

Functional Training: Muscle Structure, Function, and Performance in Older Women

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Specificity of training is a well-recognized principle of exercise physiology. Muscles adapt to the specific training stimulus imposed upon them: swimmers train by swimming, weight lifters train by lifting weights, football players train by running and throwing. These strategies have proven effective for athletes; however, they have not been applied to exercise regimes aimed at improving daily activities in older adults. Traditional exercise programs use endurance training, single joint strength training, or a combination of the two, but often they do not produce an improvement in functional measures (3). This traditional approach to exercise is prescribed for the general public with the intention of improving activities of daily living, yet they do not incorporate these activities into the training. Although resistance training using machines strengthens each muscle in isolation, these programs do not incorporate integrated performance into the training regime. Functional performance requires the integration of multiple joints and several muscle groups. In an activity such as stair climbing, the integrated strength of the quadriceps, hamstrings, hip flexors and extensors, and plantar flexors is involved. Merely strengthening each muscle in isolation does not train the muscles to work in a coordi-

Response to physical training at the cellular and whole muscle level has been established in older adults. However, the underlying molecular mechanism responsible for change has not been described nor have the relationships between change in muscle structure and functional performance been established. The purpose of this research study is to evaluate the changes of muscle ultrastructure, muscle strength, and whole body functional performance as a result of a functionally directed exercise program (stair climbing). Women (65–83 years old) selected either the control (no exercise; N = 6) or exercise (N = 7) group. The 1-year functionally based exercise program was both aerobic (75% heart rate reserve) and resistive (weighted stair climbing). Muscle ultrastructure, determined by quantitative morphometry of the vastus lateralis tissue, and maximal step-height achieved by each subject were related to isokinetic strength and muscle morphology. Changes in myofibrillar area accounted for 48% of the variance in muscle strength changes. Change in muscle contractile protein was the underlying basis for change in thigh strength which, in turn, was the basis for functional performance. These data provide evidence that, in older women, a mild functionally based training program results in improved muscle structure and performance of the lower body.

Key Words: muscle ultrastructure, physical function, elderly

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ated fashion to accomplish a multi-muscle task, such as stair climbing.

Recent research using resistive training in the older adult has been shown to increase muscle fiber area (4–7) and strength (4–7,12). The changes in strength with resistive training have been attributed to both neural adaptation (12) and muscle hypertrophy (4–7). However, the ultrastructural basis for changes in fiber area has not been investigated in the elderly nor has the ability of

these programs to translate into functional performance been reported.

The purpose of this study is to evaluate the ability of exercise training to change physical function and to investigate the underlying muscular changes responsible for change in function. This program used stair climbing with weighted backpacks as a resistive training stimulus. Muscle morphology, strength, and lower extremity functional performance were evaluated. Our evaluation incorpo-

tubing for upper body strengthening (20 minutes combined with stair walking), and 10 minutes of warm-up and stretching. Cardiovascular work was performed at 75% of the subjects' heart rate reserve [(maximum heart rate - resting heart rate) \times 75% + resting heart rate]. The sessions were held three times/week, for 60 minutes/session, for 50 weeks (5).

Muscle Biopsy

The vastus lateralis was chosen because of the high involvement of the knee extensors in both the ascent and descent on stair climbing (11).

Ultrastructural analysis Pretraining and posttraining muscle biopsy tissue from the vastus lateralis was embedded and analyzed for six control and seven exercise subjects. At the time of biopsy, the tissue was divided for light and electron micros-

