber diameter (67), as would be expected in the more oxidative hamstring muscles (50,68). Although the collagen content also varies between Type I and Type II fibers, we consider that fiber diameter variations are the more likely explanation for this effect. This is because the collagen of the extracellular matrix is more randomly than longitudinally oriented at resting muscle lengths (69). As noted by Karampinos *et al.* (70), it would thus not contribute strongly to diffusion anisotropy.

Inter-class Correlations

We observed a large number of statistically significant correlations among the quantitative parameters. These correlations existed despite the very low dynamic ranges of values that we encountered for most of the variables, attesting to the data quality. The range of f_{fat} values was well below that expected to influence diffusion measurements (71); thus, we have not reported these correlations. Also, we do not discuss the correlations among the relaxation rates further, because they either constitute inherent intra-class correlations (for instance, between R_{1f} and $R_{1\text{obs}}$) or are predictable from proton relaxation theory (such as those between R_1 and R_2).

Correlations were noted among several of the diffusion parameters, including FA, λ_2 , λ_3 , and $\text{SD}(\mathbf{v}_1)$. Although the diffusion parameters are estimated from the same set of measurements and the same analysis procedure, they are not necessarily related to each other, as revealed by the absence of correlation between FA and λ_1 . Because of this and the arguments made already, we conclude that these relationships did not result from image artifacts or noise. The data in Table 3 and Figure 6 indicate that in healthy skeletal muscle, the variations in FA result from variations in λ_2 and λ_3 only. Also, we note that FA and $\text{SD}(\mathbf{v}_1)$ were inversely related. While data from pathological cardiac muscle suggest that the relationship between FA and $\text{SD}(\mathbf{v}_1)$ could have arisen from within-voxel variations in muscle fiber orientation (72), it is unlikely that such disarray would exist in healthy subjects who abstained from severe exercise prior to the study. The only remaining possibility of which we are aware is that there may have been inter-muscle variations in free water content, which would increase the randomness of the first eigenvector (increasing $\text{SD}(\mathbf{v}_1)$) and hence the isotropic quality of the diffusion (decreasing FA). Variations in free water content would also directly affect the magnitude of the diffusion coefficients. The data in Figures 6 and 7 are consistent with this prediction.

These data, along with the correlations among relaxation, diffusion, and qMT parameters, suggest the influence of a shared biophysical mechanism for the variations in all of these parameters. Modest correlations were observed between PSR and λ_2 , λ_3 , FA, and $\text{SD}(\mathbf{v}_1)$.

These relationships are similar to those from Ou *et al.* in rat models of multiple sclerosis (73,74) and Bryant *et al.* in models of muscle inflammation (75). The relationship between the diffusion properties and PSR may be caused by a single property, such as water content. The data from the BSA phantom studies show that variations in protein content affect PSR and the water diffusivities in a similar fashion to the *in vivo* relationships. Or, it may be that two separate properties affect the diffusion properties and PSR through separate mechanisms, but happen to co-vary in a way that causes λ_2 , λ_3 , FA, and $SD(\mathbf{v}_1)$ to be correlated to PSR. Similar arguments could be made about the inverse correlations between the relaxation rates and the diffusion coefficients. These experiments were not intended to identify the mechanism for these relationships. However, all of the relationships discussed here are consistent with the existence of a common mechanism in which a greater proportion of free water reduces PSR, reduces R_1 and R_2 , increases the diffusion coefficient in all directions, and increases the isotropic character of water diffusion.

Because of the importance of free water and fat content on these parameters, and given the high frequency of inflammation and fat replacement of muscle in diseased muscles, we consider it likely that the relationships among these parameters will vary in diseased muscle. Such variations might occur in the strength, slope, and/or intercept of the relationships. Examining these relationships might therefore provide a useful basis for characterizing individual components of muscle pathology more specifically.

