Muscle activation and its distribution within human triceps surae muscles

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Muscle activation and its distribution within human triceps surae muscles. J Appl Physiol 99: 1149–1156, 2005. First published May 12, 2005; doi:10.1152/japplphysiol.01160.2004.—The purposes of this study were 1) to quantify the volume of activated parts within a whole muscle and 2) to examine activated area distributions along the length of muscle. Seven male subjects performed five sets of 10 repetitions of a single-leg calf-raise exercise with the knee fully extended. Transverse relaxation time (T2)-weighted spin echo images were acquired before and immediately after the exercise. A range of muscle functional magnetic resonance imaging; T2; activation; skeletal muscle

MUSCLE FORCE IS A FUNCTION of the amount of activation of the muscle fibers, which are arranged in three dimensions (18, 34) and are distributed across neighboring muscles (6, 12, 38). Because motor units are activated in the order of their size (14), it is conceivable that submaximal contractions, involving incomplete activation of all motor units, would affect only part of the volume of the activated muscle. One should therefore be able to characterize muscle activation with respect to its distribution in three-dimensional (3-D) space.

Muscle functional magnetic resonance imaging (mfMRI) has been frequently used to examine the intensity and/or pattern of muscle activation. This method relies on an exercise-induced increase in the proton transverse (spin-spin) relaxation time (T2) in MR images of muscle. Exercise-induced shifts in T2 values correlate with integrated electromyography (iEMG) activity (1, 19), force induced by electrical stimulation (2), and workload (1, 19). This has enabled investigators to use a thresholding method to map activity in functionally related regions of muscles, although mainly only one or a few axial MR images were examined (1, 3, 20, 26, 27, 29). Given the architectural complexity of muscle fibers and the distribution of motor unit activation, it would seem necessary to collect data from the entire length of a muscle in three dimensions to fully characterize its activation. But to date there have been very few reports on the activation of a whole muscle (22, 29), and no reports in which whole-muscle activation was examined in three dimensions. Such information on the 3-D distribution of muscle activation would further our understanding of the physiological characteristics of human skeletal muscles and could suggest new approaches to physical rehabilitation.

3-D imaging, such as computed tomography, MRI, and ultrasoundography, was developed to extract improved qualitative and quantitative information about an object or object system from images obtained with multiple modalities. For instance, 3-D MRI has been shown to be more accurate than two-dimensional (2-D) imaging for the quantification of tissue volumes (32), and segmentation of volume images at selected sections can be used to identify and delineate objects. Moreover, because 3-D MRI has the ability to provide maximum-intensity projections, which can be useful for evaluating complex 3-D structures, the visualization of small muscle regions without the use of paramagnetic contrast material may be possible.

The combination of 3-D MRI and mfMRI, hereafter referred to as 3-D mfMRI, should have clear advantages over conventional 2-D MRI and provide insight into the functional and anatomical properties of skeletal muscle. Bearing that in mind, our aims in the present study were to use 3-D mfMRI to quantify the volumes of activated muscles and examine the distributions of activated area along the lengths of those muscles. Our focus was on the triceps surae (TS) muscles, and we hypothesized that because TS muscles have different architectural and functional characteristics (17), the spatial characteristics of their activation would also differ.

MATERIALS AND METHODS

Subjects

Seven healthy young men participated in this study. Physical characteristics of the subjects are as follows: age 23.9 ± 2.0 yr, height 169.7 ± 6.9 cm, weight 62.8 ± 3.5 kg (mean and SD). All subjects were in good health with no orthopedic abnormalities and no neurological or motor disorders before testing. Each provided written, informed consent after the procedures and purposes of the study as well as the benefits and risks of participating in the study were explained. This study was approved by the ethical committee of the university for research involving human subjects.

