Nonuniform Muscle Hypertrophy: Its Relation to Muscle Activation in Training Session

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ABSTRACT

Wakahara, T., A. Fukutani, Y. Kawakami, and T. Yanai. Nonuniform Muscle Hypertrophy: Its Relation to Muscle Activation in Training Session. *Med. Sci. Sports Exerc.*, Vol. 45, No. 11, pp. 2158–2165, 2013. **Purpose:** Muscle hypertrophy in response to resistance training has been reported to occur nonuniformly along the length of the muscle. The purpose of the present study was to examine whether the regional difference in muscle hypertrophy induced by a training intervention corresponds to the regional difference in muscle activation in the training session. **Methods:** Twelve young men participated in a training intervention program for the elbow extensors with a multijoint resistance exercise for 12 wk (3 d wk −1). Before and after the intervention, cross-sectional areas of the triceps brachii along its length were measured with magnetic resonance images. A series of transverse relaxation time (T2)-weighted images was recorded before and immediately after the first session of training intervention. The T2 was calculated for each pixel within the triceps brachii. In the images recorded after the session, the number of pixels with a T2 greater than the threshold (mean + 1 SD of T2 before the session) was expressed as the ratio to the whole number of pixels within the muscle and used as an index of muscle activation (percent activated area). **Results:** The percent activated area of the triceps brachii in the first session was significantly higher in the middle regions than that in the most proximal region. Similarly, the relative change in cross-sectional area induced by the training intervention was also significantly greater in the middle regions than the most proximal region. **Conclusion:** The results suggest that nonuniform muscle hypertrophy after training intervention is due to the region-specific muscle activation during the training session. **Key Words:** TRANSVERSE RELAXATION TIME, CROSS-SECTIONAL AREA, MAGNETIC RESONANCE IMAGING, TRAINING INTERVENTION

Chronic resistance training causes gains in muscle size and strength. Studies have reported that the gain in muscle size (hypertrophy) in response to resistance training occurs nonuniformly along the length of the muscle (4,8–10,12,16–18,22). For example, Narici et al. (18) demonstrated that relative increases in cross-sectional area (CSA) of the quadriceps femoris were greater in the proximal than that in the distal regions after knee extension training. Two possible reasons for the regional difference in muscle hypertrophy have been proposed as follows: 1) differences in muscle activation and 2) differences in contractile protein synthesis (17). As for the muscle activation, we assessed transverse relaxation time (T2) of magnetic resonance (MR) images taken at various locations along the triceps brachii and examined its association to region-specific hypertrophy of the muscle after a training intervention (22). The results indicated that the region-specific changes in T2 of the triceps brachii induced by one session of elbow extension training corresponded to the regional difference in muscle hypertrophy after 12 wk of the training intervention. The change in T2 of muscle tissue after high-intensity exercise is indicative of muscle activation (2,7,14,15,23). Hence, our finding (22) suggests that the nonuniform muscle hypertrophy after a training intervention can be attributed to the region-specific muscle activation during the training session. However, the previous study (22) investigated the T2 change and muscle hypertrophy separately in different groups of subjects. It is still unclear whether the regional difference in muscle activation during a training session has a causative effect on the difference in muscle hypertrophy.

The muscle activation of the agonists during a training session can vary with exercise modalities (5,6). Escamilla et al. (6) compared the EMG activity of the rectus femoris, vastus lateralis, and vastus medialis during single-joint knee extension, multijoint squat, and leg press exercises. They demonstrated that the EMG activity of the rectus femoris was greater in the knee extension than the squat and leg press exercises, whereas EMG activities of the vastus lateralis and medialis were greater in the squat and leg press than the knee...
extension exercises. In addition, increases in the signal intensity of T2-weighted MR image immediately after single-joint knee extension and multijoint leg press exercises were different among the quadriceps femoris (5). These findings indicate that the muscle activation of the synergists is different between single- and multijoint exercises.

