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Diffusion Tensor Imaging of the Calf Muscles in Subjects With and Without Diabetes Mellitus

Masoud Edalati, PhD^{1,*}, Mary K. Hastings, PT, DPT², Christopher J. Sorensen, PhD², Mohamed Zayed, MD, PhD³, Michael J. Mueller, PT, PhD², Charles F. Hildebolt, PhD¹, and Jie Zheng, PhD¹

¹Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri, USA

²Program in Physical Therapy, Washington University School of Medicine, St. Louis, Missouri, USA

³Department of Surgery, Section of Vascular Surgery, Washington University School of Medicine, St. Louis, Missouri, USA

Abstract

Background: Diffusion tensor imaging (DTI) has been used to characterize calf skeletal muscle architecture.

Purpose: To assess the diffusional properties of the calf muscles of subjects with and without diabetes, at rest and during isometric plantarflexion exercise.

Study Type: Prospective.

Subjects: Twenty-six subjects in two groups: 13 healthy and 13 subjects with type 2 diabetes (DM); each group consisted of seven females and six males.

Field Strength/Sequence: 3T/2D single-shot spin echo planar imaging.

Assessment: Fractional anisotropy (FA), mean diffusivity (MD), diffusion eigenvalues, and fiber tracking indices were obtained from the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL) muscles of the calf at rest and during isometric plantarflexion exercise.

Statistical Tests: We used a combination of nonparametric (Wilcoxon) and parametric (*t*-test) statistical assessments.

Results: The medial gastrocnemius muscle had more indices with significant differences between the two groups (six indices with $P < 0.05$) than did the lateral gastrocnemius (three indices with $P < 0.05$) and soleus muscles (only one index with $P < 0.05$). While the healthy group showed elevated MD values from rest to exercise (MG = 5.83%, LG = 13.45%, and SOL = 11.68%), the diabetic MD showed higher increases (MG = 19.74%, LG = 29.31%, and SOL = 20.84%) that were different between groups (MG: $P = 0.009$, LG: $P = 0.037$, and SOL: $P = 0.049$).

* Address reprint requests to: M.E., Mallinckrodt Institute of Radiology, Washington University School of Medicine in St. Louis, 4525 Scott Ave., St. Louis, MO 63110. masoud@wustl.edu.

Data Conclusion: Our results indicate considerable diffusional changes between healthy subjects and subjects with diabetes at rest and during isometric plantarflexion exercise in the calf muscles. The medial gastrocnemius muscle displayed the most diffusion sensitivity to diabetes-related microstructural changes.

Diabetes mellitus (DM) is a metabolic disease that results in hyperglycemia due to insulin resistance in tissues and a relative deficiency of insulin secretion.^{1,2} Reduced skeletal muscle contractile capacity has been associated with diabetes, with the highest burden in older individuals.³ Adults with diabetes produced lower maximal isometric contraction power and had greater muscle fatigue compared with age-matched healthy nondiabetic controls.⁴ In diabetic mice, skeletal muscles showed increased susceptibility to injury and reduced muscle function and contractile force.⁵ This is consistent with the observation in humans that muscle strength or muscular function is consistently lower in older adults with diabetes and may contribute to the development of physical disability.⁶

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique that has the ability to characterize the microstructure of tissues noninvasively.^{7,8} DTI can measure and reconstruct muscle fiber bundle in the calf muscles, and it has been proposed that diffusion pathways associated with: 1) the principal eigenvalue are oriented along fiber length, 2) the second largest eigenvalue lies within the endomysium (perpendicular to the fiber length), and 3) the third eigenvalue lie within a cross-section of muscle fiber.⁹ Diffusion differences have been reported between rest and active contraction in healthy calf muscles.^{10–12} Significant elevations of mean diffusivity and three eigenvalues have been demonstrated in the medial gastrocnemius from rest to plantarflexion exercise in healthy calf muscles.¹³

DTI has been used to characterize calf skeletal muscle architecture and to assess healthy and damaged tissue.^{14–16} Hence, in this study we aimed to investigate diffusion differences in the calf muscles at rest and during isometric exercise in both healthy subjects and subjects with DM. We hypothesized that the diffusion and fiber tracking indices that reflect tissue microstructure (eg, mean diffusivity) of the calf muscles can differentiate subjects with DM (DM group) from healthy subjects (healthy group).

