3D fascicle orientations in triceps surae

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Rana M, Hamarneh G, Wakeling JM. 3D fascicle orientations in triceps surae. J Appl Physiol 115: 116–125, 2013. First published May 2, 2013; doi:10.1152/japplphysiol.01090.2012.—The aim of this study was to determine the three-dimensional (3D) muscle fascicle architecture in human triceps surae muscles at different contraction levels and muscle lengths. Six male subjects were tested for three contraction levels (0, 30, and 60% of maximal voluntary contraction) and four ankle angles (−15, 0, 15, and 30° of plantar flexion), and the muscles were imaged with B-mode ultrasound coupled to 3D position sensors. 3D fascicle orientations were represented in terms of pennation angle relative to the major axis of the muscle and azimuthal angle (a new architectural parameter introduced in this study representing the radial angle around the major axis). 3D orientations of the fascicles, and the sheets along which they lie, were regionalized in all the three muscles (medial and lateral gastrocnemius and the soleus) and changed significantly with contraction level and ankle angle. Changes in the azimuthal angle were of similar magnitude to the changes in pennation angle. The 3D information was used for an error analysis to determine the errors in predictions of pennation that would occur in purely two-dimensional studies. A comparison was made for assessing pennation in the same plane for different contraction levels, or for adjusting the scanning plane orientation for different contractions: there was no significant difference between the two simulated scanning conditions for the gastrocnemii; however, a significant difference of 4.5° was obtained for the soleus. Correct probe orientation is thus more critical during estimations of pennation for the soleus than the gastrocnemius due to its more complex fascicle arrangement.

fascicle sheet orientation; 2D ultrasound; muscle force-length interaction

Pennation angle of muscle fascicles is an important structural parameter that is related to the muscle function. The pennation allows fascicles to shorten at velocities less than that of the muscle belly in a process known as gearing (3), it determines the component of muscle fascicle force that acts in the line of action of the muscle (35), and it has been used to calculate the physiological cross-sectional area that is an indication of the muscle strength (26, 35). Pennation angles are commonly defined as the angle between the fascicles and the aponeurosis (7, 11, 49) and depend on the muscle length and force (11, 29, 34). In vivo muscle fascicle architecture has been extensively studied using B-mode ultrasound in two dimensions (2D); however, a large range of pennation angles has been reported for the triceps surae muscles. This may partially be due to differences in the position and orientation of the ultrasound probes used for detection. Regional measures of three-dimensional (3D) orientations of the muscle fascicles will help to standardize the measures of the fascicle orientations; however, such data have not previously been reported for contracting muscle.

Muscle fascicles have been considered to be arranged in 2D fascicle planes in the previous architectural studies (21, 25, 29, 45, 46). In previous 2D ultrasound studies, it has been important to align the imaging plane with the fascicle planes, to image complete fascicles (5, 20, 24, 27). However, muscle fascicles may not be aligned in planes (42), particularly in muscles with a nonuniform shape. Instead, fascicles may lie along curved surfaces in 3D space. This idea is supported by the observation of fascicles arranged in curved “fascicle sheets” in human vastus lateralis (42), where it was suggested that the fascicle sheets were arranged like the layers of an onion. During contraction, bulging of the muscle in a direction that is perpendicular to the fascicle sheets may result in bulging of those sheets, and thus local changes in the orientations of the fascicle sheets. With the arrangement of fascicles in curved sheets, the orientations of fascicles may not lie in one plane and, therefore, cannot be fully explained in 2D. A few diffusion tensor magnetic resonance imaging and 3D ultrasound studies have examined the in vivo fascicle orientation in 3D. Diffusion tensor magnetic resonance imaging studies focused on the measurement of the pennation angle from the fascicles tracked in passive muscle (18, 23, 43), and 3D ultrasound studies quantified fascicle architecture from a few fascicles selected in passive muscle (4, 9, 10, 22, 31). None of these earlier studies have described a complete set of in vivo fascicle orientations in 3D for different contraction levels and muscle lengths. A purpose of this study was to quantify the 3D fascicle orientation and orientation of fascicle planes across the triceps surae muscles in humans using 3D ultrasonography.

