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Quantitative assessment of skeletal muscle activation using muscle functional MRI

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Abstract

The purpose of the present study is to determine whether muscle functional MRI (mfMRI) can be used to obtain three-dimensional (3-D) images useful for evaluating muscle activity, and if so, to measure the distribution of muscle activity within a medial gastrocnemius (MG) muscle. Seven men performed 5 sets of 10 repetitions of a calf-raise exercise with additional 15% of body-weight load. Magnetic resonance images were obtained before and immediately after the exercise. To threshold images, only those pixels showing transverse relaxation time (T2) greater than the mean+1 S.D. of the entire regions of interest (ROIs) in the preexercise image and T2 lower than the mean+1 S.D. of the entire ROIs in the postexercise image were identified. The survived pixels showing T2 are defined as active muscle. Those thresholded images were 3-D reconstructed, and this was used to determine area of active muscle along transverse, longitudinal and vertical axes. At the exercise level used in the present study, the percentage volume of activated muscle in the MG was 62.8±4.5%. There was a significant correlation between percentage volume of activated muscle and integrated electromyography ($r=.78$, $P<.05$). Percentage areas of activated muscle were significantly larger in the medial than in the lateral region, in the anterior than in the posterior region and in the distal than in the proximal region ($P<.05$). These results suggest that mfMRI can be used to evaluate the muscle activity and to determine intramuscular variations of activity within skeletal muscle.

Keywords: Muscle; Activation; T2; EMG; Human

1. Introduction

Magnetic resonance imaging (MRI) is being used with increasing frequency in research involving acquisition of anatomical information. One unique aspect of MRI is that exercise induces signal changes resulting primarily from increases in the transverse relaxation time (T2) of tissue water. Exercise is known to produce changes in the amount and distribution of water within skeletal muscle, and at the present time, a shift in water distribution is the purported mechanism for changes in T2 [1]. These T2 changes are well correlated with integrated electromyography (iEMG) [2,3], increase with exercise intensity [2–4] and relate to torque evoked by electrical stimulation [5]. Moreover, T2 and EMG show similar changes during exercise at different joint angles [6]. This technique, which is referred to as muscle functional MRI (mfMRI), is useful for assessing the extent of muscle activation while performing a task.

Given the architectural and topographical complexity of muscle fibers [7] and motor units [8,9] and the nonuniform distribution of innervation territories of motoneurons, it would seem necessary to collect data from the entire length of a muscle in three dimensions to fully characterize its activation. Thus, the principal advantage of mfMRI is that it overcomes some of the limitations of surface EMG. With surface EMG, for example, it is difficult to detect muscle activity over large regions or in regions deep within the muscle, and it is virtually impossible to detect the activity of an entire muscle of interest and/or limit cross talk between muscles. By contrast, mfMRI readily enables one to study the activity in an entire muscle and to noninvasively obtain three-dimensional (3-D) images of muscles. Meyer and Prior [10] stated that, as compared to surface EMG, the most notable feature of mfMRI is its potential for use in mapping the spatial variations in activity within a muscle, which can enable

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determination of the spatial distribution of activated muscle fibers during a task. The first application of mfMRI to map spatial variations in activity within a muscle was by Adams et al. [5], but theirs was a 2-D analysis. Indeed, because most studies reported thus far have investigated muscle activity in only two dimensions, the patterns of 3-D variation in muscle activity and how those patterns relate to muscle function remain largely unknown.

The purpose of the present study, therefore, was to determine whether mfMRI can be used to obtain 3-D images useful for evaluating muscle activity, and if so, to measure the distribution of muscle activity within human skeletal muscle. We studied the activation of the medial gastrocnemius (MG) muscle, testing the following three hypotheses: (1) that mfMRI can be used to create 3-D images that enable one to distinguish active from inactive muscle; (2) that muscle activity, as evaluated by mfMRI, reflects the iEMG; and (3) that mfMRI can be used to determine the distribution of activity within a muscle.