If the nonuniform muscle hypertrophy after a training intervention is due to the region-specific muscle activation during the training session, the correspondence between the regional differences in muscle hypertrophy and muscle activation would also be found in a different exercise modality from that used in our previous study (single-joint elbow extension) (22). The purpose of the present study was to test the possibility for the triceps brachii by comparing training-induced hypertrophy with regional variations of T2 changes with a multijoint exercise.

METHODS

Subjects. Twenty-four healthy young men voluntarily participated in the present study. They were sedentary or recreationally active, and none had been involved in a regular resistance training program for the upper limbs for 6 months before the beginning of the experiment. They were allocated to either the training group \( n = 12, 26.9 \pm 3.4 \text{ yr}, 172.4 \pm 4.1 \text{ cm}, 64.6 \pm 7.0 \text{ kg (mean \pm SD)} \) or the control group \( n = 12, 25.3 \pm 2.3 \text{ yr}, 172.2 \pm 5.9 \text{ cm}, 67.6 \pm 9.0 \text{ kg} \). Before intervention, there was no significant difference in the age, body height, body mass, or maximal CSA of the triceps brachii between the two groups. Each subject was fully informed of the purpose and risks of the study and gave their written informed consent. The present study was approved by the Ethics Committee on Human Research of Waseda University.

Experimental design. The subjects of the training group participated in 12 wk of resistance training program for the left upper limb. The participants of the control group did not perform resistance training but maintained their normal activities during the period. Before and after the 12 wk of intervention period, muscle strength of elbow extension, one-repetition maximum (1RM) of the training exercise, CSA, and thickness of the triceps brachii were measured. The T2 change of the triceps brachii was quantified in the MR images acquired before and immediately after the first session of training intervention in the training group. The T2-weighted MR imaging was adopted because it allows us to quantify the muscle activation noninvasively within a muscle in the same region as the measurement of CSA and is suited to examine the correspondence between region-specific muscle activation and muscle hypertrophy.

Resistance training. The training exercise was a dumbbell press-type movement, which is a multijoint exercise that involved forearm extension and arm flexion in the sagittal plane (Fig. 1A). The subjects lay supine on a bench so that the elbow of the subjects did not reach the floor and lifted a dumbbell vertically from just above the chest until the elbow was fully extended (for 2 s), and then lowered it (for 2 s). They were instructed to adjust lifting cadence on the basis of verbal cues and a metronome set to one beat per second (two beats for each phase). 1RM was determined by gradually (1.0–1.5 kg) increasing the mass of the dumbbell until the subjects were unable to perform the full extension of the elbow joint. Usually, three to five trials were required to determine the 1RM, although more attempts were performed when necessary. Measurement of 1RM was performed 2 to 5 d before the first training session and every 2 wk to adjust the load throughout the training period. The training session consisted of five sets of eight repetitions at a load of 80% of 1RM, with a rest period of 90 s between sets. The session was repeated three times per week for 12 wk, usually with 1 or 2 d of rest between sessions. In some cases, however, successive training sessions were performed on consecutive days or with 3 d of rest, when the subjects could not make it convenient to take 1 or 2 d of rest. These program variables of the training were the same as those in our previous study (22), except for the training exercise. All training sessions were supervised by one of the investigators. If the subjects could not lift the dumbbell during the session, they were assisted slightly by the investigator, while they maintained their maximum effort, so that the required number of repetitions could be reached.