Experiment 1

Protocol. The lower leg length was measured, and the one-fourths and three-fourths distances from the origin of the patella to the lateral malleolus were marked on the subject’s skin with a pen. Because of
the limited range of the imaging coil, mfMR images were not available over the entire length of the TS muscles. To resolve this problem, MR imaging was carried out at two sessions, i.e., upper and lower parts of the lower leg based on these marks, and these two sessions were separated by at least 2 days and the order of testing was randomized. Subjects were positioned supine with the lower leg within a MR device, and then consecutive axial MR images were collected. Thereafter, subjects performed repetitive calf.Raise exercises. The exercise was executed outside the magnet bore, after which subjects immediately moved into the magnet for imaging.

Exercise. Each subject stood on a columnar box (height, 11 cm; diameter, 29 cm) and performed a calf.Raise exercise that involved raising and lowering one’s body unilaterally for five sets of 10 repetitions. As described previously (4, 19), the subject’s body was raised by flexing the ankle joint concentrically to a fully plantar flexed position in 2 s, and then lowered by dorsiflexing the ankle joint eccentrically so that the ankle resumed its initial position in 2 s (Fig. 1). During the exercise, the heel was positioned above the ground to facilitate exercise, and the knee joint angle was set at a fully extended position throughout the exercise. There was no rest period between the raising and lowering of one’s body. Use of a chair permitted subjects to take a 1-min rest interval between sets. Subjects were allowed to put their hands on a wall for balance. All subjects were familiarized with the calf.Raise exercise before participating in the study and were supervised by an investigator to ensure that the exercise was performed correctly.

mfMR imaging and analysis. MRI scans were carried out before and within 1 min after completion of the exercise using a 0.3-T AIRIS imaging system (Hitachi Medical). A T2-weighted spin-echo sequence (repetition time = 2,500 ms, echo time = 25, 80 ms, 256 × 256 matrix, scan time = 5 min 30 s) was used to collect 22 mfMR images using an extremity body coil; the images were 10 mm thick and 0 mm apart and provided a 27-cm field of view. The subjects lay on their backs with the knee and ankle kept at 180° (full extension) and 90° (anatomical position), respectively.

The mfMR images were transferred to a personal computer (G3, Apple) using fixed software on the MR system. Mean muscle T2 values (±SD) were calculated from reconstructed two-dimensional T2 images on a pixel-by-pixel basis from the two magnitude images with the assumption of a single-exponential decay using a modified version of the public domain National Institutes of Health (NIH) Image program (written by Wayne Rasband at the NIH and available via the Internet by anonymous ftp://rsbweb.nih.gov). The T2 images analyzed extended from one obtained just inferior to the origin of the MG (first image) through one that included the distal end of the soleus (Sol; last image) (Fig. 2). A region of interest (ROI) in each image was defined by manually tracing around the individual muscles and then subcutaneous and intramuscular fat, tendon, aponeuroses, and vessels were excluded by tracing. The active muscle regions were defined by the ranges of pixels with T2 values greater than the mean + SD of the entire ROI in each preexercise image and lower than the mean + SD of the entire ROI in each postexercise image, as referred by Adams et al. (2). In addition, five of the seven subjects were randomly selected and retested on a different day to ensure reproducibility of the T2 measurements.

The 3-D visualization was performed on Amira 3.0 software package (Template Graphics Software). The package has an intuitive user interface and can simultaneously display the orthogonal view of the 3-D image, multiple surface sections, and multiple 3-D surface renderings from any angle. The colors and transparency of the surfaces can be edited to allow the user to display one surface inside another. After the contours of the individual muscle in each image were defined, the active muscle area was defined using the aforementioned thresholding method and displayed in red. All contours were reviewed by superimposing them on the corresponding image slice and, if necessary, corrected using simple manual point-and-check operations within the platform. The surfaces of the muscle and its activated parts were then reconstructed (Fig. 3), and their volumes calculated with a surfacing algorithm that took advantage of the full 3-D data set. A Gauss filter was used to remove image noise (39).