2. Materials and methods

2.1. Subjects

Seven male subjects participated in the study: age, 24±2 years; height, 1.70±0.07 m; weight, 63±4 kg (mean±S.D.). All subjects were in good health, with no orthopedic abnormalities. Subjects were fully informed of the procedures to be used as well as the purpose of the study, and written informed consent was obtained from all subjects. The experiments were carried out according to the guidelines laid down by the Ethical Committee of the University.

2.2. Magnetic resonance image acquisition and analysis

Axial MR image was collected on an AIRIS 0.3-T MR imaging machine (Hitachi Medical, Tokyo, Japan) using extremity coil (40-cm diameter) and a T2-weighted spin-echo sequence (repetition time, 2500 ms; echo time, 25 and 80 ms; field of view, 270 mm; 22 slices; slice thickness, 10 mm; slice gap, 0 mm; matrix 256×256; scan time, 5 min 30 sec). The subjects were lying supine in the magnet with the leg held steady using a bitemporal clamp while the knee joint kept at 180° (full extension). The MRI scans were carried out before the exercise and stated within 1 min of the end of exercise.

Because of the limited range of the imaging coil, each subject participated in two imaging sessions separated by at least 10 days. Images were obtained from either between the proximal of the patella and midcalf (at proximally 50% of the distance between the proximal of the patella and the distal end of the tibia) or between the midcalf and the distal end of the tibia. The order of the acquired MR images was random. A permanent water-soluble marker was used on the subjects' skin as an anatomic reference point to ensure that images were collected from the same location for repeated scans. This reference point was also used to align collected MR images from the two sessions.

MR images were transferred to a personal computer. Activated areas were determined from MR images using a modified version of the public domain NIH image software. A region of interest (ROI) was defined by manually tracing around the MG. Care was taken to exclude subcutaneous and intramuscular fat, aponeurosis and vessels from the traced regions. The T2 (mean±S.D.) and the total number of pixels were determined for the ROI in each slice. As previously described [5], ranges of pixels with a T2 greater than the mean+1 S.D. of the entire ROI in each preexercise image and a T2 lower than the mean+1 S.D. of the entire ROI in each postexercise image were determined. Survived pixels showing T2 are defined as active muscle and indicated red color.

Thresholded images were 3-D reconstructed using Amira 3.0 software (Mercury Computer Systems, San Diego, CA), from which the activated muscle volume was then determined. To reduce the level of noise within the images, we smoothed the images using a gaussian filter (radius of 2 pixels), which redrew the images and averaged the pixel values according to a gaussian function [11]. This filter did not affect the determination of regions of muscle activation. To examine the 3-D distributions of muscle activity, we used reconstructed 3-D images to determine areas of "active muscle" along transverse (x-axis), longitudinal (y-axis) and vertical (z-axis) axes. The first step was to segment the x-, y- and z-axes of each voxel in the 3-D images using a semiautomated segmentation technique. The area of active muscle was then quantified for every 1 cm along each of the three axes. These measurements were carried out separately by two individuals. The interday reproducibility of the T2 ($n=5$) and volume ($n=8$) measurements was tested on separate days.

To validate the volume determination reconstructed from the x-, y- and z-axes, we carried out mfMRI using a known volume filled with water ($n=5$). The water portion was reconstructed in 3-D using Amira and oriented with respect to a cutting plane through each pixel along the x-, y- and z-axes. The volume was then calculated by multiplying the sum of the pixels in each plane. The error in the volume measurements was calculated using the following equation: [error (%)=(measured volume − actual volume)×100/actual volume].