Summary and Conclusions

In conclusion, a number of quantitative MRI methods, including relaxation (T_1 and T_2), qMT (PSR), fat/water content, and DT-MRI (λ_{1-3} , FA, ADC, and $SD(\mathbf{v}_1)$) have been determined in healthy human thigh muscles. A good repeatability was obtained for all measurements except PSR(FS). In young, healthy subjects, the normative values and between-muscle differences in these indices were evaluated and the relationships among the parameters were discussed. Recognizing the limitations resulting from the relatively small range of fat contents and states of muscle health that were studied, the results of these analyses emphasize the importance of free water content in determining the values for these quantitative MRI parameters. This work enables the evaluation of pathophysiological changes in muscle diseases objectively and quantitatively; and it supports and informs approaches that examine changes to individual quantitative MRI parameters as well as the relationships among them.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations and Symbols

AD	Adductor magnus/longus muscles
ADC	Apparent diffusion coefficient
ANOVA	Analysis of variance
B₀	Static magnetic field
B₁+	Radiofrequency magnetic field
BF	Biceps femoris muscle
BMI	Body mass index
BSA	Bovine serum albumin
b-value	Diffusion weighting value
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
f_{fat}	Fraction of tissue occupied by fat
FOV	Field of view
FS	Fat signal suppression
HSD	Honestly significant difference
ICC	Intra-class correlation coefficient
k_{mf}	Exchange rate from the macromolecular pool to the free water pool
MRI	Magnetic resonance imaging
MTR	Magnetization transfer ratio
MT	Magnetization transfer
n	Number of subjects
N_{EX}	Number of excitations
p	Probability of a Type I statistical error
PSR	Pool size ratio
qMT	Quantitative magnetization transfer
R_{1f}	Longitudinal relaxation rate of the free water pool

R_{10bs}	Observed longitudinal relaxation rate
RC	Repeatability coefficient
RF	Rectus femoris muscle
ROI	Region of interest
SD	Standard deviation
SENSE	Sensitivity encoding
S_f	Inversion ratio of the free water pool
SIR	Selective inversion recovery
SM	Semimembranosus muscle
ST	Semitendinosus muscle
T₁	Longitudinal relaxation time constant
T₂	Transverse relaxation time constant
TD	Pre-delay
TE	Echo time
TI	Inversion time
TR	Repetition time
TSE	Turbo spin-echo
VI	Vastus intermedius muscle
VL	Vastus lateralis muscle
VM	Vastus medialis muscle
λ₁	First eigenvalue of the diffusion tensor
λ₂	Second eigenvalue of the diffusion tensor
λ₃	Third eigenvalue of the diffusion tensor

Figure 1.

T₁-weighted images from Scan 1 (a) and Scan 2 (b) of a representative subject. All of the representative data presented in the following figures are from this subject. Quantitative indices were analyzed in the eight muscles indicated in panel (a), including those of the quadriceps muscle group (rectus femoris (RF); vastus lateralis (VL); vastus intermedius (VI); vastus medialis (VM)), those of the hamstring muscle group (biceps femoris (BF) semitendinosus (ST); semimembranosus (SM)), and the adductor magnus (AD). The four BSA standards are also present on the right of the thigh.

Figure 2.

Results of fat/water imaging studies. (a) Representative fat fraction (f_{fat}) map from scans 1.

(b) The Bland-Altman plot of f_{fat} values. The RC of less than 0.02 indicates a good repeatability of the three-point Dixon sequence, and the ICC of 0.886 indicates good reliability of the two scans across these subjects.

Figure 3.

Results of multiple spin-echo studies. (a) Example multiple spin-echo data and curve-fitting results of a single pixel in the VL muscle. FS data are indicated with red points and non-FS data are indicated with black points. (b) Example B_1^+ map from this subject, with the colorbar indicating the percentage of the nominal flip angle. (c) Representative T_2 parameter map from scan 1; the T_2 (FS) maps are not shown. The units of T_2 and T_2 (FS) are ms. (d) The Bland-Altman plot of T_2 and T_2 (FS) values. The low RC values indicate good repeatability of the multi-echo sequence in healthy subjects. The ICC values were 0.860 for T_2 and 0.854 for T_2 (FS), indicating good inter-scan reliability.

