Functional properties of human muscle fibers after short-term resistance exercise training

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Abstract

The aim of this study was to assess the relationships between human muscle fiber hypertrophy, protein isoform content, and maximal Ca$^{2+}$-activated contractile function following a short-term period of resistance exercise training. Six male subjects (age 27 ± 2 yrs) participated in a 12 week progressive resistance exercise training program that increased voluntary lower limb extension strength by > 60%. Single chemically skinned fibers were prepared from pre- and post-training vastus lateralis muscle biopsies. Training increased the CSA and peak Ca$^{2+}$-activated force ($P_o$) of fibers containing type I, IIa, or IIa/IIx myosin heavy chain by 30-40% without affecting fiber specific force ($P_o$/CSA) or unloaded shortening velocity ($V_o$). Absolute fiber peak power rose as a result of the increase in $P_o$ while power normalized to fiber volume was unchanged. At the level of the cross-bridge, the effects of short-term resistance training were quantitative (fiber hypertrophy and proportional increases in fiber $P_o$ and absolute power) rather than qualitative (no change in $P_o$/CSA, $V_o$, or power/fiber volume).

Key Words: resistance training, strength training, muscle hypertrophy, myosin heavy chain, contractile properties.
Skeletal muscles fibers have a remarkable ability to alter their phenotype in response to environmental stimuli or perturbations. An example of this capacity for adaptive change, or plasticity, is the cell hypertrophy that occurs after resistance exercise training. There is a general consensus that resistance training causes hypertrophy of all muscle fiber types, with fast fibers often showing a somewhat greater response than slow fibers (2, 11, 13, 18, 23, 31). There is also a growing body of knowledge detailing resistance exercise-induced changes in contractile protein isoform content, where the most significant alteration appears to be an up-regulation of the type IIa myosin heavy chain (MHC) isoform coupled with a down-regulation of the type IIx isoform (1, 31, 38).

Despite this progress in characterizing resistance training-induced changes in cell morphology and protein isoform content, relatively little information exists regarding the functional consequences of these adaptations. It is generally assumed that force is proportion to fiber cross-sectional area (CSA) and that the MHC isoform composition of a fiber, or its histochemically determined fiber type, is an accurate index of the fiber’s shortening velocity. However, the specific relationship between fiber CSA and force is dependent on fiber myofibrillar density, a variable that can vary between slow and fast fibers (33). Fiber maximal shortening velocity, while a function of cell MHC isoform content (25), is modulated by other sarcomeric proteins, such as the myosin light chains (3, 15, 32).

Chronic changes in the level of physical activity can alter the functional properties of individual muscle cells. For example, endurance training has little effect on fiber specific force (force/fiber CSA) but alters fiber myosin light chain isoform content and increases the unloaded shortening velocity \( (V_u) \) of slow fibers by ~ 20% (27, 36). In contrast, sprint training may decrease the specific force of fast fibers (20) without affecting slow or fast fiber shortening velocity (12).
Knowledge of the relationship between cell hypertrophy, protein content, and contractile function is therefore an important step in understanding the adaptive responses of skeletal muscle to resistance exercise training. However, no clear consensus has emerged as to the effects of resistance training on muscle fiber function. Romantowski et al. (26), studying 60-70 yr olds, observed resistance training-induced reductions in the specific force of slow fibers and a decrease in the \( V_o \) of fast fibers. Trappe et al. (34, 35), observed an increase in the specific force of type IIa fibers from elderly females and an increase in the \( V_o \) of type I and IIa fibers from elderly males. However, unlike younger subjects, fast fibers obtained from the elderly participants in these studies often showed no evidence of hypertrophy (26, 35). Also, since aging affects both fiber specific force and \( V_o \) (9, 19), it is not clear whether the observed responses represent an effect of resistance training per se or an interaction between aging and training.

The purpose of the present study was to assess the relationships between fiber hypertrophy, protein isoform content, and contractile function following 12 weeks of progressive resistance exercise training. The training program induced significant hypertrophy of all major groups of slow and fast muscle fibers. Measurements conducted on chemically skinned, maximally \( Ca^{2+} \)-activated muscle fibers indicated that resistance training altered quantitative aspects of fiber function, such as absolute peak force and absolute peak power, but did not affect qualitative or intrinsic mechanisms of contraction, such as specific force, \( V_o \), or peak power/fiber volume.