Numerous studies on muscle architecture and function have used 2D ultrasound to study the in vivo changes in fascicle length, pennation angles, and curvatures (11, 20, 25, 29, 32, 33, 50). To obtain accurate measures of pennation angle and fascicle lengths from 2D ultrasound studies, it is important to match the orientation of the scanning plane with the fascicle planes (20, 24). A typical way of achieving this is to place the probe perpendicular to the skin and find the fascicle plane orientation by rotating the probe until the imaged fascicles appear continuous between aponeuroses (5). It was hypothesized that the orientation of the scanning plane relative to the muscle fascicles will affect the calculated fascicle orientations and pennation. A purpose of this study was to compare the estimated pennation angles that would be calculated using 2D vs. 3D methods.

Muscle is a 3D entity with varying shape across the length of the muscle (14) and changes shape in the form of muscle bulging. Both experimental (29, 48) and modeling (3, 24, 35, 45) studies have shown that muscle thickness depends on the contraction level. However, increases in thickness do not occur in all muscles: for example the lateral gastrocnemius (LG)
shows an increase up to 40% at maximal voluntary contraction (MVC), while the medial gastrocnemius (MG) does not increase in thickness at MVC (29, 34). Changes in muscle shape influence the orientation of the fascicles within the muscle (3, 48). In 2D ultrasound studies, muscle thickness is reported as the distance between aponeuroses in the 2D image plane (aligned with the fascicle plane). However, muscles can change shape in the direction perpendicular to the scanning plane, and this cannot be captured by 2D imaging modality. Muscle can bulge in the direction perpendicular to the fascicle plane, keeping the muscle thickness constant in the fascicle plane, despite the changes in muscle belly length. This out-of-plane bulging is predicted to counteract the increases in pennation that would otherwise occur as the muscle belly shortens (3). In addition to bulging, muscles are predicted to undergo 3D changes in shape involving twisting during shortening (6), and this may further affect the 3D fascicle orientation.

Examinations of 3D fascicle architecture in dissected rat soleus (44) and equine longissimus dorsi (40) have shown regional variations in the 3D architecture. Stark and Schilling tracked fascicles in 3D and reported local pennation angles as the angles of the fascicles with the muscle line of action (44), while Ritruechai and coworkers reported local orientations of the fascicles in perpendicular planes to obtain a 3D representation of fascicle orientations (40). A 3D study on formalin-fixed soleus muscle in humans has also shown regional variation in the muscle architecture (1). It was hypothesized that the 3D orientations of the fascicles would vary with changes to the muscle length and force, and these 3D orientations may also be regionalized within the muscle.

METHODS

Data collection and experimental design. The muscles were imaged in a relaxed state and during isometric torques at a range of ankle angles and torque levels. Experimental data were obtained from six male subjects (age 28.4 ± 6.2 yr, height 183.1 ± 8.9 cm, mass 79.9 ± 20.1 kg); subjects were athletic and able to maintain stable and constant ankle torques during imaging. Subjects gave written, informed consent that followed the guidelines of, and was approved by, the Simon Fraser University Office of Research Ethics.

The scanning process was identical to that used in our previous study (38) with ultrasound images obtained using a linear ultrasound probe (Echoblaster, Telemed, LT) recording at 20 Hz. A grid was drawn on the leg to facilitate the scanning of the whole triceps surae volume. The subjects knelt in a water tank while the muscles were imaged using a sweeping motion of the ultrasound probe across the right leg. 2D position and orientation information from ultrasound images were transformed to 3D information using the position and orientation of a 3D optical position sensor (Certus, Optotrak, NDI, Ontario, Canada) attached to the ultrasound probe (38). Subjects were asked to perform maximal voluntary plantar flexion contractions that were at fixed ankle angles (−15, 0, 15, and 30° with negative angles representing dorsiflexion and positive for plantar flexion), at a fixed knee angle of 135°, and at three torque levels (0, 30, and 60% MVC) that were relative to the MVC for each ankle angle. Data were not collected beyond 60% MVC, because the scanning times last for 2 min for each trial, and it is not possible to sustain higher torques for longer durations. The position of the medial and lateral tibial condyles and the medial and lateral malleoli were obtained using an optical pointer and later used to define the segmental coordinate system for the lower leg.