2.3. Exercise protocol

Subjects performed calf-raise exercises for 5 sets of 10 repetitions. Exercise leg was randomly chosen. The subject's own body weight with additional 15% of body-weight load was raised by flexing the ankle joint to a fully plantar flexed position in 2 s and then lowered by dorsi flexing the ankle joint so that the ankle resumed its initial position in 2 s, as described previous studies [3,12]. There were no rest periods between the plantar flexion and dorsi flexion of the ankle or between repetitions of the movement. During the 1-min rest interval between sets, the subjects were seated in a chair. The knee remained in full extension throughout the

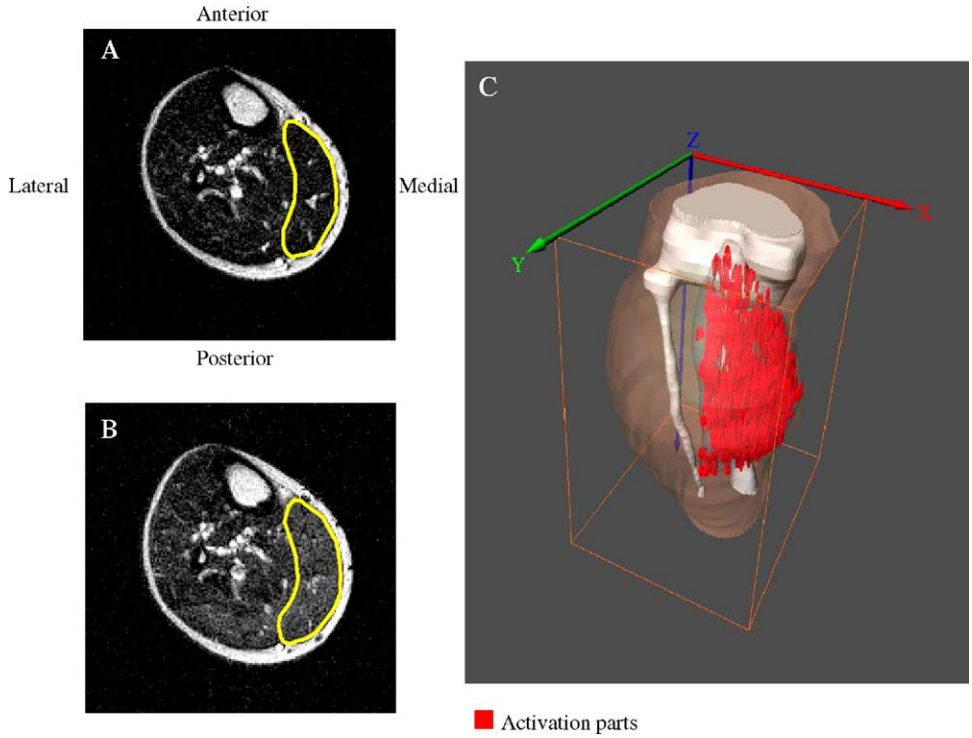

Fig. 1. Representative axial plane T2-weighted (S.E.; TR/TE, 2500/25 and 80) MR images where the location is in the midbelly of the calf muscle obtained before (A) and immediately after exercise (B), and 3-D active muscle image (C) for the MG from one subject. Yellow line indicated contour of the MG. In 3-D active muscle image, semitransparent color represents the muscle and the red regions indicate muscle regions with range pixels with a transverse relaxation time threshold, which is considered to be activated parts due to exercise.

exercise and they were allowed to put their hands on the wall to provide balance but not support. The exercise was performed outside the magnet bore, after which, subjects immediately moved into the magnet for imaging.

2.4. Electromyography

EMG activity was recorded from the midbellies of MG using preamplified surface electrodes (DE-2.1, Delsys, Boston, MA). After careful abrasion of the skin, the electrodes were placed at the same locations in each session with use of permanent spot marks. The reference electrode was placed over the patella. The EMG signals were acquired continuously throughout the calf-raise exercises with a band-pass filter between 15 and 500 Hz and were analog-to-digital converted (Mac Lab/16s, ADInstruments, Nagoya, Japan) at a sampling rate of 1000 Hz. The EMG was full-wave rectified and integrated for the duration of the exercise (40 s) to give integrated EMG (iEMG). A calculation period of 40 s was employed because it corresponded to the time required to complete each set of exercise. The iEMGs were then averaged over five sets.