Materials and Methods

Study Population

This pilot study was approved by the institution's Human Subjects Review Committee. A total of 29 subjects, 14 healthy subjects and 15 subjects with type 2 DM without any peripheral arterial disease history, were prospectively enrolled after written informed consent was obtained, from January, 2017 to January, 2018. The inclusion criteria for healthy subjects included: age = 18 years old, no symptomatology or documented evidence of any cardiovascular disease, nonsmokers and free of diabetes, metabolic, and musculoskeletal diseases. For subjects with type 2 DM, the inclusion criteria included: documented type 2 DM history, no history of peripheral arterial disease. The exclusion criteria included: hemodynamically unstable, pregnant, claustrophobic, or another contraindication to MR scanning, over 400 lbs, having comorbidities that severely limited the subject's ability to

perform a modest contraction test, history of peripheral vascular disease, or of smoking cigarettes within the last 12 months. Of these, three subjects (a healthy, and two DMs) were excluded from the analysis due to excessive motion artifacts. Overall, 26 subjects: 13 healthy subjects (seven females and six males, age range 43–71 years) and 13 subjects with DM (seven females and six males, age range 40–75 years) were included in analysis. Their demographic characteristics including mean ($\pm$ standard deviation; SD) age, body mass index (BMI), HbA1c, diabetes duration, treatment, and neuropathy (based on Semmes-Weinstein monofilament test) are listed in Table 1.

MRI Acquisition Protocol

MRI calf scans were performed on a whole-body 3T MRI scanner (Magnetom Trio; Siemens Medical Systems, Erlangen, Germany) with maximum gradient strength of 45 mT/m (maximum slew rate of 200T/m/s) using a four-channel flex coil that was wrapped around a leg. A custom-made MRI-compatible ergometer was used consisting of a brake-like pedal with an adjustable pressure gauge (set to 10 psi) that was attached to a pneumatic cylinder.¹⁷ For the rest scan, subjects laid in a supine position, feet first, and care was taken to support the leg sustainably along the main magnetic field. For the isometric plantarflexion exercise, a subject was instructed to depress the pedal (aligned with the anterior-posterior direction of the magnet) until it reached the stopper (ankle joint flexed at about 20°) and hold it steady during the scan. The knee was kept straight and secured (by straps placed at mid-calf) that allowed the posterior compartment muscles to be contracted throughout isometric plantarflexion exercise. The percentage of maximal voluntary contraction (MVC) required during the exercise was estimated based on the torque required to fully depress the pedal of the exercise device according to previous measurements of plantar flexor MVC in healthy but untrained subjects.¹⁸ Based on the geometry of the pedal and pneumatic cylinder of the exercise device, as well as increased pressure when the pedal was fully depressed, the isometric contraction was estimated as 25% of MVC.¹⁷

Prior to DTI scans, scout images were acquired for calf localization. Short-axes T₁-weighted images were obtained for anatomical depiction and specifying the desired muscles in axial slices. *in vivo* single-shot DTI data of the human calf were collected by application of dual refocusing pulses¹⁹ with reversal diffusion gradients to reduce the eddy current distortions and using spin echo planar imaging (EPI) acquisition in a stack of axial slices (perpendicular to the longaxis of the calf) at rest and during isometric exercise. Axial images were selected in such a way that the largest cross-sectional area of the calf was approximately positioned in the center of the stack. The following acquisition parameters were used: repetition time / echo time (TR/TE) = 4500/70 msec, 20 contiguous slices, slice thickness of 6 mm, 75% rectangular field of view (FOV) with acquisition matrix 96 × 72 interpolated to 192 × 144, pixel size 1.14 × 1.14 mm², 6/8 partial Fourier acquisition along the phase-encoding direction, and 4 averages. To increase signal to noise ratio (SNR) and improve fat suppression uniformity, a water excitation technique with spectral spatial pulses was used to only excite water without affecting fat (nulling fat signal). Water excitation binomial RF pulses consisted of three short (1–2–1) RF pulses separated by ~1.2 msec interpulse delays (at 3T) to achieve 180° phase shift between fat and water protons. Insensitivity to B₁ field inhomogeneity with application of binomial composite RF pulses in a water excitation

method can avoid undesired fat signals (or its regrowth during acquisition) that result in ghosting artifacts from fat contents in diffusion-weighted images.^{20,21} Two b-values of 0 and 500 s/mm² along 12 noncollinear gradient directions were used for diffusion sensitizing. Each DTI scan time was ~4 minutes.

Image Analysis

Raw images were exported and processed offline to derive the diffusion tensor for each image pixel. Prior to the diffusion tensor calculation, all diffusion-weighted images (DWI) were registered to the corresponding non-DWI followed by motion correction, eddy current distortion correction, and data averaging. Diffusion data analysis was performed using an open-source diffusion analysis software (<http://dsi-studio.labsolver.org>) and MatLab (MathWorks, Natick, MA). Accordingly, primary (λ_1), secondary (λ_2), and tertiary (λ_3) eigenvalues (10^{-3} mm²/s) with their associated eigenvectors were derived after diffusion tensor diagonalization for each image pixel. Mean diffusivity (MD; 10^{-3} mm²/s) and fractional anisotropy (FA; dimensionless) maps were then obtained.

