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Long-Term Resistance Trained Human Muscles Have More Fibers, More Myofibrils, and Tighter Myofilament Packing Than Untrained

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BASIC SCIENCES

ABSTRACT

MAEO, S., T. G. BALSHAW, B. MÄRZ, Z. ZHOU, B. HAUG, N. R. W. MARTIN, N. MAFFULLI, and J. P. FOLLAND. Long-Term Resistance Trained Human Muscles Have More Fibers, More Myofibrils, and Tighter Myofilament Packing Than Untrained. *Med. Sci. Sports Exerc.*, Vol. 56, No. 10, pp. 1906–1915, 2024. **Introduction:** Increases in skeletal muscle size occur in response to prolonged exposure to resistance training that is typically ascribed to increased muscle fiber size. Whether muscle fiber number also changes remains controversial, and a paucity of data exists about myofibrillar structure. This cross-sectional study compared muscle fiber and myofibril characteristics in long-term resistance-trained (LRT) versus untrained (UNT) individuals. **Methods:** The maximal anatomical cross-sectional area (ACSAmax) of the biceps brachii muscle was measured by magnetic resonance imaging in 16 LRT (5.9 ± 3.5 yr' experience) and 13 UNT males. A muscle biopsy was taken from the biceps brachii to measure muscle fiber area, myofibril area, and myosin spacing. Muscle fiber number, and myofibril number in total and per fiber were estimated by dividing ACSAmax by muscle fiber area or myofibril area, and muscle fiber area by myofibril area, respectively. **Results:** Compared with UNT, LRT individuals had greater ACSAmax (+70%, $P < 0.001$), fiber area (+29%, $P = 0.028$), fiber number (+34%, $P = 0.013$), and myofibril number per fiber (+49%, $P = 0.034$) and in total (+105%, $P < 0.001$). LRT individuals also had smaller myosin spacing (−7%, $P = 0.004$; i.e., greater packing density) and a tendency toward smaller myofibril area (−16%, $P = 0.074$). ACSAmax was positively correlated with fiber area ($r = 0.526$), fiber number ($r = 0.445$), and myofibril number (in total $r = 0.873$ and per fiber $r = 0.566$), and negatively correlated with myofibril area ($r = -0.456$) and myosin spacing ($r = -0.382$) (all $P < 0.05$). **Conclusions:** The larger muscles of LRT individuals exhibited more fibers in cross-section and larger muscle fibers, which contained substantially more total myofibrils and more packed myofilaments than UNT participants, suggesting plasticity of muscle ultrastructure. **Key Words:** MUSCLE HYPERTROPHY, HYPERPLASIA, PROLIFERATION

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Skeletal muscle size/mass is a key determinant of muscle function (i.e., strength and power) and sport performance (1–4), as well as health and quality of life (5,6). The size of a whole muscle is determined primarily by the number and size of individual muscle fibers (7), which are composed of thousands of myofibrils, with each myofibril in turn containing several hundred myofilaments (Fig. 1). Increases in skeletal muscle size, known as muscle hypertrophy, occur in response to functional overload, that is, resistance training (RT), especially after prolonged systematic exposure (8). Indeed, numerous studies spanning five decades indicate that long-term resistance-trained (LRT) individuals have substantially larger muscles (+~40%–80%) than untrained (UNT) individuals (9–14), as well as larger muscle fibers (+~20%–60%) (12–14). However, it

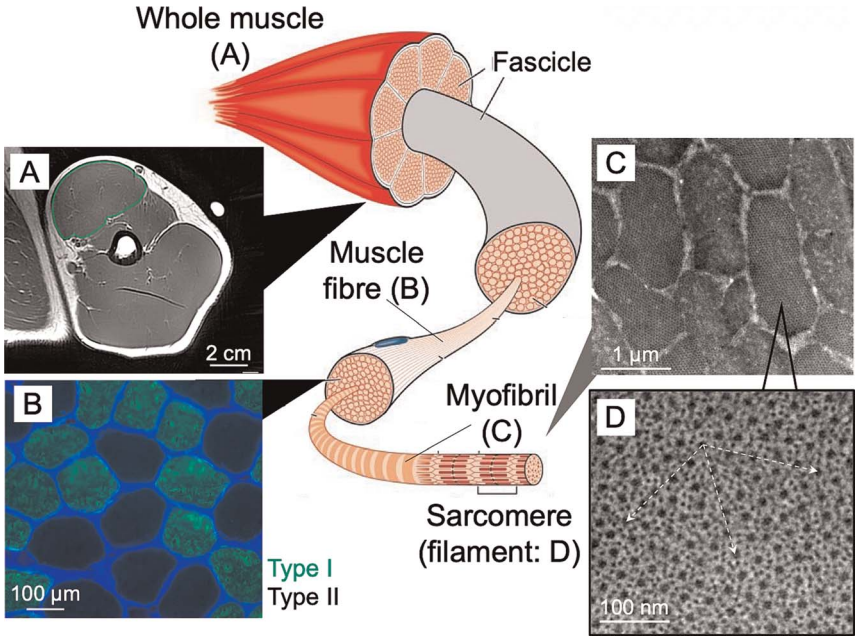

FIGURE 1—Illustration of skeletal muscle structure and obtained example images of ACSAmax of the biceps brachii (A), muscle fiber (B), myofibril (C), and myofilament (D). The arrows in D show how the myosin spacing (distance) was measured. The illustration of skeletal muscle structure was purchased from Dreamstime.com (file ID: 80735107 created by Legger) and adapted with permission.

remains controversial whether larger muscle size of LRT is accompanied by/associated with a greater number of muscle fibers (15,16). Furthermore, although structural adaptations to the size and number of myofibrils may contribute to increased muscle fiber size, and thus also whole muscle size (17,18), a paucity of data is available on the plasticity of myofibrils induced by RT (8,16). A better understanding of the adaptations to RT is a first step in elucidating how these adaptations are produced.

