3D fascicle orientations in triceps surae

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Abstract

The aim of this study was to determine the 3D muscle fascicle architecture in human triceps surae muscle at different contraction levels and muscle lengths. Six male subjects were tested for three contraction levels (0%, 30% and 60% of MVC) 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 2D 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 gastrocnemii due to its more complex fascicle arrangement.

Keywords

Fascicle sheet orientation, 2D ultrasound, muscle force-length interaction
Introduction

In-vivo muscle fascicle architecture has been extensively studied using B-mode ultrasound in two dimensions (2D). Fascicle orientation is typically measured as the pennation angle that has been defined as the angle between the fascicles and the aponeurosis (7, 10, 46). Pennation angle depends on the muscle length and relative torque levels (10, 27, 31). In 2D muscle architecture studies muscle fascicles are considered to be arranged as planes that are called fascicle planes (20, 24, 27, 42, 43). In previous 2D ultrasound studies it has been important to align the imaging plane with the fascicle planes in order to image complete fascicles (20, 24, 27, 5). However, muscle fascicles may not be aligned in planes (42), particularly in a muscle with a non-uniform 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 (39) 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 (DT-MRI) and 3D ultrasound studies have also looked at the in-vivo fascicle orientation in 3D. However, the DT-MRI studies focused on the measurement of the pennation angle from the fascicles tracked in passive muscle (17, 22, 40) and the 3D ultrasound studies quantified fascicle architecture from a few fascicles selected in passive muscle (4, 8, 9, 21, 28). None of these earlier studies have quantified 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 man 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 (10, 19, 24, 27, 29, 30, 47). In order 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 (24, 20). 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). The orientation of the scanning plane relative to the muscle fascicles will affect the calculated fascicle orientations and pennation, and a purpose of this study was to compare the estimated pennation angles that would be calculated using 2D versus 3D methods.

Muscle is a three dimensional entity with varying shape across the length of the muscle (13) and changes shape in the form of muscle bulging. Both experimental (27, 45) and modelling (3, 23, 32, 42) 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, whilst the medial gastrocnemius, MG, does not increase in thickness at MVC (27, 31). Changes in muscle shape influence the orientation of the fascicles within the muscle (3, 45). In 2D ultrasound studies muscle thickness is reported as the distance between aponeurosis 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.

3D fascicle architecture from dissected rat soleus (41) and equine longissimus dorsi (37) have shown regional variations in the 3D architecture. Stark and Schilling tracked the fascicles in 3D and reported local pennation angles as the angles of the fascicles with the muscle line of action (41), while Ritruechai and co-workers reported local orientations of the fascicles in perpendicular planes to obtain a 3D representation of fascicle orientations (37). A 3D study on formalin-fixed soleus muscle in man has also shown regional variation in the muscle architecture (1). It would be expected that the 3D orientations of the fascicles changes as the muscle changes its length and force, and these 3D orientations may also be regionalized within the muscle.
third purpose of this study was to identify relations between contraction level, muscle length and regionalization on the 3D orientations of the muscle fascicles.

Methods

Data collection and experimental design

The purpose of this study was to identify the 3D orientations of fascicles and fascicle sheets in the triceps surae muscles at different ankle angles and torque levels. The muscles were imaged for 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 years, height 183.1±8.9cm, mass 79.9±20.1 Kg); subjects were athletic and experienced with specific sporting movements to ensure that stable and constant ankle torques could be generated during imaging. Subjects gave their informed, written consent to participate in accordance with the Simon Fraser University’s Office of Research Ethics policy on research using human subjects.

The scanning process was identical to that used in our previous study (35) with ultrasound images obtained using a linear ultrasound probe (Echoblaster, Telemed, LT) recording at 20 Hz. 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) attached to the ultrasound probe (35). Subjects were asked to perform maximal voluntary plantarflexion contractions, MVC, that were at fixed ankle angles (-15°, 0°, 15° and 30° relative to neutral), a fixed knee angles of 135°, and at three torque levels (0, 30%, 60% MVC) that were relative to the MVC for each ankle angle. Data were not collected beyond 60% of MVC because the scanning times last for two minutes for each trial and it is not possible to sustain the relative torques for that duration. 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 plantarflexion contractions in a water tank (35). 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 in order to adjust to different leg lengths of the subjects. The foot plate was connected to a strain gauge to obtain the ankle torque and visual feedback for torque was provided to the subjects. The ankle torque data were collected at 2000 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 (34, 35) to obtain the muscle fascicle orientation in 3D. 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 in each ultrasound image (34). 2D orientations in the image plane were combined with the 3D position and orientation of the ultrasound scanning plane in order to obtain the local 3D fascicle orientation corresponding to the respective pixels in 3D.