**Methods**

*Subjects.* This study was approved by the Institutional Review Board at Oregon State University. Six males volunteered to serve as subjects after being informed of the nature of the study and after providing their consent in writing. Their mean (± SE) age, height, and body mass
at the beginning of the study were 27 ± 2 yr, 178 ± 2 cm, and 82.3 ± 4.2 kg. Health questionnaire responses indicated that all subjects were non-smokers, were free of any apparent signs or symptoms of neuromuscular disease, and were not taking medications or drugs, including anabolic steroids, that could affect their response to exercise training. None of the subjects had participated in a strength or endurance training program for a minimum of one year preceding the study.

**Resistance exercise training program.** All subjects completed a resistance exercise training program consisting of 36 exercise sessions performed three times per week on non-consecutive days. The training program used free-weight and machine based exercises designed to overload the major lower (squats, knee extension, knee flexion, calf raises), upper (bench press, lat pull down, shoulder press, triceps press, biceps curl, seated row), and abdominal muscle groups.

During each training session, subjects completed three sets of 5-10 of the exercises listed above (divided approximately equally between those targeting the upper and lower body). Subjects performed 12 repetitions per set during the first two weeks of the training program. Thereafter, one weekly session was performed at 10 repetitions per set, the second session at 8 repetitions per set, and the third weekly session at 6 repetitions per set. During all sessions, the training resistance was adjusted so that subjects were able to complete only the specified number of repetitions, plus or minus one repetition. This non-linear periodized program was used in order to maximize training adaptations (17). All exercise sessions were supervised by one of the investigators or by a trained assistant.

**Evaluation of voluntary strength and body composition.** Subjects reported to the laboratory on two or three separate occasions before the start of the training program. These pre-training visits were used to obtain a pre-training muscle biopsy (see below), to teach subjects
proper exercise technique, to determine their six-repetition maximum voluntary strength for leg press and bench press exercise, and to assess body composition using an air displacement densitometry plethysmograph (Life Measurement Instruments, BOD POD; Concord, CA) and the Siri equation (29). Six-repetition maximum voluntary strength was re-evaluated every 4 weeks throughout the training program. Post-training body composition was assessed in the week following the last training session.

**Muscle biopsy.** A pre-training muscle biopsy was obtained from the left vastus lateralis during the subjects’ initial visit to the laboratory. The biopsy was obtained prior to any other data collection, physiological testing, or training. To minimize the possibility of studying fibers that may have been damaged by the last bout of exercise, the post-training biopsy was obtained 3-4 days following the final training session, after we had ensured that subjects were not experiencing any delayed muscle soreness. The post-training sample was obtained from the right leg in order to eliminate the possibility of studying regenerating fibers at the pre-training biopsy site. All pre- and post-training muscle samples were obtained from similar anatomical sites located mid-way between the greater trochantor and the patella.

**Composition of the solutions for in vitro experiments.** The composition of the relaxing and the Ca\(^{2+}\)-activating solutions were determined using the computer program described by Fabiato (7) with apparent stability constants adjusted for temperature, pH, and ionic strength (6). Both solutions contained 7.0 mM EGTA, 20.0 mM imidazole, 1 mM free Mg\(^{2+}\), 4 mM MgATP, 14.5 mM creatine phosphate, and 15 U·ml\(^{-1}\) creatine kinase. The free Ca\(^{2+}\) concentration of the relaxing and activating solutions was adjusted to pCa 9.0 and pCa 4.5, respectively (where pCa = - log [Ca\(^{2+}\)]), using a 100 mM CaCl\(_2\) standard solution (Calcium Molarity Standard, Corning Incorporated, Corning, NY). In both solutions, pH was adjusted to 7.0 with KOH and total ionic strength to 180 mM with KCl. A dissection solution was made from relaxing solution and a
protease inhibitor cocktail prepared according to the manufacturer (Complete EGTA-Free Protease Inhibitor, Boehringer Mannheim, Indianapolis, IN). The skinning solution consisted of equal volumes of dissection solution and glycerol.

**In vitro measurement of fiber contractile properties.** Pre- and post-training muscle biopsies were immediately placed in cold (4°C) dissection solution where they were longitudinally divided into small bundles of fibers. The fiber bundles were stored in skinning solution maintained at 4°C for 24 hours and then being transferred to fresh skinning solution and stored at –20°C.

Over the next three weeks, bundles were transferred to relaxing solution where single muscle fiber segments were isolated using fine forceps. A single fiber segment was mounted between an isometric force transducer (Model 400, Aurora Scientific, Aurora, Ontario) and the arm of a high-speed motor (Model 308B, Aurora Scientific) by securing the fiber ends in small stainless steel troughs using 4-0 monofilament posts and 10-0 suture (36). The motor was controlled by a high speed servomechanism and circuit (model 300-FC1, Positron Development, Inglewood, CA) operating either in length (for slack tests) or force (for isotonic contractions) mode.