Generation of new muscle fibers, called hyperplasia, occurs during developmental growth in mammals (19). In human muscles, early work by MacCallum (20) in 1898 suggested that hyperplasia does not occur after postnatal growth. However, later studies have found evidence that the number of fibers may change over the long term with a relatively slow process, such that it increases in response to daily functional demands/overload (21) and decreases with aging/reduced physical activity (22). Nevertheless, whether hyperplasia occurs following RT remains an open question. For example, similar muscle fiber numbers have been found before and after 12 wk of RT in young adults (23) and 6 months of RT in older adults (24) when estimated by dividing muscle anatomical cross-sectional area (ACSA) by fiber CSA. Similarly, MacDougall et al. (12) found no significant difference in muscle fiber numbers between young LRT and UNT males. On the contrary, Sale et al. (14) found a significant correlation between muscle fiber number and muscle size. This inconsistency might arise from the fact that the training interventions of the longitudinal studies (23,24) (12 wk to 6 months) were not of sufficient duration to induce distinct changes in muscle structures and/or due to small sample sizes (groups with $n \leq 8$) in both longitudinal (23) and cross-sectional (12) studies that reported no change/difference in fiber numbers.

There is limited literature on whether RT changes the ultrastructure of skeletal muscle. In humans, MacDougall (25) observed a 16% increase in myofibril CSA after 6 months of RT. However, a subsequent study reported typically no changes in myofibril CSA after short-term RT (18 wk) (26). Furthermore, neither study reported any measure of myofibril number (per fiber or in total). Thus, even the most basic questions, such as whether larger muscles/fibers following RT are accounted for by larger size and/or increased number of myofibrils, have not been resolved (8,16). In addition, preliminary studies with small participant numbers and short-duration RT interventions have reported the distance between myofilaments within a myofibril, indicative of myofilament packing density, to decrease after 10 wk ($n = 3$) (27), but not after 6 wk ($n = 8$) (28), of RT. Reduced myosin spacing could indicate a packing strategy by which more contractile filaments are contained in a given area, which would be expected to increase specific tension and thus strength irrespective of muscle size (29). Based on the limited evidence to date, further research is clearly needed to examine this possibility. Furthermore, individuals who have completed several years of RT might be expected to exhibit pronounced adaptations, where these are physiologically possible, and thus may reveal the potential for human ultrastructural adaptations.

The present study compared muscle fiber and myofibril characteristics in LRT versus UNT individuals. We hypothesized that, compared with UNT, LRT individuals would have 1) larger and a greater number of muscle fibers, 2) larger and a greater number of myofibrils (per fiber and in total), and 3) smaller myofilament spacing (greater packing density). We also hypothesized that the size and number of muscle fibers and myofibrils, as well as myofilament spacing, would be correlated with muscle ACSA.

METHODS

Participants

Sixteen LRT (5.9 ± 3.5 yr of RT experience) and 13 UNT participants were recruited and completed the study. All participants were required to be healthy males aged 18–40 yr, with no history of taking anabolic or androgenic steroids, asymptomatic at the time of testing and with no major injuries within the last 3 months. LRT participants needed to have an extensive history of upper arm RT, specifically ≥2 sessions per week for ≥10 months·yr^{-1} and ≥3 yr, as well as elbow flexion maximum voluntary torque (MVT) of >90 N·m and MVT·kg^{-1} body mass of >1.1 (N·m)·kg^{-1} (9,10,30). UNT had no systematic physical training history of any kind. Physical activity levels were assessed with the International Physical Activity Questionnaire (IPAQ, short format (31)). RT routines of the LRT individuals were assessed via a detailed questionnaire. LRT participants had performed both single-joint and multijoint RT exercises for the elbow flexors (e.g., dumbbell biceps curl, pull-ups, bench pull, single-arm pull), with their RT over the last year involving near maximum loads (1–5 repetition maximum [RM]), heavy loads (6–14 RM), and moderate loads (≥15 RM) accounting for 38% ± 27%, 49% ± 25%, and 13% ± 10% of training time, respectively. All participants received written and verbal information about the experimental protocol before providing written informed consent. This study was approved by Loughborough University Ethical Advisory Committee (R17-P174) and was conducted according to the Declaration of Helsinki. All participants were asked to refrain from performing exercise of the upper arm muscles in the 48 h before measurements.

Magnetic Resonance Imaging

T1-weighted axial magnetic resonance imaging of the nondominant arm was obtained with a 3T scanner (Discovery MR750w; GE Healthcare, Chicago, IL) and a receiver 16-channel flex coil. Axial images (perpendicular to the humerus) were obtained from the humeral head to below the elbow joint in three overlapping blocks, aligned with the humerus, using the following parameters: time of repetition, 600 ms; time of echo, 12.8 ms; field of view, 180 × 180 mm; image matrix, 260 × 260; pixel size, 0.69 × 0.69 mm; slice thickness, 5 mm; and interslice gap, 5 mm, with the PROPELLER mode to remove motion artifact due to breathing (32) (Fig. 1A). Participants were scanned while in the supine position, after maintaining the same position for ~10 min to allow fluid to equilibrate, with the elbow joint fully extended and relaxed. Oil filled capsules were placed on the skin along the humerus to facilitate alignment between the blocks during analysis. ACSA of the biceps brachii muscle was segmented (as one mass for the long and short heads) along the whole of the muscle using a public domain DICOM software (Horos, v3.3.6; https://horosproject.org), and its maximum value (i.e., the maximal ACSA, ACSAmax) was used for further analysis.