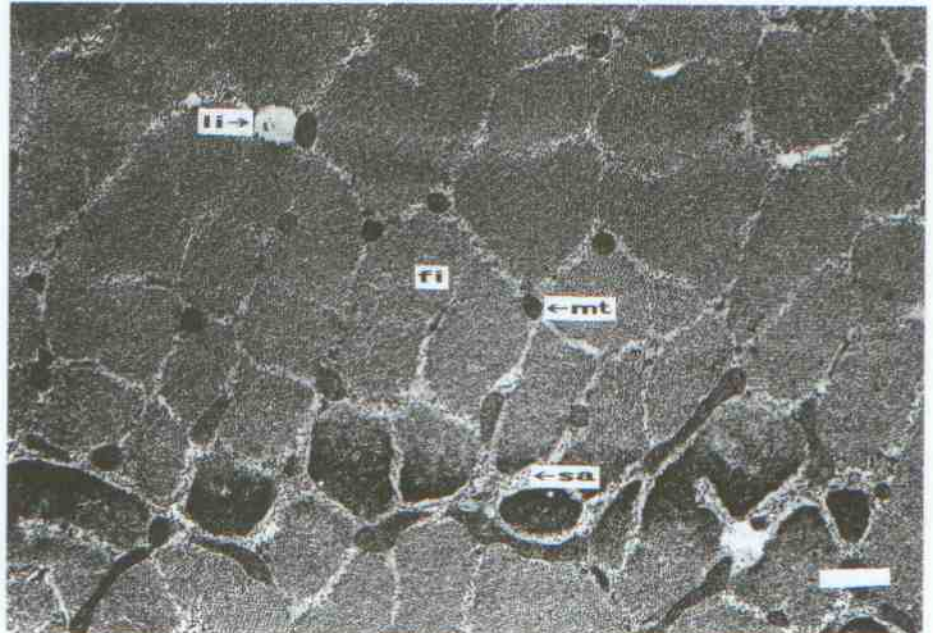


FIGURE 2. Electron photomicrograph of the vastus lateralis muscle biopsy. Magnification = 27,200 \times . li = lipid. mt = mitochondria. sa = sarcoplasm. fi = fiber. bar = 2 μ m.

Pretraining and posttraining muscle biopsy tissue from the vastus lateralis was embedded and analyzed for six control and seven exercise subjects.

copy. The tissue was trimmed of any fat or fascia, and fibers were aligned before being fixed in 4% buffered glutaraldehyde rinsed in a cacodylate-sucrose mixture. The samples were then processed in 1% osmium-tetroxide, dehydrated, and infiltrated with 50% propylene oxide and 50% Epon (A&B) resin. Ultra-thin transverse sections were cut and stained with uranyl acetate and lead acetate (8). Photomicrographs were taken on plate film using a Philips 410 elec-

tron microscope (The Netherlands), and the negatives were analyzed at a final magnification of 27,200 \times . Four fields were randomly selected on each of 10 micrographs to yield 40 fields for analysis of each sample. The following structures were quantified by point-counting using a B36 grid (144 test points): mitochondria, myofiber, sarcoplasm, and lipid (8, 14) (Figure 2). The point-counts yield area ratios (eg., mitochondrial point-count/total point-count) that are estimates of the volume ratios, according to standard stereological methods. Point-count density is expressed as a percent. According to geometric probability theory (14), an absolute area of each component results from the product of these volume densities and the cross-sectional area of the fiber expressed as micrometers squared. A representative photomicrograph is shown in Figure 2.

Histochemical analysis Histochemical and ultrastructure analyses were done from the same muscle biopsy tissue sample. Muscle tissue was obtained from the midsection of the vastus lateralis. Muscle fibers were aligned and quick-frozen in liquid

nitrogen; sections were cut on a cryostat microtome; ATPase reaction at pH 9.4 after preincubation at pH 4.3, 4.6, and 10.3 was used to determine fiber type (2); and the cross-sectional area was determined by planimetry. At least 100 fibers were analyzed on each sample, and the investigator was blinded to the sample identification. Further details of the histochemical preparation are reported in Cress et al (5).