A custom-made frame was used to perform the plantar flexion contractions in the water tank (38). The frame had two parts: a foot plate to strap the right foot of the subject, and a leg support to support the right thigh and maintain a fixed knee angle throughout the experiment. The leg support could be moved in relation to the foot plate to adjust to different leg lengths of the subjects. The foot plate was connected to a strain gauge to measure the ankle torque, and visual feedback for torque was provided to the subjects. The ankle torque data were collected at 2,000 Hz via a 16-bit A/D converter (USB-6210, National Instruments, Austin, TX) using a LabView software environment (National Instruments, Austin, TX) and synchronized to the ultrasound images.

Determination of fascicle orientations. Images were processed using the methods described in our previous studies (37, 38) to obtain the muscle fascicle orientation in 3D. The region of interest (muscle region) was obtained by manually digitizing the muscle boundary in the images. The selected images were then processed using automated methods to determine the muscle fascicle orientations. In brief, 2D images obtained during the scanning process were filtered using multiscale vessel enhancement filtering followed by wavelet analysis to obtain the local 2D orientations of the muscle fascicles (37). Multiscale vessel enhancement filtering reduces any non-like structures in the image (8), and the presence of other line-like structures like blood vessels and nerves was avoided by manually selecting the images for fascicle orientation determinations. Polarized wavelets of 39 × 39 pixel dimensions were convolved with the local image region around each pixel to obtain a map of fascicle orientations across the whole image. 2D orientations in the image plane were combined with the 3D position and orientation of the ultrasound scanning plane to obtain the local 3D fascicle orientation corresponding to the respective pixels in 3D.

The representative muscle volume was divided into voxels of 5 × 5 × 5 mm³, and then a representative fascicle orientation was chosen for each voxel. During the scanning process, multiple scans of the calf muscles were obtained from different orientations of the ultrasound probe, resulting in imaged planes with different orientations of the same region (Fig. 1). A voxel in the 3D volume can contain multiple pixels of the 2D imaged planes. These pixels may belong to the same or different scanning planes. The representative orientation in a voxel was obtained from the weighted mean of the orientations from all of the pixels in that voxel. This step was slightly different from the previous work (38). Rather than taking the fascicle orientation from the pixel with the maximum convolution value from the wavelet analysis, a weighted mean of fascicle orientation was considered. The weights were based on the convolution values obtained from the wavelet analysis for a particular pixel and the distance of the pixel from the center of the voxel. The weight function for the convolution (w₁) and for the distance (w₂) were given by

![Fig. 1. Three-dimensional (3D) voxels in the scanned muscle volume can contain pixels from multiple two-dimensional (2D) images. Shown is the representation for one such 2D image plane intersecting a 3D voxel.](http://jap.physiology.org/DownloadedFrom)
where \( \text{conv} \) is the convolution value at a particular pixel, \( \text{conv}_0 \) is the maximum convolution value over all the trials for a subject, \( [x,y,z] \) is the 3D location of the pixel center, \( [x_0,y_0,z_0] \) is the voxel center, \( \sigma \) is the spread of an isotropic Gaussian distribution and was chosen to be 2.5 mm in this case (half-width of the isotropic voxel), and \( \theta \) is the weighted mean at pixel center.

**Determination of fascicle sheets orientations.** The orientations of fascicle sheets were represented by the normals to local regions within those sheets. Ultrasound images represent fascicle planes when the fascicles appear as long continuous curvilinear structures in the images. An image with the continuous fascicular structure gives a high convolution number during wavelet analysis. This quality was used to select the fascicle plane orientation in each voxel. Analogous to the fascicle orientation, the representative orientation for each voxel was obtained as the weighted mean of the orientation of the normals to the fascicle planes lying in that region, with the convolution value being the weighting factor. The planes were defined to have constant orientation in a voxel region, so the weight factor was only based on the convolution value and not on the distance.