Once mounted, the fiber could be rapidly transferred between small wells milled into a stainless steel dip-plate. The plate was mounted to the stage of an inverted microscope where the fiber could be observed during data collection. Sarcomere length was adjusted to 2.5 µm using a calibrated ocular micrometer (600X). Fiber length (FL) was measured with a digital micrometer. Fiber width was read from the ocular micrometer while the fiber was briefly suspended in air (< 5 s). Three separate measurements were obtained at ~ 0.25, 0.50, and 0.75 of FL, with the fiber returned to relaxing solution between each measurement. Fiber CSA was calculated by assuming the mounted fiber forms a cylinder when suspended in air (24, 36). The mean of the three CSA
values was taken as the final fiber CSA. The temperature of the relaxing and activating solutions was continually monitored during data collection by a small thermocouple inserted into the solution bathing the fiber. Solution temperatures were maintained at 15°C during the experiments.

Output from the motor and force transducer were monitored on a digital oscilloscope, amplified (model 300-DIF2H, Positron Development), digitized (5 kHz), and interfaced to a personal computer (AT-MIO-15E-1, National Instruments, Austin, TX). Data reduction, analysis, and storage were performed by programs written in our laboratory (LabView, National Instruments).

Peak Ca\(^{2+}\)-activated force and \(V_o\) were evaluated using a slack test procedure. Fibers were activated, allowed to attain peak force, and then subjected to a rapid reduction in length (90% complete in ~ 1 ms). Force dropped to zero as the fiber shortened under no external load (see Figure 1-A). When the fiber has shortened to the point where it was no longer slack, there was a rapid re-development of tension. The fiber was returned to relaxing solution, re-extended to its original FL, and the entire procedure repeated at a different slack step length. In this study, the longest slack step imposed on the fiber averaged 15.3 ± 0.1 % of FL and never exceeded 20% of FL. Peak Ca\(^{2+}\)-activated force was calculated as the difference between maximal force and the force baseline during unloaded shortening. Unloaded shortening velocity was taken as the slope of the relationship between the time required for tension re-development and slack step length (Figure 1-B).

Fiber force-velocity-power relationships were determined from a series of isotonic contractions (Figure 1-C). Contractions were performed in sets of three (each contraction 50-100 ms in duration) in which total shortening never exceeded 20% of FL. Shortening velocity was calculated from the motor position record obtained over the last 25-50 ms of each contraction.
Fiber force was calculated over similar time points, using the force obtained during a slack step imposed immediately after the last isotonic contraction as a baseline. Data were fit by the Hill equation (14), \((P + a)(V + b) = (P_o + a)b\), where \(P_o\) is peak force and the constants \(a\) and \(b\) have dimensions of force and velocity, respectively (Figure 1-D). The parameters describing the relationship, \(V_{max}\) (the velocity-axis intercept), \(a/P_o\) (a unitless parameter describing the curvature of the relationship), and peak Ca\(^{2+}\)-activated force, were used to calculate fiber peak power (39).

**Determination of fiber myosin isoform composition.** Following the physiological measurements, the fiber segment was removed from the transducer and motor, hydrolyzed in 30 µl of an SDS sample buffer (containing 62.5 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 5% beta-mercaptoethanol, 0.001% bromophenol blue), denatured for 4 minutes at 95°C, and stored at −80°C. Later, a portion of the fiber solute was loaded on a gel system consisting of a 7% polyacrylamide separating gel and a 3.5% stacking gel (8). Electrophoresis was carried out on Bio-Rad mini-Protean 3 electrophoresis cells running at 70 V for 22-24 hours (4°C). Protein bands were visualized using the silver staining procedure described by Shevchenko et al. (28) modified in that the silver nitrate incubation was carried out at room temperature instead of 4°C. Myosin heavy chain isoforms in the single fiber segments were identified by comparison with human myosin standards that were run on one or more lanes of each gel. The myosin standards were made by extracting myosin from human vastus lateralis muscle biopsy samples. Figure 2 is an example of a gel illustrating separation of the three MHC isoforms present in adult skeletal muscle and the identification of the MHC isoform composition of single muscle fiber segments.