Isokinetic Strength

Pretraining and posttraining isokinetic strength measures are reported as isokinetic work at 180 $^{\circ}$ /sec (work180). Isokinetic work is the integrated area under the peak torque curve. As isokinetic measures evaluate individual muscle groups, we use principal components to statistically integrate these measures into a single summary score (1). A dynamic strength index was determined through principal components statistical analyses (1) of all isokinetic data (peak torque and work for knee extension/flexion at 60, 180, 240, and 300 $^{\circ}$ /sec). This method of weighted

rates muscle fiber structure of the vastus lateralis and thigh muscle strength in septuagenarian women (5). We have examined the ultrastructural components of the muscle fiber and maximal achieved step height, a functional measure of

The ultrastructural basis for changes in fiber area has not been investigated in the elderly.

whole body performance. These measures are added to expand our understanding of the relationship between changes in local muscle morphology and strength and how that relationship can influence whole body performance. Figure 1 illustrates the study design for analysis of the relationships from ultrastructural morphology to whole body performance. We first characterize the ul-

trastructural changes underlying the gross fiber cross-sectional area increases found in subjects in the exercise group. Next, we evaluate the relationship between the myofibrillar area and the isokinetic strength of thigh extension and an integrated measure of thigh strength. Finally, we determine the relationship between maximal thigh strength and maximal achieved step height.

We address three questions:

- 1) what is the ultrastructural basis for muscle fiber changes occurring with training in the elderly?
- 2) what is the relationship between changes in morphology (ultrastructure and fiber area) and local muscle strength?
- 3) what is the relationship between local muscle properties and whole body performance?

METHODS

Subjects

Healthy, older women, from 65 to 83 years of age, with no known cardiovascular, neuromuscular, or metabolic disease volunteered as part

of a larger study (5). All procedures were approved by the Medical School Human Subjects Research Committee at the University of Wisconsin-Madison, Madison, WI, and all subjects signed an informed consent form. As electron microscopy processing is expensive and labor-intensive, we analyzed a subset of the original group. For electron microscopy, all control paired (preintervention and postintervention) samples with good tissue samples were processed ($N = 6$). To evaluate the hypothesis, change in ultrastructure as a result of the training program, we made an *a priori* decision to process samples from seven exercise subjects that demonstrated the greatest changes in strength. Demographic data and selected subject characteristics are shown in Table 1.

Exercise Training

The program combined aerobic and resistive training. Weighted stair climbing provided resistance to the legs as subjects carried 10% of their body weight in a backpack. Stair climbing (ascent and descent) was chosen because it is a functional exercise that recruits the vastus lateralis in eccentric and concentric movement in the last 30° of extension (11). Each flight of stairs consisted of 24 steps, with risers 15 cm high. Each subject ascended and descended the stairs eight times per session. In addition to stair climbing, each session included endurance dance (30 minutes), resistive exercises using elastic

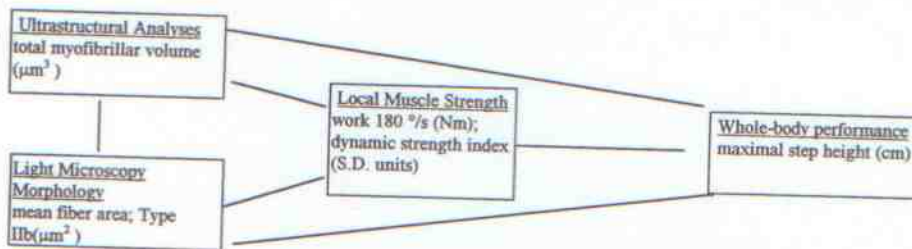


FIGURE 1. Study design.

	Control				Exercise			
	Preintervention N = 6		Postintervention N = 6		Preintervention N = 7		Postintervention N = 7	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
Age (years)	73.3	7.0	—	—	70.1	4.3	—	—
Weight (kg)	62.3	8.2	60.3	6.4	66.0	10.7	64.8	9.7
Height (cm)	156.8	3.2	—	—	160.2	5.8	—	—
Mean fiber area (μm^2)	338.1	88.2	299.4	87.6	276.7	74.0†	308.7	46.8
Work 180* (Nm)	43.7	7.4	38.7	5.72	38.9	10.4†	46.7	10.3

* Isokinetic work at 180°/sec.

† Significantly different from control ($p < .05$).

TABLE 1. Preintervention and postintervention subject characteristics for the electron microscopy subgroup.