Figure 4.

Results of SIR experiments. (a) Example data and fitting curves of SIR data from a pixel in the VL muscle. The bi-exponential behavior is observed from the sub-panel. (b, c) Representative T_1 and PSR maps from scan 1; the T_1 (FS) and PSR(FS) maps are not shown. The units of T_1 and T_1 (FS) are s. (d) The Bland-Altman plot of T_1 and T_1 (FS) values (black and red points, respectively). The low RC values indicate good repeatability of the SIR method for T_1 estimation. The ICC values were 0.739 (T_1) and 0.696 (T_1 (FS)). (e) The

Bland-Altman plots of PSR and PSR(FS). A high RC value, relative to the mean, was observed. The ICC values were 0.820 (PSR) and 0.001 (PSR(FS)).

Figure 5.

Results of diffusion imaging studies. (a) Representative λ_3 map from scans 1. Note that the FS pulses were similarly effective in all regions of subcutaneous fat. The unit of λ_3 is $10^{-3} \text{ mm}^2\text{s}^{-1}$. (b) Representative ADC map from scan 1. The unit of ADC is $10^{-3} \text{ mm}^2\text{s}^{-1}$. (c) Representative FA map from scans 1. (d) The Bland-Altman plot of λ_3 values, indicating good repeatability; the ICC value was 0.786, indicating good reliability between the two scans across all subjects. (e) The Bland-Altman plot of ADC values, indicating good repeatability; the ICC was 0.713, indicating good reliability between the two scans across all subjects. (f) The Bland-Altman plot of FA values, indicating good repeatability; the ICC value was 0.860, indicating good reliability between the two scans across all subjects.

Figure 6.

Inter-relationships among the diffusion tensor indices in five subjects. (a) There was not a significant correlation between FA and λ_1 ($p > 0.05$). (b, c, d) Negative correlations were observed between FA and λ_2 ($r = -0.72$, $p < 0.05$), FA and λ_3 ($r = -0.85$, $p < 0.05$), and FA and $SD(v_1)$ ($r = -0.64$, $p < 0.05$). The unit of λ_{1-3} is $10^{-3} \text{ mm}^2\text{s}^{-1}$. The unit of $SD(v_1)$ is degrees.

Figure 7.

Inter-relationships among the PSR and diffusion tensor indices in five subjects. Low-to-moderate, negative correlations existed between PSR and λ_1 (panel a; $r = -0.170$, $p > 0.05$), λ_2 (panel b; $r = -0.477$, $p < 0.05$), λ_3 (panel c; $r = -0.491$, $p < 0.05$), and the SD of the first eigenvector (panel d; $r = -0.510$, $p < 0.05$). The unit of λ_{1-3} is $10^{-3} \text{ mm}^2\text{s}^{-1}$.

Figure 8.

Inter-relationships among the ADC and relaxation indices in five subjects. Negative correlations existed between ADC and R_2 (panel a; $r = -0.304$, $p < 0.05$), ADC and $R_2(\text{FS})$ (panel b; $r = -0.395$, $p < 0.05$), ADC and $R_{1\text{obs}}$ (panel c; $r = -0.344$, $p < 0.05$), and ADC and $R_{1\text{obs}}(\text{FS})$ (panel d; $r = -0.34$, $p < 0.05$). The unit of R_2 , $R_2(\text{FS})$, R_1 , and $R_1(\text{FS})$ is Hz. The unit of ADC is $10^{-3} \text{ mm}^2\text{s}^{-1}$.

