

Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training

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McCall, G. E., W. C. Byrnes, A. Dickinson, P. M. Pattany, and S. J. Fleck. Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *J. Appl. Physiol.* 81(5): 2004–2012, 1996.—Twelve male subjects with recreational resistance training backgrounds completed 12 wk of intensified resistance training (3 sessions/wk; 8 exercises/session; 3 sets/exercise; 10 repetitions maximum/set). All major muscle groups were trained, with four exercises emphasizing the forearm flexors. After training, strength (1-repetition maximum preacher curl) increased by 25% ($P < 0.05$). Magnetic resonance imaging scans revealed an increase in the biceps brachii muscle cross-sectional area (CSA) (from 11.8 ± 2.7 to 13.3 ± 2.6 cm²; $n = 8$; $P < 0.05$). Muscle biopsies of the biceps brachii revealed increases ($P < 0.05$) in fiber areas for type I (from $4,196 \pm 859$ to $4,617 \pm 1,116$ μm²; $n = 11$) and II fibers (from $6,378 \pm 1,552$ to $7,474 \pm 2,017$ μm²; $n = 11$). Fiber number estimated from the above measurements did not change after training ($293.2 \pm 61.5 \times 10^3$ pretraining; $297.5 \pm 69.5 \times 10^3$ posttraining; $n = 8$). However, the magnitude of muscle fiber hypertrophy may influence this response because those subjects with less relative muscle fiber hypertrophy, but similar increases in muscle CSA, showed evidence of an increase in fiber number. Capillaries per fiber increased significantly ($P < 0.05$) for both type I (from 4.9 ± 0.6 to 5.5 ± 0.7 ; $n = 10$) and II fibers (from 5.1 ± 0.8 to 6.2 ± 0.7 ; $n = 10$). No changes occurred in capillaries per fiber area or muscle area. In conclusion, resistance training resulted in hypertrophy of the total muscle CSA and fiber areas with no change in estimated fiber number, whereas capillary changes were proportional to muscle fiber growth.

weight training; muscle adaptation; fiber number; fiber type; muscle cross-sectional area

MUSCLE ENLARGEMENT as a result of resistance training is both documented and evident in athletes who chronically participate in heavy resistance training (6, 9, 19). However, a controversy exists as to whether hypertrophy of existing muscle fibers entirely determines muscle enlargement or whether muscle fiber hyperplasia also plays a role (4, 30). Although some animal models have provided evidence of a role for hyperplasia in hypertrophy of muscle (4), support for this phenomenon in humans is limited.

The majority of human cross-sectional studies of elite resistance-trained athletes supports a role for muscle fiber hypertrophy in muscle enlargement (19, 30). Indirect evidence of increased fiber numbers has been observed in elite resistance-trained athletes (20); however, other investigations of these athletes have found no increases in estimated fiber number (19, 25). Some investigations have reported significant positive correlations between estimated fiber number and muscle cross-sectional area (CSA) (1, 19, 25). However, cross-sectional studies are not able to evaluate whether

greater than normal fiber number was determined genetically or increased by prolonged training.

Human longitudinal resistance training studies of muscle hypertrophy vary considerably in design with some (6, 9), but not all (2, 9, 10, 29), reporting hypertrophy of the trained muscles. Several studies report muscle fiber hypertrophy, particularly of the type II fiber population (6, 13, 29); however, others have failed to induce muscle fiber hypertrophy (8, 10). Those studies that failed to induce muscle fiber hypertrophy consisted of short training duration (10) and/or purely concentric muscle actions (8). Only one human longitudinal resistance training study evaluated muscle fiber hyperplasia, finding no hyperplasia in elite body builders during a period of controlled training (2). However, because additional hypertrophy was not evident in either whole muscle CSA or muscle fiber areas, conclusions from this study concerning hyperplasia are tenuous.

Another approach for investigating muscle hypertrophy in humans, although not induced by resistance exercise training, was taken by Sjostrom et al. (28). Using data from cadavers, they suggested that muscle fiber hyperplasia may occur as a result of the chronic stress of daily activities; however, genetic predispositions could not be ruled out as an explanation (28).

An issue related to muscle hypertrophy resulting from resistance training is the effect of muscle hypertrophy on the capillary density. Cross-sectional investigations of elite resistance-trained athletes have usually found no change in the number of capillaries per fiber, resulting in a decrease in capillary density expressed per fiber area (27) and per muscle area (32). However, one cross-sectional study reported an increase in the number of capillaries per fiber, with capillary density expressed per muscle area unchanged (26). Results of human longitudinal resistance training studies investigating capillary density changes are also equivocal. Some studies report no change in capillaries per fiber or per muscle area (18, 31). However, Hather et al. (13) reported increases in capillaries per fiber, with capillaries per fiber area unchanged in the resistance training protocol that produced the most significant muscle fiber hypertrophy and increased in the training conditions that produced less or no muscle fiber hypertrophy.

In summary, results of human investigations are equivocal with respect to both the determinant(s) of muscle hypertrophy and the effects of muscle hypertrophy on capillary density. Therefore, the purpose of this study was to evaluate the contributions of muscle fiber hypertrophy and hyperplasia to overall muscle enlargement resulting from resistance training as well as the effects of changes in these parameters on capillary density.

METHODS

Subjects. Subjects were college men between 18 and 25 yr old who had recreational resistance training backgrounds. Recreational lifters were identified by using a training history questionnaire and defined as those who lifted weights regularly but without formally structured training regimens and/or specific goals related to weight training. Recreational lifters were utilized to minimize the influence of neuromuscular adaptations, which have been shown to predominate during the first 3–5 wk of training in subjects initiating resistance training, with the adaptations from muscle hypertrophy predominating as training continues (21). Of the 28 potential subjects who volunteered to participate in the study, 15 met the specified criteria. After subjects were selected, informed consent documents (approved by the University's Human Subjects Committee) were signed by all subjects.

