Quantitative diffusion tensor MRI-based fiber tracking of human skeletal muscle

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1Institute of Imaging Science, 2Department of Biomedical Engineering, 3Department of Radiology and Radiological Sciences, 4Department of Electrical and Computer Engineering, 5Interdisciplinary Graduate Program in the Biomedical Sciences, and 6Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee

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Lansdown DA, Ding Z, Wadington M, Hornberger JL, Damon BM. Quantitative diffusion tensor MRI-based fiber tracking of human skeletal muscle. J Appl Physiol 103: 673–681, 2007. First published April 19, 2007; doi:10.1152/japplphysiol.00290.2007.—Diffusion-tensor magnetic resonance imaging (DT-MRI) offers great potential for understanding structure-function relationships in human skeletal muscles. The purposes of this study were to demonstrate the feasibility of using in vivo human DT-MRI fiber tracking data for making pennation angle measurements and to test the hypothesis that heterogeneity in the orientation of the tibialis anterior (TA) muscle’s aponeurosis would lead to heterogeneity in pennation angle. Eight healthy subjects (5 male) were studied. T1-weighted anatomical MRI and DT-MRI data were acquired of the TA muscle. Fibers were tracked from the TA’s aponeurosis by following the principal eigenvector. The orientations of the aponeurosis and muscle fiber tracts in the laboratory frame of reference and the orientation of the fiber tracts with respect to the aponeurosis were determined. The muscle fiber orientations, when expressed relative to the laboratory frame of reference, did not change as functions of superior-to-inferior position. The sagittal and coronal orientations of the aponeurosis did not change in practically significant manners either, but the aponeurosis’ axial orientation changed by ~40°. As a result, the mean value for the pennation angle (θ) was decreased from 16.3 (SD 6.9°) to 11.4° (SD 5.0°) along the muscle’s superior-to-inferior direction. The mean value of θ was greater in the deep than in the superficial compartment. We conclude that pennation angle measurements of human muscle made using DT-MRI muscle fiber tracking are feasible and reveal that in the foot-head direction, there is heterogeneity in the pennation properties of the human TA muscle.

human; dorsiflexors; biomechanics

THE GEOMETRIC ARRANGEMENT of fibers within a muscle influences the muscle’s maximum force production and shortening velocity values. This is most clearly seen in pennate muscles, in which the fibers insert into an aponeurosis at an oblique angle. This packing arrangement increases the number of sarcomeres in parallel, increasing force production. Indeed, the physiological cross-sectional area (PCSA), which accounts for pennation, is a better predictor of muscle force than the anatomical cross-sectional area (15). However, the increase in force production occurs at the expense of shortening velocity, because a component of the shortening occurs orthogonally to the muscle’s long axis.

For many years, the only technique available for measuring pennation was direct measurement of cadaver specimens (42). This method was subject to errors from fixation artifacts and the likelihood that the subjects were unrepresentative with regard to age and health status. Subsequently, brightness-mode ultrasound (US) was introduced as a method for measuring pennation angles in vivo (38). These measurements are noninvasive, can be performed in real time, and are well suited to studying both isometric and dynamic contractions. These studies have provided quantitative information about muscle-tendon kinematics during contraction, revealing that tendons and aponeuroses elongate, fascicles shorten, and pennation angles increase during isometric contractions (13, 29). Other studies have demonstrated that pennation is a muscle architectural parameter that is modifiable by training (23, 24) and perhaps also by disuse (26, 33).

One limitation of US-based pennation measurements is that in some muscles pennation angles are heterogeneous (1, 7, 21, 40), but US measurements are localized to one site at a time. Also, almost all of the applications of US to study pennation so far have used two-dimensional (2D) imaging. A notable exception is a preliminary report describing the use of three-dimensional (3D) US to identify, in three subjects, pennation heterogeneity in the tibialis anterior (TA) muscle, with values ranging from ~15° to ~7° at the distal extent (21).