	Control						Exercise					
	Preintervention N = 6		Postintervention N = 6		Change		Preintervention N = 7		Postintervention N = 7		Change	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
Mitochondria (%)	2.7	0.7	3.4	0.8	0.7	0.5	3.4	0.9	3.0	0.8	-0.39	1.3
Myofibril (%)	74.1	3.6	72.1	4.2	-2.0	4.6	72.0	2.9	76.3	4.3	4.2	4.5*
Sarcoplasm (%)	22.7	4.1	24.0	4.3	1.3	4.3	23.9	2.7	19.0	4.7	-3.93	4.2
Lipid (%)	0.4	0.2	0.4	0.2	0		0.6	0.4	0.6	0.4	0	

* Significantly different from control ($p < .05$).

TABLE 2. Ultrastructural volume density measurements for control and exercise groups preintervention and postintervention.

averaging is an integrated measure of thigh strength reported in standard deviation units. The summary score known here as the dynamic strength index integrates all isokinetic measures rather than having to select one dependent variable (ie., knee extensor strength) to represent thigh strength. The dynamic strength index more nearly approximates functional leg capacity than any one measure alone. The statistical procedure is detailed in Cress et al (5).

Stair Performance

Stair ascent was used to evaluate functional performance because the knee extensor muscles play a dominant role in stair climbing (11). Following the training period, maximal attainable riser height was determined by using eight stair sets, ranging in height from 22 to 74 cm (riser increments of 7.5 cm). Each stair set had two steps with black sides and a top and a 2-cm wide white strip marking the bottom and front edge of each step. All subjects participated in the measurement of their stair performance. The stair sets were placed in a semicircle, with step-heights in random order. Standing alone in the center of the semicircle, the subject identified and attempted the staircase with the greatest riser height she thought she could climb without outside support or use of the hands. If the subject failed in the first attempt, she was directed to the next lower staircase to make a second attempt. This procedure was repeated until

the subject met with success. If the subject was able to climb the first staircase easily, she was led to the next higher case until she was unable to climb the stair in a bipedal fashion without outside aid. Of the eight stairs available to the subjects, only three heights (22.86, 35.56, and 46.99 cm) in the midrange of those available were achieved by the subjects. The details of the procedure are described in Konczak et al (9).

Statistical Analysis

The *a priori* selected variables were the only variables analyzed. Statistical procedures were performed using SPSS version 6.0 (SPSS, Inc., Chicago, IL). Prechanges and postchanges within each group (control and exercise) were compared using a paired *t* test. Pearson correlation was used to establish relationships on all dependent and independent variables. Means \pm standard deviations are reported unless otherwise noted. One-way analysis of variance (ANOVA) was used to determine significant differences between groups and for the mean strength and morphology variables at each stair height. The η^2 from the ANOVA procedure was used to show the strength of the association between stair height and strength (work180, dynamic strength index) and morphology (myofibrillar area). η^2 provides information regarding unique variance between interval (strength) and ordinal (stair height) scales. It is used here to provide in-

formation commonly reported as R^2 when relating two interval scales. A probability of $p < 0.05$ was chosen for statistical significance. Effect size, a procedure used in meta-analysis studies, was determined by subtracting the change in the control group from that of the exercise group and dividing by the standard deviation of the change (effect size = Δ exercise - Δ control/standard deviation of change) (10). Effect size is a useful method of expressing the net value of an intervention, taking into account not only changes from baseline in the exercise group but also prevention of loss in the control group over the same time period.

RESULTS

Subject Characteristics

Selected physiological characteristics of the subjects are listed in Table 1. For these same characteristics, the subset of subjects reported here did not differ statistically from the larger group from which they were selected in either baseline or posttraining measures (5). However, at baseline, the exercise group subset had significantly ($p < .05$) lower mean fiber area and strength than the control group. Program compliance was 86%, with subjects attending 89% of all sessions.