Training protocol. Subjects trained under supervision by using dynamic constant resistance (free weights and weight machines) for 12 wk, on Monday, Wednesday, and Friday mornings. The eight exercises in the training regimen included all major muscle groups; however, four exercises emphasized muscles that flex the arm at the elbow. Subjects began training by using a resistance equal to their 10 repetitions maximum (10 RM) for each particular exercise. Subjects performed three sets for each exercise with a 1-min rest between sets and exercises. Subjects were instructed to lift until concentric failure for every set and to use a spotter's assistance to complete 10 repetitions or when necessary for safety reasons. The resistance for the next training session was increased by 5% and rounded to the nearest 2.27 kg when the subject was able to complete 12 repetitions unassisted for any set or at least 10 repetitions unassisted for all sets or if the subject had been using the same resistance for five training sessions. If the subject was unable to complete at least eight repetitions unassisted for all three sets, the resistance was decreased by 5% for the next session. Throughout the posttraining testing, maintenance training was employed in which resistance was not increased but remained at the resistance at which the subject could complete at least 8, but no more than 12, unassisted repetitions for each of the three sets.

Dietary evaluation. During the course of training, periodic dietary evaluations occurred in which the subjects kept a detailed 3-day record of their dietary intake encompassing 2 weekdays and 1 weekend day. This information was computer analyzed (Nutritionist III, N² Computing, San Bruno, CA) to assess whether the subjects were ingesting adequate protein amounts to allow for muscle growth. The subjects were counseled by a registered dietitian to increase protein intake if they were consuming <1.5 g protein/kg body wt because this amount has been shown to be necessary to promote optimal muscle hypertrophy (16).

Criterion measurements. Before and after training the subjects underwent three testing sessions, which occurred in the following order: skinfold measurements and determination of upper arm muscle CSA by nuclear magnetic resonance imaging (MRI); muscle biopsy of the biceps brachii to assess selected morphological characteristics; and forearm flexor one-repetition maximum (1-RM) strength. Additionally, 1-RM strength was measured every 3 wk during training.

Skinfold measurements were obtained by using calipers (Lafayette Instruments, Lafayette, IN) at seven sites as described previously (23). Body mass was determined weekly by the researchers using the same physician's scale.

For the MRI protocol, the subjects were supine with their arm extended next to their side as the MRI scan was

performed on the nondominant upper arm by using a 1.5-T MRI system (Picker International, Highland Heights, OH). The mean distance from three measurements was used to locate the scan site one-third of the distance from olecranon to acromion processes. The alignment for the scan was perpendicular to either the humerus or the arm itself and was held constant for all measurement periods for a given subject. A gradient echo technique was used, which allows for good visualization and delineation of different muscle groups in the arm. The scan parameters were as follows: 26-ms frequency-encoding time, 350-ms repetition time, 20-cm field of view, 5-mm slice thickness, 192 × 256 image matrix, and 11 slices. The area (cm²) of the biceps brachii, brachialis, combined biceps brachii and brachialis, triceps brachii, and total arm was measured by using the image obtained from the central (i.e., 6th slice) MRI scan. A computerized digitizer with a trackball was used to trace each area as displayed on the computer's monitor by using software provided by the manufacturer. The mean of two measurements was used for each area. Each subsequent determination was accomplished without the visual display of the tracing from the preceding measurement. Two investigators completed all measurements, with the mean value used for statistical analysis.

The muscle biopsies were obtained 1–5 days after the MRI scan. An experienced investigator performed a single-site needle muscle biopsy of the biceps brachii by using standard procedures as modified by Evans et al. (12). If the first sample did not appear adequate, a second, and occasionally a third, sample was obtained by using the same incision. The post-training sample was taken within ~1 cm of the pretraining sample and at the same depth. Biopsy samples were promptly frozen in either freon or isopentane cooled in liquid nitrogen and then stored at –70°C until further analysis. Samples were coded before storage, and all further analyses were accomplished blinded to subject identity and sample time.

The muscle tissue from the biopsy was mounted in OCT medium while in a cryostat at –20°C, and 10- μ m-thick serial cross sections were cut and placed on coverslips. Histochemical analysis for composition of type I and II fibers was done by adenosinetriphosphatase (ATPase) staining procedures by using an alkaline preincubation at pH 10.3 (5). Capillary densities were determined by endothelial cell stains by using a lectin system of biotinylated *Ulex europaeus* I (22). A Zidas computerized digitizer (Carl Zeiss, Thornwood, NY) in combination with a Zeiss microscope fitted with a drawing tube (Carl Zeiss) were used for morphological measurements. The scale factor for the digitizing system was fixed for all measurements by measuring a known distance from a micrometer (Bausch and Lomb, Rochester, NY) with the mean of 20 calibration trials used as the scale factor. All calibrations and measurements were done at ×10 magnification. To reduce variability due to heterogeneity of fiber distribution within a single muscle site (11), the mean of three fields was used to compute the fiber composition, capillary density per muscle area, and interfiber space.

Composition of type I and type II fibers was determined from projection of ATPase stains by using a microprojector (Bioscope 500 series, Southern Precision Instrument, San Antonio, TX). Fields were selected to include ~100 fibers with the best integrity possible. Fascicles were used to define the fields whenever possible; however, if there was poor integrity of an area within a fascicle, fields were defined by continuous intact areas.