A more accurate representation of a muscle’s architecture than that provided by 2D methods could potentially be found with the 3D fiber reconstruction attainable using diffusion-tensor magnetic resonance imaging (DT-MRI). DT-MRI is based on the correspondence between the principal direction of water diffusion and the local cellular geometry in tissues such as skeletal (6, 7, 20) and cardiac (36) muscle and the white matter tracts of the central nervous system (3). By measuring diffusion in six or more noncollinear directions, this diffusion can be described using a tensor model. The tensor has three eigenvalues, which describe the magnitude of the diffusion coefficient in three orthogonal directions, and three eigenvectors, which specify those directions. The eigenvector corresponding to the largest eigenvalue is coincident with the longitudinal axis of the cell, and by following the direction of greatest diffusion and adding points at regular intervals (e.g., one imaging voxel width), the local fiber trajectories can be reconstructed.

In 2002, we (7) demonstrated that DT-MRI-based tracking of skeletal muscle fibers is feasible and showed that the...
MUSCLE PENNATION MEASURED WITH DT-MRI

Innovative Methodology

penetration angle measurements obtained using DT-MRI fiber tracking agree with those made using direct anatomical inspection. Heemskerk et al. (19) used DT-MRI fiber tracking of mouse muscle to measure the PCSA, penetration angle, and fiber length directly (19) and showed subsequently that these measurements change in the expected manner when the foot angle is changed (18). Recently, it has been demonstrated that DT-MRI fiber tracking is feasible in human muscle studies as well (41). DT-MRI may additionally be useful for studies of muscle injury (17, 44) and muscle microarchitecture, with potential sensitivity to such parameters as fiber diameter (16, 39).

While the use of DT-MRI to study heterogeneous patterns of human muscle architecture and their impact on muscle mechanics would greatly improve our understanding of muscle structure-function relationships in health, aging, and disease, there are a number of practical complications to performing these studies. These include the short transverse relaxation time constant (T2) of muscle (~40 ms at 3T; unpublished observations), describing a rapid signal decay that lowers the signal-to-noise ratio (SNR) and limits the amount of diffusion weighting that can be obtained within a reasonable echo time; the radiofrequency (RF) coil length and the size of the static magnetic field isocenter (both smaller than a typical limb muscle); limitations imposed by planar region-of-interest (ROI) definition as the seed surface for fiber tracking; and imaging artifacts caused by limb motion, chemical shift artifact, and spatial distortions in methods such as echo-planar imaging. In this work, we present an image acquisition and analysis strategy that overcomes these limitations and apply it to study the architecture of tibialis anterior (TA) muscle. We used this method to test the hypotheses that pennation angles within the TA muscle are spatially heterogeneous and that this heterogeneity arises from variations in the orientation of the TA’s central aponeurosis in the axial plane.

METHODS

Subjects

Eight apparently healthy subjects (5 male), height 177.4 cm (SD 7.4), mass 73.3 kg (SD 14.3), and age 23.0 yr (SD 2.7), volunteered to participate in the study. By self-report, seven of the subjects were right leg dominant. All procedures were approved by the Vanderbilt University Institutional Review Board, and written informed consent of the risks, benefits, and procedures was obtained from each subject prior to participation.

MRI Data Acquisition

The subjects lay supine in a 3T Philips Intera Achieva MR Imager with the dominant foot strapped into a custom-built exerciser. Initially, the inferior portion of the leg was placed into a rigid ~16 cm, six-element phased array inner diameter knee coil. Foam inserts placed inside the coil and the fixation of the foot to the exercise device both served to reduce motion artifacts. The knee coil was used to increase the SNR of the images, but its length prohibited imaging all of the TA muscle in a single acquisition. Therefore anatomical imaging was performed using two overlapping slice packets (superior and inferior; Fig. 1). To coregister the two data sets in postprocessing, MnCl2 capsules (1 cm inner diameter, 3 cm length) were fixed to the skin and used as fiducial markers. One marker each was placed at the superior and inferior ends of the muscle, as identified by an initial visual inspection and palpation during a light contraction, and three markers were placed at the midpoint of the muscle (Figs. 1 and 2). Because these markers were larger than the voxel dimensions of the anatomical images (see below), ambiguity concerning the marker location was reduced. For the DT-MRI acquisitions, the superior and inferior sections were further divided into two equal and contiguous halves (Fig. 1).