Reconstructed 3-D images were segmented into eight portions to quantify the volume of regions of active muscle using a semiautomated segmentation technique. The first step in this method is to divide the 3-D images of each muscle into eight regions so as to orientate the cutting plane with respect to the center of the x-, y-, and z-axes, respectively. The volumes of these muscle regions were quantified for every four of the eight regions, enabling comparison of deep and superficial regions, medial and lateral regions, and proximal

![Fig. 1. Schematic illustration of calf-raise exercise. Exercise was performed in a standing position and involved raising and lowering one’s body unilaterally for 5 sets of 10 repetitions. Subject’s body was raised by flexing the ankle joint concentrically to a fully plantar flexed position in 2 s (B), and then lowered by dorsiflexing the ankle joint eccentrically (C) so that the ankle resumed its initial position in 2 s (A).](http://jap.physiology.org/)

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and distal regions (Fig. 4). The accuracy of the volume determination made by Amira was checked by measuring a known volume filled with water, and the error of measurements was calculated using the following equation: 

\[
\text{error (\%)} = \left( \frac{\text{measured volume}}{\text{actual volume}} \right) \times 100 \%
\]

The validity of 3-D mfMRI for estimating active muscle area was tested by comparing the 3-D mfMRI data with conventional EMG measurements, looking at the effects of workload. Subjects \((n = 6)\) performed seven sets of 10 unilateral plantar flexion exercises at three different resistances of increasing magnitude: 25%, 50%, and 75% of their 12 repetition maximum (RM). Sufficient rest (3 days) was allowed between bouts to exclude the effect of fatigue. Activated areas and iEMG activity were collected from the midbelly of the medial gastrocnemius (MG) muscle using 3-D mfMRI and EMG, respectively. Calculation of the activated area was similar to the abovementioned, and iEMG values during respective workloads were normalized to the corresponding values (%iEMG) obtained during the 12-RM test.

Experiment 2

Protocol. On the basis of the results of experiment 1, all subjects further participated in an EMG study. A maximal voluntary contraction (MVC) test was conducted after a familiarization session, from which the torque and EMG were determined. After a sufficient rest period (~10 min) after completion of the MVC measurement, subjects performed five sets of 10 repetitions of the calf-raise exercises, during which the EMG activity was measured.

Electromyography. With the use of preamplified surface electrodes (DE-2.1; interelectrode distance 10 mm; Delsys), EMGs were recorded from the proximal and distal parts of the MG and the midbelly of the lateral gastrocnemius (LG) and Sol muscles during the MVC and calf-raise exercises. The Sol electrode was placed as described by McLean and Goudy (23), centered at a location two-thirds the distance from the calcaneus to the head of the fibula, which corresponds to the length of the Sol. Care was taken to ensure that this site was located between the Achilles tendon and the inferior border of the LG, and a preliminary cross-correlation analysis revealed negligible cross-talk among EMG channels. After the skin was carefully cleaned, the electrodes were placed at locations previously marked with a permanent marker. The permanent marks enabled us to place the electrodes at the same spot in each session. Reference electrodes were placed over the patella of both legs. The EMG signals were amplified \((\times 1,000)\) and band-pass filtered \((15–500 \text{ Hz})\).

Torque. Maximal isometric plantar flexion torque was measured on an electrical dynamometer (Biodex System 3, Sakai Medical). Subjects were placed in a comfortable, upright, seated position (hip at 90° flexion) on the dynamometer chair and were secured using thigh, pelvic, and torso straps so as to minimize extraneous body movements. The ankle joint was set at 90° (anatomical position), with the knee joint at full extension and the foot securely strapped to a footplate connected to the lever arm of the dynamometer. After a warm-up and submaximal contractions, the subjects were required to exert maximal plantar flexor force for 2–3 s. The task was repeated twice per subject with at least 5 min between trials. During the measurement of isometric torque, each subject was required to fold his arms across his chest and was given verbal encouragement in an attempt to achieve a maximal voluntary effort level. All procedures and verbal encouragement were administered by the same investigator for all subjects.

Data Analysis

The EMG and torque signals were stored on a personal computer after analog-to-digital conversion at a sampling frequency of 1 kHz (16 bit, Mac Lab/16, ADInstruments). For the MVC trials, the torque was quantified as the peak value. The EMG signals were full-wave rectified and then determined as the integral value (i.e., iEMG) over the duration of contraction (i.e., 40 s). A calculation period of 40 s was employed because it corresponded to the time required to complete each set of calf-raise exercises. The iEMGs recorded during calf-raise exercise were then averaged over five sets. Each iEMG recorded during MVC and calf-raise exercise was divided by the respective duration, and average iEMG during calf-raise exercise was expressed as a percentage of the corresponding values (%iEMG) obtained during the MVC.