**Statistical analysis.** Data are presented as mean ± SE. Fiber segments were grouped according to their MHC isoform composition for analysis. In order to compare the morphological and functional properties of fibers differing in their myosin heavy chain composition, pre-training fibers were analyzed with a two-way ANOVA (MHC isoform composition x subject)
and subsequent Tukey post-hoc test. To investigate changes in fiber morphology or function as a result of resistance exercise, pre- and post-training fibers were analyzed using a two-way ANOVA with main effects of subject and training status. In all analyses, each fiber was treated as a single observation. Pre- and post-training characteristics of the subjects were compared with a repeated measures ANOVA. Statistical significance was accepted at P < 0.05.

Results

General adaptations to training. Lean body mass rose 4% over the course of the training program (from 63.7 ± 2.8 to 66.4 ± 2.3 kg; P < 0.05) while total body mass was unchanged. Lower body neuromuscular strength, as assessed by the six-repetition maximum for leg press exercise, rose from a pre-training value of 1524 ± 99 N to 1791 ± 69 N at the 4th week (P > 0.05 vs. pre-training), 2241 ± 117 N at the 8th week (P < 0.05 vs. pre-training), and 2532 ± 115 N at the 12th week (P < 0.05 vs. pre-training) of training. Over the course of the training program, leg press six-repetition maximum strength increased 62% relative to total body mass (from 18.5 ± 0.8 to 30.0 ± 1.5 N/kg body mass, P < 0.05), or 61% relative to lean body mass (from 23.8 ± 0.7 to 38.3 ± 2.1 N/kg lean body mass, P < 0.05).

Myosin isoform composition of pre- and post-training fibers. Functional properties were determined on 204 pre- and 163 post-training vastus lateralis muscle fibers. As seen in Table 1, the relative number of fibers containing type I MHC was similar before and after training. However, the relative number of fibers containing type IIa MHC increased from 30% prior to training to 55% after training while the relative number of single fibers containing type IIa and type IIx MHC fell from 22% to 3%. Post-training fibers containing type IIx or type I/IIa MHC were relatively rare. Consequently, 94% of the pre- and 100% of the post-training fibers studied contained either type I, type IIa, or type IIa/IIx MHC.
Fiber cross-sectional area and peak Ca$^{2+}$-activated force. Before training, fibers containing type II MHC, either exclusively or in combination with another isoform (i.e. I/IIa, IIa, IIa/IIx, or IIx), were significantly larger in CSA than fibers containing the type I MHC exclusively (Table 1). The mean CSA of the type I, IIa, and IIa/IIx fibers all increased with resistance training. On an absolute basis, hypertrophy of the type IIa (+ 1989 µm$^2$) and IIa/IIx (+ 2014 µm$^2$) fibers exceeded that of the type I (+ 1596 µm$^2$) fibers by 25%. The relative increase in CSA averaged 30% for all three groups of fibers.

Pre-training fibers containing fast MHC isoform produced significantly greater force than fibers containing type I MHC (Table 2). This was due to the greater CSA of the fast fibers coupled with their significantly greater specific force. Resistance training resulted in significant increases in the absolute peak Ca$^{2+}$-activated force of fibers containing type I (+40%), IIa (+35%), and IIa/IIx (+34%) MHC. On average, training-induced increases in fiber CSA and Ca$^{2+}$-activated force were proportional, since the mean specific force of the type I, IIa, and IIa/IIx fibers was unchanged over the course of the study (Table 2). Figure 4 clearly shows a shift in the frequency distributions of CSA and peak Ca$^{2+}$-activated force of all three groups of fibers towards greater values after training. In order to examine the relationship between fiber CSA and peak Ca$^{2+}$-activated force, the individual fibers compiled in the histograms were plotted in a scattergram (Figure 4) and reference lines were drawn that represented the average pooled specific force of the type I (Panel A, 117 kN/m$^2$), type IIa (Panel B, 136 kN/m$^2$), and type IIa/IIx (Panel C, 146 kN/m$^2$) fibers. Pre- and post-training type I, IIa, and IIa/IIx fibers all clustered about their respective reference lines, showing little deviation from the mean specific force across a wide range of fiber CSA and peak Ca$^{2+}$-activated force.

All subjects experienced hypertrophy of both type I and IIa fibers, and in general, peak Ca$^{2+}$-activated force of type I and IIa fibers rose in direct proportion to the increase in fiber CSA.
(Figure 3). However, there was some inter-subject variability in this response with fibers from some subjects showing small increases and other small decreases in specific force. Closer examination reveals that these changes were in directions that reduced the variability of the population as a whole. For example, pre-training average specific force for the six subjects ranged from 107-134 kN/m² for the type I fibers and from 119-153 kN/m² for the type IIa fibers. After training, these ranges were reduced by ~50%, to 115-128 kN/m² and 131-146 kN/m², respectively.