Five-point rank score was utilized by two MRI scientists (M.E., J.Z., more than 5 years experience in DTI postprocessing and musculo-skeletal imaging) who independently (reviewers were blinded to each other's evaluation) evaluated images for uniformity of fat suppression and DWI image quality. The fat uniformity was scored as follows: 5 = excellent; 4 = mild nonuniformity / mildly impacting analysis; 3 = moderately impacting analysis, but trustable; 2 = marked fat artifact, but not all structures; 1 = poor/inadvertent water signal suppression, whereas DWI quality scoring was on the following basis: 5 = excellent; 4 = above average; 3 = adequate; 2 = suboptimal; 1 = poor.

Regions of interest (ROIs) were manually drawn (by the two above-mentioned authors together) in the posterior part of the calf using the axial T₁-weighted image to delineate the desired muscles (Fig. 1), including the medial gas-trocnemius (MG), the lateral gastrocnemius (LG), and the soleus (SOL) muscle compartments. To minimize the negative effects of intramuscular fat and partial volume on the DTI metrics, ROIs were selected with minimal intramuscular fat and voxels primarily consisting of muscle contents were included in analysis (excluding muscle boundaries). Fiber tracking was performed in each identified ROI with minimum FA set at 0.1 and an angle threshold of 30° as the fiber termination criteria. Mean fiber tract length (entire muscle volume; mm), fiber density (number of fibers within an ROI per pixel), and fiber angle (mean fiber angle deviated from the long-axis of the calf; in degrees) were determined. The normal vector representing the long-axis of the calf was individually determined from axial slices covering the entire calf. Mean fiber angle was determined as the average angle between all tracts in an ROI and the associated normal vector.

Statistical Analysis

The diffusion and fiber tracking indices consisted of FA, MD, λ_1 , λ_2 , λ_3 , fiber length, fiber density, and fiber angle. To test for differences within and between groups, we used a combination of nonparametric and parametric statistical assessments. Statistical significance level was set at $P < 0.05$ for all analyses. Data distributions were tested for normality with

the Shapiro–Wilk W test. Means, standard deviations, and 95% confidence intervals were used for descriptive statistics. For each group, the differences between the two levels (rest vs. exercise) were determined for the three muscles (MG, LG, and SOL). If the differences were normally distributed, the paired t -test was used to assess statistical significance. If they were nonnormally distributed, the Wilcoxon signed rank test was used. To assess how these values differed between groups, the equality of variances was assessed with the O'Brien, Brown-Forsythe, Levene, Bartlett, and two-sided F -tests. Differences among group means for normally distributed data with equal variances were tested with the t -test. If the data for the two groups were normally distributed but the variances were not equal, the Welch t -test was used. If one or both data distributions was nonnormal, the nonparametric Wilcoxon rank sums test was used. To assess interreviewer variability, Cohen's κ statistics was used to compare fat uniformity and DWI quality evaluated by the two independent reviewers (M.E., and J.Z.). Spearman rank correlation was also used to further assess the relationship between reviewers' scores. Statistical analyses were performed with JMP Pro 13.2.1 (SAS Institute, Cary, NC).

Results

For all subjects, scans were successfully performed with uniform fat suppression and sufficient DWI quality by visual reviewing. The overall quadratic weighted κ statistics for interreviewer variability showed strong agreement for both fat suppression uniformity ($\kappa = 0.71$, $P < 0.001$) and DWI quality ($\kappa = 0.77$, $P < 0.001$). In correlation analyses, good positive correlations were evident between observers' scores for fat suppression uniformity ($\rho = 0.75$, $P < 0.001$) and DWI quality ($\rho = 0.84$, $P < 0.001$). The averaged SNR of the three muscle compartments from all subjects were MG = 70.17 ± 14.78 , LG = 72.87 ± 20.50 , and SOL = 63.99 ± 15.16 . Figure 2 illustrates water excitation T_1 -weighted images, FA, and MD maps of a healthy subject and a subject with DM at rest and during isometric exercise. Both healthy and DM groups displayed reversed trends in FA and MD values from rest to exercise for the examined muscles (Fig. 3). The opposite changes were apparent by FA reduction and MD increase for the muscles from rest to exercise. Table 2 summarizes mean FA, MD, three eigenvalues, fiber length, density, and angle of the three different muscles at rest and during the exercise for both healthy and DM groups. Mean diffusion indices of the three calf muscle compartments during exercise are displayed by the boxplot in Fig. 4, for both healthy and DM groups.

Table 3 shows the statistical analysis results within each of the two groups of subjects. The intergroup statistical analysis of diffusion and fiber tracking metrics between the healthy and the DM groups are listed in Table 4. The MG muscle had the highest number of diffusion and fiber tracking indices that were statistically different between the healthy and the DM groups. In contrast, the least number of significant statistical differences of the indices between the groups was found for the soleus muscle.

Within each of the two groups, MD differences between rest and exercise values were different ($P < 0.05$ for all the muscles), where higher exercise MD values were found in the DM group. For all the examined muscles, the MD was the only index that was significantly different between groups with greater MD changes in the DM group: MG (5.83% in healthy,

19.74% in DM; $P = 0.009$), LG (13.45% in healthy, 29.31% in DM; $P = 0.037$), and SOL (11.68% in healthy, 20.84% in DM; $P = 0.049$).