Statistical Analysis

Means \((\pm \text{SE})\) were calculated for all variables. Reproducibility of measurement of T2 value was tested by regression analyses and
Student’s t-test. Statistical significance of the relationship between active muscle area and EMG or workload was studied by regression analysis, and Pearson product-moment correlations (r) were calculated. Differences in muscle measures [muscle volume, cross-sectional area (CSA), and volume of activated muscle], normalized iEMG, and %T2 change between different muscles and regions were tested by one-way analysis of variance. Statistical significance of the relationship between volume of actual muscle and activated muscle was checked by the regression analysis. All statistical analyses were performed using the Stat View software package (Hulinks), and significance was accepted at P < 0.05.

RESULTS

The correlation coefficient between the first and second measurements of T2 for five subjects was \( r = 0.89 \) for the rest value and \( r = 0.86 \) for after exercise value. There was no significant difference in T2 value between mean values of the two measurements. The respective coefficient of variations was calculated to be 6.1 and 7.0%.

The reconstructed volume was found in good agreement with the actual volume from phantom \( (r = 1.00; P < 0.01) \).
The error ranged between −0.36% and −0.14%. Active muscle area determined by 3-D mfMRI was significantly correlated with both EMG and workload with correlation coefficients of 0.84 and 0.85, respectively.

Table 1 shows the muscle volume, activated muscle volume, and the relative values for individual TS muscles. The Sol was found to have the largest muscle volume (430.2 ± 12.2 ml), followed by the MG (247.0 ± 16.8 ml) and LG (130.3 ± 6.5 ml; *P < 0.05), and these values were similar to those obtained in a previous study (11). During calf-raise exercise, the percentage volume of individual activated muscle varied. Those most activated muscle were the MG (45.7 ± 2.3%), followed by the LG (34.9 ± 1.6%) and Sol (34.9 ± 1.3%). There was a significantly difference between MG and LG or Sol (*P < 0.05). The absolute activated volume of muscle was linearly related to the muscle volume (r = 0.94, *P < 0.05).

The anatomical and activated CSA along the length of each TS muscle are shown in Fig. 5. The location of the largest activated CSA was observed in the mid-belly of each muscle, and thus individual TS muscles had similar locations for the largest anatomical and activated CSA. Figure 6 shows the relative activated CSA along the length of each muscle. The distal portion of the MG had a larger percent activated CSA than the proximal portion (*P < 0.05). In contrast, there was no regional difference in the LG and Sol.

The relative activated volume in every portion for each TS muscle is shown in Fig. 7. In the MG, there was a significant difference in percent activated volume between distal and proximal portions (*P < 0.05). However, the LG, Sol, and another portion of the MG did not show such partial differences.

Table 2 shows the maximal isometric torque, normalized iEMG, and percent change of T2 in the individual TS muscles. The normalized iEMG in the distal parts of the MG was greater than the LG, Sol, and proximal parts of the MG as well (*P < 0.05). MG had the largest %T2 change among TS muscles (*P < 0.05).

**DISCUSSION**

When implementing a new measuring technique, it is important to confirm its accuracy, reproducibility, and validity. The results of the present study demonstrate that 3-D mfMRI is both highly accurate and highly reproducible. Muscle volumes were measured to accuracies within −0.3%, which is remarkable given the imaging protocol employed 10-mm-thick slices. The reproducibility of this technique was demonstrated by repeated measurements that yielded a coefficient of variation of ~7%. It is also noteworthy that there was a significant correlation between the workload-related changes in active muscle area measured using 3-D mfMRI and iEMG activity.

Taken together, these findings indicate that the 3-D mfMRI technique described here is sufficiently accurate, reproducible, and valid for use in determining active muscle area as a measure of the amount of activation.