The muscle volume was divided into voxels of $5 \times 5 \times 5$ mm$^3$ 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 (figure 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 the pixels in that voxel. This step was slightly different from the previous work (35). 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 ($w_c$) and for the distance ($w_d$) were given by
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 and \( \sigma \) 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).

**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 (5, 20, 24, 26). An image with the continuous fascicular structure gives a high convolution number during wavelet (34). This quality was used to select the fascicle plane orientation in each voxel (35). 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**

In order 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 lab 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 is 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 (figure 2). The origin of the muscle coordinate system was set at the mean point in the muscle. Due to the semi-cylindrical shape of the soleus (1), a different coordinate system was used with its \( z \)-axis as the vector joining the mean co-ordinate of the knee joint centers with the mean co-ordinate 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 centre 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 \( \beta_f = \cos^{-1}(\delta_z) \) that was the angle between the vector parallel to the local fascicle direction and the \( z \)-axis, and an azimuthal angle \( \varphi_f = \tan^{-1}(\delta_y/\delta_x) \) 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 \( \beta_f \) 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 \( \beta_{fp} \) and \( \varphi_{fp} \). A 90° value for \( \beta_{fp} \) and \( \varphi_{fp} \) 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 \( \beta_{fp} \) represents the change in rotation of the plane about the long axis of the muscle and a change in \( \varphi_{fp} \) 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 in order to compare the pennation angles that would be obtained by different scanning protocols.
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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 be line like for 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. $\beta_f$ 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) $\beta_f$ was calculated in 3D as described above, (2) $\beta_{cfp}$ 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) $\beta_{vfp}$ 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 (figure 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 relative torque level on fascicle orientation; with the polar angle $\beta$ and azimuthal angle $\phi$ as the dependent variables, subject identity as a random factor, muscle region, ankle angle and relative torque level as fixed factors. Post-hoc Tukey tests were performed to determine the effects of regions, relative torque levels and ankle angles 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 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 greatest variations in the fascicle orientations along the length of the muscle (similar to the LG). $\beta_f$ increased from 11.3° to 19.0° and $\varphi_f$ 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 $\varphi_{fp}$ were along the width of muscle with the values decreasing from 226.0° to 218.7°.

The changes $\beta_f$ in soleus were similar in magnitude to those in the gastrocnemius muscle 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 $\varphi_f$ 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 LG architecture (Table 1). $\beta_f$ decreased from 13.1° to 11.8° and $\varphi_f$ decreased from 98.5° to 96.5° with the increase in ankle angle from -15° to 0°. $\beta_{fp}$ increased slightly with increase in ankle angle and $\varphi_{fp}$ decreased from ankle angles of -15° to 0° and then increased from 0° to 30°. $\beta_f$ and $\varphi_f$ increased slightly (less than a degree) with the increase in torque from 0 to 60%. $\beta_{fp}$ and $\varphi_{fp}$ changed less than 0.5° with increases in torque from 0% to 60% MVC.

There was a significant effect of the ankle torque and the ankle angle on MG architecture (Table 2). As the ankle angle increased from -15° to 30° $\beta_f$ increased from 14.2° to 16.9° and $\varphi_f$
decreased from 99.1° to 97.9°. $\beta_{fp}$ increased with increase in ankle angle while $\varphi_{fp}$ increased from ankle angles of -15° to 15° and decreased from 15° to 30°. With the increase in torque the changes in $\beta_f$, $\varphi_f$, $\beta_{fp}$ and $\varphi_{fp}$ were less than one degree.