**Determination of muscle-based coordinate system.** To study the regionalization of the orientations, the 3D position for each voxel, the muscle fascicle orientations, and the fascicle plane orientations were all transformed from the laboratory-based coordinate system to the muscle-based coordinate system as follows. Three major axes, \( x, y, \) and \( z \), were determined for the gastrocnemius muscles using eigenvalue decomposition of the points inside the muscle volume. The major axes correspond to the major anatomical axes of the muscle: the \( z \)-axis lies the major (longitudinal) axis of the muscle, the \( y \)-axis lies across the width of the muscle (medial-lateral axis), and the \( x \)-axis lies across the depth (deep-superficial axis) of the muscle (Fig. 2). The origin of the muscle-based coordinate system was set at the mean point in the muscle. Due to the semi-cylindrical shape of the soleus, a different coordinate system was used with its \( z \)-axis as the vector joining the mean coordinate of the knee joint centers with the mean coordinate of the muscle-tendon junction markers, the \( y \)-axis along the width of the muscle, and the \( x \)-axis along the depth of the muscle. The origin of the soleus was taken to be 60% of the total distance between the knee and the muscle tendon junction from the knee, because this approximated the center of the muscle.

Both the local fascicle orientations and the local fascicle plane orientations were transformed from a Cartesian to a spherical coordinate system. The 3D orientations that were represented by direction cosines \( [\delta x, \delta y, \delta z] \) in Cartesian coordinates were transformed to a polar angle \( \beta_p \) that was the angle between the vector parallel to the local fascicle direction and the \( z \)-axis, and an azimuthal angle \( \varphi_p \) that was the angle between the projection of fascicle in the \( x-y \) plane (transverse plane for the muscle) and the \( x \)-axis. The polar angle \( \beta_p \) can be considered as the pennation angle for a local segment of the muscle fascicles.

The orientations of the normals to the fascicle sheets were similarly represented by \( \beta_n \) and \( \varphi_n \). A 90° value for \( \beta_n \) and \( \varphi_n \) represents a plane parallel to the \( y = 0 \) plane, is parallel to the long axis of the muscle, and perpendicular to the width of the muscle. Any change in \( \beta_n \) represents the change in rotation of the plane about the long axis of the muscle, and a change in \( \varphi_n \) represents the tilting of the plane.

**Pennation angle representation.** The position and orientation of the ultrasound probe can influence the measured 2D pennation angle. Any change in the position and orientation of the ultrasound probe with respect to the muscle may alter the measured values. In this study, the 3D orientations of fascicles and fascicle sheets were used to simulate the effect of 2D ultrasound scans along different directions to compare the pennation angles that would be obtained by different scanning protocols. Ultrasound takes a slice through 3D objects, and the structures appearing in the slice represent sections through the 3D object. If ultrasound scans an infinitesimally thin wire, the image will contain a line when the scanning plane contains the wire (or a portion of it) and is parallel to the plane containing the wire. For any other orientation, a point will appear at the intersection of the imaging plane with the wire. If the wire has a finite thickness, it can be considered as a thin cylinder. When scanned in a plane parallel to the plane containing the long axis of the cylinder, the 3D cylinder will be imaged as a 2D line of certain thickness parallel to the long axis of the cylinder. For any difference between the orientation of the plane containing the longitudinal axis of the cylinder and the scanning plane, the cylinder will be imaged as an elliptical structure, with the major axis inclined to the longitudinal axis of the cylinder. The elliptical structure will line like small deviations, and the length of the imaged section will decrease for larger deviations.

Ultrasound images of muscle contain fascicular structures with finite thickness. The thickness of the fascicles was up to 5 pixels and hence can be considered as small cylinders in each voxel, and the local regions in an image can be considered as the slices of the fascicles in the image plane. The locally sliced fascicles are 2D representations of 3D fascicles in the ultrasound image plane and may result in a pennation angle different from that measured from the 3D fascicle, depending on the position and orientation of the scanning plane.

Pennation angle was calculated as the angle the fascicle makes with the \( z \)-axis of the muscle-based coordinate system. \( \beta_p \) was measured as the angle the fascicle makes with the long axis of the muscle. In a typical 2D ultrasound scan, the orientations are measured in the image plane, and, in this study, analogous calculations of the fascicle orientation were made from the sliced fascicles in the image plane. The orientations of the sliced fascicles were obtained by projecting the 3D fascicle orientations in the mean fascicle plane. This was done in three different ways. 1) \( \beta_p \) was calculated in 3D as described above. 2) \( \beta_{cp} \) was measured as the angle between the long axis of the muscle and the sliced fascicle in the mean fascicle plane (calculated from the 0% torque level and 0° ankle angle condition). This is analogous to collecting 2D ultrasound scans with the probe strapped in a fixed position over the muscle for all the trials. 3) \( \beta_{cph} \) was measured as the
angle between the long axis of the muscle and the projection of the fascicle when it was projected in the mean fascicle plane for each trial. This is analogous to adjusting the ultrasound probe to lie in the fascicle plane for each trial. The soleus was bigger than LG and MG and more complex in architecture than the gastrocnemii (1), so the mean fascicle plane orientations were determined for medial and lateral sides of the soleus, and the fascicle orientations were projected in the respective directions.