The major finding of this study was that the areas of activated muscle accounted for ~46% of the total MG and 35% of the total LG and Sol, respectively. From a muscle architecture perspective, the MG was characterized by shorter fascicle lengths and larger fascicle angles than the LG (17) and thus contains more fibers within a given volume (11). Consequently, the MG has a greater force-producing capacity than the LG or Sol. Our results are corroborated by data reported for
ankle plantar flexor exercise, in which the MG showed higher iEMG (19, 28, 36, 37) and T2 values (4, 19, 28, 42) than the LG and Sol at several workload settings. We therefore conclude that the relative areas of activation differ among TS muscles during our exercise protocol.

The Sol showed the largest activated area among the TS muscles. Although previous studies have reported that the Sol is certainly active during planar flexion with the knee fully extended, minimal activation was observed using a resistance setting that yielded 50 repetitions (36). The normalized iEMG and %T2 value obtained in the present study are consistent with those earlier ones. Moreover, our other calculations indicate that the Sol made up 53% of the total volume of the TS muscles, followed by the MG (31%) and LG (16%). Total muscle volume strongly correlated with the volume of activated muscle in the present study, and thus the larger activated volume seen in the Sol is likely related to the overall size of the muscle.

We found that, in the MG, relative activated area in the distal portion of the muscle was larger than that in the proximal portion. This region-specific difference was consistent with the present EMG data, suggesting muscle activation is distributed unevenly along the length of the MG. The existence of such heterogeneity is reflected by the architecture of the MG muscle (18); the fiber pennation angles in the distal portion are significantly smaller than in the proximal portion. That there is a significant negative correlation between the specific tension and the fiber pennation angle (16) means that the distal portion of the human MG has a greater force-generating capacity.

The existence of compartmentalization within muscles has been demonstrated in the MG of the rat (8, 15), where there is a load-related activation differential between the proximal and distal compartments. On the other hand, direct dissection of cadaver specimens revealed no neuromuscular compartmentalization in the human MG (40). It is known that neuromuscular compartmentalization is dependent on the innervation ratio and muscle architecture (35). However, muscles in embalmed cadavers change their morphological characteristics due to factors such as shrinkage (10), which makes it difficult to acquire precise information about muscle architecture. Furthermore, the fact that nonuniform architecture along the length of a muscle can play a key role in creating different regions of muscle activation (41) is consistent with the idea that human MG possesses neuromuscular compartmentalization, as has been shown for the LG (35). Motor units located in the distal portion of the rat MG have a greater fiber CSA than those in the proximal portion (7). If these differences exist in the human MG, activated areas would differ along the length of the muscle. Indeed, heterogeneity in the distribution of fiber CSA has important implications for the

![Fig. 6. Relative activated CSA along the length of the MG, LG, and Sol muscles. Percentage of activated CSA is expressed relative to the anatomical CSA. Distance 0 was identified for each subject by the proximal edge of the MG muscles. Each bar represents the mean (±SE) for all subjects. *Significantly different from the 2nd value; #significantly different from the 3rd value.](image-url)

![Fig. 7. Relative activated volume of deep and superficial regions, medial and lateral regions, and proximal and distal regions in the MG, LG, and Sol muscles. Percent activated volume is expressed relative to the whole muscle volume. Percent activated volume is compared with deep and superficial regions, medial and lateral regions, and proximal and distal regions, respectively. Each bar represents the mean (±SE) for all subjects. *Significant difference.](image-url)
mechanical and functional properties of different regions of a skeletal muscle. Unfortunately, the distribution of fiber CSA in the human MG is not yet known; thus a more detailed study is warranted. Still, our findings suggest that the differences in activation between the distal and proximal portions of the MG are likely related to the differences in muscle architecture and neuromuscular compartmentalization and/or fiber distribution.