Eigenvalue indices were greater during exercise compared to rest in the DM group. In the healthy group, eigenvalue differences between rest and exercise ($P < 0.05$ for all the muscles) were also greater during exercise compared to rest, except for λ_1 (MG and SOL). The DM group showed higher eigenvalue increases (from rest to exercise) in all muscle compartments ($\lambda_1 = 16.61\%$, $\lambda_2 = 29.46\%$, and $\lambda_3 = 27.23\%$) than those obtained from the healthy group ($\lambda_1 = 2.80\%$, $\lambda_2 = 17.20\%$, and $\lambda_3 = 15.85\%$). Changes in λ_2 and λ_3 showed larger relative increases compared with λ_1 for either of the two groups. Intergroup comparisons of the eigenvalues also showed greater increases in the DM group (compared to the healthy) and were significant in the MG (for all the indices) and the LG (only for λ_1 and λ_3), but not in the SOL muscle.

FA reductions from rest to exercise were observed within each of the two groups ($P < 0.05$ for all the muscles). Between the healthy and the DM groups, FA revealed no difference in the muscles: MG (-26.21% in healthy, -23.84% in DM, $P = 0.396$), LG (-22.79% in healthy, -13.86% in DM, $P = 0.191$), and SOL (-33.35% in healthy, -25.48% in DM, $P = 0.055$).

Fiber tracking analysis revealed fiber length and density reductions and fiber angle increases from rest to exercise that were different for all muscles within each of the two groups (except fiber angle of the SOL muscle in the DM group). The DM group showed greater fiber length reductions (healthy: -42.14% , DM: -48.79%) and increased fiber angle (healthy: 65.62% , DM: 88.84%) than the healthy group averaged over all the muscles, where only the MG muscle was statistically different between groups ($P = 0.010$ for fiber length and $P = 0.002$ for fiber angle). Although fiber density revealed a greater reduction in the DM group (-57.83%) than the healthy group (-54.06%), no significant statistical difference was found between groups for the muscles: MG ($P = 0.298$), LG ($P = 0.678$), and SOL ($P = 0.281$).

Examples of the changes in fiber tracts of the MG muscle from rest to exercise (the same two subjects as in Fig. 2) are shown in Fig. 5, demonstrating elevated MD during exercise. The MG muscle fibers were color-coded by MD values and scaled for better visualization. It was also evident that the MG muscle of the DM patient illustrated much more severe fiber disarrays with loss of fiber bundle coherency than the MG of the healthy subject during the exercise.

Discussion

In this study we employed DTI and fiber tracking to evaluate the characteristics of three muscles (MG, LG, and SOL) of the calf in healthy and DM subjects. Our focus was on the quantitative changes of various diffusion and fiber tract indices from rest to isometric plantarflexion exercise. The gastrocnemius muscle (fast twitch muscle), which is responsible for phasic type activity, displayed more diffusion and fiber tracking indices with significant statistical changes than the soleus muscle (slow twitch muscle) from rest to plantarflexion

exercise between groups. This was consistent with our hypothesis that diffusional indices can differentiate diabetic calf muscles from healthy muscles during plantarflexion exercise (specifically in the gastrocnemius muscles).

DTI allows noninvasive in vivo assessment of microstructures of tissues, by exploiting the properties of water molecule diffusion. Significant diffusional differences of resting human skeletal muscles between healthy and patients with hematoma and muscle tear injuries in the lower leg showed increased mean diffusivity, decreased diffusion anisotropy, and nonuniform muscle fiber arrangement in patients with muscle injury.¹⁴ Dynamic changes of DTI indices in response to acute ischemia-reperfusion injury were studied in skeletal muscle of mice, where the mean diffusivity increased mainly due to increases in orthogonal diffusivity coefficients during the reperfusion phase, and these coefficients correlated with histological measures supporting the changes obtained from MRI observations.¹⁵

SNRs were computed with a previously described method under the assumption that MR noise has a Rayleigh distribution.^{22,23} Based on a previous study²⁴ that determined the minimum required SNR to ensure the accuracy of diffusion measures, our SNR measurements of all the subjects had 1% and ~1–3% accuracy and precision criteria for a given bvalue between 435–725 s/mm².

The resting MD values (10⁻³mm²/s) of the healthy group were in good agreement with published values^{12,25–27} for the same muscles (MG = 1.64 ± 0.10, LG = 1.67 ± 0.09, SOL = 1.56 ± 0.07). Although the healthy group showed elevated MD values from rest to exercise (MG = 5.83%, LG = 13.45%, and SOL = 11.68%), MD in the DM group showed more increase in comparison with those in the healthy counterparts (MG = 19.74%, LG = 29.31%, and SOL = 20.84%). Impaired extracellular matrix (ECM) remodeling in diabetic muscle tissues leads to a greater volume of the ECM.² Although the molecular diffusion process is not solely governed by the collagen contents within ECM, it is linked by degradation and loss of cellularity of the muscle, resulting in increased diffusion coefficient.²⁸ Therefore, this may be the basis for increased mean diffusivity in DM (compared with healthy muscles) because the amount of ECM collagen inversely correlated with the diffusion coefficient.