Areas of type I and type II fibers were measured from the capillary stain while a projection of the serial section of the ATPase stain for identification of the fiber types was simultaneously viewed. Weighted mean fiber area was determined

from the areas of the type I and type II fibers in conjunction with the fiber composition data by using previously described formulas (19). To determine the number of fiber measurements required for an accurate determination of average fiber area for an individual, a sequential estimation analysis was carried out for each fiber type as described previously for a similar evaluation of adipose cell size (7). In the present study, the sequential estimation analysis indicated a leveling off of the mean and SD after measurement of the areas of 50 fibers for both fiber types pre- and posttraining. After 50 fiber measurements, the individuals' mean area correlations with their means from 100 measurements were ≥ 0.97 for both fiber types pre- and posttraining, indicating the individual means had stabilized. Calculations of mean type I and II fiber areas included all the fiber areas measured for an individual, with a minimum of 75 fibers measured for each fiber type.

Interfiber space measurements were also made from the capillary stains by superimposing a square-shaped field (25 cm²) onto the digitizing tablet, measuring the areas of the muscle fibers appearing in the field, and then measuring the area of the field. The relative interfiber space was calculated by subtracting the cumulative muscle fiber area from the total muscle CSA within the square field and expressing it as a percentage of the total muscle CSA. Only areas with good integrity were included within a field. For 15 of the 66 fields measured, a smaller size square field (9 cm²) was used because there was not a large enough tissue area with good integrity. Each field evaluated was measured three times and the mean used for statistical analysis.

For estimated fiber number, the biceps brachii CSA from the MRI scan was first corrected for interfiber space as calculated from the muscle biopsy to derive the corrected biceps brachii muscle CSA by using the following formula

biceps brachii CSA

$$= [\text{biceps brachii CSA} \cdot (\text{relative interfiber space}/100)]$$

The estimate of fiber number was subsequently computed and corrected to account for sarcomere shortening as described previously (19).

Capillary density was expressed as capillaries per fiber (both type I and II), capillaries per fiber area (both type I and II), and capillaries per muscle area. Capillaries per fiber were determined separately for type I and type II fibers by counting the number of capillaries around each individual fiber and then computing the mean. Sequential estimation analyses were also carried out to determine the number of measurements required to be representative of an individual's number of capillaries per type I and II fibers (7). This analysis indicated a leveling off of the mean and SD after measurement of 25–30 fibers for both fiber types, pre- and posttraining. After 50 fiber measurements, the individuals' mean capillaries per fiber correlations with their means from 100 measurements were ≥ 0.96 for both fiber types pre- and posttraining, indicating that individual means had stabilized.

The number of capillaries per fiber area (μm^2) was calculated by dividing the number of capillaries bordering each fiber by the area of the fiber to which they were adjacent. The mean number of capillaries per fiber area was then computed for each fiber type. The number of capillaries per muscle area (mm^2) was determined for the same fields used to determine the interfiber space.

Maximal strength testing occurred 4–7 days after the muscle biopsy. 1-RM strength was determined to the nearest 1.13 kg during the concentric phase of the seated preacher curl exercise and was evaluated pre- and posttraining and at 3, 6, and 9 wk of training. The supinated close-grip position on

a curl bar (small fingers 6 in. apart) was utilized. For the initial 1-RM testing session, subjects were asked to estimate their 1 RM, and 70% of that amount was used for three to four warm-up repetitions. The resistance was increased by a researcher to obtain the 1 RM in five to six trials. Subjects were allowed a 2-min rest between attempts and performed only one repetition per trial after the initial warm-up. For subsequent testing periods, the previous session's 1 RM was attempted on the third trial. The weights were covered to help control for any motivational factors that might occur if the subjects were aware of the amount of weight being attempted. Subjects were verbally encouraged during all strength testing. The same investigator conducted all of the strength testing sessions. Weights used for the 1-RM tests were verified by using a certified scale.

After the initial 1-RM testing for the preacher curl, 10-RM strength was determined to the nearest 2.27 kg for each of the exercises in the training regimen to establish the initial training resistances. This was also accomplished in five to six trials for each exercise, with a 2-min rest between each trial. Hand and feet positions were standardized and controlled throughout training.

Evaluation of methodologies. To evaluate reliability in locating the site for the MRI scan, seven subjects were remeasured for scan site location and were scanned a second time within the same testing session. There were no significant differences for the combined biceps brachii and brachialis muscle CSA between the means of the two scan times, with a 0.99 ($P < 0.05$) correlation between the two scan times.

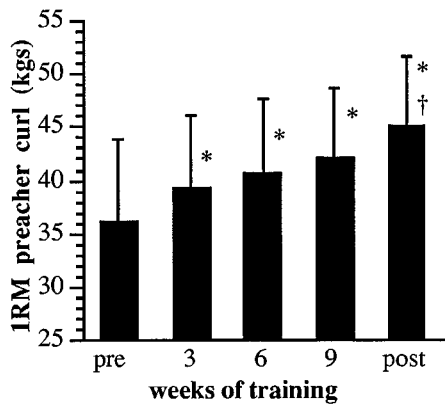
Intrainvestigator reliability and interinvestigator objectivity were evaluated for MRI scan and the histochemical stain measurement procedures. There were no significant differences in mean values within or between investigators for any measurements, with the exception of the intrainvestigator evaluation of area measurements using the Zidas system, in which means differed by no more than 3.28%. The correlations between investigators were 0.99 ($P < 0.05$) for both fiber area and muscle CSA measurements.