Prior to all acquisitions, the static field homogeneity was optimized in the volume of interest using an automated, second-order shimming routine. Sixty fast spin-echo T1-weighted anatomical images, covering the inferior section of the muscle, were obtained with 2.5-mm-thick axial slices; inter-slice gap = 0; field of view (FOV) = 18 × 18 cm repetition time (TR)/echo time (TE) = 500/16 ms; echo train length = 3; an acquired matrix of 256 × 256 (reconstructed at 512 × 512); and number of excitations (NEX) = 2. Then DT-MRI images were obtained in two separate acquisitions, which together coincided with the inferior anatomical imaging segment. For each acquisition, ten 7.5-mm-thick slices were obtained using TR/TE = 5,000/42 ms, FOV = 18 × 18 cm, acquired matrix = 64 × 64 (reconstructed at 128 × 128), inter-slice gap = 0, a 60.3% partial Fourier acquisition, and NEX = 2. Fat suppression was performed using a 200-Hz spectral width, spectrally selective adiabatic inversion recovery (SPAIR) pulse applied 160 ms prior to the excitation pulse. The phase encoding gradient was set so as to shift residual chemical shift artifact by ~10 pixels anteriorly, so that it did not interfere with the fiber tracking procedures. The 160-ms inversion time and the 200-Hz spectral width were optimized in pilot studies so as to provide a maximum contrast-to-noise ratio between the water and residual chemical shift artifact. A non-diffusion-weighted image plus six diffusion-weighted images (with weighting equal to 500 s/mm2 applied along the X, Y, Z, XY, XZ, and YZ directions) were obtained. The coil was then repositioned, taking care to hold the leg as still as possible, and the procedures were repeated in the superior segment.

MRI Data Analysis

Calculation of the diffusion tensor and derived measures. Initial processing of the DT-MRI images involved distortion correction and calculation of the diffusion tensor and derived measures. First, the Philips Research Imaging Development Environment (PRIDE) Diffusion Registration tool (version 0.4) was used to correct spatial distortions in the diffusion-weighted images by registering them to their corresponding non-diffusion-weighted images using an affine

![Fig. 1. Placement of slice packets in anatomical image and diffusion-tensor magnetic resonance imaging (DT-MRI) acquisitions. The shaded oval represents the position of the tibialis anterior (TA) muscle, marked proximally, distally, and at the midpoint by fiducial markers (black rectangles). The black lines and arrows represent image acquisitions over the superior segment of the muscle and the shaded lines and arrows represent image acquisition over the inferior segment. Two anatomical image slice packets, overlapping at the middle set of fiducial markers and indicated by the lines and arrows above the leg, were obtained. Four DT-MRI slice packets, adjacent within each anatomical image segment and overlapping at the fiducial markers, were obtained and are indicated below the leg.](image_url)
transformation. Then version 6.0a1 of the PRIDE Fiber Tracking tool was used to calculate, for each voxel in the DT-MRI data sets, the diffusion tensor and fractional anisotropy.

**Registration of image data sets.** These and all subsequently described image processing procedures were performed using Matlab v. 7.01 (The Mathworks, Natick, MA). By identifying the middle set of MnCl₂ markers in both sets of images, we calculated the coregistration in the superior-inferior, left-right, and anterior-posterior directions, yielding a single anatomical data set. We expect that this approach would produce a fiducial registration error of one-half the in-plane and through-plane resolutions of the anatomical images (0.35×0.35 mm and 2.5 mm, respectively). No rotation of the image sets with respect to each other was observed. To assemble the DT-MRI data sets, a single inferior slice packet was formed from the two most inferior DT-MRI data acquisitions. A superior DT-MRI segment was formed in a likewise manner. Finally, a single DT-MRI data set was created using the coregistration calculated for the anatomical data sets, adjusting appropriately for the differences in slice thickness and matrix size.

**Mask formation.** To provide a stop criterion for fiber tracking, a mask was formed around the TA muscle. To do so, a semi-automated boundary-finding algorithm was implemented as previously described (8). Briefly, this algorithm requires the user to initially hand define an ROI in the first of a series of slices. This ROI is then used as a prior shape definition in the next slice. By using this initial shape guess and applying a smoothness constraint, the ROI is deformed until an intensity-gradient-based cost function is minimized. The user then hand corrects the control points as necessary, and 200 points between each pair of successive control points are smoothly interpolated using a Catmull-ROM spline routine (32).