Unloaded shortening velocity. Before training, fibers containing type IIa MHC shortened 5-fold faster than fibers containing type I MHC while fibers containing type IIx fibers shortened 1.6 times faster than the type IIa fibers (Table 3). Fibers containing two MHC isoforms had shortening velocities that were intermediate to those of fibers containing one or the other of the isoforms. Resistance training had no effect on the mean unloaded shortening velocity of fibers containing type I, type IIa, or type IIa/IIx MHC.

Force-velocity-power relationships. Figure 5 shows composite force-velocity-power relationships of groups of type I, IIa, and IIa/IIx fibers. Fibers used in the force-velocity-power experiments represented a sub-set of the fibers subjected to the slack test procedure (see Table 4 for the number of fibers per mean). As can be observed from Figure 5, the mean peak Ca²⁺-activated force of the pre- and post-training fibers were almost identical to the values obtained during the slack test procedure. In agreement with the slack test results, pre-training V_{max} (determined by extrapolation of the force-velocity relationship) was significantly greater (P < 0.05) in fibers containing type I/IIa (1.65 ± 0.25 FL/s), IIa (1.77 ± 0.09 FL/s), IIa/IIx (1.82 ± 0.09 FL/s), and IIx (2.15 ± 0.38 FL/s) MHC than in fibers containing the type I (0.64 ± 0.02 FL/s) MHC isoform. However, V_{max} did not differentiate between sub-groups of type II fibers or fibers containing multiple MHC isoforms. Resistance training had no significant effect on the V_{max} of
the type I, IIa, or IIa/IIx fibers.

The parameter $a/P_o$ was significantly less ($P < 0.05$) for pre-training type I fibers ($0.033 \pm 0.001$) than pre-training type I/IIa ($0.045 \pm 0.008$), IIa ($0.054 \pm 0.003$), IIa/IIx ($0.084 \pm 0.008$), or IIx ($0.091 \pm 0.015$) fibers, an indication that faster fibers had force-velocity relationships with progressively less curvature. Resistance exercise altered $a/P_o$ for the type IIa fibers only, reducing this parameter by $0.007$ units ($P < 0.05$). Since resistance training had relatively small effects on $a/P_o$, there was no pre- to post-training difference ($P > 0.05$) in the fractional force or velocity that elicited peak power for the type I ($0.149 \pm 0.002$ vs. $0.143 \pm 0.002$), IIa ($0.181 \pm 0.004$ vs. $0.172 \pm 0.002$), or IIa/IIx ($0.208 \pm 0.007$ vs. $0.199 \pm 0.008$) fibers.

Due to their greater $P_o$, $V_{max}$, and $a/P_o$, peak power was 4- to 14-fold greater for type II vs. type I fibers (Table 4). Resistance training increased the absolute peak power of the type I and type IIa fibers by 30 and 42%, respectively. Absolute peak power of the type IIa/IIx fibers rose almost 14% after training, but this change was not statistically significant. Resistance training had no effect on peak power per unit fiber volume for any group of fibers.

**Discussion**

The CSA of vastus lateralis muscle fibers containing type I, IIa, or IIa/IIx MHC increased by an average of 30% after 36 resistance exercise training sessions. These data are consistent with the resistance training-induced increases in slow and fast fiber CSA reported in the histochemical literature (13, 18, 22, 23). Note that direct comparisons between the present data and histochemical results must take into account the 20% swelling in fiber diameter that occurs during the chemically skinning process (10). Once adjusted by a factor 1.44, the absolute CSA’s of the slow and fast fibers reported here fall well within the range of values determined by enzyme histochemistry for sedentary and resistance trained young male subjects (13, 18, 22, 23,
The novel findings of the present study concern the physiological properties of the post-training, hypertrophied muscle fibers. We found that peak Ca\textsuperscript{2+}-activated force rose in direct proportion with muscle fiber hypertrophy since, 1) the mean specific forces of pre- and post-training fibers similar, and 2) the relationships between fiber CSA and peak Ca\textsuperscript{2+}-activated force did not appear to be altered by the training protocol. Thus, the short-term training program used in this investigation had no effect on the average specific force of fibers containing type I, IIa, or IIa/IIx MHC. Since the single fiber segments were maximally activated with Ca\textsuperscript{2+}, their force is proportional to the number of cross-bridges in parallel. Because force increased proportionally with fiber hypertrophy, the most direct conclusion drawn from these results is that cross-bridge density was unaltered by the training protocol. Our physiological results therefore support ultrastructural studies showing that the myofilament density (4) and the percentage of cell volume occupied by myofibrils (2, 21) are not altered in skeletal muscle fibers of young men by progressive resistance exercise training.