There was a significant effect of the ankle torque and the ankle angle on soleus architecture (Table 2). $\beta_f$ decreased from 9.4° to 8.5° with increase in ankle angle from -15° to 0° followed by an increase from 8.5° to 9.8° with the increase in ankle angle to 30°. $\varphi_f$ increased from 111.6° to 117.3° with increase in ankle angle from -15° to 0° followed by a decrease to 110.9° with the increase in ankle angle to 30°. $\beta_{fp}$ increased from 96.2° to 105.3° with increase in ankle angle from -15° to 15° followed by a decrease to 98.7° with increase in ankle angle to 30°. $\varphi_{fp}$ showed statistically significant but very small changes.

There was also a significant interaction between ankle torques and ankle angles on the fascicle orientation (figure 7). As shown in figure 7, the relation between ankle angle and orientation is altered by torque level. In LG $\beta_f$ decreased as the ankle angle increased for 0% MVC but for higher torque levels this trend gradually vanished. Furthermore, in MG and soleus there was a greater increase in $\beta_f$ with ankle angle at higher torque levels. In LG $\varphi_f$ had an opposite trend with increasing ankle angles for 0% and 60% torque levels.

**Effect of 2D and 3D scanning directions on the estimated pennation angles**

Pennation angles calculated from the local 3D measures of $\beta_f$, projected fascicle orientations in a constant fascicle plane ($\beta_{fcp}$) and projected fascicle orientations in a variable fascicle plane ($\beta_{fvp}$) were significantly different (figure 8). The planes of projection were similar to the scanning planes that would be chosen in 2D ultrasound scanning. Both $\beta_{fcp}$ and $\beta_{fvp}$ were underestimated in LG and MG compared to $\beta_f$ by a small value of less than 1°. However, in soleus the mean difference between $\beta_{fcp}$ and $\beta_{fvp}$ was 4.5°, $\beta_{fcp}$ and $\beta_f$ was 10°, and $\beta_{fvp}$ 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, 10, 20, 25). In this study, the 3D quantification was made possible by the novel protocol for ultrasound data collection and analysis (34, 35). The major findings of this study were: 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 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

Regionalisation of architecture has been related to regional changes in activation patterns in the pig masseter muscle (14). Herring and co-workers showed a varying line of action of the muscle with a phasic activity pattern and this was co-related to the change in fascicle orientation in the muscle. Regionalisation of activation patterns has previously been reported in cycling studies in the gastrocnemii in man (44) where distal to proximal and medial to lateral changes in activation patterns were observed. In this current study similar patterns were observed for fascicle orientations (figure 4-7 and table 1). Wakeling (2009) reported greater intensity of electromyography (EMG) in the distal region of LG and MG for high cadence than the proximal region (44). $\beta_f$ is greater in the distal region of the muscle (figure 4 and 5, table 1 ) with a greater increase in $\beta_f$ at higher torque levels in the distal region compared to that in the proximal region (figure 9). A greater pennation angle ($\beta_f$) may allow for greater rotation of fascicles leading to a smaller change in fascicle length (greater gearing) facilitating the high velocity contractions in an active muscle (3, 45).

The fascicle orientation depends on ankle joint angle and joint torque, and thus on muscle length and force. $\beta_f$ for both MG and soleus increased at shorter muscle lengths whereas $\beta_f$ decreased for shorter lengths of the LG. The decrease in $\beta_f$ for LG was prominent at 0% MVC.
but was not observed at 60% MVC (table 2). Pennation angle has been shown to increase with shorter muscle lengths in 2D studies, however, increase in pennation for LG have been small compared to a greater increase in MG and soleus (10, 20, 27). It has also been shown in some studies that the pennation angle depends on the contraction level with a greater pennation angles occurring at higher muscle forces (20, 27). In this study, $\beta_r$ increased significantly with increasing torque at more plantarflexed positions, not at the more dorsiflexed positions (figure 7). A similar trend has been shown for submaximal contractions in the vastus lateralis, with a significantly greater pennation angle at greater knee angles compared to no change for smaller knee angles (10, 11). The changes in $\beta_r$ may seem different from a previous study in which the pennation angle increased with torque level across a range of ankle angles (27), but it has to be noted here that Maganaris and co-workers tested at MVC while in this study the maximum torque level was 60% MVC.