Statistical analysis. Paired *t*-tests were used to compare differences between pre- and posttraining. Two-way analysis of variance (ANOVA) was used to compare differences between type I and II fibers as well as between pre- and posttraining. An χ^2 analysis was used to compare differences in fiber area distributions between pre- and posttraining. Repeated-measures one-way ANOVA was used to evaluate differences throughout training for dietary intake and 1-RM strength. Paired and unpaired *t*-tests were utilized to evaluate measurement reliability and objectivity for the MRI and fiber area measurements. Pearson product-moment correlation coefficients were utilized to evaluate relationships between selected variables. For all statistical analyses, the 0.05 level of significance was used. Statistical analyses were carried out on an Apple-Macintosh microcomputer using Statview statistical software.

RESULTS

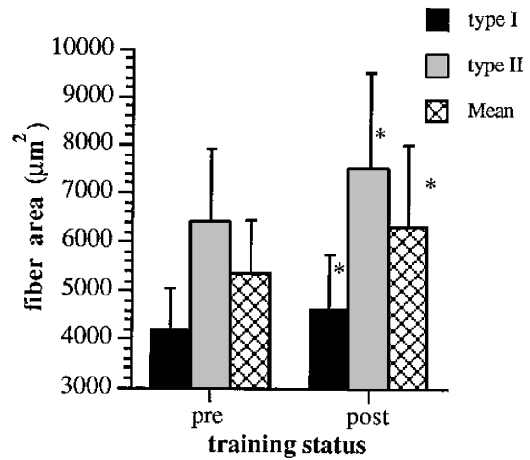
Twelve subjects completed the study; however, one subject was not biopsied because of concern regarding his health history. The average number of training sessions completed was 33.25 ± 0.75 , with all subjects completing ≥ 32 sessions. The changes in body weight (73.65 ± 6.80 kg pretraining; 74.46 ± 7.60 kg posttraining) and sum of skinfolds (77.33 ± 20.01 pretraining; 73.78 ± 17.84 posttraining) were not significant.

Dietary analysis indicated that mean protein intake was unchanged throughout training and never fell



* significantly different from pretraining ($p < 0.05$)
 † significantly different from 9 wk ($p < 0.05$)

Fig. 1. One-repetition maximum (1-RM) preacher curl strength values at pretraining (pre), posttraining (post), and every 3 wk during training. Values are means \pm SD.



* significantly different from pretraining ($p < 0.05$)

Fig. 2. Fiber area values for type I, II, and mean muscle fiber areas pre- and posttraining. Values are means \pm SD; $n = 11$ subjects.

below the goal of at least 1.5 g/kg body wt. One subject was counseled to increase protein intake during the course of the study. The mean percent protein kilocalories remained relatively constant (15.6–17.7% of total kilocalories) throughout training. Total kilocalorie intakes also did not change significantly throughout training and were considered adequate given that none of the subjects decreased body weight during the course of the investigation.

1-RM strength. Changes in 1-RM strength are illustrated in Fig. 1. The 1 RM increased significantly between pretraining and all of the subsequent testing sessions ($P < 0.05$); however, significant increases between adjacent testing sessions occurred only from pretraining to 3 wk and from 9 wk to posttraining ($P < 0.05$).

Muscle CSA. The results for changes in muscle CSA (cm^2) are presented in Table 1. In four of the subjects, the delineation between the biceps brachii and brachialis muscles on the pre- and/or posttraining MRI scans was not clear enough to be determined by at least one of the investigators; therefore, the results for eight subjects were used for the biceps brachii and brachialis muscles. As a result of training, significant increases ($P < 0.05$) occurred in the CSA of the biceps brachii (12.6%), combined biceps brachii and brachialis (9.9%), triceps brachii (25.1%), and total arm (14.6%); however, the increase in the brachialis (7.7%) was not significant.

Fiber areas. The results for fiber areas pre- and posttraining are illustrated in Fig. 2. A repeated-measures 2×2 ANOVA (fiber type by training status)

indicated significant main effects ($P < 0.05$) for both training status and fiber type, with no interaction between fiber types between pre- and posttraining ($P = 0.09$). Therefore, type II fiber area was significantly greater than type I fiber area, and training resulted in significant fiber hypertrophy in type I (10%) and II (17.1%) fiber areas. Although the ANOVA interaction term was not significant between fiber types, a paired t -test indicated the type II/I area ratio increased significantly ($P < 0.05$) from 1.53 ± 0.31 pretraining to 1.63 ± 0.29 posttraining. Additionally, a paired t -test indicated a significant increase ($P < 0.05$) in mean fiber area (17.7%).

χ^2 Analysis indicated that both type I and II fiber area distributions were significantly changed as a result of training (Fig. 3, A and B). There were greater frequencies of larger fibers after training for both type I and II fibers. In addition, the pattern of hypertrophy differed between the type I and II fibers. In the type I population the hypertrophy occurred in the medium size fibers, whereas the entire range of fibers underwent hypertrophy in the type II population. Finally, the distribution of type II fibers was much wider than that of type I fibers both before and after training.

Fiber composition. For pretraining determination of fiber composition, a mean of 131 ± 38 fibers were counted for each of the three fields. For posttraining, the mean number of fibers counted per field was 122 ± 26 . No differences were found between the fields that contributed to the determination of type I fiber composition (ranges: from 50.1 to 52.3% pretraining and from

Table 1. Upper arm muscle CSA values measured from MRI scans pre- and posttraining

Training Status	Biceps Brachii ($n = 8$)	Brachialis ($n = 8$)	Combined* ($n = 12$)	Triceps Brachii ($n = 12$)	Total Arm ($n = 12$)
Pretraining	11.78 ± 2.70	8.35 ± 1.25	22.22 ± 4.55	24.75 ± 6.50	67.07 ± 10.84
Posttraining	$13.26 \pm 2.56^\dagger$	8.99 ± 2.51	$24.42 \pm 4.21^\dagger$	$30.96 \pm 8.24^\dagger$	$76.86 \pm 12.09^\dagger$

Values are means \pm SD; n , no. of subjects. CSA, cross-sectional area; MRI, magnetic resonance imaging. * Combined biceps brachii and brachialis. † Significantly different from pretraining, $P < 0.05$.