Table 1

Descriptive and reproducibility data for f_{fat} , T_2 , $T_2(\text{FS})$, T_1 , $T_1(\text{FS})$, PSR, and PSR(FS) in eight human thigh muscles. For each variable, the mean and SD are given in the top row; significant differences are indicated in the middle row; and the absolute and relative RC values are given in the bottom row. The units of T_2 and $T_2(\text{FS})$ are ms and the units of T_1 and $T_1(\text{FS})$ are s. A hyphen (-) indicates lack of significance. For f_{fat} , $n = 4$.

	RF	VL	VI	VM	BF	ST	SM	AD
f_{fat}								
Mean (SD)	0.028 (0.003)	0.050 (0.014)	0.032 (0.014)	0.022 (0.006)	0.043 (0.004)	0.037 (0.009)	0.033 (0.007)	0.031 (0.006)
Significance:	<VL, BF	>RF, VL, VM, SM, AD	<VL, BF	<VL, BF, ST	>RF, VL, VM, SM, AD	>VM	<VL	<VL, BF
RC (% of Mean)	0.003 (10.7%)	0.013 (26.0%)	0.020 (62.5%)	0.009 (40.9%)	0.016 (37.2%)	0.011 (29.7%)	0.025 (75.8%)	0.005 (16.1%)
T_2								
Mean (SD)	30.1 (0.7)	31.7 (1.0)	31.4 (1.1)	31.5 (1.1)	32.3 (1.2)	31.5 (1.4)	31.9 (1.3)	31.8 (1.0)
RC (% of Mean)	1.2 (4.0%)	1.1 (3.5%)	1.6 (5.1%)	1.2 (3.8%)	1.2 (3.7%)	1.2 (3.8%)	1.4 (4.4%)	1.0 (3.2%)
Significance:	<VL, VM, BF, ST, SM, AD	>RF	-	>RF	>RF	>RF	>RF	>RF
$T_2(\text{FS})$								
Mean (SD)	29.8 (0.8)	30.9 (1.0)	30.8 (1.0)	30.8 (1.1)	30.6 (1.1)	29.9 (1.2)	30.9 (1.4)	30.7 (1.3)
Significance:	<VL	>RF	-	-	-	-	-	-
RC (% of Mean)	0.7 (2.3%)	1.1 (3.6%)	1.1 (3.6%)	1.4 (4.6%)	1.2 (3.9%)	1.1 (3.7%)	1.6 (5.2%)	1.2 (3.9%)
T_1								
Mean (SD)	1.36 (0.03)	1.33 (0.02)	1.36 (0.02)	1.36 (0.04)	1.32 (0.03)	1.33 (0.03)	1.33 (0.05)	1.34 (0.03)
Significance:	>BF	-	>BF	>BF	<RF, VL, VM	-	-	-
RC (% of Mean)	0.06 (4.4%)	0.06 (4.5%)	0.05 (3.7%)	0.05 (3.7%)	0.07 (5.3%)	0.04 (3.0%)	0.04 (3.0%)	0.04 (3.0%)
$T_1(\text{FS})$								
Mean (SD)	1.37 (0.03)	1.35 (0.02)	1.38 (0.02)	1.37 (0.04)	1.34 (0.03)	1.36 (0.03)	1.35 (0.04)	1.36 (0.03)
Significance:	-	-	-	-	-	-	-	-
RC (% of Mean)	0.07 (5.1%)	0.06 (4.4%)	0.04 (2.9%)	0.06 (4.3%)	0.04 (2.9%)	0.03 (2.2%)	0.09 (6.6%)	0.03 (2.2%)
PSR								
Mean (SD)	0.094 (0.01)	0.094 (0.007)	0.091 (0.008)	0.101 (0.020)	0.103 (0.010)	0.116 (0.027)	0.094 (0.007)	0.109 (0.028)
Significance:			<ST, SM	<ST, SM	-	>VL, VM	>VL, VM	-
RC (% of Mean)	0.024 (27.3%)	0.012 (13.6%)	0.008 (9.3%)	0.024 (27.9%)	0.027 (29.0%)	0.026 (27.7%)	0.038 (40.4%)	0.017 (18.5%)
PSR (FS)								
Mean (SD)	0.094 (0.005)	0.092 (0.004)	0.091 (0.006)	0.092 (0.007)	0.094 (0.004)	0.101 (0.006)	0.100 (0.008)	0.109 (0.025)
Significance:	<SM	<SM	<SM	-	-	-	>RF, VL, VI	-
RC (% of Mean)	0.021 (23.1%)	0.032 (34.4%)	0.02 (22.7%)	0.014 (15.9%)	0.02 (22.2%)	0.021 (22.3%)	0.048 (48.0%)	0.02 (21.3%)