Ultrastructure Volume Density and Area

The preintervention to postintervention volume densities for muscle

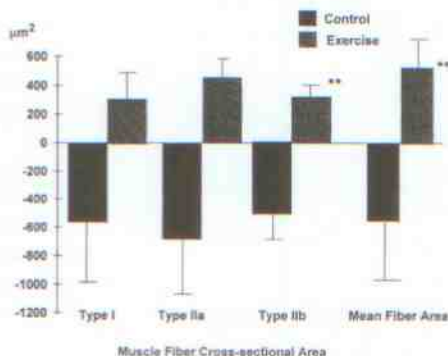


FIGURE 3. Change from baseline to posttraining in mean muscle fiber cross-sectional area by light microscopy in micrometers squared (** = $p < .05$).

organelles in both the control and exercise groups are listed in Table 2. No intergroup differences were detected in either preintervention or postintervention samples. However, change in myofibrillar volume density for the exercise group was significantly ($p < .05$) larger than that of the control group.

The mean fiber area was calculated from the mean cross-sectional areas of Type I, Type IIa, and Type IIb. The area was calculated in the following manner: (Type I area \times Type I N) + (Type IIa area \times Type IIa N) + (Type IIb area \times Type IIb N)/total N of fibers = mean fiber area. The mean \pm SEM change in the mean fiber area from baseline is shown in Figure 3. Geometric probability theory allows one to estimate volume density based on the ratio of areas of the structure of interest and the surrounding structures (14). We determine the area of the subcellular components of interest by multiplying the volume density of the component by the subject's mean fiber area determined from light microscopy (eg., myofiber % \times mean fiber area = myofibrillar area). The change in area (μm^2) for each ultrastructural category is shown in Figure 4. A significant change ($p < .05$) in the myofibrillar area was seen in the exercise group with training (exercise = $411.11 \pm 160 \mu\text{m}^2$; control = $-426 \pm 312 \mu\text{m}^2$; $p = .03$), but no significant change following the training

program was seen in any other organelle. In the exercise group, the decrease in mitochondrial volume density ($\Delta 0.39\%$; Table 2) was compensated for by an increase in mean fiber area (average of all fiber types) ($\Delta 404 \mu\text{m}^2$), so that no net change in the total mitochondrial content of the muscles occurred with this training program (Figure 4). Both the myofibrillar density ($\Delta 4.2\%$) and myofibrillar area ($\Delta 411 \mu\text{m}^2$) increased with training in the exercise group. Accounting for a large fraction of the variance (38%) in the change in the myofibrillar area was the change in Type IIb fiber area, which agrees well with the 42% contribution of the change in Type IIb fibers to the change in mean fiber area following training (5). The effect size of 1.196 for the exercise intervention indicates that the intervention increased the mean fiber area greater than 1 standard deviation over that of the controls. For instance, the effect size for myofibrillar area was 3.5, indicating that the intervention increased the contractile protein content 3.5 standard deviations over that of the control subjects.

Strength

Significant and opposite directional changes from baseline were seen between the exercise and control groups for isokinetic work at $180^\circ/\text{sec}$ for extension (work180). In

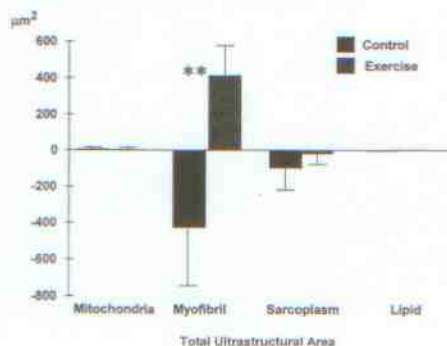


FIGURE 4. Change from baseline to posttraining in ultrastructural area in micrometers squared (** = $p < .05$).