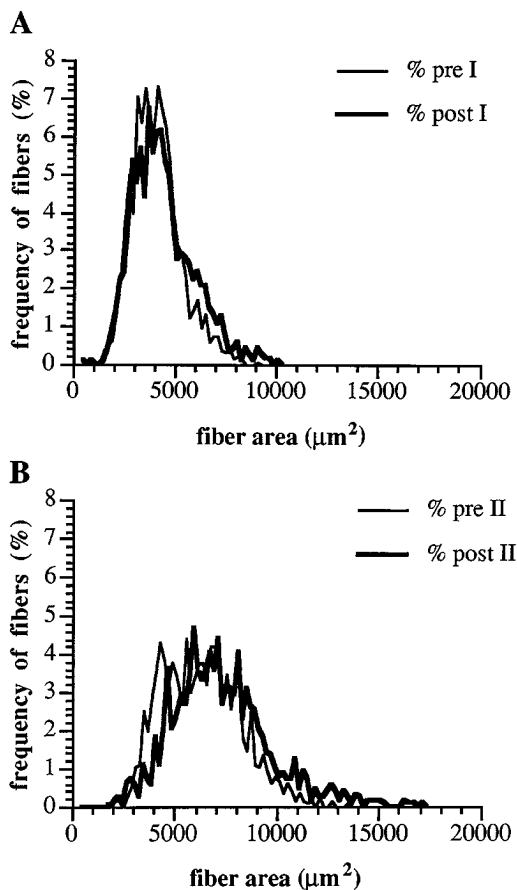


Fig. 3. Pooled fiber area frequency distributions pre- and posttraining for type I (A) and type II (B) fibers.

42.9 to 49.3% posttraining). No significant change occurred for percent type I fiber composition as a result of training ($51.11 \pm 9.58\%$ pretraining; $45.55 \pm 10.09\%$ posttraining; $P = 0.07$).

Interfiber space. The relative interfiber space did not change as a result of training. Interfiber space was $9.39 \pm 1.68\%$ pretraining and $8.76 \pm 2.3\%$ posttraining.

Fiber number estimate. Estimates of fiber number were possible only for the eight subjects whose biceps brachii CSA could be measured both pre- and posttraining. There was no change in the estimated number of fibers in the biceps brachii as a result of training. When corrected for sarcomere shortening, estimates of fiber number were $293.2 \pm 61.5 \times 10^3$ pretraining and $297.5 \pm 69.5 \times 10^3$ posttraining.

Capillary density. The results of the capillary density data are presented in Table 2. One subject was excluded from the results because of an inability to

measure adequate populations of capillaries per fiber from the posttraining sample. A repeated-measures 2×2 ANOVA (capillaries per fiber type by training status) indicated a significant main effect ($P < 0.01$) for the increase in type I (12.7%) and II (22.6%) capillaries per fiber after training. No main effect occurred between fiber types ($P = 0.12$), nor was there interaction between fiber types between pre- and posttraining ($P = 0.10$). Capillaries per fiber area did not change as a result of training for either type I or II fibers. Capillaries per muscle area (mm^2) were also unchanged as a result of training ($P = 0.08$), although 7 of the 10 subjects had increases for this measure of capillary density.

DISCUSSION

Determinants of muscle hypertrophy. Despite similar increases in the group's averages for mean fiber area (14.5%) and biceps brachii CSA (13.3%; corrected for interfiber space) for the eight subjects whose biceps brachii CSA could be measured, no correlation existed between the increases in biceps brachii CSA and the mean fiber area ($r = 0.191$). Additionally, no correlation existed between the increase in biceps brachii CSA with either type I fiber area ($r = 0.197$) or type II fiber area ($r = 0.353$). Therefore, although estimated fiber number was unchanged after training for these subjects, the overall muscle hypertrophy was unrelated to the magnitude of muscle fiber hypertrophy.

The absence of correlation(s) could conceivably result from inherent limitations in the procedures used to obtain the biceps brachii CSA and fiber area measurements. Thus, although the relative increases in the group means may be similar, limitations in the procedures used to obtain these measurements could have introduced variability in the individual values, resulting in the absence of a relationship between the two indexes of hypertrophy. Because the consequences of these methodological limitations were realized at the outset, procedures were designed to, at best, limit their impact and, at minimum, evaluate their consequences.

With regard to the MRI scan used to obtain the biceps brachii CSA, the location of the scan site and alignment of the scan were demonstrated to be reproducible, as indicated in the results. Additionally, the ability of an investigator to obtain a reliable and objective measurement of muscle CSA was demonstrated. Therefore, the procedures used to obtain the measurement of the biceps brachii CSA for an individual were found both valid and reliable.