Muscle Biopsy

Muscle biopsies were taken from the distal biceps brachii of the nondominant arm to avoid the nerves and blood vessels under local anesthesia (5 mL of 1% lidocaine) using the conchotome technique. Muscle samples were dissected of any visible fat and connective tissue and blotted dry before being split into two portions. One portion was immediately embedded in a mounting medium (Tissue-Tek O.C.T. Compound; Sakura Finetek Europe, Alphen aan den Rijn, the Netherlands), frozen in liquid nitrogen-cooled isopentane, and stored at −80°C for immunohistochemistry (muscle fiber) analysis. The other portion was immersed in 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) with 5 mM $CaCl_2$ and stored at 4°C overnight. The tissue was then washed in cacodylate buffer and stored at 4°C before further processing for transmission electron microscopy (ultrastructure) analysis.

Muscle fiber analysis. Transverse serial sections (8 μm) were obtained using a cryotome and placed onto poly-L-lysine–coated glass slides. Sections were washed with tris-buffered saline (3 min × 3 times), fixed for 10 min in 3.7% paraformaldehyde at room temperature, washed again with tris-buffered saline (3 min × 3 times), and then blocked with tris-buffered saline containing 2% bovine serum albumin, 5% goat serum, and 0.2% Triton for 1 h at room temperature. After removing the blocking solution by tipping the slides, serial muscle sections were then incubated with a primary antibody for myosin heavy chain I (A4.951; Developmental Studies Hybridoma Bank, Iowa City, IA) diluted 1:200 in the blocking solution for 1 h at room temperature. Sections were then washed with tris-buffered saline (3 min × 3 times) and incubated for 2 h at room temperature with an appropriate secondary antibody consisting of goat anti-mouse Alexa Fluor 488 (A11029; Fisher Scientific, Pittsburgh, PA) diluted 1:500 and wheat germ agglutinin Alexa Fluor™ 350 Conjugate (W11263; Fisher Scientific) diluted 1:20 in the blocking solution. Following incubation, sections were washed with tris-buffered saline (3 min × 5 times), and then coverslips were mounted with Fluoromount aqueous mounting medium (F-4680; Sigma-Aldrich, St. Louis, MO).

Images were captured using a fluorescence microscope (Leica DM2500) at ×20 magnification (Fig. 1B). Image analysis was undertaken using Fiji (v.2.0.0) software (33), and the investigator was blinded to the participant code of each sample. Only transversely sectioned fibers were included in the analysis; that is, any fiber cross sections that were clearly oblique or not transverse to the long axis of the fiber were excluded. Fiber cross-sectional area was assessed by manually drawing around the perimeter of each fiber for 200 different fibers per participant. In four participants (three in LRT and one in UNT), only 145–165 fibers were analyzed for area because of insufficient number of clear images/fiber perimeters. In addition to the fibers analyzed for area, a total of 500 fibers per participant were counted as type I or type II fibers. Fiber type composition was expressed in two ways: 1) percentage by fiber number, that is, the number of fibers of each type relative to the total number of fibers counted (n = 500), and 2) percentage by fiber area, that is, the summed area of the fibers of each type relative to the total area of fibers analyzed (n = 200). The total number of fibers within the whole biceps brachii muscle

TABLE 1. Descriptive characteristics of LRT and UNT individuals.

Variable	LRT	UNT	P	Cohen's d
Age (yr)	23.2 ± 3.6	20.5 ± 2.3	0.012*	0.89 [large]
Height (m)	1.82 ± 0.08	1.77 ± 0.07	0.152	0.56 [medium]
Humerus length (cm)	33.9 ± 2.1	33.3 ± 1.4	0.394	0.33 [small]
Body mass (kg)	88.2 ± 12.9	74.6 ± 11.4	0.006*	1.12 [large]
IPAQ value (MET·min·wk^{-1})	5150 ± 2655	2689 ± 1452	0.006*	1.15 [large]

Data are group mean ± SD. n = 16 for LRT and n = 13 for UNT.
*Significant difference between groups.

was estimated by dividing muscle ACSAmax by average fiber area, where average fiber area was calculated as follows (12):

$$[(\%\text{ type I number})(\text{mean type I area}) + (\%\text{ type II number})(\text{mean type II area})]/100$$

Ultrastructure analysis. Fixed tissue was rinsed in phosphate buffer, secondary fixed in osmium tetroxide, dehydration and resin embedded before cross-sectioning using an ultramicrotome to prepare sections of 50 nm thickness for transmission electron microscopy examination. Full protocols by McMillan and Eisenback (34) were followed. Ultrastructural images of the sample were taken using a FEI Tecnai F20 transmission electron microscope at magnifications of ×4000 (for myofibril area, Fig. 1C) and ×19,500 (for myosin spacing, Fig. 1D). Image analysis was undertaken using Fiji software with the investigator blinded to the participant code of each sample, and only transversely sectioned fibers were included in the analysis. Myofibril cross-sectional area was assessed by manually drawing around the perimeter of 200 individual myofibrils per participant throughout the entire sections. The numbers of myofibrils per fiber and per muscle were calculated by dividing muscle fiber area or muscle ACSAmax, respectively, by the average myofibril area. Finally, myosin spacing was assessed for 300 interspace distances per participant, by measuring the average distance across six myosin filaments in a line (i.e., 5 interspace distances, the length of an arrow in Fig. 1D, divided by 5) in three separate directions starting from a randomly chosen point (i.e., 15 distances starting from one point), and two random points for one myofibril (i.e., 30 distances for one myofibril) and for 10 different myofibrils (i.e., 300 distances in total) (28).