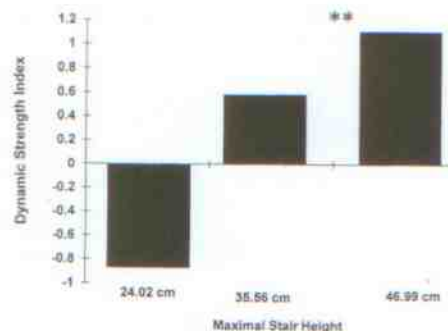


FIGURE 5. Maximal stair height and dynamic strength index (** = $p < .05$).

the control group, baseline work was $38.8 \pm 3.2 \text{ Nm}$, which decreased by $5.0 \pm 1.4 \text{ Nm}$. In the exercise group, baseline was $43.7 \pm 2.0 \text{ Nm}$, which increased by $7.8 \pm 1.5 \text{ Nm}$ ($p < .05$). The effect size was 6.3, indicating that the positive change in the exercise group (7.8) was greater than 6 standard deviations above the loss in the control group (-5.0) over the year of the study. For both groups, the change in Type IIb fiber area (over 1 year) was significantly ($p < 0.05$) correlated with the change in work180 ($r = 0.42$) and with the change in the integrated thigh strength ($r = 0.55$). No significant correlation was found at baseline between myofibrillar area and the work ($r = 0.346$; $p = 0.08$) or dynamic strength index ($r = 0.330$; $p = 0.09$) strength measures. However, change in the myofibrillar area (pretraining to posttraining period) was significantly associated with strength (work180, $r = 0.69$; dynamic strength index, $r = 0.59$). Even though the baseline values for the fiber cross-sectional area and strength (work180) were significantly different, the focus of this paper is the relationships and the magnitude of the changes in these muscle characteristics.

Stair Performance

The riser height that each subject could climb was determined after the training period. Of the eight possible risers from which the subjects could choose, all subjects' maximal achieved heights were one of three

riser heights (22.86, 35.56, and 46.99 cm). The muscle fiber properties, leg strength, and thigh strength at the highest achieved riser height were compared by analysis of variance. Work180 was significantly ($F = 10.23$; $p = .003$) different for the lowest to the highest riser height (28.0 ± 1.0 , 43.6 ± 4.9 ; 47.0 ± 9.6 , respectively), with strength accounting for 65% ($\eta^2 = .6509$) of the performance. This same pattern was seen in the dynamic strength index and is illustrated in Figure 5, with strength accounting for 60% ($\eta^2 = .5966$) of performance ability. The underlying structural properties and myofibrillar area (μm^2) were also significantly different among the achieved riser heights ($F = 13.18$; $p = .015$), indicating that 73% ($\eta^2 = .7357$) of the variance in stair performance is due to underlying contractile protein.

DISCUSSION

This functionally based training program, including weighted stair climbing, produced significant effects in the muscle fiber cross-sectional area and strength measures. We show that the increase in myofibrillar area

The increase in myofibrillar area was the underlying basis for the change in fiber cross-sectional area of the vastus lateralis.

was the underlying basis for the change in fiber cross-sectional area of the vastus lateralis. In addition, we find that these fiber and strength properties explain more than 60% of the maximal step height performance (Figure 6).

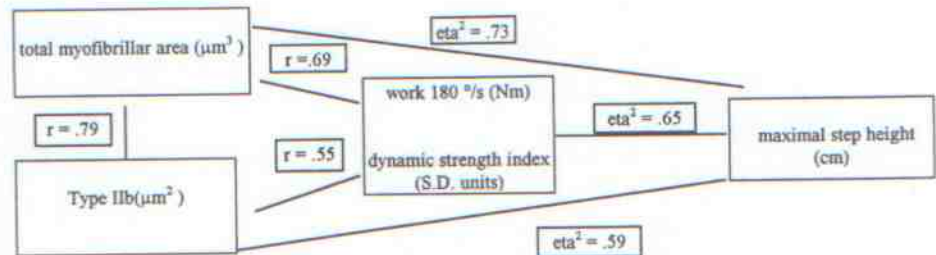


FIGURE 6. Study outcomes.

Ultrastructural Changes

What were the subcellular morphological changes that accompanied training, and how did these changes relate to the fiber cross-sectional area increases in these septuagenarian women? At the beginning of training, these women had mitochondrial volume densities in their vastus lateralis similar to those reported for younger females (8). The pretraining mitochondrial volume densities averaged $3.1 \pm 0.8\%$ for the combined groups, which is similar to the value ($3.9 \pm 0.4\%$) for 31-year-old women but smaller than the value ($4.7 \pm 0.3\%$) reported for 28-year-old men (8).