**DT-MRI fiber tracking procedures.** To define a suitable seed surface for fiber tracking and to enable pennation angle measurements based on the fiber tract data, the aponeurosis dividing the two superficial and deep compartments of the muscle was identified and digitized in the anatomical images. Five to ten points were used to define the aponeurosis in each slice. A 3D mesh with 300 rows and 100 columns was constructed by smoothly interpolating the in-plane and through-plane positions of the points in each row and each column of the mesh (Fig. 2).

To initiate fiber tracking, the mesh was shifted by +1 voxel width into the deep compartment. Fibers were tracked beginning at each point of intersection along the mesh in the direction of the principal eigenvector, at 1-voxel-width increments. A fiber stopped tracking if it met one of three conditions: a fractional anisotropy value <0.1 or >0.5, an orientation change between points >90 degrees, or an attempt to track outside of the defined mask. Then this procedure was repeated in the superficial compartment by shifting the position of the mesh by ~1 voxel width.

**Pennation, Fiber Orientation, and Aponeurosis Orientation Measurements**

**Pennation angle measurements.** We calculate the pennation angle, θ, as the angle between any point \( p \) on the fiber tract and the plane tangent to the seed point \( s \) from which that fiber tract emerges. For each row in the mesh, the in-plane \( Y \) positions were fit as a function of the in-plane \( X \) positions to a 14th-order polynomial (which was the minimum order necessary to avoid structured residuals in all slices). For each column in the mesh, the through-plane \( Y \) positions were fit as a function of the through-plane \( Z \) positions to a third-order polynomial. With the use of these curves, a tangent plane and its normal unit vector \( \hat{n} \) were defined. \( \theta \) was calculated as \( \theta = \arcsin(r \cdot \hat{n}) \), where \( r \) is the direction vector between \( s \) and \( p \). Figure 3 depicts this measurement graphically. The mean value of \( \theta \) along the entire fiber tract was calculated. This analysis was repeated for each fiber tract in the superficial and deep compartments.

**Fiber and aponeurosis orientation in the laboratory frame of reference.** To determine the source(s) of heterogeneity in pennation (see below), the muscle fiber and aponeurosis orientations were also characterized in the laboratory frame of reference. To determine the muscle fiber orientations, identical procedures to those used to measure the pennation angle were employed, except that the projections of the position vector \( r \) in the \( XY \) plane (corresponding approximately to the axial anatomical plane), \( XZ \) (coronal) plane, and \( YZ \) (sagittal) plane were determined rather than the orientation with respect to the aponeurosis. The calculations were made by converting the \((x,y),(x,z),\) and \((y,z)\) Cartesian coordinates to polar angles. To determine the aponeurosis orientations, direction vectors \((v)\) were defined between each successive pair of points in each mesh row. The projections of each vector in the \( XY \) plane, the \( YZ \) plane, and the \( XZ \) plane were calculated in a similar manner to that described for the fiber orientations. The frame of reference for these measurements is illustrated in Fig. 2. These analyses were performed for the seven right leg-dominant subjects only.

**Statistical Analysis**

Statistical analysis was performed using SPSS v. 15 (SPSS, Chicago, IL) using discrete characterizations of the muscle’s aponeurosis.

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**Fig. 2.** A: sample high-resolution anatomical image from mid-portion of the muscle, indicating the points used to digitize the location of the TA central aponeurosis. The middle set of fiducial markers is also seen. B: sample high-resolution anatomical image from the distal portion of the muscle. The local direction vector (red arrow) and the frame of reference for the fiber and aponeurosis orientations are indicated, with the \(+Z\)-axis emerging from the plane of the image. Note that the images in A and B have been individually sized to maximize detail. C: aponeurosis mesh from this subject generated at reduced density (40 rows × 20 columns). The points of intersection along the mesh were used as the seed points for fibers.
orientation, fiber orientation, and pennation properties. For each analysis, the aponeurosis was divided into five equal-length vertical segments. The mean values for aponeurosis orientations in the $XY$, $XZ$, and $YZ$ planes were compared using the General Linear Model (GLM) with repeated measures on the aponeurosis Location. The mean $XY$, $XZ$, and $YZ$ muscle fiber orientations were calculated in each vertical segment, separately for the two muscle compartments. Also, the mean pennation angle was calculated in the deep and superficial compartments of each muscle segment. For each variable, the mean values were compared using the GLM (compartment > location, with repeated measures on each factor). When required, multiple comparisons were made using 95% confidence intervals adjusted using a Bonferroni procedure. Three measurements were used to calculate the overall pennation properties of each compartment: the arithmetic mean, the weighted mean (weighted by the relative aponeurosis area corresponding to the fiber tract), and the value for $\theta$ at the muscle’s maximum cross-sectional area. The mean compartmental values were compared as part of the GLM procedure (arithmetic mean) or by Student’s $t$-test. Descriptive statistics include the mean and standard deviation (SD). Statistical significance was accepted at $P < 0.05$.