Strength testing. Maximum isometric strength of elbow extension was measured with an isokinetic dynamometer (CON-TREX; CMV AG, Dubendorf, Switzerland). The subjects lay supine on a bed of the dynamometer with the shoulder flexed at 90° (Fig. 1B). The wrist of the subjects was fixed to a lever arm of the dynamometer with a belt. The elbow joint was fixed at 70° (full extension = 0°), because the isometric elbow extension torque was reported to be highest at this angle (13). The rotation axes of the elbow joint and dynamometer were visually aligned as closely as
possible. The maximum voluntary isometric torque of elbow extension was measured twice for each subject. A rest period of 2–3 min was provided between trials. The higher value of the two torques was used for further analyses. The torque signal was transferred to a computer via an A/D converter at 1000 Hz (PowerLab/16SP; ADInstruments, Bella Vista, NSW, Australia). The coefficient of variation (CV) of the repeated measurements for isometric torque of elbow extension was 1.8% ± 2.2%. The intraclass correlation coefficient (ICC) of the repeated measurements was 0.988 (90% confidence interval (CI), 0.978–0.994).

**Measurement of CSA.** An MR scanner (Signa 1.5T; GE Healthcare, Waukesha, WI) with a whole-body coil was used to acquire a series of T1-weighted cross-sectional images of the left upper arm (Fig. 2) (echo time, 11 ms; repetition time, 520 ms; slice thickness, 1 cm; matrix, 256 × 192; field of view, 18 cm). All the subjects were instructed to refrain from doing strenuous exercise and drinking alcohol 1 d before MR image recordings. The MR imaging after training intervention was performed at least 6 d after the last training session. The subjects lay prone in the magnet bore. Two scans were performed for the proximal and distal regions of the upper arm. Scanned MR images were transferred to a computer. The CSA of the triceps brachii was measured in each image in which the muscle was visible by manually tracing the outline of the muscle with software (ImageJ; National Institutes of Health, Bethesda, MD). Care was taken to exclude intramuscular fat and blood vessels. The tracing was repeated twice for each slice. The mean of the two values was used for subsequent analyses. The CV of the two measurements for CSA was 0.6% ± 1.0%. The ICC of the measurements was 0.9995 (90% CI, 0.9994–0.9995). The relative change in CSA of the triceps brachii from a baseline value (CSA of the triceps brachii before the training intervention) was computed on five regions (at 4, 10, 16, 22, and 28 cm from the elbow joint) to reduce the effect of the baseline value. The muscle volume of the triceps brachii was calculated as the product of slice thickness (1 cm) and the sum of CSA along its length. In our pilot study (n = 7, 26.9 ± 3.9 yr, 172.1 ± 5.5 cm, 65.5 ± 6.3 kg), the CV and ICC of the repeated measurements for the maximal CSA with the same procedure as in the present study was 2.2% ± 1.3% and 0.979 (90% CI, 0.913–0.995), respectively.

**Measurement of muscle thickness.** Measurement of muscle thickness was carried out according to previous studies (1,11). A B-mode ultrasound apparatus (SSD-6500; Aloka, Tokyo, Japan) was used to measure the muscle thickness with a linear array probe (UST-5712, Aloka). The probe with water-soluble transmission gel was placed at the posterior site of the upper arm and perpendicular to the skin surface at 60% of the upper arm length from the acromial process to the lateral epicondyle of the humerus. During the measurements, the subjects stood with their arm extended and relaxed. Muscle thickness of the triceps brachii was determined as the distance from subcutaneous adipose tissue–muscle interface to muscle–bone interface. The long and medial heads of the triceps brachii were included in the thickness measured. In addition, thickness of the long head was measured.

**FIGURE 2—Examples of T1-weighted MR images at the distal (4 cm from the elbow joint), middle (16 cm from the elbow joint), and proximal (28 cm from the elbow joint) regions of the upper arm before (upper) and after (lower) the 12 wk of training intervention. White broken line indicates the boundary of the triceps brachii.**
at 60% of the upper arm length (1) and slightly medial to the site where the thickness of the triceps brachii was measured. The thickness of the long head was determined as the distance from subcutaneous adipose tissue–muscle interface to the aponeurosis.