Table 2. Capillary density values pre- and posttraining

Training Status	Capillaries per Fiber		Capillaries per Fiber Area $\times 10^{-3}$, μm^2		Capillaries per Muscle Area, mm^2
	Type I	Type II	Type I	Type II	
Pretraining	4.9 ± 0.63	5.09 ± 0.76	1.22 ± 0.164	0.868 ± 0.153	285.39 ± 39.93
Posttraining	$5.52 \pm 0.69^*$	$6.24 \pm 0.72^*$	1.26 ± 0.246	0.916 ± 0.209	$320.29 \pm 64.81^\dagger$

Values are means \pm SD for 10 subjects. *Significantly different from pretraining, $P < 0.01$. $^\dagger P > 0.05$ but < 0.10 for difference from pretraining.

With regard to muscle fiber area measurements, as well as other parameters determined from the muscle biopsy, the central issue is the appropriateness of a single-site biopsy for determination of muscle characteristics, because several investigators have concluded that human muscle is heterogeneous in fiber composition (11, 14, 17). Although it is unknown whether fiber areas also differ between sites within a muscle, variability in the fiber composition would influence the calculation of the mean fiber area. To reduce the variability of fiber composition between locations of the muscle, sampling of three to five biopsy sites has been recommended (11). Although Elder et al. (11) theorized the between-site variability contributed the greatest portion of the total variance, inspection of their data reveals similar values for between- and within-site variance. Therefore, we proposed that, by taking the mean of several areas within a single site, the reduction of total variability would approach that of multiple biopsy sites. Interestingly, no within-site variability was evident in the present study, perhaps because our biopsy sample contained a smaller population of fibers compared with the average sample size in the cadaver study conducted by Elder et al. Thus heterogeneity of muscle fiber composition in some subjects may have contributed to variability in the calculation of mean fiber area in the present study despite efforts to localize the site and depth of pre- and posttraining biopsies. For example, in one subject (*subject 23*), a decrease in type I fiber composition (Table 3) caused mean fiber area to

increase despite small decreases in both type I and II fiber areas. Shifts in human fiber composition, especially in this direction after muscle overload, are not supported by previous data (24), and such shifts are likely the result of variability between the pre- and posttraining biopsy sites.

Another issue relevant to obtaining individual fiber area values is the population of fibers that must be measured to accurately reflect the average fiber area of the biopsy site and presumably the entire muscle. The present investigators could find only one other study that addressed this issue; however, highly trained body builders served as the subjects (1). Sequential estimation analyses indicated that the number of fibers measured during our study exceeded the point where the individuals' rolling cumulative means and SDs became constant. Thus the determination of each individual's average type I and II fiber area was not biased by an inadequate fiber population. Additionally, the procedures used to obtain the fiber area measurements were found to be objective, and the reliability of the investigator who made the fiber area measurements was considered acceptable.

Therefore, within the limitations of our methods, muscle fiber hyperplasia was not evident in our study. However, one should be cautious about ruling out any possibility of fiber hyperplasia in human skeletal muscle despite the lack of change in estimated fiber number in the present study, particularly in light of the lack of correlation between the muscle fiber hypertrophy and

Table 3. Pre- and posttraining values for all subjects, subjects with greater relative type II fiber hypertrophy, and subjects with less relative type II fiber hypertrophy

Subject No.	Type I Area, μm^2		Type II Area, μm^2		Mean Fiber Area, μm^2		Corrected Biceps Brachii CSA, cm^2		Type I Composition, %		Estimated Biceps Brachii Fiber Number, $\times 10^3$	
	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training	Pre-training	Post-training
<i>LH (n = 8)</i>												
14	3,726	3,861	6,719	6,333	5,505	5,348	8.39	9.03	40.6	39.9	207.3	229.6
20	3,535	3,406	4,626	5,342	3,993	4,320	8.77	11.48	58.0	52.8	298.6	361.3
26	4,913	4,920	7,548	8,796	5,989	6,860	10.17	12.10	59.1	50.0	230.9	239.8
13	4,017	3,558	4,195	4,224	4,094	3,770	11.30	11.22	57.0	68.2	375.6	404.8
25	5,201	6,166	6,800	7,328	6,249	6,917			34.5	35.3		
16	4,820	5,290	7,333	8,939	6,042	7,550			51.3	38.1		
27	3,881	4,319	6,189	7,149	6,249	6,917	9.49	11.71	37.2	36.0	329.3	353.2
23	3,560	3,419	7,886	7,692	5,373	5,648	9.37	10.32	58.1	47.8	237.2	248.6
Mean	4,207	4,367	6,412	6,975	5,437	5,916	9.58	10.98	49.5	46.0	279.8	306.2
\pm SD	± 666	$\pm 1,009^{\text{b,e}}$	$\pm 1,347$	$\pm 1,625^{\text{b,e}}$	± 917	$\pm 1,367^{\text{b}}$	± 1.04	$\pm 1.13^{\text{b}}$	± 10.4	± 11.2	± 65.5	$\pm 75.6^{\text{b}}$
<i>GH (n = 3)</i>												
21	2,997	4,486	5,134	7,768	4,108	6,274			48.0	45.5		
24	5,807	6,807	9,023	11,899	7,124	10,089	16.44	17.04	58.6	35.5	313.8	229.7
12	3,700	4,556	4,700	6,742	4,106	5,604	10.66	12.90	59.3	52.1	352.9	313.0
Mean	4,168	5,283	6,286	8,803	5,112	7,323	13.55	14.97	55.3	44.4	333.4	271.4
\pm SD	$\pm 1,462$	$\pm 1,320^{\text{c,e}}$	$\pm 2,381$	$\pm 2,730^{\text{c,e}}$	$\pm 1,742$	$\pm 2,420^{\text{b}}$	± 4.09	± 2.93	± 6.3	± 8.3	± 27.6	± 58.9
<i>All (n = 11)</i>												
Mean	4,196	4,617	6,378	7,474	5,348	6,300	10.57	11.98	51.1	45.5	293.2	297.5
\pm SD	± 859	$\pm 1,116^{\text{c,e}}$	$\pm 1,552$	$\pm 2,017^{\text{c,e}}$	$\pm 1,104$	$\pm 1,707^{\text{b}}$	± 2.56	$\pm 2.36^{\text{c}}$	± 9.5	$\pm 10.1^{\text{d}}$	± 61.5	± 69.5