RESULTS

DT-MRI Fiber Tracking

Figure 4 shows sample fiber tracking results for the same subject whose mesh definition was depicted in Fig. 2. Figure 4A shows the DT-MRI fiber tracts of the deep compartment, illustrated as gold lines; Fig. 4B illustrates the DT-MRI fiber tracts of the superficial compartment as green lines. The color variations among the fiber tracts exist only to provide contrast. Taken together, the anatomical images, fiber tracts, and mesh illustrate the bipennate arrangement of the TA’s fibers, as it is seen that the fiber tracts attach at oblique angles to the aponeurosis and outward and superiorly from the aponeurosis. Note that in this case, there are several areas without tracked fibers, probable reasons for which are provided in the DISCUSSION.

Fiber and Aponeurosis Orientations—Laboratory Frame of Reference

The $XY$, $XZ$, and $YZ$ muscle fiber orientations are plotted as functions of superior-inferior muscle location for the deep and superficial compartments in Figs. 5, A and B, respectively. There are no significant differences in muscle fiber orientation as a function of superior-inferior position in either compartment. Data for the aponeurosis are illustrated in Fig. 5C. It is
directed anteriorly and medially throughout its superior-inferior extent and these orientations do not change in a practically significant manner. However, its axial orientation shifts significantly from being oriented anteromedially at its most superior location to being located almost entirely in the coronal plane at its most inferior locations.

**Pennation Angle Measurements**

Figure 6 contains a map of the spatial distribution of $\theta$ for the subject whose fiber tracking data were shown in Fig. 4. The distributions are plotted separately for the deep and superficial faces of the TA’s central aponeurosis. Figure 5D characterizes the group mean $\theta$ data at five locations along the aponeurosis and reveal two forms of pennation heterogeneity. The first was between compartments and, as summarized in Table 1, was detected only when the pennation properties of the entire muscle were considered: the arithmetic mean value of $\theta$ (GLM main effect for compartment, $P = 0.011$) and weighted mean value of $\theta$ (paired Student’s t-test, $P = 0.044$) were significantly different between the two compartments, whereas the mean compartmental values of $\theta$ at the muscle’s maximum cross-sectional area (paired Student’s t-test, $P = 0.058$) were not significantly different. Also, the GLM revealed a significant main effect for location (GLM main effect, $P = 0.04$); as indicated in Fig. 5D, $\theta$ tended to decrease in the more distal portions of the muscle. The compartment×location interaction was not significant, indicating a similar tendency in both compartments.

**DISCUSSION**

In this work we described quantitative DT-MRI fiber tracking human muscle, including measurements of the pennation angle. These measurements confirm the preliminary report of Hiblar et al. (21) concerning the magnitude and heterogeneous nature of $\theta$ in the human TA muscle. They concur with previous ultrasound data that indicated that $\theta$ is similar in the two compartments at the muscle’s maximum cross-sectional area (29). They also indicate that the values differ between compartments when the entire muscle’s pennation properties are considered. More generally, the image acquisition and analysis strategy that we presented here will allow characterization of the pennation properties of in vivo human muscle in a variety of healthy and clinical states. Moreover, the fiber tractography data can provide the basis for developing a detailed understanding of the relationships among muscle structure, stress-strain distributions, perfusion, and metabolism.
Technical Challenges to Muscle DT-MRI Overcome