We did observe small changes in the average specific force of fibers from some subjects. Importantly, these changes were always in a direction that reduced the overall variability of the population at large. Thus, for previously sedentary male subjects, the specific force of both slow and fast fibers appears to be more tightly regulated after a period of resistance exercise training.

The unloaded shortening velocities of the type I, IIa, and IIa/IIx fibers were not altered by resistance training. These results are similar to those of Harridge et al. (12) who reported no change in fiber shortening velocity after 6 weeks of training involving very brief (3 second), high intensity cycle sprint bouts. In contrast, endurance training has been found to increase the \( V_o \) of type I fibers by 20\%, an adaptation that appears to be related to alterations in fiber myosin light chain isoform content (27, 36). Taken together, fibers containing type I MHC seem to respond
differently to high- vs. low-intensity exercise, but whether this is due to the exercise intensity per se, i.e. the force of the muscular contractions, or to other factors related to the specific exercise protocols is not known.

In the absence of resistance training-induced changes in unloaded or maximal shortening velocity, or in the shape of the force-velocity relationships, the peak power of the type I and IIa fibers increased roughly in proportion to changes in their peak Ca\textsuperscript{2+}-activated force. Normalization of power to fiber volume, which negates the effects of the training-induced change in fiber CSA, confirms this interpretation. The elevated power of the post-training fibers was therefore due to their ability to attain greater force. Thus, fiber hypertrophy was directly and solely responsible for the increased peak power of the post-training slow and fast fibers.

The adaptations to resistance training are complex, involving both neural (5 ) and peripheral mechanisms (16). It is therefore difficult to state with any degree of confidence to what extent the changes in Ca\textsuperscript{2+}-activated muscle fiber function reported here affect neuromuscular performance. A reasonable interpretation of the present data, in regards to its effect on muscular function, is that short-term resistance training alters the potential of muscle fibers to produce torque and power in the direction and magnitude reported here.

Based on this argument, it seems likely that the increased neuromuscular power observed after strength training is due, at least in part, to the greater potential of individual muscle fibers to produce power. The contribution of the type II fibers would be particularly important in this regard since they produce 6-fold greater power than the type I fibers. Like previous studies (1, 38), we found an overall reduction in type IIx MHC isoform content as indicated by, 1) the absence of post-training fibers containing the type IIx MHC exclusively, 2) a reduction in the relative number of fibers containing both type IIx and type IIa MHC, and 3) a corresponding increase in fibers containing type IIa MHC exclusively. A loss in “pure” type IIx fibers would be
expected to reduce overall power, although the magnitude of the reduction is unclear because of the relatively rare occurrence of these fibers. The data in Figure 5 and Table 4 show that the training-induced shift from hybrid IIa/IIx fibers towards IIa fibers may have a minor impact on muscular power potential since the peak power of these groups of fibers are identical ($P = 0.90$). In this case, fiber hypertrophy was sufficient to compensate for a loss in fiber power that would likely have occurred due to a training-induced shift in MHC content towards a slower isoform.

Finally, we studied the responses of relatively young, previously sedentary subjects to training in order to maximize the generalizability of our results. Previous studies examining contractile properties of skinned muscle fibers following short-term resistance training have reached different conclusions regarding the effects of resistance training on muscle fiber function. For instance, one group found that 12 weeks of resistance exercise training increased the specific force of type IIa fibers obtained from female subjects by 5% (35) and elevated the $V_o$ of type I and IIa fibers from male subjects by 75% and 45%, respectively (34). Others have reported reductions in slow fiber specific force with resistance training (26). It is noteworthy that all of these studies were conducted on fibers obtained from subjects averaging 74 yrs of age in one case (34, 35) and 60-70 years old in the other (26). Slow and fast skeletal muscle fibers from elderly subjects have lower specific force and substantially reduced unloaded shortening velocities in comparison to fibers obtained from young to middle-aged subjects (9, 19). Since there was no young or middle aged control group in these previous training studies, it is not clear whether the reported changes in fiber specific force and $V_o$ represent the effect of resistance training per se, an interaction between aging and resistance training, or a generalized effect of increased physical activity on muscle fiber function of the elderly. Along similar lines, hindlimb suspension reduces the specific force of rat type I soleus fibers, but resistance exercise performed during hindlimb suspension is effective in returning this variable to weight bearing levels (37).
Taken together, a reasonable interpretation is that short-term resistance training has no effect on specific force or $V_o$ of slow or fast fibers, unless these functional properties have been altered as a result of other interventions or processes.