Statistical Analysis

All data on the whole cohort of participants were first checked for outliers by the median absolute deviation method (35), using a threshold value of 3 (very conservative (36)). One participant (LRT) for myofibril number per fiber was found to be an outlier, but was nevertheless included in the analysis because inclusion/exclusion of these data resulted in the same interpretation, and the data seemed biologically plausible. All analyses were performed using SPSS (version 25: IBM, Armonk, NY). Significance was set at an alpha level of 0.05. Data were checked for normality using Shapiro–Wilk tests in each group. Age, type I fiber area, myofibril area, and myosin spacing were nonnormally distributed, and thus, we used Mann–Whitney U tests to compare groups for these variables. For the other variables, unpaired t-tests were used. The effect sizes of between-group differences were calculated as Cohen's d values and interpreted as large (≥0.80), medium (0.50–0.79), small (0.20–0.49), or trivial (<0.20). Bivariate relationships were assessed with Pearson's product–moment correlations to assess the relationship of muscle ACSAmax with the structural variables of fibers and myofibrils on the whole cohort data. In addition, the coefficient of determination (R^2) was calculated for these relationships. All data are presented as mean ± SD in the text, and individual data together with box and whisker plots are shown within the figures (detailed in each figure).

RESULTS

Group characteristics. Descriptive characteristics of the groups are shown in Table 1. LRT individual were slightly (~2 yr) older than UNT (P = 0.012). There were no significant between-group differences in height or humerus length (P = 0.152–394), but body mass was 18% larger for LRT than UNT (P = 0.006). The IPAQ value was 92% greater for LRT than UNT (P = 0.006).

Muscle ACSAmax. Biceps brachii muscle ACSAmax was 70% greater for LRT than UNT (18.4 ± 2.7 vs 10.8 ± 2.2 cm^2, P < 0.001, d = 3.07 "large"; Fig. 2). The large difference in ACSAmax remained similar (63%) when normalized to

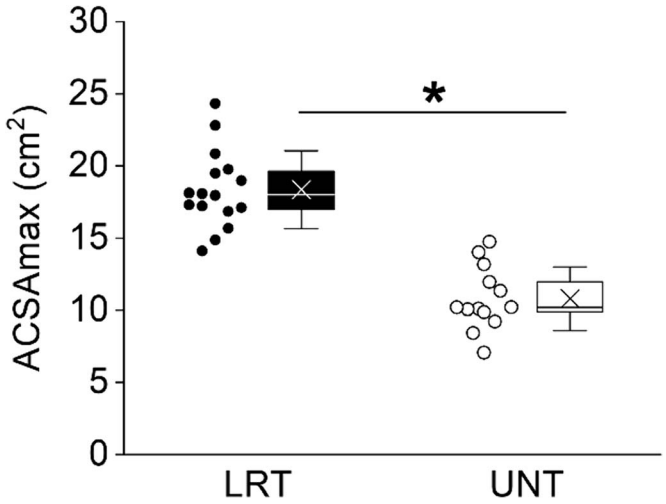

FIGURE 2—ACSAmax of LRT and UNT males. * denotes a significant difference between groups (P < 0.05) by a t-test. Individual plots together with box (lower–upper quartile) and whisker (SD) plots are shown. The cross mark and line in the box indicate the mean and median, respectively.

humerus length^2 (1.60 ± 0.22 vs 0.98 ± 0.20, $P < 0.001$, $d = 2.90$ "large") to account for any possible influence of body size on ACSAmax. Considering the similarity of between-group differences in absolute and normalized ACSAmax, data are only reported as absolute values (cm^2) to avoid redundancy. In addition to ACSAmax, muscle size was also assessed as ACSA at the location the biopsy was taken, but these two measures of muscle size produced identical findings, and therefore, data are only reported as ACSAmax to avoid redundancy.

Muscle fiber type composition, size, and number. Muscle fiber type composition did not significantly differ between LRT and UNT when expressed by the proportion of either number (type I: 48.3% ± 8.1% vs 45.7% ± 5.8%, type II: 51.7% ± 8.1% vs 54.3% ± 5.8%, $P = 0.348$, $d = 0.36$ "small"; Fig. 3A) or area (type I: 44.7% ± 9.0% vs 43.9% ± 6.2%, type II: 55.3% ± 9.0% vs 56.1% ± 6.2%, $P = 0.796$, $d = 0.10$, "trivial"; Fig. 3B). Fiber area was significantly larger in LRT than UNT for both type I (+27%, 6707 ± 1613 vs 5281 ± 1389 μm^2, $P = 0.022$, $d = 0.95$ "large"; Fig. 3C) and type II (+31%, 8422 ± 2760 vs 6419 ± 2137 μm^2, $P = 0.041$, $d = 0.81$, "large"; Fig. 3D) fibers. Consequently, the average fiber area (of both fiber types) was significantly larger for LRT than UNT (+29%, 7588 ± 2134 vs 5881 ± 1726 μm^2, $P = 0.028$, $d = 0.88$, "large"; Fig. 4A). Fiber number (i.e., in total within the biceps brachii ACSAmax) was also greater for LRT than UNT (+34%, 260 ± 77 × 10^3 vs 194 ± 50 × 10^3, $P = 0.013$, $d = 1.02$, "large"; Fig. 4B). Both the average fiber area ($r = 0.526$, $R^2 = 0.277$, $P = 0.003$) and fiber number ($r = 0.445$, $R^2 = 0.198$, $P = 0.015$) were significantly correlated with muscle ACSAmax (Figs. 4C, D).

Myofibril size and number. There was a tendency toward smaller myofibril area (−16%, 0.628 ± 0.204 vs 0.748 ± 0.192 μm^2, $P = 0.074$, $d = 0.61$, "medium"; Fig. 5A) for LRT versus UNT. The number of myofibrils per fiber (+49%, 13.1 ± 5.6 × 10^3 vs 8.8 ± 4.1 × 10^3, $P = 0.034$, $d = 0.87$, "large"; Fig. 5B) and the total number of myofibrils within the whole muscle (i.e., per ACSAmax) (+105%, 32.0 ± 10.7 × 10^8 vs 15.6 ± 5.6 × 10^8, $P < 0.001$, $d = 1.91$, "large"; Fig. 5C) were greater for LRT than UNT. Myofibril area ($r = -0.456$, $R^2 = 0.208$, $P = 0.015$), number of myofibrils per fiber ($r = 0.566$, $R^2 = 0.320$, $P = 0.002$), and within the whole muscle ($r = 0.208$, $R^2 = 0.762$, $P < 0.001$) were all significantly correlated with ACSAmax (Figs. 5D–F).