The resting eigenvalues (10⁻³mm²/s) in our healthy group agree with those previously reported,^{26,27} with resting eigenvalues of the LG muscle being reported as: $\lambda_1 = 2.13 \pm 0.04$, $\lambda_2 = 1.56 \pm 0.03$, $\lambda_3 = 1.29 \pm 0.02$ and our values for the LG muscle being: $\lambda_1 = 2.18 \pm 0.11$, $\lambda_2 = 1.54 \pm 0.12$, $\lambda_3 = 1.29 \pm 0.07$. The exercise eigenvalues in the healthy group were within a range of that reported for the calf muscles²⁹ and that reported for the MG muscle only.¹³ The DM group showed statistically higher increases in eigenvalues from rest to exercise (compared with the healthy group) in the gastrocnemius muscles, except for λ_2 of the LG muscle; $P = 0.081$, but not in the soleus muscle. We assume that during exercise the eigenvalues in the DM group were more influenced by the rise of interstitial fluid contents and ECM due to reduced structural constraints, which resulted in increased diffusion eigenvalues.

In this study, mean resting FA values of the healthy muscles showed good agreement with published values in one study,²⁶ and in another study¹⁴ FA values in MG muscle for healthy

subjects were in the range of these values in our study. However, slightly lower resting FA values (compared with our results) for healthy muscles have been demonstrated,^{27,30} and higher values have also been reported.^{25,29} Similar mean FAs were reported between edematous and normal thigh muscles but without statistically significant differences,³¹ with these findings being supported in two studies that demonstrated no significant difference in FA, despite apparent changes of MD and eigenvalues in the calf muscles.^{32,33} Because the extent of fractional anisotropy differences between groups were not statistically significant, this implies that comparable increases of the eigenvalues (λ_1 , λ_2 , and λ_3) from rest to exercise between groups may resemble diffusion ellipsoids that dilate approximately equally along all the axes (in axial and radial directions).

Reconstructed fiber tracking revealed that fiber length reduction (shortening) occurred during the exercise for all the muscles in this study, confirming previous findings.¹³ The resting in vivo skeletal muscle length or fiber would shorten based on a capacity to displace a load during an isometric contraction.³⁴ The largest fiber length reductions from rest to exercise in the healthy group were seen in the SOL (−50.51%) and in MG and SOL of DM patients (−52.53% and −52.36%, respectively). Loss of fiber bundle uniformity and coherency was expected as an immediate response to active muscle contraction.¹³ It has been demonstrated that the resting calf muscle fiber organization was clearly perturbed in the injured area (muscle hematoma and tear injuries).¹⁴ In our fiber tracking results, we visually observed only small variations in fiber orientations at resting muscles between healthy and DM groups. During exercise in the DM subjects resulted in severe fiber disarray that can be distinguished from the healthy group based on visual appraisal.

The largest angle deviations from the calf long-axis were seen in the SOL muscle for both healthy and DM groups, which is in agreement with a previous study.²⁵ In the MG, mean fiber angle from rest to exercise significantly increased in each group and was significant between healthy and DM groups as well. Fiber angle results showed no statistical differences for the LG and SOL muscles; however, fiber angles for both muscles were increased from rest to exercise. We also found that the change in the fiber angle of the healthy MG muscle between rest and isometric plantarflexion exercise was 57.66%, indicating good agreement with a previous study that reported 46.15%.²⁹ Our fiber length and angle results confirm that microstructural integrity of the diabetic MG muscle is affected more by isometric exercise than the healthy MG muscle; this may be due to more contraction load during exercise on the MG muscle compared with the other muscles; however, the interpretation of fiber tracking results is complex and requires further investigations.

It has been demonstrated that fiber density (within ROI per pixel) reduction, as an index of fiber tracts passing through a region, can be an indicator of abnormalities of brain white matter.³⁵ In addition to more pronounced fiber length and angle changes in DM versus healthy subjects, fiber density variations also showed a greater reduction in the DM group. These changes may be reflecting the existence of damaged skeletal muscle in DM.

As stated earlier, the MG muscle had the highest number of different diffusion indices between the healthy and DM groups, and this may be attributed to a higher oxygen

consumption rate in the MG during exercise. It has been previously shown that the oxygen consumption rate and extraction fraction in the calf muscles significantly increase during isometric plantarflexion exercise, with the greatest increase in the MG.¹⁷ The differences in diffusion indices found in the MG may also be related to greater intermuscular adipose tissue stored in the MG for those with DM compared with those without DM.^{36,37} Future work will explore the mechanisms of changes in MG for oxygen consumption, diffusion properties, and DTI fibers.