Table 2

Descriptive and reproducibility data for DTI data in eight human thigh muscles. For each variable, the mean and SD are given in the top row; significant differences are indicated in the middle row; and the absolute and relative RC values are given in the bottom row. The units of the diffusivities are $10^{-3} \text{ mm}^2/\text{s}$ and the units of $\text{SD}(\mathbf{v}_1)$ are degrees. A hyphen (-) indicates lack of significance.

	RF	VL	VI	VM	BF	ST	SM	AD
λ_1	2.11 (0.08)	2.18 (0.10)	2.21 (0.09)	2.08 (0.07)	2.10 (0.06)	2.15 (0.07)	2.11 (0.06)	2.07 (0.06)
Significance:	-	>VM, BF, AD	>VM, BF, AD	<VL, VI	<VL, VI	>AD	>AD	<VL, VL, ST, SM
RC (% of Mean)	0.17 (8.1%)	0.24 (11%)	0.2 (9.3%)	0.18 (8.8%)	0.19 (9.2%)	0.14 (6.6%)	0.21 (10%)	0.11 (5.5%)
λ_2	1.67 (0.08)	1.90 (0.14)	1.83 (0.12)	1.73 (0.09)	1.57 (0.07)	1.55 (0.08)	1.64 (0.09)	1.69 (0.07)
Significance:	>BF, ST<VL, VI	>RF, VM, BF, ST, SM, AD	>RF, BF, ST, SM, AD	>BF, ST, SM	>AD; <RF, VL, VI, VM	<RF, VL, VI, VM, AD; >SM	<VL, VI, VM; <ST	>ST; <VL, VI, BF
RC (% of Mean)	0.13 (7.8%)	0.42 (22.1%)	0.2 (11.2%)	0.13 (7.7%)	0.16 (10.3%)	0.16 (10.5%)	0.18 (11.1%)	0.08 (4.9%)
λ_3	1.51 (0.05)	1.64 (0.08)	1.64 (0.09)	1.56 (0.06)	1.42 (0.07)	1.40 (0.07)	1.48 (0.06)	1.54 (0.06)
Significance:	>BF, ST<VL, VI	>RF, VM, BF, ST, SM, AD	>RF, BF, ST, SM, AD	>BF, ST	<RF, VL, VI, VM	<RF, VL, VI, VM, AD	<VL, VI	>ST<VL, VI
RC (% of Mean)	0.11 (7.3%)	0.22 (13.3%)	0.14 (8.8%)	0.08 (5.2%)	0.15 (10.6%)	0.16 (11.6%)	0.13 (8.8%)	0.10 (6.7%)
ADC	1.76 (0.07)	1.91 (0.10)	1.89 (0.10)	1.79 (0.07)	1.70 (0.07)	1.70 (0.06)	1.75 (0.07)	1.77 (0.06)
Significance:	>ST<VL, VI	>RF, VM, BF, ST, SM, AD	>RF, VM, BF, ST, SM, AD	>BF, ST, SM; <VI	>AD; <VL, VI, VM	<RF, VL, VI, VM; >SM	<VL, VI, VM; <ST	<VL, VI, BF
RC (% of Mean)	0.13 (7.4%)	0.28 (14.7%)	0.17 (9.2%)	0.12 (6.8%)	0.14 (8.4%)	0.14 (8.3%)	0.16 (9.2%)	0.08 (4.7%)
FA	0.18 (0.01)	0.14 (0.02)	0.16 (0.02)	0.15 (0.01)	0.21 (0.02)	0.23 (0.02)	0.19 (0.01)	0.16 (0.01)
Significance:	>VL, VI, VM, AD; <BF, ST	<RF, BF, ST, SM	<RF	<RF, BF, ST, SM	>RF, VL, VM, AD; <ST	>RF, VL, VM, BF, SM, AD	>VL, VM, AD; <ST	<RF, BF, SM; <ST
RC (% of Mean)	0.03 (16.7%)	0.04 (28.6%)	0.03 (18.8%)	0.03 (20.0%)	0.06 (30.0%)	0.05 (21.7%)	0.02 (10.5%)	0.03 (18.8%)
$\text{SD}(\mathbf{v}_1)$	8.1 (2.0)	14.9 (5.2)	10.2 (4.0)	9.2 (2.9)	4.7 (1.8)	3.5 (0.1)	5.4 (2.9)	9.5 (3.5)
RC (% of Mean)	10.8 (133%)	17.1 (114%)	6.1 (59.8%)	9.2 (100%)	2.7 (57.5%)	2.1 (60.0%)	3.7 (68.5%)	11.3 (119%)