**Summary and Conclusions.** Twelve weeks of progressive resistance exercise training, sufficient to increase neuromuscular strength by > 60%, resulted in significant hypertrophy of fibers containing type I, IIA, or IIA/IIX MHC. Peak Ca$^{2+}$-activated force and absolute peak power rose in direct proportion with the increase in fiber CSA while unloaded shortening velocity and power per fiber volume were unaffected by training. Short-term strength training altered the functional properties of slow and fast vastus lateralis muscle fibers obtained from previously sedentary young male subjects in a quantitative manner, i.e. related directly to an increase in the number of cross-bridge, without affecting the density of cross-bridges or their intrinsic contractile properties.
Acknowledgments

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References


Figure Legends

Figure 1. Examples of methodology. A) Superimposed position and force records of two slack tests conducted on a fiber segment. In record “a”, the fiber attained peak force and was subjected to a rapid slack length step. The fiber shortened under no load until it had taken up the slack, at which point there was a redevelopment of force. In contraction “b” the fiber was subjected to a longer slack step resulting in a greater duration of unloaded shortening. Calibration bars represent 200 µm, 0.2 mN, and 100 ms. B) The duration of unloaded shortening from 5 slack steps, including contractions “a” and “b” from panel A, have been plotted against the corresponding slack step length. The slope of the first order least squares regression (R² = 0.992) fit to the data points represents the fibers unloaded shortening velocity (0.47 fiber length/s). C) A series of three isotonic contractions terminated by a slack step. Isotonic force and the corresponding shortening velocity were determined over the last half of each contraction. Calibration bars represent 200 µm, 0.2 mN, and 100 ms. D) Force and velocity data points from 9 contractions were plotted and fit by the Hill equation. Data points obtained from the records in Panel C are indicated by the open symbols. In this example, maximal shortening velocity was 1.70 FL/s, peak force was 0.96 mN or 129 kN/m², and a/P₀ was 0.050.

Figure 2. A portion of a silver stained polyacrylamide gel illustrating the identification of myosin heavy chain (MHC) isoforms in single muscle fiber segments. Lane 2 was loaded with a human myosin standard. Portions of single fiber segments were loaded in the remaining lanes (one segment per lane). Fibers loaded in lanes 1, 3, and 4 contained type IIa, type I, and type IIa/IIx MHC, respectively. Overall, 94% of the pre- and 100% of the post-training fibers were classified as containing either type I, IIa, or IIa/IIx MHC.
**Figure 3.** Training responses of fibers from each of the six subjects. Left, middle, and right panels represent the average fiber CSA, peak Ca^{2+}-activated force, and peak specific force, respectively, of pre- and post-training fibers obtained from each subject. Each subject is represented by a unique symbol. A) Fibers containing type I MHC. B) Fibers containing type IIa MHC.

**Figure 4.** Histograms and scattergrams of fiber cross-sectional area and peak Ca^{2+}-activated force. Pre-training fibers are indicated by the gray bars and symbols. Post-training fibers are indicated by the black bars and symbols. A) Fibers containing type I MHC. The line indicates the relationship between cross-sectional area and peak force that corresponds to a specific force of 117 kN/m^2, the mean specific force of the pooled pre- and post-training type I fibers. B) Fibers containing type IIa MHC. The line indicates the relationship between cross-sectional area and peak force that corresponds to a specific force of 136 kN/m^2, the mean specific force of the pooled pre- and post-training type IIa fibers. C) Fibers containing type IIa/IIx MHC. The line indicates the relationship between cross-sectional area and peak force that corresponds to a specific force of 146 kN/m^2, the mean specific force of the pooled pre- and post-training type IIa/IIx fibers.

**Figure 5.** Force-velocity and force-power relationships of pre- and post-training fibers. Dashed lines indicate pre-training relationships; solid lines represent post-training relationships. A) Force-velocity-power relationships for fibers containing type I MHC. B) Force-velocity-power relationships for fibers containing type IIa MHC. C) Force-velocity-power relationships for fibers containing type IIa/IIx MHC. D) Force-power relationships for fibers containing type I, IIa, or IIa/IIx MHC. The relationship of the pre-training type IIx fibers has been included for
comparison.
Table 1. Cross-sectional area of fibers obtained before and after 12 weeks of resistance training.