Fatty infiltration of calf muscles (especially in those with DM) results in chemical shift artifacts in EPI-acquired data and consequently affects measurements of the DTI and fiber tracking indices.³⁸ In contrast to conventional fat saturation or water excitation methods with a 1–1 binomial pulse pair, higher-order binomial RF pulse fashions such as 1–2–1 (as used here) or 1–3–3–1 are considered to perform better, with broader fat suppression efficiency and uniformity.³⁹ To qualitatively evaluate the effect of fat infiltration, all fat-suppressed T₁-weighted images were individually inspected for the quality of fat suppression within the studied muscles. Although diabetic muscles are known to have a higher intermuscular adipose tissue than healthy muscle,^{36,37} in this cohort of patients we observed minimal intermuscular fat infiltration in the examined muscles (MG, LG, and SOL) of any DM subject. This low intermuscular fat infiltration could be due to a well-controlled DM group (HbA1c = 7.29%).

There are a few limitations in this study. Besides the relatively small sample size, there were slight shifts in slice locations between resting and isometric exercise. To mitigate this negative effect, the participant's leg was kept as stable as possible, using straps during the scan. Image acquisition and analysis focusing on all 20 axial slices rather than a single slice may further reduce variability of slice locations. Force of the muscle contraction during exercise was not normalized to the participants. This could result in variations in the measured diffusion and fiber tracking metrics during calf exercise. Nonetheless, the variations of the measured indices at rest was like those during exercise for the healthy group, which indicates that this error would have been very limited in this study. Choice of a reliable fat suppression technique to employ in musculoskeletal imaging is a very difficult task to do due to field inhomogeneities and variant-induced susceptibilities.²¹ A selective water excitation technique efficiently reduced the effect of fat contaminations, but it may not be able to fully suppress fat contents due to the B₀ field inhomogeneity and low spatial resolution. Care was taken to manually identify apparent fat residuals within the ROIs and exclude them from analyses. Nonetheless, this undesirable fat effect on DTI indices requires extensive study in the future. DTI pre- and postprocessing procedures, eg, calf muscles segmentation, were relatively time-intensive, which is challenging for its application to clinical practice.

In conclusion, DTI indices demonstrated considerable diffusional differences in the calf muscles at rest and during isometric plantarflexion exercise in healthy participants and patients with DM. The MG muscle provided the most difference with statistical significance between healthy and diabetic subjects, suggesting more sensitivity than other muscles to the changes of muscle microstructure in people with diabetes. Overall, DTI was demonstrated to be a sensitive tool for characterization of microstructural changes in the skeletal muscle and

may have the potential to differentiate between healthy and diabetic calf muscles based on their diffusional properties and facilitate drug development and evaluation in diabetes.

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FIGURE 1:

Illustration of anatomical sagittal (right; showing a stack of 20 axial slices) and mid-calf axial (left) images of a subject's calf. Axial calf image shows manual ROI muscle segmentations in a resting healthy subject. MG = medial gastrocnemius, LG = lateral gastrocnemius, and SOL = soleus.

FIGURE 2:

Axial calf maps of water excitation T₁-weighted (top row), fractional anisotropy (FA; middle row), and mean diffusivity (MD; bottom row) from a healthy subject and a subject with diabetes (DM). Posterior compartments (red line) of calf muscles displayed FA reduction and MD increase between resting and exercise within each individual group. Visual inspections of exercise maps for healthy and DM subjects indicate higher MD values in the diabetic muscles. FA is a dimensionless quantity, whereas the MD map is represented by $10^{-3}\text{mm}^2/\text{s}$.

FIGURE 3:

Effects of isometric plantarflexion exercise of the calf muscles on FA and MD indices. Line plots demonstrate opposite changes in mean FA and MD values in transition from rest to exercise for all healthy subjects (solid line) and subjects with DM (dashed line).

FIGURE 4: Boxplot comparisons of exercise eigenvalues (a–c), MD (d), and FA (e) between healthy and diabetic (DM) groups for all the calf muscles: MG, LG, and SOL. The DM group resulted in relatively higher eigenvalues and MD compared with the healthy group for all the muscles. Mean FA showed minor changes in the intergroup analysis for all the muscles during exercise. Lower (Q1), median (Q2), and upper (Q3) quartiles are represented for all the muscles in each group.

FIGURE 5:

Fiber tracts differences (MD color-coded) of the MG muscle for the example subjects as depicted in Fig. 2. Fiber tracts were reconstructed and obtained by manual drawing of the ROIs (yellow) in the medial gastrocnemius region. The resting healthy and diabetic MG muscles show slight differences in their mean diffusivity (difficult to distinguish between them), as also visualized in Fig. 2. The MG muscle fibers varies from rest to exercise (left to right), indicating loss of fibers coherence due to muscle contraction. Exercise fiber tracts appear more affected in the diabetic muscle, where healthy fibers are majorly dominated by uniform fibers; well oriented in the long-axis of the calf (corresponding to superior-inferior axis of subjects). With exercise, diabetic MG muscle appear to show a greater fiber loss, fiber length shortening, and density reduction compared with values under a condition of rest. Due to the loss of relatively lower mean diffusivity fibers (mostly in the superior part), the diabetic MG muscle (bottom right panel) had higher MD values compared with the healthy MG muscle (top right panel) during exercise.