Myosin spacing. LRT had a modest but significantly smaller myosin spacing than UNT (−7%, 39.2 ± 3.3 vs 42.4 ± 2.5, $P = 0.010$, $d = 1.08$, "large"; Fig. 6A), and this was negatively correlated with ACSAmax ($r = -0.387$, $R^2 = 0.150$, $P = 0.042$; Fig. 6B).

DISCUSSION

The main findings of this study were that LRT individuals who had 70% larger muscles than UNT, also had 1) larger size (+29%) and a greater number (+34%) of muscle fibers within the ACSAmax, 2) a tendency for smaller myofibrils (−16%) but more myofibrils per fiber (+49%) and in total (+105%),

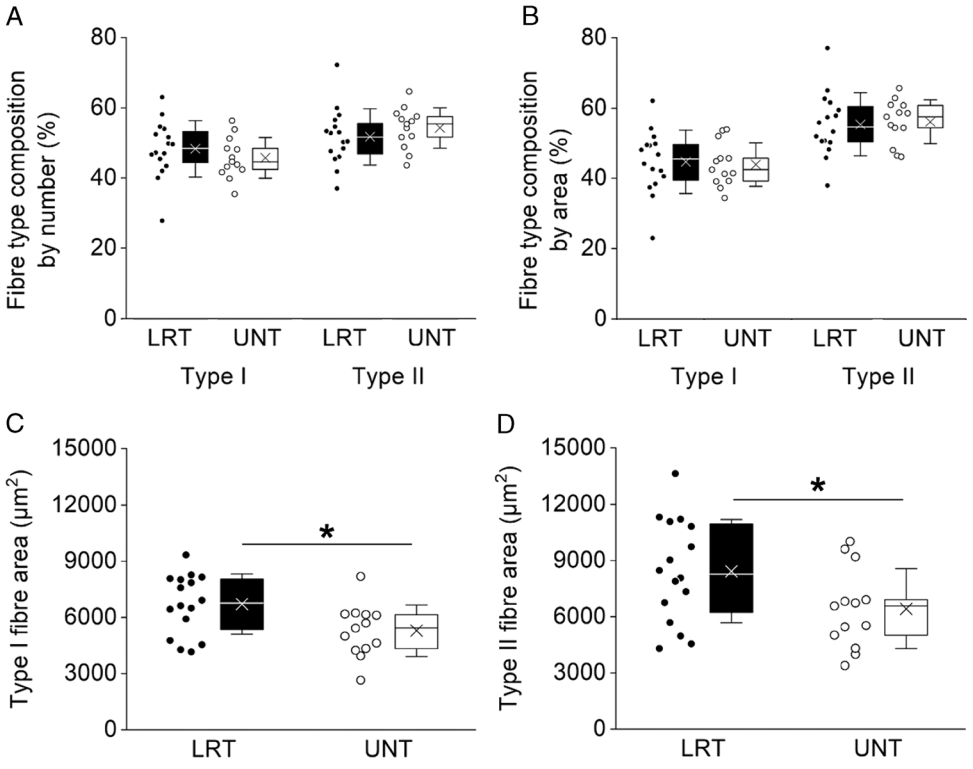

FIGURE 3—Muscle fiber type composition by number (A) and by area (B), and type I fiber area (C) and type II fiber area (D) of LRT and UNT males. * denotes a significant difference between groups ($P < 0.05$). To compare the groups, Mann–Whitney *U* test (C) and *t*-tests (A, B, D) were used. Individual plots together with box (lower–upper quartile) and whisker (SD) plots are shown. The cross mark and line in the box indicate the mean and median, respectively.

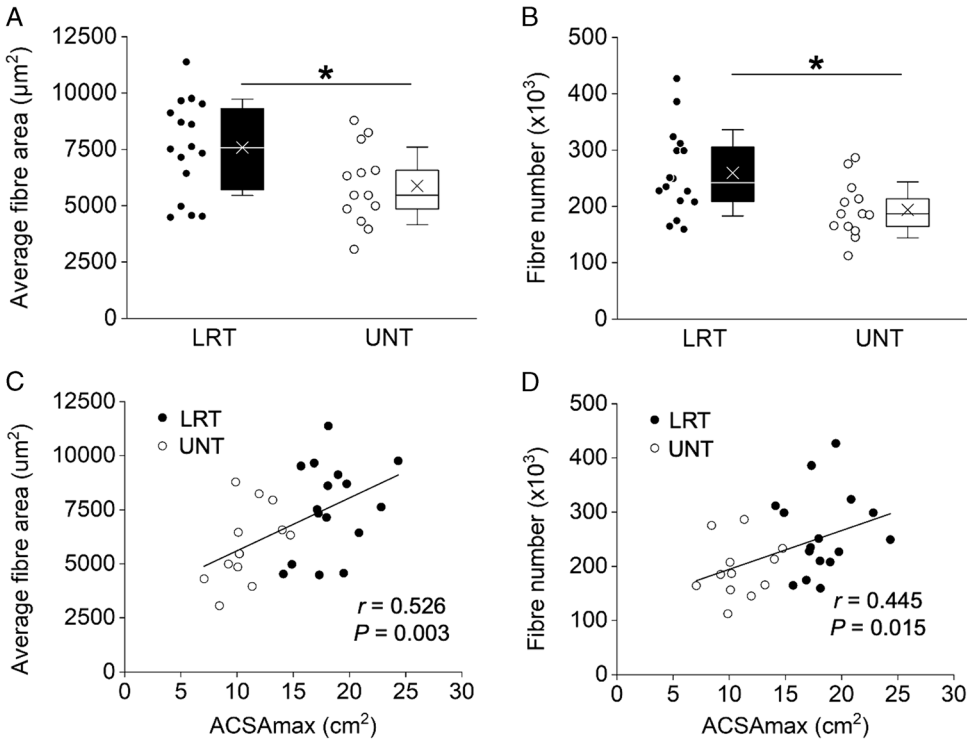

FIGURE 4—Average fiber area across type I and II fibers (A) and estimated fiber number (B), and relationships of ACSAmax with average fiber area (C) and fiber number (D) of LRT and UNT males. * denotes a significant difference between groups ($P < 0.05$) by a *t*-test. Individual plots together with box (lower–upper quartile) and whisker (SD) plots are shown. The cross mark and line in the box indicate the mean and median, respectively.