As discussed above, implementing DT-MRI in human muscle physiology studies has been hindered by a number of technical challenges. In this study, problems related to the short $T_2$ of muscle were addressed by using the small diameter knee coil and gradients capable of achieving strengths of 80 mT/m, allowing us to obtain sufficient diffusion weighting at short TE. Limb motion was limited by using foam inserts placed within the coil and by fixing the foot to the exercise apparatus. Problems associated with the restricted RF coil and static field homogeneity geometries were overcome by using restricted-length slice packets, reassembled using coregistration of fiducial markers in postprocessing. Imaging artifacts such as image distortions and chemical shift artifact were reduced by using restricted-length slice packets for the DT-MRI scans, by optimizing shimming and fat suppression options, and by correcting distortions in the diffusion-weighted images in postprocessing. Finally, a novel definition of the seed surface for fiber tracking was introduced. These developments enabled us to appreciate the architecture of the TA muscle-tendon unit at very high spatial resolution, in three dimensions.

The Three-Dimensional Nature of Muscle Fiber Architecture

An activated muscle fiber exerts a fiber type-specific force proportional to its cross-sectional area onto the aponeurosis of insertion (4, 35). The amount of force applied along the muscle’s line of action depends on the relative orientations of the fibers and aponeurosis, with the result that quantitatively accounting for these orientations yields improved force predictions (14, 35). It is obvious but important to note that both the fibers’ and aponeurosis’ spatial positions vary in three dimensions. The result of this is that the pennation angle is fully described only when the measurement technique is sensitive to both of its components: the elevation angle (the angle made by the longitudinal component of the fiber and aponeurosis positions) and the azimuthal angle (the relative orientations of the fibers and the transverse axis of the aponeurosis).

To be sensitive to both $\lambda$ and $\phi$, a technique must have depth sensitivity and a consistent frame of reference. Two-dimensional measurement techniques, such as planar US, offer the advantage of a short acquisition time, making it suitable for studies of contracting muscles (13, 29). However, there is a narrow axial resolution for planar US (0.47 mm at 5 MHz; (5)).

Table 1. Pennation properties of the tibialis anterior muscle characterized using three single-site measurements

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Arithmetic Mean, °</th>
<th>Weighted Mean, °</th>
<th>Maximum CSA, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>12.0 (6.0)</td>
<td>9.5 (3.8)</td>
<td>16.3 (8.6)</td>
</tr>
<tr>
<td>Deep</td>
<td>12.8*(6.1)</td>
<td>11.9*(4.3)</td>
<td>18.4*(8.9)</td>
</tr>
</tbody>
</table>

Values are means (SD). CSA, cross-sectional area. *Significant differences ($P < 0.05$) between the mean values for the deep and superficial compartments.
recently for fascicle length measurements and, consistent with the limitations of narrow axial resolution just discussed, was found to result in greater measured fascicle lengths than 2D US measurements (27). Following correction for probe rotations, 3D US was also used in a recent preliminary report to measure θ in the TA muscle, with the finding that θ decreases from ~15 to ~7° along the superior-to-inferior direction of the muscle (21).

The θ measurements from the quantitative DT-MRI technique agree with this finding. In addition, we explain the source of this heterogeneity. Figure 5 showed that the muscle fiber orientations in the laboratory frame of reference do not vary at all as functions of superior-to-inferior position; a similar observation was reported by Sinha et al. (41) for human calf muscles. Also, whereas the coronal and sagittal orientations of the aponeurosis do not change in a spatially heterogeneous manner as functions of superior-to-inferior position, the axial orientation of the aponeurosis changes considerably (~40°). Through exclusion, it can only be the changes in axially aponeurosis orientation that cause the observed heterogeneity in θ.

The overall picture that emerges is that the muscle fibers are vertically stacked on top of one another, inserting into a structure whose axial orientation changes dramatically. The result is that λ is constant and that φ and θ change in the more distal locations of the muscle.

Moreover, the technique builds on the qualitative demonstration of the feasibility of DT-MRI fiber tracking of human skeletal muscle recently presented by Sinha et al. (41). A key component of the method presented here is the definition of the aponeurosis as the seed surface for fiber tracking. As noted by Heemskerk et al. (19), multiple planar regions of interest must be specified to capture all of a pennate muscle’s fiber tracts. This is problematic because the two fiber tractography data sets will not overlap perfectly, and as a consequence it is not possible to synthesize the two fiber tractography data sets without generating redundant information in some portions of the muscle. The red and green fiber tracts shown in Fig. 5C of Heemskerk et al. (19), which were generated from two separate planar ROI definitions, illustrate this point. The use of the 3D mesh representation of the aponeurosis enables fiber tracking of the entire muscle from a single region of interest, avoiding the redundancy issued described above. It also provides a salient reference structure for the architectural measurements and thus allows the quantitative characterization of pennation in an automated, objective manner (i.e., without manual specification of tangent lines).