Pennation angle has been studied extensively using 2D ultrasound but the azimuthal angle ($\phi_i$) is an important component to describe the 3D orientation of the fascicles that has not been described before. A helical arrangement of axial muscle fibres in fish has previously been suggested to be important for transmission of force in and maintenance of optimal strain rates (12, 18). During the very fast startle response in fish, the helical arrangement of the white muscle fibres enables optimal strain rates during the startle response compared to much larger strain rates in the longitudinal red fibers (2, 38). 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 azimuthal angle of fascicles as observed in this study may result in a partial helical arrangement of the muscle fascicles and this may affect the strain rates during contraction.

3D architectural information is important for 3D muscle models in order to understand force generation in healthy and diseased muscle (15, 33). If the regional differences in architecture are not considered, it could lead to errors in estimation of fascicle lengths and force transmission in the muscle. Muscle force is not just transmitted at the muscle tendon junction rather there are additional pathways for the force transmission (15). It has been shown in experimental (16) and modeling studies (36) that, even after removing the tendon from a muscle, a significant fraction of the force can be transferred to the bone via non muscle-tendinous
3D fasc orientation

The non-tendinous pathways include lateral transmission of force to neighbouring fascicles, the epimysium and to neighbouring muscles. Pennation angle measurements are useful to study the transmission of force to aponeurosis or the muscle tendon junction while azimuthal angle will provide information for the lateral transmission of forces. 3D orientation will be helpful to understand the mechanism of this fascicular transmission of intramuscular force. This will also be important for 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 ($\beta_{fp}$, $\varphi_{fp}$) and these are architectural parameters which have not previously been described. Fascicle sheet orientation is important to understand the arrangement of fascicles as a group and will have implications to the spread of force to the neighbouring fascicles. Larger variations were observed in $\varphi_{fp}$ than $\beta_{fp}$ in each of the three muscles, which means that the fascicle sheets stay at a similar angle relative to the long axis of the muscle but change their tilt with the maximum change being observed along the width of the muscle (medial to lateral sides of the muscle).

Sejerested and co-workers (1984) have reported curved fascicle layers with higher curvature of the outer fascicle sheets in the human vastus lateralis and have suggested that all the layers would take up the curvature of the bone at high levels of relative torques (39). In this study, the fascicle sheet orientations were not uniform across the muscle, which confirms that the fascicles were arranged in curved sheets. Further, in soleus, the $\varphi_{fp}$ indicate that the fascicle sheet orientations from the medial and lateral sides of the muscle are almost the mirror images of each other. Agur and co-workers (2003) have shown in human cadavers that the soleus is divided by a septum in the middle of the muscle and fascicles insert on the aponeurosis as well as the septum (1). The mirror image configuration of fascicle sheets further supports that observation. It is important that in 2D ultrasound scans of soleus that care is taken to scan the fascicles in the correct plane.
The varying orientations of the fascicle sheets have an implication to appropriate 2D ultrasound scanning protocols. As has been identified in previous ultrasound studies, it is important to scan the fascicles in their perceived fascicle planes (5, 20). Pennation angles that were estimated from different scanning planes differed by less than a degree in the gastrocnemii and the trends of change in pennation angle with force and ankle angle were same in spite of the small magnitude differences (figure 8). It can be concluded from these results that both scanning protocols, i.e. securing the ultrasound over the muscle in the same place for different force and joint angle trials or finding different optimal scanning plane for each trial, would result in similar results. However, larger differences in the estimated pennation were found between the different scanning planes in the soleus: this can be attributed to a greater change in the fascicle plane orientations across ankle angles and torque levels in the soleus than in the gastrocnemii and greater variation in the architecture across the muscle. Due to larger variation in architecture across the muscle the errors obtained by projecting or scanning fascicles into a single plane would be greater for the soleus. This is relevant to 2D scanning studies because 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 relative torque levels in muscles with complex architecture like 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 determination of fascicle plane orientation has important implications not just in 3D muscle architecture and function but also in the way that 2D measurements of architectural parameters are made. The regionalisation of 3D architecture in both the fascicle orientations and the normals to the fascicle planes imply that the fascicles and fascicle sheets may be curved in 3D.
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Figure captions

Figure 1 3D voxels in the scanned muscle volume can contain pixels from multiple 2D images. The figure above shows the representation for one such 2D image plane intersecting a 3D voxel.

Figure 2 Representation of the muscle based coordinate system shown for the LG from posterior and lateral view.