and 3) smaller myosin spacing (−7%, i.e., greater packing density), than UNT. In addition, considering the whole cohort (i.e., both groups), muscle size (ACSAmax) was positively correlated with muscle fiber size ($r = 0.526$) and number ($r = 0.445$), as well as myofibril number (per fiber $r = 0.566$ and in total $r = 0.873$), and negatively correlated with myofibril size ($r = -0.456$) and myosin spacing ($r = -0.382$). These findings mostly supported our hypotheses and suggest that the larger muscles of LRT individuals were characterized as having muscle fibers of greater size and number in cross section, which likely contain smaller but more myofibrils as well as more tightly packed myofilaments.

Whole muscle and muscle fibers. Muscle ACSAmax was 70% greater for LRT than UNT (Fig. 2). This between-group difference was similar to previous studies (57%–76%

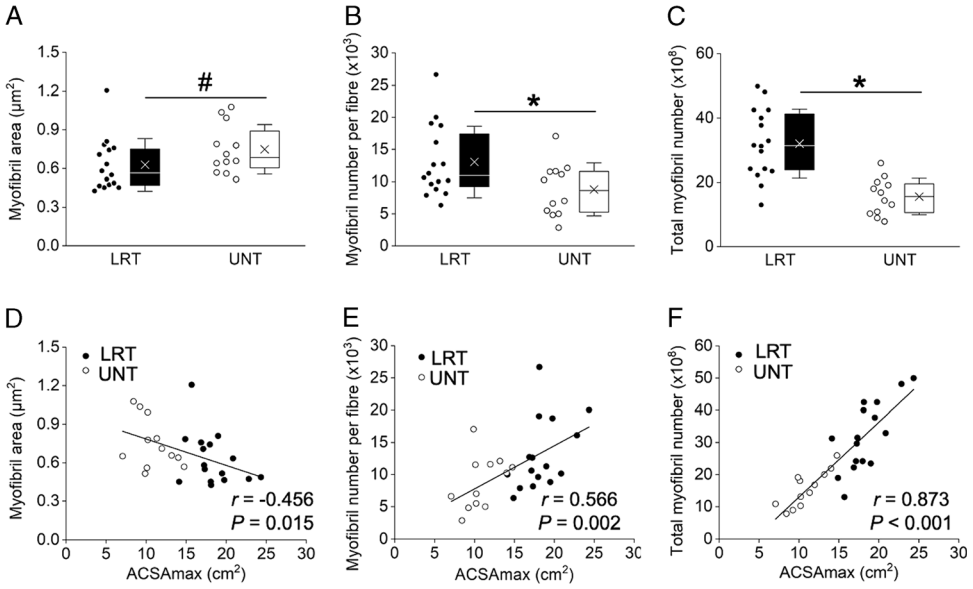

FIGURE 5—Myofibril area (A), myofibril number per fiber (B), and total myofibril number (C), and relationships of ACSAmax with myofibril area (D), myofibril number per fiber (E), and total myofibril number (F) of LRT and UNT males. * denotes a significant difference between groups ($P < 0.05$) by a *t*-test, and # denotes a tendency toward significance ($P = 0.072$) by Mann–Whitney *U* test. Individual plots together with box (lower–upper quartile) and whisker (SD) plots are shown. The cross mark and line in the box indicate the mean and median, respectively.

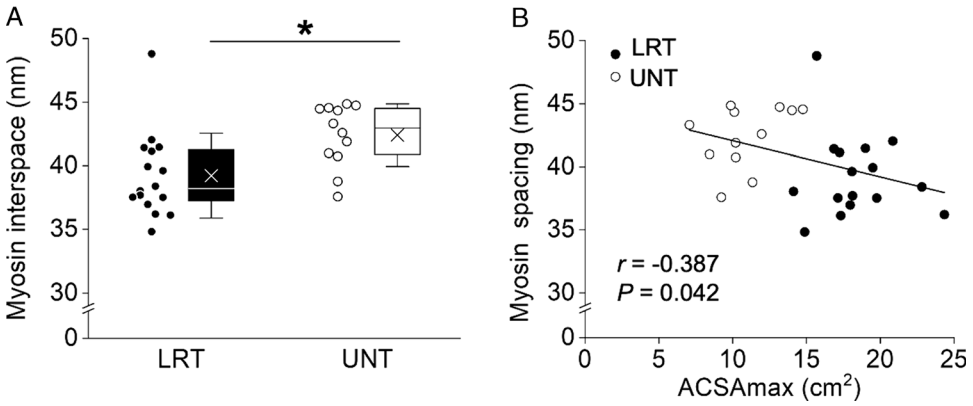

FIGURE 6—Myosin interspace (A) and its relationship with ACSAmax (B) of LRT and UNT males. * denotes a significant difference between groups ($P < 0.05$) by Mann–Whitney *U* test. Individual plots together with box (lower–upper quartile) and whisker (SD) plots are shown. The cross mark and line in the box indicate the mean and median, respectively.

difference) (12,14) that compared the size of the biceps brachii between LRT (elite bodybuilders) and UNT males. Thus, the LRT participants recruited in this study can be considered as having substantially larger biceps brachii muscles compared with UNT, a widely documented adaptation to prolonged RT.