2.5. Statistics

Descriptive statistics are means±S.D. Statistical significance of the relationship between percentage volume of activated muscle, which is expressed relative to the whole muscle, determined by mfMRI and iEMG was studied by regression analysis, and Pearson product–moment correlations (r) was calculated. Accuracy of reconstructed volume determination and reproducibility of T2 and volume measurements were tested by regression analyses and Student's t test. Difference in area of activated muscle between different regions was tested by one-way analysis of variance. The probability level accepted for statistical significance was set at $P<.05$.

3. Results

The reconstructed volume determined from segments along the x-, y- and z-axes was found to be in good agreement

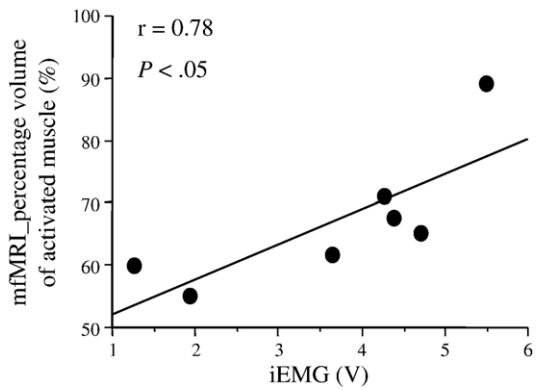

Fig. 2. The relationship between percentage volume of activated muscle determined by mfMRI and iEMG of the MG in each subject.

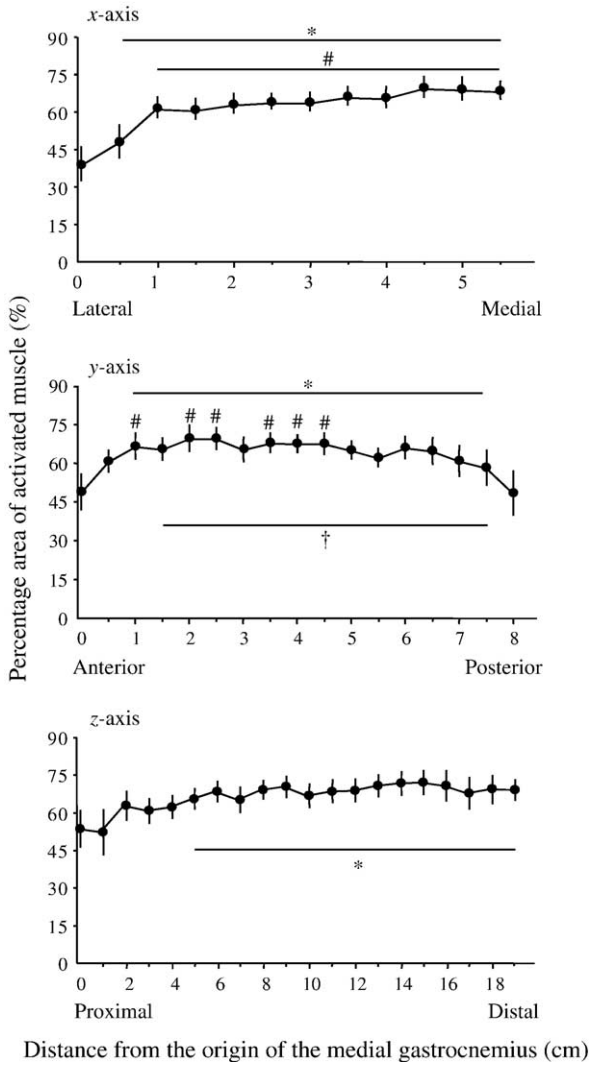

Fig. 3. Percentage area of activated muscle, which is expressed relative to the anatomical cross-sectional area, along the x-axis (transversely), y-axis (longitudinally) and z-axis (vertically) of the MG, respectively. Distance 0 was identified for each subject by the lateral, anterior and proximal edges of the MG. Each bar represents the mean (±S.D.) for all subjects. *Significantly different from the 1st value, $P<.05$; #significantly different from the 2nd value, $P<.05$; †significantly different from the 17th value, $P<.05$.