**Statistical analysis.** The muscle was divided into the following regions (Fig. 3): three along the length of the muscle (z-axis), proximal, central, and distal; two along the depth of the muscle (x-axis), deep and superficial; and two along the width of the muscle (y-axis), medial and lateral. General linear model ANOVA was used to test the effects of muscle region, ankle angle, and ankle torque on the fascicle orientation, with the polar angle $\beta$ and azimuthal angle $\varphi$ as the dependent variables, subject identity as a random factor, and muscle region, ankle angle, and torque as fixed factors. Post hoc Tukey tests were performed to determine the effects of regions, ankle angles, and torque on the dependent variables. The results obtained were considered significant for $P$ value < 0.05. Mean differences were calculated for the pennation angles calculated in the section above using the matched pair $t$-test to compare the effect of different scanning protocols.

**RESULTS**

The methods were used to obtain 3D fascicle orientations for the triceps surae muscle. The orientations were represented as direction cosines and plotted as a vector map. Figures 4–6 show the vector grid from representative subjects from different view points, for an extreme ankle angle and torque level used in the experiment. The figures show the regionalization of both pennation angle and azimuthal angle in the three muscles, along with the changes in architecture with change in torque level and ankle angle.

**Regionalization of fascicle orientation and fascicle plane orientation.** Both the fascicle orientation and the fascicle plane orientation were regionalized in all three muscles (Table 1).

In LG, the maximum variations in $\beta_f$ and $\varphi_f$ were along the length of the muscle. $\beta_f$ increased from 11.2 to 14.5°, and $\varphi_f$ increased from 96.3 to 99.2° from the proximal to the distal end. The orientations of the normals to the fascicle planes changed across the muscle regions, with maximum change along the muscle width, and there was a greater variation in $\varphi_{fp}$ than $\beta_{fp}$. $\varphi_{fp}$ decreased from the lateral to medial sides of the muscle from 262.3 to 254.4°, and $\beta_{fp}$ changed by less than a degree change across the different regions.

The MG showed the greatest variations in the fascicle orientations along the length of the muscle (similar to the LG). $\beta_r$ increased from 11.3 to 19.0°, and $\psi_r$ increased from 94.2 to 103.4°. The fascicle planes varied in both $\beta_{fp}$ and $\varphi_{fp}$. The biggest variations in $\beta_{fp}$ were along the length of the muscle, with the increase from the proximal to the distal region from 93.2 to 98.5°, and in $\varphi_{fp}$ they were along the width of muscle, with the values decreasing from 226.0 to 218.7°.

The changes of $\beta_r$ in soleus were similar in magnitude to those in the gastrocnemii muscles, but the variation between lateral to medial sides from 12.0 to 7.3° was similar to the change from 10.3 to 7.6° from the proximal to distal ends. The $\varphi_r$ changed from 95.6 to 124.0° from the lateral to medial region. Similar trends were reflected in the fascicle plane orientations. The $\varphi_{fp}$ values changed from 70.2 to 111.2° between the lateral and medial regions, indicating that the planes’ arrangements were a reflection between the medial and lateral sides of the muscle.

**Effect of ankle torque and ankle angle on fascicle orientations and fascicle plane orientation.** There was a significant effect of the ankle torque and the ankle angle on the triceps surae architecture (Table 2, Fig. 7). $\beta_r$ increased in all three muscles with increase in ankle torque from 0 to 60% MVC at 30° ankle angle; however, this increase was not observed at $-15°$ ankle angle. $\varphi_r$ increased in the soleus with increase in ankle torque from 0 to 60% MVC at both $-15$ and 30° ankle angles. $\varphi_r$ increased from 0 to 60% MVC at 30° ankle angle in LG; however, it showed no change in MG. The increase in $\varphi_r$ from 93.9 to 97.6° in LG and 106.3 to 110.2° in soleus indicates that the fascicles tilted toward sagittal plane with increase in contraction level, while this increase was not observed in MG (Table 2).