n, No. of subjects; All, all subjects; GH, greater relative type II fiber hypertrophy; LH, less relative type II fiber hypertrophy. ^an = 6 for LH subgroup; n = 2 for GH subgroup; n = 8 for All group. ^bSignificantly different from pretraining, P ≤ 0.05. ^cSignificantly different from pretraining, P < 0.01. ^dP > 0.05 but < 0.10 for difference from pretraining. ^eMain effect for training status as indicated by 2 × 2 analysis of variance.

the increase in total muscle CSA. The preceding discussion offered one possible explanation for this lack of a relationship. Another possible explanation is that fiber hyperplasia was a determinant of muscle hypertrophy in some, but not all, subjects. After inspection of the data from all 11 subjects, we offer an alternative interpretation based on the magnitude of the subjects' type II fiber hypertrophy. Three individuals exhibited a greater degree of type II fiber hypertrophy [greater hypertrophy (GH) subgroup; range of increase from 31.9 to 51.3%] compared with the other eight subjects [less hypertrophy (LH) subgroup; range of increase from -5.7 to 21.9%]. Individual subject data divided by the degree of type II fiber hypertrophy is presented in Table 3. To help provide a basis for the merit of this alternative explanation, statistical comparisons were made to evaluate the influence of the GH subjects on the group means. Although the increases in biceps brachii CSA were of similar magnitude between the LH ($15.1 \pm 11.5\%$) and the GH ($12.3 \pm 12.3\%$) subgroups, the increase was significant only for the LH subgroup, because the statistical power was severely limited by the small sample size of the GH subgroup. The percent increases in 1-RM strength were significant and comparable for the two subgroups ($27.5 \pm 15.0\%$ for LH; $27.9 \pm 10.6\%$ for GH). In both subgroups a 2×2 ANOVA (fiber type by training status) indicated significant main effects for fiber hypertrophy after training.

With respect to fiber number estimates, exclusion of the two GH subjects whose fiber numbers were estimated resulted in a significant increase in the estimated fiber number (from $279.8 \pm 65.5 \times 10^3$ pretraining to $306.2 \pm 75.6 \times 10^3$ posttraining) in the remaining six subjects. Therefore, although muscle fiber hypertrophy was a determinant of overall muscle enlargement for all subjects, the contribution of muscle fiber hyperplasia may have been dependent on the magnitude of type II fiber hypertrophy. These contrasting results suggest there may be differences in how individuals achieve similar degrees of muscle hypertrophy.

This post hoc analysis must be viewed cautiously, especially in regard to a type I statistical error. The calculation of estimated fiber number has the combined limitations of the procedures used to determine muscle CSA and fiber areas that were discussed above. Each of these limitations combines to increase the variability of values for the estimate of fiber number. There is no reason to expect any systematic error operated between pre- and posttraining in the measurement of any variable. Therefore, because the consequence of large variability is decreased statistical power to find an actual difference when one exists (i.e., increased likelihood of type II error), we feel confident that the significant increase in estimated fiber number in LH subjects is not the result of a type I error.

The existence of individual differences in the determinants of muscle hypertrophy was unexpected and is speculative. Therefore, the potential mechanism(s) responsible cannot be obtained from this study. However, a brief discussion of this issue relative to the present data should help with future research endeavors. One

might anticipate that preexisting characteristics of the subjects could affect the determinant(s) of muscle hypertrophy. Although our subject population was not homogeneous for pretraining variables, the degree of muscle fiber hypertrophy and/or hyperplasia after training did not appear to be dependent on the pretraining values for muscle fiber area(s), biceps brachii CSA, or 1-RM strength. For example, the pretraining fiber areas were similar when subjects were divided by the magnitude of hypertrophy (see Table 3).

Muscle fiber hypertrophy. The muscle fiber hypertrophy in the present study is in agreement with longitudinal resistance training studies that also used both concentric and eccentric actions (6, 13). Other resistance training studies using only concentric actions have either failed to find fiber hypertrophy (8) or only found hypertrophy of the type II fibers (13). Recently, Hather et al. (13) reported that hypertrophy of type I fibers only occurred when training included both concentric and eccentric actions. Therefore, the results of the present study confirm that type I and II muscle fibers hypertrophy in response to resistance training that includes both concentric and eccentric actions.

The frequency distributions of fiber areas indicate a much greater range of areas for the type II fibers compared with the type I fibers both pre- and posttraining (see Fig. 3, A and B). This greater distribution of type II fiber areas was also found in a cross-sectional study of male body builders (1). In the present study, both type I and II fiber area distributions were significantly changed after training, with the pattern of hypertrophy differing between the two fiber types. Although the entire range of type II fibers hypertrophied, the hypertrophy in type I fibers occurred primarily in the medium-size fibers.

In addition to differences in the distributions of fiber areas between type I and II fibers, differences also existed between fiber types for the relative degree of hypertrophy both before and after training. There was a greater relative hypertrophy of type II fibers as a result of training, as indicated by the significant increase in type II-to-I fiber area ratio. Other longitudinal resistance training studies using dynamic constant resistance have also reported increases in type II-to-I fiber area ratio (6, 13). The recreationally trained subjects in the present study had similar type II-to-I fiber area ratios (1.53 pretraining; 1.63 posttraining) as previously reported for the biceps brachii of male body builders (range 1.50–1.57) (1, 2, 25). Additionally, the subjects in the present study had greater type II/I fiber area ratios compared with values reported for untrained men (range 1.10–1.38) (2, 25). Therefore, the subjects may have achieved some preferential type II fiber hypertrophy from participating in resistance training before the study, and this ratio was augmented by the training regimen of the present study.