The result is a single data set containing a highly spatially resolved description of the muscle’s architectural properties. This anatomical data set can serve as a scaffold on which other mechanical and physiological information can be overlaid and the relationships among a muscle’s anatomical, mechanical, and physiological properties understood. For example, a number of functional aspects of triceps surae muscle contraction have been shown to be spatially heterogeneous, including strain (11, 12, 22, 28), pennation angle changes (31), and hemodynamic and metabolic responses (31, 37). The dorsiflexors have not been studied as extensively, but we have observed regional heterogeneities in the T2-weighted signal intensity time course within the TA muscle and between the TA and the extensor digitorum longus muscles that are consistent with mechanical, hemodynamic, and/or metabolic heterogeneity as well (9). These heterogeneities may result from regional variations in motor unit activation due to neuromuscular compartmentation (10, 43), fascicle curvature (25, 34), or through regional differences in pennation (1, 22; present study). Spatial heterogeneity in pennation could influence the muscle’s mechanical behavior (by affecting the principal direction and amount of strain) and regional perfusion values (through the generation of intramuscular pressure gradients). Moreover, this spatial heterogeneity would be expected to be further modified downstream by the nonuniform material properties of the tendon (30). Particularly when combined with other functional information obtainable through MRI and MR spectroscopic imaging, DT-MRI fiber tracking’s ability to recognize and quantify the 3D aspects of muscle-tendon architecture represents a significant new tool for relating a muscle’s architecture to its mechanical and physiological behavior.

**Muscle DT-MRI: Remaining Challenges**

While DT-MRI represents an important new tool for in vivo structural analysis of muscles, a close examination of the fiber tracts shown in Fig. 4 reveals that there are several technical obstacles that remain to be resolved. The fiber tracts exhibit some tortuosity, which probably resulted from noise effects. In the present study, we reduced their influence by taking the mean pennation angle for the entire fiber tract, but for studies such as those suggested above, additional signal-to-noise ratio improvements would be required. The outer portions of the muscle seemed to lack fiber tracts in this subject, and some portions of the aponeurosis also appeared to be devoid of fiber tracts entirely. The most likely explanation for both of these results is premature stopping of the fiber tracking procedure due to the muscle’s anatomical features or the user-selected stop criteria. Although this result was our worst (rather than a typical) fiber tracking result, it was selected for display because it highlights a difficulty with the tensor model of diffusion, which is that it can only recognize a single principal axis of diffusion within a voxel. While multiple muscle fiber orientations within individual muscles are not expected to exist, particularly given the findings presented in Fig. 5, A and B, an under-specification of diffusion properties would result when there are both muscle fibers and intramuscular fat contained in a voxel. When the amount of intramuscular fat is large, there would potentially be sufficiently isotropic diffusion that there would be an early termination of the fiber tracking procedure, leaving succeeding portions of the muscle unaccounted for. A useful approach for addressing this issue in future studies would be to use high-angular resolution diffusion imaging (2), which uses more complex models of diffusion to allow identification of multiple diffusion components. In particular, this approach is probably necessary for studies of aging or diseased muscle, where larger amounts of noncontractile tissue are expected.

**Conclusions**

We presented a strategy for the acquisition and analysis of anatomical and DT-MRI images, enabling the highly spatially resolved characterization of the architecture of human muscles. These data agree with previous 3D imaging-based characterizations of the pennation properties of this muscle. Although the amount of pennation heterogeneity observed for this muscle was small, the method itself is applicable to a wide range of...
human muscles, including those in which pennation angles are considerably larger. DT-MRI fiber tracts may also form an architectural scaffold on which other functional properties of muscles can be overlaid and understood relative to the local muscle architecture. DT-MRI is therefore an important tool for muscle architectural research in health and disease, capable of providing insights into muscle structure-function relationships not available through other noninvasive measurement techniques.

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