Region-related differences in activation were not observed in the LG or Sol. English and coauthors (35, 41) reported that the LG, which contains three anatomically distinct heads, exhibits functional partitioning. However, when Wolf et al. (41) recorded task-oriented EMG activity from the LG, they found no statistically significant differences in EMG activity between the proximal-distal and medial-lateral regions when the subject performed a standing unilateral plantar flexion with non-weight bearing. Our findings are consistent with those and suggest task-dependent nonuniform activation might occur in the LG. To our knowledge, however, no regional variations in Sol activation have yet been found. Further studies would be necessary to clarify the activation difference in the Sol.

So why does the distal portion of the muscle show greater activation than the proximal portion in the MG, but not in the LG or Sol? One possibility is that, because the MG acts as an agonist muscle among plantar flexors, greater activation in the distal portion is effective to force transmission to the ankle joint. Zuurbier et al. (43) found that the largest extension of the MG aponeurosis occurred in the most distal part of the muscle during contraction in humans, which suggests the greatest agonistic action of a muscle might be located in the region nearest a joint. On the other hand, Muramatsu et al. (25) reported that the aponeurosis of the human MG is not heterogeneously stretched along the muscle length during contraction. To resolve this discrepancy, additional studies will need to be carried out under conditions in which the knee joint is flexed.

There are two main limitations to the noninvasive measurement of muscle activation using 3-D mfMRI. First, the active muscle area was estimated based on thresholded T2 values. Given the strong correlation they observed between the number of pixels showing an increase in T2 and the observed force output, Adams et al. (2) concluded that T2 values greater than the mean ± SD from the values in corresponding preexercise images can be considered indicative of active muscle. To measure the area of nonmuscle tissues within muscle, they were defined as the areas with resting T2 values greater than 35 ms subtracted from the postexercise images. Although several studies have used this thresholding method to map the location of muscle activity (1, 3, 20, 26, 27, 29), it is somewhat equivocal because resting T2 values are affected by the magnetic field and scan parameters (for a review, see Ref. 24), as well as by the fiber type (5) and the intramuscular fat content (13, 31). For example, there is a difference in the resting T2 values between our recently published data (21) and Akima’s data (3), although the muscles and the physical characteristics of the subjects were similar. For that reason, to accurately quantify the active and nonactive muscle areas, ranges of pixels with T2 values greater than the mean ± SD of the entire ROI in the preexercise image and lower than the mean ± SD of the entire ROI in the postexercise image were defined as an active muscle. The first threshold was consistent with earlier studies (1, 3, 20, 26, 27, 29), whereas the second should account for excluded areas of nonmuscle tissue, given that this threshold value is about the same as that obtained with bone marrow and that intramuscular fat has a greater T2 value than marrow. In this way, we were able to accurately exclude areas of nonmuscle tissue from areas of activated muscle. In an earlier report, Prior et al. (29) suggested that T2 values could not be used to reliably map active muscle on a pixel-by-pixel basis in normal subject. Nevertheless, in view of our finding that there is a highly significant correlation between active muscle area and EMG activity or workload, we conclude that areas of muscle activation estimated using 3-D mfMRI are representative of the actual amount of muscle activation.

Second, because exercise-induced increases in T2 depend on muscle fiber type (30), differences in T2 values among TS muscles cannot be directly interpreted as a difference in muscle activation. The gastrocnemius muscle, for example, contains a considerably higher percentage of fast-twitch fibers than does the predominantly slow Sol (9). The T2 increase is greatest in a muscle characterized by a higher percentage of fast-twitch fibers than low oxidative capacity and high glycolytic capacity (31). Fortunately, fiber types and their distributions in human muscles do not dramatically compare with those in the corresponding muscles of rodents and other quadrupeds (33). Therefore, Prior et al. (30) concluded that semiquantitative comparisons among muscles can be justified irrespective of fiber type. In fact, human TS muscles have frequently been used in studies in which mfMRI was used to measure muscle activation (4, 19, 28, 42).

In conclusion, our data show that the MG had the largest percent activated areas (45.7%), followed by the LG (34.9%) and Sol (34.9%). There was a nonuniform distributed pattern of muscle activation in the MG, but not in the LG and Sol. These results suggest that the size of the activated region and its distribution would be different among TS muscles, thus indicating that individual TS muscles play different functional roles during contraction.

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