with the actual volumes ($r=1.00$, $P<.01$). The errors ranged from −2.0% to −4.9%, and there were no significant differences between the reconstructed and actual volumes.

The correlation coefficients between the first and second measurements were $r=.89$ ($P<.01$) for rest T2, $r=.86$ ($P<.01$) for after exercise T2 and $r=.97$ ($P<.01$) for volume. There were no significant differences in the T2 and volume between mean values of the two measurements.

Fig. 1 presents axial MR images that were acquired before (A) and immediately after exercise (B) and 3-D active muscle images (C) of the MG from one subject. Following exercise, the muscles that were activated appear hyperintense on these T2-weighted images (B). The red regions indicate the muscle portions, which are activated due to exercise (C).

Fig. 2 shows the relationship between percentage volume of activated muscle determined by mfMRI and iEMG of the MG in each subject. There was a significant correlation between the percentage volume of activated muscle and iEMG ($r=.78$, $P<.05$).

At the exercise level used in the present study, the percentage volume of activated muscle in the MG was 62.8±4.5%. The percentage areas of activated muscle expressed relative to the anatomical cross-sectional areas along the x-, y- and z-axes of the MG are shown in Fig. 3. Percentage areas of activated muscle were significantly larger in the medial than in the lateral region, in the anterior than in the posterior region and in the distal than in the proximal region ($P<.05$). Collectively then, our findings indicate that the intramuscular distribution of muscle activity within the exercised MG can vary along the transverse, longitudinal and vertical axes, resulting in a three-dimensionally nonuniform distribution of muscle activity.

4. Discussion

The results of the present study demonstrate a novel method for assessing muscle activity in 3-D space. Previous studies have shown strong correlations among the exercise-induced shift in T2 and iEMG [2,3], the force induced by electrical stimulation [5] and the exercise intensity [2–4]. Moreover, there is a relative agreement between the exercise-induced shift in T2 and the EMG during plantar flexion exercise at different knee joint angles [6]. The muscle activation data evaluated using the T2 threshold in the present study are consistent with those previous studies. The volume of activated muscle within the MG correlated significantly with iEMG, which is noteworthy as information extracted from the surface EMG is often considered to be a global measure of motor unit activity. This means that because it is the activity of individual motor units that induces the activation of muscles or muscle groups, regions of muscle activity measured with mfMRI can be considered to represent regions of neurally evoked activation.

The novel finding in the present study was that there is a substantial variation in the muscle activation within the MG. Activation was greatest in the medial, anterior and distal regions along the transverse, longitudinal and vertical axes, respectively, and was distributed in a parabolic fashion longitudinally, but in a more linear fashion transversely and vertically. Apparently, the medial, anterior and distal portions of the MG are the most susceptible to contraction and may thus generate more force and work than other regions of the muscle do.

As implied above, one possible interpretation of these nonuniform patterns of muscle activity relates to the innervation pattern of nerve fiber. Wolf and Kim [13] showed that patterns of nerve ramification are variable. The human MG has one primary nerve that divides into two secondary branches and then into highly variable numbers

of tertiary and quaternary branches. Patterns of nerve ramification previously have been mapped in three dimensions within a volume using 3-D computer modeling [14], but we did not map the pattern of innervation of the MG. An alternative explanation for the nonuniform pattern of muscle activity is that motor units are arranged topographically within some muscles [8,9] and are activated in the order of their size [15]. This would enable contraction to be evoked in discrete regions of a muscle [16]. In either case, it is conceivable that submaximal contractions, involving incomplete activation of all motor units, would activate only some areas of the muscle.