<table>
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<tr>
<th>MHC isoform</th>
<th>no. of fibers</th>
<th>fiber CSA, $\mu$m$^2$</th>
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<tr>
<td>I</td>
<td>86 (42%)</td>
<td>69 (42%)</td>
</tr>
<tr>
<td>I/IIa</td>
<td>8 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>IIa</td>
<td>62 (30%)</td>
<td>90 (55%)</td>
</tr>
<tr>
<td>IIa/IIx</td>
<td>45 (22%)</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>IIx</td>
<td>3 (2%)</td>
<td>0</td>
</tr>
</tbody>
</table>

No. of fibers indicates the number of fibers isolated from pre- and post-training muscle biopsies with the corresponding percentage of total pre- or post-training fibers in parentheses. Fiber cross-sectional area values are mean ± SE. An asterisk indicates a significant difference (P < 0.05) between pre- and post-training fibers containing similar MHC isoforms. For the pre-training fibers, any two means with different superscripts are significantly different (P < 0.05).

Abbreviations: CSA, cross-sectional area.
Table 2. Peak Ca\textsuperscript{2+}-activated force of fibers obtained before and after 12 weeks of resistance training.

<table>
<thead>
<tr>
<th>MHC isoform</th>
<th>Fiber force, mN</th>
<th>Fiber force, kN/m\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>I</td>
<td>0.60 ± 0.02 a</td>
<td>0.84 ± 0.03 *</td>
</tr>
<tr>
<td>I/IIa</td>
<td>0.93 ± 0.06 b</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>0.89 ± 0.02 b</td>
<td>1.20 ± 0.03 *</td>
</tr>
<tr>
<td>IIa/IIx</td>
<td>0.95 ± 0.03 b</td>
<td>1.27 ± 0.06 *</td>
</tr>
<tr>
<td>IIx</td>
<td>1.11 ± 0.13 b</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE. Number of fibers per mean same as in Table 1. An asterisk indicates a significant difference (P < 0.05) between pre- and post-training fibers containing similar MHC isoforms. For the pre-training fibers, any two means with no common superscripts are significantly different (P < 0.05).
Table 3. Unloaded shortening velocity of fibers obtained before and after 12 weeks of resistance training.

<table>
<thead>
<tr>
<th>MHC isoform</th>
<th>pre</th>
<th>post</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.58 ± 0.02 a</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>I/Ia</td>
<td>1.61 ± 0.36 b</td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>2.95 ± 0.17 c</td>
<td>2.77 ± 0.11</td>
</tr>
<tr>
<td>Ia/Ix</td>
<td>3.78 ± 0.20 c</td>
<td>3.49 ± 0.60</td>
</tr>
<tr>
<td>Ix</td>
<td>4.72 ± 0.79 d</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE fiber lengths/s. Unloaded shortening velocity was determined by the slack test procedure. Number of fibers per mean same as in Table 1. For the pre-training fibers, any two means with different superscripts are significantly different (P < 0.05).
Table 4. Peak power of fibers obtained before and after 12 weeks of resistance training.

<table>
<thead>
<tr>
<th>MHC isoform</th>
<th>peak power, µN·FL·s⁻¹</th>
<th>peak power, watts·liter⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>I</td>
<td>8.3 ± 0.3 a</td>
<td>10.8 ± 0.4 *</td>
</tr>
<tr>
<td>I/IIa</td>
<td>37.7 ± 4.4 b</td>
<td>6.10 ± 0.89 b</td>
</tr>
<tr>
<td>IIa</td>
<td>49.1 ± 1.7 b</td>
<td>69.9 ± 2.2 *</td>
</tr>
<tr>
<td>IIa/IIx</td>
<td>72.6 ± 4.0 c</td>
<td>82.4 ± 14.3</td>
</tr>
<tr>
<td>IIx</td>
<td>115.2 ± 19.5 d</td>
<td>15.51 ± 2.72 d</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Number of fibers (pre, post): type I (78, 64), type I/IIa (7, 0), type IIa (54, 80), type IIa/IIx (37, 5), type IIx (3, 0). An asterisk indicates a significant difference (P < 0.05) between pre- and post-training fibers containing similar MHC isoforms. For the pre-training fibers, any two means with different superscripts are significantly different (P < 0.05).
Figure 1.
Figure 2
Figure 3.
Figure 4.
Figure 5