**T2 change and percent activated area.** A series of T2-weighted MR images (echo times, 25, 50, 75, and 100 ms; repetition time, 2000 ms; matrix, 256 × 256; field of view, 18 cm; slice thickness, 1 cm; gap, 1 cm) was recorded before and immediately after the first session of the training intervention with the same MR scanner and coil as the T1-weighted imaging (Fig. 3). The subjects lay prone on a bed of the scanner. Ink marks were made on the skin of the upper arm and used to adjust the position of the subjects for repeated scans. The time elapsed from completion of the training session to initiation of the scanning was 97 ± 22 s. Calculation of T2 of the triceps brachii was performed with software (ImageJ, National Institutes of Health). The outline of the triceps brachii was manually selected in the images. Noncontractile tissues such as intramuscular fat and blood vessels were excluded from the analysis. The T2 was determined for each pixel within the selected area. The mean and SD of T2 within the selected area were calculated for each image obtained before the training session. In the images after the training session, the number of pixels with a T2 greater than the threshold (mean + 1 SD of T2 before the session) was counted and expressed as the ratio to the whole number of pixels of the triceps brachii (percent activated area) to reduce the effect of absolute CSA (3,22). The percent activated area of the triceps brachii was determined on five regions (at 4, 10, 16, 22, and 28 cm from the elbow joint). Although the boundaries between heads (long, medial, and lateral heads) of the triceps brachii were not clearly visible in the obtained images, a portion of each head (about 1 cm²), rather than the entire CSA, was selected in several images (four images for the long head, three images for the medial head, and one image for the lateral head). The percent activated area of each head was computed as the ratio of the number of pixels with a T2 greater than the threshold to the total number of pixels within the selected area. These analyses were conducted twice for each slice, and an averaged value was used for subsequent analyses. The CV of the measurements for percent activated area was 0.5% ± 0.6 percent. The ICC of the measurements was 0.9997 (90% CI, 0.9996–0.9997).

**Statistics.** Paired *t*-tests were used to test the significance of the difference in 1RM, maximum isometric strength, and muscle volume and thickness before and after the intervention period. One-way ANOVA with repeated measures was used to test the effect of the region (five regions) on the percent activated area of the triceps brachii and relative changes in CSA. One-way ANOVA was also used for the effect of the region on the percent activated area of the long head (four regions: 10, 16, 22, and 28 cm from the elbow joint) and medial head (three regions: 4, 10, and 16 cm from the elbow joint) and the effect of the head (three heads) on the percent activated area at 16 cm from the elbow joint.

![FIGURE 3—Examples of T2-weighted MR images at the distal (4 cm from the elbow joint), middle (16 cm from the elbow joint), and proximal (28 cm from the elbow joint) regions of the upper arm before (upper) and immediately after (lower) the first session of resistance training. White broken line indicates the boundary of the triceps brachii. Note that the contrast in a specific portion of the triceps brachii does not change in the middle region. This portion corresponds to the long head of the triceps brachii.](image-url)
Two-way ANOVA with repeated measures was used to analyze the effects of time (before and after intervention) and region (five regions) on the absolute values of CSA. The ANOVA was followed by post hoc tests with Bonferroni correction. Statistical significance of the tests was set at \(P < 0.05\). As indices of effect size, \(r\) (for t-tests and post hoc comparisons) and partial \(\eta^2\) (for ANOVA) values were reported with \(P\) values. The analyses were performed using the statistical software package (SPSS 12.0J for Windows; SPSS Inc., Chicago, IL).