Table 3

Inter-correlations among all measured parameters. Correlations were calculated for 40 data points, calculated from the between-day means for all eight muscles in the five subjects for whom there were suitable qMT data. Correlations in bold text are statistically significant ($p < 0.05$). Data are not presented for f_{fat} because the range of values encountered was very small.

	R_2	R_2 (FS)	R_{1f}	R_1	PSR	k_{mf}	R_{1f} (FS)	R_1 (FS)	PSR (FS)	k_{mf} (FS)	FA	ADC	λ_1	λ_2	λ_3	$SD(v_1)$
R_2	1.000	0.900	0.491	0.520	0.269	0.474	0.581	0.592	0.214	0.402	0.070	-0.304	-0.309	-0.329	-0.191	-0.273
R_2 (FS)		1.000	0.655	0.686	0.329	0.292	0.690	0.716	0.371	0.261	0.244	-0.395	-0.304	-0.431	-0.325	-0.367
R_{1f}			1.000	0.985	0.160	0.219	0.913	0.912	0.194	-0.211	-0.049	-0.285	-0.442	-0.216	-0.188	-0.069
R_1				1.000	0.329	0.199	0.913	0.924	0.299	-0.137	0.040	-0.349	-0.453	-0.290	-0.264	-0.155
PSR					1.000	-0.069	0.216	0.285	0.644	0.348	0.508	-0.436	-0.172	-0.477	-0.491	-0.506
k_{mf}						1.000	0.461	0.429	-0.175	0.276	-0.386	-0.151	-0.475	-0.082	0.053	0.110
R_{1f} (FS)							1.000	0.993	0.164	-0.003	-0.126	-0.328	-0.542	-0.255	-0.179	-0.081
R_1 (FS)								1.000	0.275	0.025	-0.081	-0.344	-0.526	-0.279	-0.206	-0.128
PSR(FS)									1.000	0.234	0.361	-0.216	0.013	-0.270	-0.277	-0.424
k_{mf} (FS)										1.000	-0.105	0.100	0.105	0.034	0.166	-0.101
FA											1.000	-0.652	-0.096	-0.735	-0.836	-0.639
ADC												1.000	0.809	0.978	0.951	0.759
λ_1													1.000	0.694	0.623	0.433
λ_2														1.000	0.948	0.838
λ_3															1.000	0.732
$SD(v_1)$																1.000