Capillary density. There were no differences in the overall pattern of responses for capillary density when the GH and LH subgroups were considered separately, indicating that the training responses of the capillaries were concordant with the muscle fiber hypertrophy.

Although the increases in capillaries per fiber were not statistically significant in the GH subgroup (15.1% type I; 38.3% type II), most likely because of the reduced statistical power of the smaller subject population, the magnitude of increases was actually greater than in the LH subgroup (12.5% type I; 17.7% type II; $P < 0.01$).

Differences in methodologies confound comparisons with results of previous studies because, even when capillary density is seemingly being expressed the same, reporting of the specific methodologies is often vague or not referenced (13, 31, 32). Some expressions of capillary-to-fiber ratios do not discriminate by fiber type or the composition of the sampling area (26). This could have an impact on the capillary-to-fiber ratio if either the fiber composition is not homogeneous or the numbers of capillaries surrounding the type I and II fibers differ (3). The present study has significantly improved on previous studies by performing rigorous evaluations of the methodological issues for obtaining accurate capillary density values and also by expressing the capillary density by using three methods. Additionally, the capillary stain used in the present study produces a superior visualization of capillaries compared with the commonly used periodic acid-Schiff stain (22).

Even with the limitations of previous studies, the present investigation does concur with recent findings from a longitudinal study of increases in capillary number around both type I and II fibers in the vastus lateralis as a result of resistance training (13). In the present study, the magnitude of the increase was proportional to the fiber growth such that capillaries per fiber area did not change for either major fiber type. The same results were found by Hather et al. (13) but only for the resistance training condition that included both concentric and eccentric actions. Other longitudinal resistance training studies have failed to find changes in capillary density expressed as either capillaries per fiber or per muscle area (18, 31). However, both of these studies also failed to produce increases in muscle fiber area, and therefore conclusions regarding the effects of muscle hypertrophy on capillary density are tenuous.

Results of cross-sectional studies investigating capillary density in elite resistance-trained athletes are equivocal (26, 27, 32). Some have speculated there may be more of a stimulus to increase capillary number per fiber and thereby maintain capillary density per fiber area and/or muscle area when a body builder-type regimen compared with an Olympic- or power lifter-type regimen is used (26, 32). The present study utilized a body builder-type regimen and supports observations from such studies. Future investigations are needed to elucidate the mechanism(s) responsible for capillary number increases in response to different regimens of resistance training.

Fiber composition. The majority of the previous human research does not support a change in gross fiber composition as a result of resistance training (6, 13, 26). However, some have suggested there may be transformations within major fiber types I and II as a result of

either "aerobic" or "anaerobic" training (15) and of resistance training (8). Additionally, animal models of increased muscle use find an increase in the percentage of slow muscle fibers (type I) in the overloaded muscle(s), with decreased muscle use producing the opposite results (24). Our results support no change in fiber composition after resistance training; however, 9 of 11 subjects decreased in type I fiber composition after training (see Table 3). Although not statistically significant ($P = 0.07$), this tendency is puzzling and inconsistent with evidence from other mammalian models (24).

Interfiber space. The relative interfiber space did not change significantly as a result of training (9.39% pretraining; 8.76% posttraining). Therefore, because of the muscle hypertrophy, there was an increase in the absolute amount of interfiber space after training. With use of a stereological point-counting technique, cross-sectional studies have reported similar relative amounts of "collagen and other noncontractile tissue" in the biceps brachii of both untrained men [13.4% (Ref. 19); 14.4% (Ref. 25)] and male body builders [12.1–13.0% (Ref. 19); 12.0% (Ref. 25)]. Thus it appears that increases of interfiber space and muscle fiber area are coupled during resistance training.

Summary and conclusions. Muscle fiber hypertrophy was a determinant of overall muscle enlargement as a result of resistance training. Although both type I and II fibers hypertrophied, the type II fibers demonstrated a greater capacity for hypertrophy, were more varied in their range of sizes, and were larger than type I fibers both pre- and posttraining. In the group of subjects whose fiber numbers could be estimated, there was no evidence of muscle fiber hyperplasia; however, there was also no relationship between muscle fiber hypertrophy and total muscle hypertrophy. This might be attributed to inherent limitations in the use of a single-site muscle biopsy.

Alternatively, the potential for fiber hyperplasia as a determinant of muscle enlargement may have been influenced by the magnitude of type II fiber hypertrophy. In those subjects with relatively less type II fiber hypertrophy, muscle fiber hyperplasia may have been an additional determinant of overall muscle enlargement. In contrast, a few subjects exhibited greater relative type II muscle fiber hypertrophy and no hyperplasia, despite undergoing comparable increases in muscle CSA and strength. Examination of the parameters evaluated in the present study could not provide an explanation for the speculated individual differences in response to training.

The second major finding of the present study was that increases in capillary number can occur in response to muscle hypertrophy induced by resistance exercise training. The increase in capillary number was proportional to muscle fiber growth, such that the capillary density per fiber area and muscle area were unchanged.

In conclusion, resistance training resulted in hypertrophy of the total muscle CSA and fiber areas with no change in estimated fiber number, whereas capillary changes were proportional to muscle fiber growth.

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