RESULTS

One-way ANOVA revealed a significant main effect of the region \((P < 0.001, \text{partial } \eta^2 = 0.467)\) on the percent activated area of the triceps brachii in the first session of the training (Fig. 4A). The percent activated area at 10 and 16 cm from the elbow joint was significantly higher than the several other regions \((4 \text{ vs } 10 \text{ cm, } P = 0.022, r = 0.767; 10 \text{ vs } 22 \text{ cm, } P = 0.005, r = 0.826; 10 \text{ vs } 28 \text{ cm, } P = 0.045, r = 0.732; 16 \text{ vs } 22 \text{ cm, } P = 0.002, r = 0.854; 16 \text{ vs } 28 \text{ cm, } P < 0.030, r = 0.754)\). The percent activated areas of each head are shown in Figure 4B. A significant main effect of region was found for the percent activated areas of the long \((P < 0.001, \text{partial } \eta^2 = 0.477)\) and medial \((P < 0.001, \text{partial } \eta^2 = 0.685)\) heads. The percent activated area of the long head at 28 cm from the elbow joint was significantly higher than that at 16 cm \((P = 0.001, r = 0.849)\) and 22 cm \((P = 0.006, r = 0.802)\) from the elbow joint. In the medial head, the percent activated area at 4 cm from the elbow joint was significantly lower than that at 10 cm \((P < 0.001, r = 0.864)\) and 16 cm \((P < 0.001, r = 0.844)\) from the elbow joint. At 16 cm from the elbow joint, the percent activated area of the long head was significantly lower than the medial \((P < 0.001, r = 0.924)\) and lateral \((P < 0.001, r = 0.912)\) heads.

Figure 5 is the distribution of CSA of the triceps brachii along its length before and after the intervention period. In the training group, there were significant main effects of time \((P < 0.001, \text{partial } \eta^2 = 0.851)\) and region \((P < 0.001, \text{partial } \eta^2 = 0.685)\) on the absolute values of CSA.
nonuniform muscle hypertrophy and activation

In the training group, there were significant increases in the 1RM of training exercise (before, 23.6 ± 6.5 kg; after, 32.8 ± 7.5 kg, \( P < 0.001, r = 0.963 \)), the maximum isometric strength (before, 48.5 ± 12.5 N·m; after, 53.0 ± 11.2 N·m, \( P = 0.005, r = 0.723 \)), the muscle volume of the triceps brachii (before, 362.4 ± 82.6 cm³; after, 434.0 ± 92.5 cm³, \( P < 0.001, r = 0.933 \)), and the muscle thickness of the triceps brachii (before, 3.1 ± 0.3 cm; after, 3.7 ± 0.5 cm, \( P < 0.001, r = 0.882 \)) after 12 wk of intervention. No significant change was observed in the muscle thickness of the long head in the training group (before, 2.0 ± 0.3 cm; after, 2.1 ± 0.2 cm). Although the body mass was unchanged in the training group (before, 64.6 ± 7.0 kg; after, 65.1 ± 6.4 kg), it was slightly but significantly increased in the control group (before, 67.6 ± 9.0 kg; after, 68.8 ± 9.0 kg, \( P = 0.018, r = 0.642 \)). In the control group, there was no significant change in the 1RM (before, 22.3 ± 3.6 kg; after, 22.2 ± 3.9 kg), the maximum isometric strength (before, 45.5 ± 8.8 N·m; after, 47.5 ± 9.8 N·m), the muscle volume (before, 344.1 ± 73.9 cm³; after, 348.1 ± 80.6 cm³), the muscle thickness of the triceps brachii (before, 3.1 ± 0.4 cm; after, 3.2 ± 0.5 cm), or the muscle thickness of the long head (before, 1.9 ± 0.3 cm; after, 2.0 ± 0.4 cm).

DISCUSSION

The main finding of the present study was that the regional differences in percent activated area of the triceps brachii in the first session of multijoint exercise were similar to that in muscle hypertrophy after 12 wk of training intervention; i.e., both the percent activated area and relative changes in CSA of the triceps brachii were greater in the middle when compared with the most proximal region (Figs. 4A and 6). The present data are in line with the finding of our previous study that showed the correspondence between the regional differences in percent activated area and in muscle hypertrophy induced by a single-joint exercise for the triceps brachii (22), although the pattern of regional differences was different from the present one. The exercise-induced increase in T2 is indicative of muscle activation (2,7,14,23), because it is related to the exercise intensity (2,7,19), the number of repetitions of exercise with a given load (23), and the EMG of the muscle (2). Therefore, our findings strongly suggest that the nonuniform muscle hypertrophy induced by the training intervention is, at least in part, due to the regional differences in muscle activation during training session.