Table 1.

Population Characteristics

	Healthy (n = 13)	DM (n = 13)	P
Age	58.85 ± 8.06	66.54 ± 9.86	0.100
BMI (kg/m ²)	29.65 ± 9.26	30.51 ± 5.79	0.808
HbA1c (%)	5.38 ± 0.35	7.29 ± 1.38	<0.001
Diabetes duration (y)	NA	9.71 ± 6.24	NA
Diabetes treatment			
Oral (Yes/No)	NA	10/3	NA
Insulin (Yes/No)	NA	1/12	NA
Neuropathy (Yes/No)	NA	2/11	NA

Age, BMI = body mass index, HbA1c, and diabetes duration are represented as mean ± standard deviation.

DM = diabetes mellitus.

Statistical significance level was set at P < 0.05.

Table 2.

Mean Values $\pm$ Standard Deviation of FA, MD, Three Eigenvalues, Fiber Length, Density, Angle for MG, LG, and SOL Muscles at Rest and During Plantarflexion for Both Healthy Subjects and Subjects With DM

	MG		LG		SOL	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
Healthy ($n = 13$)						
FA	0.26 ± 0.02	0.19 ± 0.03	0.27 ± 0.02	0.21 ± 0.04	0.26 ± 0.03	0.17 ± 0.03
MD	1.64 ± 0.10	1.73 ± 0.07	1.67 ± 0.09	1.89 ± 0.08	1.56 ± 0.07	1.74 ± 0.09
λ_1	2.12 ± 0.11	2.09 ± 0.12	2.18 ± 0.11	2.29 ± 0.14	2.01 ± 0.05	2.09 ± 0.17
λ_2	1.52 ± 0.13	1.67 ± 0.08	1.54 ± 0.12	1.87 ± 0.12	1.46 ± 0.12	1.72 ± 0.08
λ_3	1.30 ± 0.10	1.44 ± 0.06	1.29 ± 0.07	1.49 ± 0.09	1.22 ± 0.06	1.46 ± 0.08
Fiber length	98.72 ± 14.80	63.33 ± 13.52	95.56 ± 13.22	56.78 ± 16.62	90.60 ± 22.29	42.22 ± 9.85
Fiber density	5.96 ± 2.14	2.79 ± 1.25	6.93 ± 2.39	2.82 ± 1.53	3.42 ± 2.21	1.27 ± 0.45
Fiber angle	17.26 ± 5.32	24.90 ± 5.14	13.00 ± 4.72	28.46 ± 13.29	28.60 ± 9.00	41.93 ± 4.58
DM ($n = 13$)						
FA	0.25 ± 0.02	0.19 ± 0.04	0.26 ± 0.03	0.22 ± 0.04	0.25 ± 0.02	0.19 ± 0.04
MD	1.65 ± 0.11	1.97 ± 0.29	1.72 ± 0.12	2.22 ± 0.40	1.57 ± 0.08	1.90 ± 0.23
λ_1	2.11 ± 0.13	2.37 ± 0.35	2.22 ± 0.15	2.74 ± 0.51	1.99 ± 0.10	2.27 ± 0.29
λ_2	1.50 ± 0.11	1.91 ± 0.30	1.61 ± 0.14	2.19 ± 0.43	1.49 ± 0.10	1.86 ± 0.23
λ_3	1.32 ± 0.10	1.63 ± 0.25	1.34 ± 0.11	1.74 ± 0.28	1.23 ± 0.07	1.57 ± 0.20
Fiber length	98.77 ± 7.70	47.80 ± 17.85	97.00 ± 10.24	55.76 ± 15.78	88.96 ± 16.50	41.54 ± 10.83
Fiber density	6.04 ± 1.69	2.18 ± 1.14	6.79 ± 2.80	3.12 ± 1.32	3.94 ± 1.38	1.42 ± 0.72

	MG		LG		SOL	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
Fiber angle	16.35 ± 5.21	33.82 ± 9.50	15.36 ± 4.12	28.64 ± 14.55	28.89 ± 8.58	44.17 ± 13.21

DM = diabetes mellitus, MG = medial gastrocnemius, LG = lateral gastrocnemius, SOL = soleus, FA = fractional anisotropy, MD = mean diffusivity, X = eigenvalue index.

MD, λ_1 , λ_2 , and λ_3 represented in $10^{-3}\text{mm}^2/\text{s}$. Fiber indices units are as follows: length (mm), density (number of fibers within ROI per pixel), and angle in degrees as measured with respect to the long-axis of the calf.

Table 3.