Muscle fiber type composition was similar between LRT and UNT, with both groups having 44%–48% type I fibers and 52%–56% type II fibers when expressed either by number or area (Figs. 3A, B), which is in line with the previous reports indicating no clear effect of LRT on fiber type composition (12,14). The muscle fiber area of LRT was similarly greater than UNT for both type I and type II fibers (+27% and +31%, Figs. 3C, D). This supports the previous finding of consistent fiber type hypertrophy within the biceps brachii of LRT versus UNT males (14) and suggests that the common observation of preferential type II fiber hypertrophy after short-term RT (37,38) does not persist with prolonged RT. The average fiber area (of both fiber types) was +29% larger for LRT than UNT (Fig. 4A) and was positively correlated with muscle ACSAmax (Fig. 4C). This agrees with widely acknowledged findings that enlarged muscle fibers explain, at least partly, enlarged muscle size after RT (see reviews (16,39) for details).

Furthermore, the between-group difference of muscle fiber size (29%) was found to be much smaller than that of muscle ACSAmax (70%). As a result, the estimated muscle fiber number within the ACSAmax of LRT was +34% greater than UNT (Fig. 4B). Therefore, there was a bigger percentage difference and effect size for fiber number than fiber area between LRT and UNT (+34% vs +29%; $d = 1.02$ vs $d = 0.88$). Fiber number was also positively correlated with muscle ACSAmax in the whole cohort, but with a slightly weaker correlation coefficient than for fiber area ($r = 0.445$ vs $r = 0.526$; Figs. 4C, D). The current findings are in agreement with previous studies that found either a significant correlation ($r = 0.60$) (14) or, although not reported as such by the authors, a tendency (i.e., $r = 0.35$, $n = 25$, $P < 0.10$) (12) between muscle fiber number and muscle size. However, these same studies also reported no differences in muscle fiber number between LRT/elite bodybuilders and UNT individuals (12,14), perhaps because these studies included three groups and smaller participant numbers per group than the current investigation, potentially reducing their statistical power for finding differences.

There are two possible adaptive explanations for the observation of greater fiber number in the muscle cross section of prolonged RT individuals. First, it could indicate human muscle fiber hyperplasia after prolonged RT (i.e., an increase in the total number of muscle fibers within the whole muscle), in accordance with the adaptations to years of daily functional demands (21). Alternatively, in pennate muscles such as the vastus lateralis, changes in fiber/fascicle length could also increase the apparent number of fibers with a given cross section of the muscle, as well as increasing muscle CSA and volume (40). Although the current study did not assess fascicle length, we deliberately chose to examine the biceps brachii owing to its fusiform architecture, to minimize the potential confounding influence of differences in muscle architecture between groups (12,14). Therefore, because of the limited pennation of the biceps brachii, a genuine increase in fiber number may be the most likely explanation for the greater number of fibers in the muscle cross section of LRT individuals. Finally, given the cross-sectional nature of this study, we cannot rule out the possibility that the LRT participants had an inherently greater number of muscle fibers than their UNT counterparts, perhaps a consequence of a selection bias, and longitudinal studies of sufficient RT duration are needed to resolve this uncertainty.

Myofibrils and myofilaments. Myofibril area showed a tendency to be smaller (−16%) in LRT than UNT individuals (Fig. 5A), and there was a significant negative correlation between myofibril area and muscle ACSAmax (i.e., larger muscle, smaller myofibrils; Fig. 5D). This contradicted our second hypothesis and a previous RT study (25) that found a 16% increase in myofibril size after 6 months of RT. This discrepancy cannot be explained with any certainty, although average myofibrillar size may be the result of the combined effects of both myofibril growth, which increases myofibril size, and proliferation/splitting, which instead reduces myofibril size. After LRT (mean, 6 yr in the current study), myofibrillar proliferation/splitting could be sufficient to counter any myofibrillar growth resulting

in no overall change in average myofibrillar area. As a result of the tendency toward smaller myofibrils in LRT, together with their larger muscle fiber area and muscle ACSAmax, myofibril number was greater for LRT than UNT by +49% per fiber and +108% in total (Figs. 5B, C), and both were correlated with ACSAmax (Figs. 5E, F). A recent study by Jorgenson et al. (41) used high-resolution immunofluorescence microscopy and revealed that a relatively brief 7-wk RT period increased the number of myofibrils in type II fibers without altering myofibril size, which agrees with the current study. Therefore, muscle hypertrophy after RT appears to be associated with a greater amount of contractile/myofibrillar material, mainly because of an increased number of myofibrils, and this may be a primary reason why LRT individuals are capable of producing significantly higher levels of force and power compared with UNT (11,14).

Myosin spacing was smaller in LRT than UNT participants and was negatively correlated with ACSAmax (Figs. 6A, B). Although the between-group difference in myosin spacing was modest (−7%), its effect size was large (Cohen's $d = 1.08$) because of the low interindividual variability of this variable. This suggests that LRT had greater myofilament packing density than UNT, and larger muscles were also associated with more packed myofilaments. As mentioned earlier, two preliminary studies have reported a decrease ($n = 3$) (27) and no change ($n = 8$) (28) in myosin spacing after short-term RT. Similar to the current findings, a recent pilot study (42) found a small group of LRT (10 ± 3 yr of experience, $n = 6$) had ~9% more myosin and actin protein content (on a per mg basis), suggestive of greater myofibrillar packing. Nonetheless, the current study provides the first visual evidence for the greater contractile filament density of prolonged RT and thus offers a mechanistic basis for increased specific tension that would be expected to contribute to strength gains irrespective of hypertrophy (29). It is also notable that small (+2%–6%) but consistent increases in muscle radiological density, suggestive of increased contractile filament packing, have been found after RT (28,43,44). Furthermore, we have previously found greater *in vivo* whole muscle–specific tension of LRT versus UNT (11), with a similar magnitude (+9%) to the difference in myosin spacing found in the current study (−7%), although a relatively small single fiber study (groups of $n = 6$) found no significant difference in fiber-specific tension of LRT versus UNT (45). Nonetheless, the current study provides the first evidence of differences in the ultrastructure of skeletal muscle tissue in LRT versus UNT that would be expected to modestly enhance specific tension.