$\beta_{fp}$ decreased in LG and soleus and increased slightly in MG with increase in contraction level from 0 to 60% MVC at both 15 and 30° ankle angles. The decrease in $\beta_{fp}$ values in LG and soleus indicated that the fascicle planes become more parallel to the long axis of the muscle with increase in the contraction level from 0 to 60% MVC. $\varphi_{fp}$ increased in LG and MG from 257.2 to 260.4° and 222.4 to 227.4°, respectively, indicating that the planes tilted toward sagittal plane with increase in contraction levels at 30° ankle angle. In the soleus, the $\varphi_{fp}$ decreased from 99.2 to 93.8°, again indicating that the fascicle planes tilted toward sagittal plane with increase in contraction level at 30° ankle angle.

**Effect of 2D and 3D scanning directions on the estimated pennation angles.** Pennation angles calculated from the local 3D measures of $\beta_r$, projected fascicle orientations in a constant fascicle plane ($\beta_{fcp}$), and projected fascicle orientations in a variable fascicle plane ($\beta_{vp}$) were significantly different (Fig. 8). The planes of projection were similar to the scanning planes that would be chosen in 2D ultrasound scanning. Both $\beta_{fcp}$ and $\beta_{vp}$ were underestimated in LG and MG, compared with $\beta_r$ by a small value of $<1°$. However, in soleus, the mean difference between $\beta_{fcp}$ and $\beta_{vp}$ was 4.5°, $\beta_{fcp}$ and $\beta_r$ was 10°,
and $\beta_{rz}$ and $\beta_f$ was 14°. The differences were of similar magnitude for different ankle torques and ankle angles.

**DISCUSSION**

This is the first study to quantify 3D muscle architecture in vivo for different contraction levels in a muscle; most previous studies on in vivo fascicle architecture have used 2D ultrasound (7, 11, 21, 27). In this study, the 3D quantification was made possible by the novel protocol for ultrasound data collection and analysis (37, 39). The major findings of this study were as follows: 1) muscle fascicle orientations and fascicle plane orientations are regionalized in each of the three muscles of triceps surae; 2) muscle fascicle orientation and the fascicle plane orientation depend on both the level of muscle contraction and muscle length; and 3) pennation angle estimates based on 2D ultrasound studies are significantly affected by the orientation of scanning plane and can be different from their actual 3D values.

**3D fascicle orientation.** Pennation angles ($\beta_f$) as reported in this study are not identical to those reported in previous 2D studies. In 2D images, the pennation angle is typically measured as the angle relative to the aponeurosis (21, 30, 34), while in this study the measurements were relative to the major axis through the muscle (38). The numbers reported in Table 2 and Fig. 7 were averaged across the different regions of the muscle and are useful to study the main effects of torque and ankle angle on the fascicle orientations. However, regionalization of the fascicle orientations (Table 1) and differences in orientation between the major ($z$-) axis of the muscle and local aponeurosis orientations may lead to discrepancies between $\beta_f$ and the pennation angles from 2D studies.

Regionalization of architecture has been related to regional changes in activation patterns in the pig masseter muscle (15). Herring and coworkers (15) showed a varying line of action of the muscle with a phasic activity pattern, and this was correlated to the change in fascicle orientation in the muscle.

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**Fig. 4.** 3D fascicle orientations in the LG from posterior and lateral views for one representative subject. The images show the regionalization of pennation angle $\beta$ and azimuthal angle $\phi$ in the muscle and the change in architecture with torque and ankle angle.
Regionalization of activation patterns has previously been reported in cycling studies in the gastrocnemii in humans (47), where distal-to-proximal and medial-to-lateral changes in activation patterns were observed. In this present study, similar patterns were observed for fascicle orientations (Figs. 4–7 and Table 1) that may be functionally related to the regional differences in the muscle activation.