Statistical Analysis of Diffusion and Fiber Indices Within Each Group (Healthy and DM)

	MG			LG			SOL		
	<i>P</i>	95% CI		<i>P</i>	95% CI		<i>P</i>	95% CI	
Healthy (<i>n</i> = 13)									
FA	0.002^b	(0.05, 0.09)		<0.001^a	(0.04, 0.08)		<0.001^a	(0.07, 0.10)	
MD	0.002^a	(-0.14, -0.04)		<0.001^a	(-0.29, -0.14)		<0.001^a	(-0.25, -0.11)	
λ_1	0.241 ^a	(-0.02, 0.06)		0.034^a	(-0.22, -0.01)		0.095 ^b	(-0.15, 0.02)	
λ_2	0.002^a	(-0.24, -0.07)		<0.001^a	(-0.42, -0.25)		0.003^b	(-0.36, -0.15)	
λ_3	<0.001^a	(-0.19, -0.09)		<0.001^a	(-0.27, -0.13)		<0.001^a	(-0.29, -0.19)	
Fiber length	<0.001^a	(26.66, 44.12)		0.002^b	(29.07, 49.33)		<0.001^a	(35.15, 61.61)	
Fiber density	<0.001^a	(2.23, 4.10)		<0.001^a	(2.47, 5.74)		0.002^b	(1.02, 3.43)	
Fiber angle	<0.001^a	(-11.32, -3.97)		0.002^b	(-22.96, -7.21)		<0.001^a	(-18.80, -7.86)	
DM (<i>n</i> = 13)									
FA	<0.001^a	(0.04, 0.08)		0.017^a	(0.01, 0.07)		<0.001^a	(0.04, 0.08)	
MD	<0.001^b	(-0.51, -0.15)		<0.001^a	(-0.74, -0.26)		<0.001^a	(-0.46, -0.19)	
λ_1	0.009^b	(-0.48, -0.05)		0.002^b	(-0.88, -0.17)		0.005^a	(-0.45, -0.10)	
λ_2	<0.001^a	(-0.58, -0.24)		<0.001^a	(-0.84, -0.31)		<0.001^a	(-0.50, -0.24)	
λ_3	<0.001^a	(-0.46, -0.16)		<0.001^a	(-0.56, -0.24)		0.002^b	(-0.47, -0.22)	

	MG		LG		SOL	
	P	95% CI	P	95% CI	P	95% CI
Fiber length	<0.001 ^b	(44.47, 58.46)	<0.001 ^a	(29.20, 53.28)	<0.001 ^a	(36.88, 57.97)
Fiber density	<0.001 ^a	(2.81, 4.73)	<0.001 ^a	(2.16, 5.18)	<0.001 ^a	(1.84, 3.19)
Fiber angle	<0.001 ^a	(-22.33, -12.20)	<0.001 ^b	(-20.69, -4.69)	0.095 ^a	(-22.40, -8.16)

P values represent assessments of differences between rest and exercise.

Statistical significance level was set at P < 0.05. CI = confidence interval.

^a Assessed with the t-test.

^b Assessed with the Wilcoxon signed rank test.

DM = diabetes mellitus, MG = medial gastrocnemius, LG = lateral gastrocnemius, SOL = soleus, FA = fractional anisotropy, MD = mean diffusivity, X = eigenvalue index. MD, λ1, λ2, and λ3 represented in 10⁻³ mm²/s. Fiber indices units are as follows: length (mm), density (number of fibers within ROI per pixel), and angle in degrees.

Table 4. Statistical Analysis of Diffusion and Fiber Indices Between Healthy and DM Groups

	<u>MG</u>		<u>LG</u>		<u>SOL</u>	
	<i>P</i>		<i>P</i>		<i>P</i>	
FA	0.396 ^c		0.191 ^a		0.055 ^a	
MD	0.005^c		0.028^b		0.047^b	
λ1	0.004^c		0.033^c		0.064 ^c	
λ2	< 0.001^b		0.081 ^c		0.342 ^c	
λ3	0.031^b		0.029^b		0.411 ^c	
Fiber length	0.010^c		0.383 ^c		0.903 ^a	
Fiber density	0.298 ^a		0.678 ^a		0.281 ^c	
Fiber angle	0.002^a		0.644 ^c		0.640 ^a	

P values were assessed for differences between rest and exercise.

Statistical significance level was set at $P < 0.05$.

^a Assessed with *t*-test assuming equal variances and normal distributions.

^b Assessed with *t*-test assuming equal variances and normal distributions.

^c Assessed with Wilcoxon rank sums test with one/both distributions being nonnormal.

DM = diabetes mellitus, MG = medial gastrocnemius, LG = lateral gastrocnemius, SOL = soleus, FA = fractional anisotropy, MD = mean diffusivity, *P* = eigenvalue index. MD, λ1, λ2, and λ3 represented in $10^{-3} \text{ mm}^2/\text{s}$. Fiber indices units are as follows: length (mm), density (number of fibers within ROI per pixel), and angle in degrees.