The relative change in CSA of the triceps brachii after the training intervention was lower in the most proximal region than that in the middle (Fig. 6). In MR images, the proximal region was almost exclusively occupied by the long head of the triceps brachii. On the other hand, all three heads were included in the middle region, and the medial head was dominant in the distal region. Therefore, the smaller relative increase in CSA in the proximal than that in the middle regions indicates that the extent of hypertrophy of the long head was lower than the other two heads. This was supported by the results that the muscle thickness of the triceps brachii increased significantly after training intervention, whereas the thickness of the long head did not. The different hypertrophy among the heads can be explained by the difference in muscle activation during the training session; the percent activated area of the long head in the first session was lower than that of the medial and lateral heads (Fig. 4B). Lower muscle activation and hence smaller mechanical and metabolic stresses in the long head would have resulted in the relatively small hypertrophy in this head after the training intervention. In contrast to the present data, the percent activated area of the long head in a single-joint resistance exercise of elbow extension (lying triceps extension) was similar to that of the medial head and higher than that of the lateral head (22). It should be noted that the long head is a biarticular muscle, whereas the medial and lateral heads are not. The T2 increase of the biarticular rectus femoris induced by a squat exercise was shown to be lower than that of the monoarticular vasti group (20). In addition, EMG activity of the rectus femoris was lower in squat and leg press than that in knee extension exercises, whereas the EMG of the vastus lateralis and medialis was greater in squat and leg press than that of the rectus femoris.
in knee extension exercises (6). Taken together, muscle activation of the synergists in a training session may be dependent on the exercise modality and the anatomical feature of each component of synergists.

Another factor that could account for the nonuniform muscle hypertrophy is the intramuscle difference in protein synthesis (17). For this possibility, the present study provides no data, but if the difference in protein synthesis was the source of the region-specific muscle hypertrophy, the hypertrophic pattern along muscle length would have been specific to the trained muscle, being independent of exercise modality. However, the hypertrophic pattern of the triceps brachii differed between the single- (22) and multijoint exercises (present study), suggesting that the intramuscle difference in the protein synthesis is not the major factor affecting the region-specific muscle hypertrophy.

In the present study, the relative change in CSA of the triceps brachii was greater in the middle regions than the most proximal region (Fig. 6). This is consistent with the data reported by Popadic Gacesa et al. (21), who observed the greatest relative increase in CSA of the triceps brachii at the second third of the muscle length after 12 wk of sitting bench press training. On the other hand, Housh et al. (9) reported significant increases in the triceps brachii CSA in the proximal and middle regions and an insignificant change in the distal region after 8 wk of concentric isokinetic training of elbow extension. Our previous study (22) demonstrated that the relative increase in CSA of the triceps brachii was prominent in its proximal region than the distal region after 12 wk of dynamic elbow extension training with a dumbbell (lying triceps extension). The program variables of the training in our previous study (22) were the same as those in the present study except for the training exercise. Therefore, the difference in hypertrophic changes of the triceps brachii between the present and previous studies is attributable to the difference in exercise modality.

The present study confirmed the correspondence between regional differences in T2 change and muscle hypertrophy induced by a different exercise modality from our previous study (22). This suggests that quantification of T2 change induced by just one training session has the potential to predict the regional difference in muscle hypertrophy along its length after a training intervention. The prediction may provide useful information for sports athletes to design resistance training programs, because the region-specific increase in muscle size could affect joint performance during sports activities by changing the force acted on the tendon and also the distribution of muscle mass within the segment.

In summary, we observed that the regional difference in T2 change of the triceps brachii in the first session of multijoint training was similar to that in relative change in CSA induced by the 12 wk of training intervention. The finding suggests that nonuniform muscle hypertrophy after a training intervention is due to the regional difference in muscle activation during the training session.

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