It should be noted that mfMRI does not directly measure muscle electrical activity; instead, it is a reflection of muscle cell metabolism and fluid uptake. The underlying cause of the T2 increase in the exercising muscle is not fully understood. But given that mfMRI is based on signals from hydrogen atoms and that the primary source of hydrogen in the human body is water, it seems likely that the exercise-induced increase in T2 seen in skeletal muscle involves movement of water into the muscle. On the other hand, the simple movement of water into the muscle does not fully explain the observed T2 change, as increase in muscle area similar in magnitude to those observed after exercise, but achieved through venous occlusion, are not associated with increases in muscle T2 [4]. Moreover, changes in osmotically active metabolites, such as lactate and phosphate, have been shown to directly correlate with the degree of T2 alteration [17,18]. It is therefore thought that increases in T2 are the result of osmotically driven shifts in muscle water, which increase the volume of the intracellular space and intracellular acidification caused by metabolic by-products [19,20]. This suggests that although increases in iEMG are indicative increases in the firing rate and/or recruitment of motor units [21], exercise-induced changes in T2 are indicative of the metabolic activity associated with force generation. Still, alteration of EMG variables is also reportedly related to accumulation of muscle lactate, intracellular acidosis, muscle conduction velocity, force output and mechanical changes (for a review, see Ref. [22]). It thus appears likely that metabolic events related to muscle activation and energy demands of the activated myocytes are responsible for the increases in T2 observed in mfMRI images obtained after exercise.

The results of the present study indicate that the accuracy and reproducibility of mfMRI are very high. The measurement error of reconstructed volumes was less than 5%, which is remarkably low given that the imaging protocol included two imaging sessions, and should be tolerable when making measurements of tissue volume. In addition, measurements made using this technique were found to be highly reproducible, as there was a strong correlation between the first and second measurements of T2 and volume.

There are nevertheless several limitations to the present study. First, mfMRI was not performed in real time during exercise; images were acquired within 7 min after the subjects finished their exercise. An exercise-induced shift in T2 is detectable after as few as two contractions and then increases to a work-rate-dependent plateau within a few minutes [23]. Recovery after exercise takes 20 min or more [4], which should have enabled us to acquire functional images following exercise performed outside the scanner room [10]. Second, because of the limited range of the imaging coil, mfMRI was carried out in two imaging sessions separated by at least 10 days. This necessitated two exercise periods, introducing the possibility that the response would differ between the first and second periods. On the other hand, the high degree of reproducibility in the volume and T2 measurements suggests that this source of error is minimal. Third, the present study was performed using a 0.3-T scanner, but the 1.5-T scanner is the one most widely used. This is likely not a problem, however, as it was previously shown that resting T2 is not significantly affected by different magnetic fields [5]. Fourth, no normalization procedure for EMG was used. To reduce the variability due to changes in the recording conditions, we usually express interference EMG relative to some standard value. The most common standard is the interference EMG recorded during a maximal voluntary contraction (MVC). Unfortunately, there was no way to properly normalize the EMG data during the present calf-raise exercise because no MVC is performed. Finally, measurements of mfMRI and EMG were not made simultaneously. As described above, the measurements of active muscle area and iEMG can be made in the same subject and at the same position, but the two measurements cannot be made simultaneously. Despite these limitations, the present findings clearly document the feasibility of using mfMRI to noninvasively and quantitatively map regions of human muscle activation in three dimensions.

In summary, mfMRI is capable of characterizing muscle function and measuring intramuscular variations of activity within skeletal muscle. Muscle functional MRI alone or in conjunction with other modalities may provide a powerful means for studying the basic science of biomechanics, allowing study of muscle pathology and adaptation and allowing for improved neuromuscular rehabilitation.

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