The fascicle orientation depends on ankle joint angle and joint torque, and thus on muscle length and force. $\beta_r$ for both MG and soleus increased at shorter muscle lengths, whereas $\beta_r$ decreased for shorter lengths of the LG. The decrease in $\beta_r$ for LG was prominent at 0% MVC, but was not observed at 60% MVC (Table 2). Pennation angle has been shown to increase for shorter muscle lengths in 2D studies; however, the increases in pennation for LG have been small compared with greater increase in MG and soleus (11, 21, 29). It has also been shown in some studies that the pennation angle depends on the contraction level, with greater pennation angles occurring at higher muscle forces (21, 29). In this study, $\beta_r$ increased significantly with increasing torque at more plantar-flexed positions, but not at the more dorsiflexed positions (Fig. 7). In a previous study on the triceps surae, it was shown that increases in pennation with increase in plantar flexion were more pronounced at 100% MVC and very small, if any, at rest.

![Diagram](http://jap.physiology.org/doi/10.1152/japplphysiol.01090.2012/fig5)

Fig. 5. 3D fascicle orientations in the MG from posterior and lateral views for one representative subject. The images show the regionalization of pennation angle $\beta$ and azimuthal angle $\phi$ in the muscle and the change in architecture with torque and ankle angle.
A similar trend has also been shown for submaximal contractions in the vastus lateralis, with a significantly greater pennation angle at greater knee angles compared with no change for smaller knee angles (11, 12).

Pennation angle has been studied extensively using 2D ultrasound, but the azimuthal angle (φ) is an important component to describe the 3D orientation of the fascicles that has not been described before. A helical arrangement of axial muscle fibers in fish has previously been suggested to be important for the maintenance of optimal strain rates (13, 19). During the very fast startle response in fish, the helical arrangement of the white muscle fibers enables optimal strain rates during the startle response compared with much larger strain rates in the longitudinal red fibers (2, 41). A helical arrangement of fascicles would result from a constant polar angle (pennation angle in this study), but with a varying azimuthal angle. Regional variations in pennation angle indicate curved fascicles in a plane. Regional variations in azimuthal angle of fascicles, as observed in this study, may result in a partial helical arrangement (nonplanar curving) of the muscle fascicles, and this may affect the strain rates during contraction. 3D architectural information is important for 3D muscle models to understand force transmission from muscles to the bones (16, 36). Muscle force is not just transmitted at the muscle tendon junction, rather there are additional pathways for force transmission (16). It has been shown in animal (17) and human studies (39) that a significant fraction of the force can be transferred to the bone via nonmuscle-tendinous pathways. Along with the connective tissue properties, the transmission of force depends on the relative arrangement of the tissue with respect to each other. Pennation angle, typically measured as the angle with respect to the aponeurosis, has been used to estimate the force transmitted to the tendon (35). Due to the nonplanar nature of fascicles, a 2D measure of orientation is inadequate to describe the transmission of force in 3D. Azimuthal angle complements the pennation angle to understand the mechanics of force transmission. This is particularly relevant for 3D modeling studies and clinical treatments involving manipulation of force transmission pathways, such as tendon transfer surgeries and aponeurotomy.

Fascicle plane orientations and effect of scanning plane on pennation angle measurements. Fascicle sheet orientations were represented by the orientation of the normal to the fascicle planes in each voxel (βfp, φfp), and these are architec-
Table 1. Regionalization of fascicle orientation and fascicle plane orientation in the triceps surae muscles