Limitations. There are some limitations in this study. First, and most importantly, this was a cross-sectional study and not a longitudinal one, which makes it impossible to attribute causality (i.e., which adaptations were due to RT and which were inherent to the participants). However, studying LRT versus UNT individuals has the advantage to be able to identify larger potential adaptations/differences (12,14) than in short/medium-term longitudinal studies (25,27,28), and this may better inform the capability for adaptation. Nevertheless, more direct longitudinal studies of sufficient RT duration (e.g., ≥1 yr), ideally with multiple measurements over the intervention period, are warranted to further develop our knowledge of RT adaptations.

In addition, it should be acknowledged that not all the muscle ACSA is muscle fiber material (12), nor is all the fiber CSA myofibrillar material (46), yet our calculations of muscle fiber and myofibril numbers assume that they are. Thus, the actual numbers of muscle fibers and myofibrils will be lower than the estimated numbers reported in this study. However, in young healthy participants, a majority (80%–85%) of both muscle ACSA and fiber CSA do consist of muscle fibers and myofibrillar material, respectively, and these proportions appear to be consistent with RT (12,25,41,44,46). Therefore, it seems likely that our findings of LRT individuals having more muscle fibers and myofibrils than UNT participants, as well as the correlations between muscle ACSA and muscle

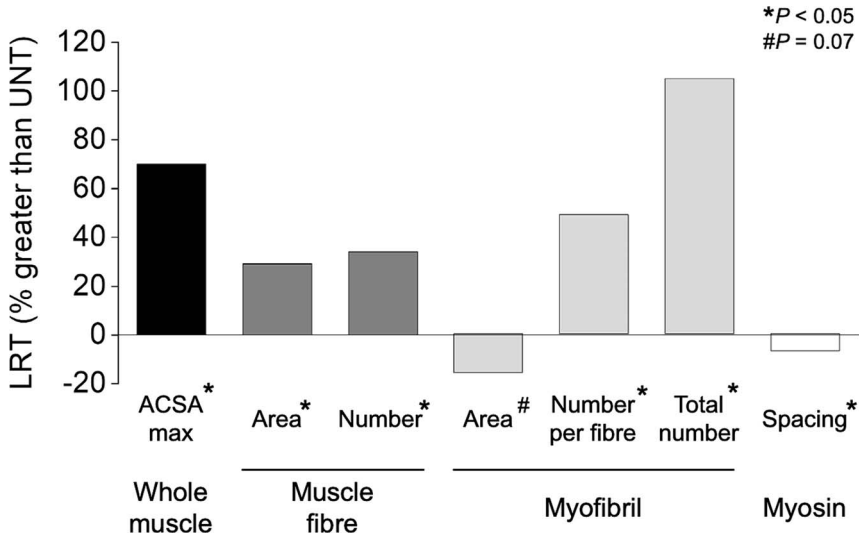

FIGURE 7—Summary of the differences in whole muscle, muscle fiber and ultrastructural variables of LRT compared with UNT males. Data are percentage differences in group mean values.

fiber or myofibril numbers, remain robust even when the noncontractile components of the biceps brachii muscle are taken into account. Nevertheless, we recommend that future studies correct for noncontractile components to estimate muscle fiber and myofibril numbers more precisely.

Finally, elements of our correlation analysis may have been confounded by circularity. For example, the relationship between muscle ACSAmax and fiber number (derived from ACSAmax divided by average fiber area) involves circularity, which may confound this relationship (47). Therefore, the between-group difference in fiber number within the muscle ACSAmax between LRT versus UNT may be more robust evidence than the relationship of fiber number with ACSAmax. In addition, we are aware that our fiber number calculation was a crude estimation mainly because of innate difficulty in generalizing single biopsy data to the whole muscle level (48); however, currently, there appears no other way to estimate fiber number of human muscles *in vivo*. Therefore, we used the same approach as previous studies (12,14) but with a larger sample size per group, and found clearer between-group differences than the previous investigations and a large effect size (d = 1.02) for greater fiber number within the muscle cross section of LRT versus UNT. Furthermore, the disproportionately greater group difference in ACSAmax (70%) compared with fiber area (29%) alone suggests that fiber number was greater for LRT than UNT. Therefore, although our approach of indirect estimation of fiber number has an inherent methodological limitation, we consider that our overall findings including fiber number add important cross-sectional data to the existing literature. Although it is impossible to directly measure the size and number of all muscle fibers *in vivo*, advancing diffusion tensor magnetic resonance imaging techniques have been shown to be promising tools to noninvasively estimate muscle fiber diameters and fiber types in areas of interest (49,50). Thus, future studies should be directed toward adopting/developing these emerging techniques, together with traditional biopsy methods, to better understand the plasticity of muscle fibers and myofibrils following RT.

CONCLUSIONS

The main findings of this study are summarized in Figure 7. Compared with UNT, LRT had a substantially larger biceps brachii muscle area, which was mediated by having not only larger muscle fiber area but also a greater number of fibers. LRT had more myofibrils per fiber and in total, with a tendency for smaller myofibrils. A novel finding of this study was the greater myofilament packing density of LRT, suggesting that skeletal muscle ultrastructure may be adaptable and could contribute to changes in specific tension and strength.

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