Over the 1-year program, Figure 4 illustrates that the ultrastructural change was principally due to change in myofibrillar area, with all other components remaining unchanged. These data support the finding of Wang et al (13) that the increase in myofibrillar volume with resistive training has the effect of "diluting the organelles" of the muscle cell in young subjects. Forty-two percent of the changes in fiber cross-sectional area were due to alterations in the Type IIb area (Figure 3). The change in Type IIb fiber area accounted for 62% ($r = .79$) of the variance in myofibrillar area (Figure 6).

Many studies of training programs for elderly subjects are only 8–12 weeks in length (6,7), and early changes in strength have been attributed primarily to neuronal changes (12). These data show that the strength training effects in the elderly also reflect underlying myofiber adaptation during this year-long pro-

gram in the elderly. The exercise program had a positive effect on both morphology (effect size = 3.5) and strength (effect size = 6.3), with the exercise group showing a marked improvement over the loss in the control group.

Muscle Structure and Strength

How are structural changes in the vastus lateralis related to parallel changes in isokinetic strength? We report knee extension strength because of the important contribution of the knee extensors in the ascent and descent of stair climbing (11). We collapsed these highly related variables (knee extension/flexion, peak torque, and work at four angular velocities) into one number, the dynamic strength index, using principal components, a method of weighted averaging (1). We thus gain an integrated measure of thigh strength, rather than relying on one isolated muscle measure to represent lower body strength. Change in contractile protein measured as myofibrillar area explained 48% ($r = .69$) of the isokinetic strength (work180) and 35% ($r = .59$) of integrated thigh strength (dynamic strength index). These findings indicate that changes in the representative fibers from a single muscle (vastus lateralis) relate most closely to the strength for the quadriceps group. However, these changes also reflect the functional changes of the thigh as a whole. By using a summary measure, we can reduce the number of comparisons that need to be made with-

out losing statistical information. The dynamic strength index and mean fiber area are summary measures of strength and morphology, respectively. The effect of the intervention on integrated thigh strength (dynamic strength index), 1.3, was of the same magnitude as the effect size on the cross-sectional area (mean fiber area, 1.2). The direction and magnitude of the changes in muscle structure and function are the primary focus of this paper. These relationships hold in spite of the fact that the baseline absolute values for the mean fiber area and work180 for the two groups were significantly different from each other (Table 1).

One of the benefits of a functionally based exercise program is to strengthen several if not all the muscles around a joint. Muscles around the same joint are all highly correlated in strength, eg., quadriceps to hamstrings strength. We used the dynamic strength index to relate thigh strength to functional performance. Our measure of integrated functional performance was maximal achieved step-height. We used maximal step-height as a functional outcome because it is similar to the essential daily function of elevating the total body weight from the floor with one leg. As indicated in Figure 6, the change in muscle contractile protein structure (myofibrillar area) was associated with the change in the fiber area (Type IIb area), which, in turn, was reflected in the change in strength (work180, dynamic strength index). The relationships of the myofibrillar area and the dynamic strength index to maximal achieved step-height were similar. Greater than 60% of the maximal step-height climbing ability is explained by thigh strength, which agrees well with 73% explained by underlying muscle structure. These data show that the effects

of structural and strength training translate to functional ability.

CONCLUSIONS

This functionally based training program resulted in muscle fiber and isokinetic performance adaptations, indicating that elderly muscle shows similar plasticity to functionally oriented tasks as it does to more specialized regimes. The adaptations of the vastus lateralis as indicated on the ultrastructural and fiber level were reflected in increased strength, which, in turn, was translated into improved whole body performance. Clearly, the specificity of the training response can be exploited at the functional level by using activities that integrate a number of muscle groups in the exercise regime. These structural and strength changes can translate in performance in activities, such as stair climbing, that are important for independent living.

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