<table>
<thead>
<tr>
<th>Depth</th>
<th>Width</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG</td>
<td>Lateral</td>
<td>Superficial</td>
</tr>
<tr>
<td>βf</td>
<td>12.4 ± 0.02</td>
<td>12.7 ± 0.02</td>
</tr>
<tr>
<td>ϕf</td>
<td>97.8 ± 0.02</td>
<td>97.5 ± 0.02</td>
</tr>
<tr>
<td>βfp</td>
<td>93.1 ± 0.03</td>
<td>93.7 ± 0.02</td>
</tr>
<tr>
<td>ϕfp</td>
<td>262.3 ± 0.03</td>
<td>257.2 ± 0.06</td>
</tr>
<tr>
<td>MG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>βf</td>
<td>14.3 ± 0.02</td>
<td>14.9 ± 0.02</td>
</tr>
<tr>
<td>ϕf</td>
<td>98.3 ± 0.02</td>
<td>98.4 ± 0.02</td>
</tr>
<tr>
<td>βfp</td>
<td>95.5 ± 0.02</td>
<td>95.5 ± 0.02</td>
</tr>
<tr>
<td>ϕfp</td>
<td>226.0 ± 0.05</td>
<td>222.4 ± 0.04</td>
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<tr>
<td>SOL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>βf</td>
<td>12.0 ± 0.02</td>
<td>9.43 ± 0.03</td>
</tr>
<tr>
<td>ϕf</td>
<td>95.6 ± 0.12</td>
<td>112.2 ± 0.27</td>
</tr>
<tr>
<td>βfp</td>
<td>95.7 ± 0.12</td>
<td>94.8 ± 0.13</td>
</tr>
<tr>
<td>ϕfp</td>
<td>70.2 ± 0.09</td>
<td>83.5 ± 0.16</td>
</tr>
</tbody>
</table>

Values are mean ± SE, in degrees. β, Pennation angle; ϕ, azimuthal angle. Orientations of the fascicles (βf, ϕf) and normal to the fascicle planes (βfp, ϕfp) across all the torque levels and ankle angle torques over approximately 40,000 points are shown. LG, lateral gastrocnemius; MG, medial gastrocnemius; SOL, soleus.

Table 2. Mean fascicle orientations and fascicle plane orientations for different ankle angles and torque levels in the triceps surae

<table>
<thead>
<tr>
<th>Angle, °</th>
<th>Torque, %MVC</th>
<th>LG</th>
<th>MG</th>
<th>SOL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−15°</td>
<td>30°</td>
<td>−15°</td>
<td>30°</td>
</tr>
<tr>
<td>βf</td>
<td>0</td>
<td>13.3 ± 0.05</td>
<td>10.5 ± 0.03</td>
<td>14.4 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12.7 ± 0.05</td>
<td>12.9 ± 0.06</td>
<td>14.2 ± 0.07</td>
</tr>
<tr>
<td>ϕf</td>
<td>0</td>
<td>99.6 ± 0.06</td>
<td>93.9 ± 0.04</td>
<td>99.2 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>98.2 ± 0.05</td>
<td>97.6 ± 0.06</td>
<td>99.2 ± 0.05</td>
</tr>
<tr>
<td>βfp</td>
<td>0</td>
<td>94.3 ± 0.04</td>
<td>94.9 ± 0.04</td>
<td>93.9 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>92.6 ± 0.06</td>
<td>93.7 ± 0.07</td>
<td>94.9 ± 0.06</td>
</tr>
<tr>
<td>ϕfp</td>
<td>0</td>
<td>259.6 ± 0.13</td>
<td>257.2 ± 0.14</td>
<td>222.1 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>258.1 ± 0.14</td>
<td>260.4 ± 0.12</td>
<td>222.7 ± 0.10</td>
</tr>
</tbody>
</table>

Values are means ± SE. Shown are the orientations of βf, ϕf, βfp, and ϕfp over 10,000 points. MVC, maximal voluntary contraction.
when a muscle contracts, it bulges, and this can change the position of the scanning plane relative to the muscle fascicles, leading to errors in the estimated pennation angles. It is thus important to optimize the ultrasound probe orientation for different joint torques in muscles with complex architectures, such as the soleus.

Conclusions. In conclusion, this study quantified the 3D muscle architecture in the triceps surae and related the architectural properties to the ankle joint angle and joint torque. The 3D fascicle orientation is regionalized across each muscle in the triceps surae and depends on the muscle length and contraction level. Azimuthal angle, a new parameter obtained as a virtue of 3D quantification methods, changed by the same magnitude as the typical 2D orientation measure of pennation angle. The regional variations of azimuthal angle represent a partial helical arrangement of fascicles, indicating a on-planar arrangement of the fascicles. The novel determination of fascicle plane orientation allows us to understand how 2D measurements of architectural parameters are related to the 3D structure of muscle. The evaluation of 2D measuring techniques against the 3D structure of these muscles has shown that muscles with greater regional variations in the fascicle plane orientations, such as the soleus, are prone to greater errors in 2D measures of fascicle orientation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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