Figure 3 Representation of the regions into which the muscle was split, viewed from different viewpoints. The axes represent the muscle based co-ordinate system used to assign the positions in the muscle and the dots represent the voxel locations from the lateral gastrocnemius in one subject. The black and grey dots were used to differentiate the regions of the muscle. Posterior view shows the regions along the length (A) and width of the muscle (B). The lateral view shows the regions along the depth of the muscle (C).

Figure 4 3D fascicle orientations in the LG from posterior and lateral views for one representative subject. The images show the regionalization of pennation angle and azimuthal angle in the muscle and the change in architecture with torque and ankle angle.

Figure 5 3D fascicle orientations in the MG from posterior and lateral views for one representative subject. The images show the regionalization of pennation angle and azimuthal angle in the muscle and the change in architecture with torque and ankle angle.

Figure 6 3D fascicle orientations in the soleus from posterior and lateral views for one representative subject. The images show the regionalization of pennation angle and azimuthal angle in the muscle and the change in architecture with torque and ankle angle.

Figure 7 Effect of torque levels on fascicle orientation and ankle angle relation in triceps surae. The dots reported are mean values of the orientations of the fascicle (βf, φf). The symbols (●) show the mean ± s.e.m. of the orientations of the fascicle (N=2500).

Figure 8 Effect of scanning plane on the measured pennation angle calculated from 3D fascicle (βf), from fascicle projected in a constant fascicle plane(βfcp), and from the fascicle projected in the variable fascicle plane (βfp), as obtained in each trial corresponding to torque level and ankle angle. The symbols (●) show the mean ± s.e.m. of the orientations of the fascicle (N=40000).

Figure 9 Regional differences increase in βf at different torque levels in LG and MG. Greater βf was obtained in the distal ends of the muscle. The symbols (●) show the mean ± s.e.m. of the orientations of the fascicle (N=10000).
Table 1 Regionalization of fascicle orientation and fascicle plane orientation in the triceps surae muscles.

<table>
<thead>
<tr>
<th></th>
<th>Lateral</th>
<th>Medial</th>
<th>Depth</th>
<th>Width</th>
<th>Length</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Central</td>
<td>Distal</td>
<td></td>
<td></td>
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<tr>
<td>LG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_f$°</td>
<td>12.41 ± 0.02</td>
<td>12.64 ± 0.02</td>
<td>12.71 ± 0.02</td>
<td>12.33 ± 0.02</td>
<td>11.25 ± 0.02</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>97.85 ± 0.02</td>
<td>96.67 ± 0.02</td>
<td>97.49 ± 0.02</td>
<td>96.97 ± 0.02</td>
<td>96.34 ± 0.02</td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>93.10 ± 0.03</td>
<td>94.34 ± 0.02</td>
<td>93.73 ± 0.03</td>
<td>93.74 ± 0.02</td>
<td>93.46 ± 0.03</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>262.27 ± 0.03</td>
<td>254.36 ± 0.06</td>
<td>257.21 ± 0.06</td>
<td>259.25 ± 0.05</td>
<td>257.29 ± 0.09</td>
</tr>
<tr>
<td>MG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_f$°</td>
<td>14.34 ± 0.02</td>
<td>15.19 ± 0.03</td>
<td>14.90 ± 0.02</td>
<td>14.62 ± 0.02</td>
<td>11.37 ± 0.03</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>98.34 ± 0.02</td>
<td>97.76 ± 0.02</td>
<td>98.42 ± 0.02</td>
<td>97.65 ± 0.02</td>
<td>94.24 ± 0.02</td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>95.14 ± 0.02</td>
<td>95.09 ± 0.02</td>
<td>95.54 ± 0.02</td>
<td>95.70 ± 0.02</td>
<td>93.16 ± 0.03</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>226.02 ± 0.05</td>
<td>218.67 ± 0.04</td>
<td>222.42 ± 0.05</td>
<td>222.24 ± 0.04</td>
<td>221.78 ± 0.06</td>
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<td>Sol</td>
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<td></td>
<td></td>
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<tr>
<td>$\beta_f$°</td>
<td>12.02 ± 0.02</td>
<td>7.31 ± 0.01</td>
<td>9.43 ± 0.03</td>
<td>8.93 ± 0.01</td>
<td>10.34 ± 0.02</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>95.6 ± 0.12</td>
<td>124.07 ± 0.19</td>
<td>112.20 ± 0.27</td>
<td>113.65 ± 0.15</td>
<td>117.81 ± 0.81</td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>95.73 ± 0.12</td>
<td>103.93 ± 0.10</td>
<td>94.84 ± 0.13</td>
<td>102.90 ± 0.09</td>
<td>99.17 ± 0.12</td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>70.19 ± 0.09</td>
<td>111.19 ± 0.12</td>
<td>83.50 ± 0.16</td>
<td>100.16 ± 0.11</td>
<td>99.22 ± 0.16</td>
</tr>
</tbody>
</table>

The values reported are mean ± standard error of mean of the orientations of the fascicles ($\beta_f$°) and normal to the fascicle planes ($\beta_{fp}$°) across all the torque levels and ankle angle torques over 40,000 points.
and normal to the fascicle planes ($\beta_{fp}$, $\phi_{fp}$) over 40,000 points.

The values reported are mean ± standard error of mean of the orientations of the fascicles ($\beta_i$, $\phi_i$) and normal to the fascicle planes ($\beta_{fp}$, $\phi_{fp}$) over 40,000 points.

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

<table>
<thead>
<tr>
<th></th>
<th>Ankle Angle</th>
<th>Relative Torque</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-15° 0° 15° 30° 0% 30% 60%</td>
<td></td>
</tr>
<tr>
<td><strong>LG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_i$°</td>
<td>13.12±0.03  13.08±0.02  12.12±0.03  11.8±0.03  12.45±0.02  12.26±0.02  12.86±0.02</td>
<td></td>
</tr>
<tr>
<td>$f_i$°</td>
<td>98.58±0.03  97.85±0.03  96.53±0.03  96.05±0.03  97.29±0.03  96.87±0.03  97.54±0.02</td>
<td></td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>92.98±0.04  93.00±0.03  93.78±0.04  93.17±0.03  93.86±0.02  93.64±0.03  93.71±0.03</td>
<td></td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>258.25±0.07 256.41±0.32 258.41±0.34 259.67±0.07 258.06±0.07 258.57±0.07 257.95±0.06</td>
<td></td>
</tr>
<tr>
<td><strong>MG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_i$°</td>
<td>14.26±0.03  13.47±0.03  14.52±0.03  16.92±0.04  14.42±0.03  14.71±0.03  15.13±0.03</td>
<td></td>
</tr>
<tr>
<td>$f_i$°</td>
<td>99.05±0.03  98.37±0.03  97.88±0.03  96.44±0.03  98.17±0.03  97.46±0.03  98.49±0.03</td>
<td></td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>94.32±0.03  93.75±0.03  95.59±0.03  99.05±0.03  95.10±0.03  95.30±0.03  96.40±0.03</td>
<td></td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>222.51±0.06 220.44±0.06 220.85±0.07 225.66±0.06 221.62±0.05 222.31±0.06 223.03±0.06</td>
<td></td>
</tr>
<tr>
<td><strong>Sol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_i$°</td>
<td>9.40±0.02   8.49±0.02   8.66±0.02   9.79±0.02   9.30±0.02   8.77±0.02   9.19±0.02</td>
<td></td>
</tr>
<tr>
<td>$f_i$°</td>
<td>111.65±0.23 117.34±0.26 113.02±0.25 110.87±0.20 108.48±0.21 113.6±0.21 118.18±0.22</td>
<td></td>
</tr>
<tr>
<td>$\beta_{fp}$°</td>
<td>96.22±0.13  103.13±0.16 105.33±0.16 98.69±0.16 102.58±0.14 101.21±0.13 98.69±0.13</td>
<td></td>
</tr>
<tr>
<td>$f_{fp}$°</td>
<td>91.11±14   103.15±0.24  94.96±0.17  94.98±0.18  93.75±0.15  96.45±0.17  97.86±0.17</td>
<td></td>
</tr>
</tbody>
</table>
Posterior view

MG

LG

Achilles tendon

Lateral View

x

y

z

z

x
(A) Posterior View

(B) Posterior View

(C) Lateral View

(A) (B) (C)
Pennation angle ($\beta$)

Torque level

Ankle Angle

Azimuthal angle ($\phi$)