Pflügers Arch (1993) 424:494–502

Pflügers Archiv
European Journal of Physiology

Muscle fiber types of women after resistance training – quantitative ultrastructure and enzyme activity

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Received September 15, 1992/Received after revision January 6, 1993/Accepted February 23, 1993

Abstract. Muscle biopsies of the vastus lateralis muscle taken before and after 18 weeks of resistance training were compared by preparing frozen cross sections for electron microscopy and using adjacent sections for fiber typing by myosin ATPase activity. Quantitative ultrastructural changes were observed in histochemically-identified muscle fiber types of twelve young women who underwent the training. The percentage of type IIB fibers decreased and IIA fibers increased. The cross-sectional area of all major fiber types increased with training. The absolute volume of myofibrils, intermyofibrillar space, and mitochondria increased with training for most major fiber types (type I, IIA and IIAB), but the relative volume percentages were not significantly changed because of corresponding fiber hypertrophy. Mean mitochondrial size for types I and IIA and myofibril size for types IIC and IIB increased significantly with training. The capillary number per fiber and density did not change with training. Activity levels were measured for selected glycolytic and oxidative enzymes. Cytochrome oxidase and hexokinase increased significantly with training, while creatine kinase, citrate synthase, phosphofructokinase, glyceraldehyde phosphate dehydrogenase and hydroxyacyl CoA dehydrogenase enzymes were not significantly altered. The results suggest that this type of high-repetition resistance training causes the intracellular components of all fiber types to increase proportionally with an increase in fiber size. In addition, the enzyme analysis indicates the muscle as a whole may increase its oxidative phosphorylation capacity in conjunction with the decreased percentage of type IIB fibers.

Key words: Skeletal muscle hypertrophy – Muscle stereology – Exercise adaptations

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Introduction

The plasticity of skeletal muscle is reflected in its ability to adapt to altered metabolic and functional demands. Such demands may be induced by resistance or endurance training programs. Resistance training consists of a few contractions at maximal capacity, while endurance training consists of many submaximal contractions. Generally, endurance training is associated with an increase in the oxidative capacity of muscle, suggested by increases in (a) the proportion of oxidative fibers [27], (b) the activity levels of oxidative enzymes, and capillary density [26], and (c) the volume percentage of mitochondria [10]. In contrast, resistance training results in an increase in muscle strength accompanied by an increase in neural adaptation (such as co-contraction of synergists or increased number of motor units activated [6]) and muscle size [35]. This increased muscular size is due to muscle fiber hypertrophy [8], which is far greater [36] than any hypertrophy caused by endurance training [2]. Although hypertrophy occurs in all fiber types, that of type II fibers is most pronounced [8, 35].

Resistance training does not cause the conversion of type I slow to type II fast fiber however, a significant decrease in percentage of type IIB and a concomitant increase in type IIA fibers in women lifters trained for both 6 and 20 weeks suggest a conversion from type IIB to IIA [35, 36]. Women appear to have the same physiological ability as males to tolerate and adapt to heavy resistance training ([5], R. S. Staron, unpublished observations). However, there have been relatively few studies concerned with the effects of heavy resistance training on the muscle fiber structure in women.

Generally, women have a smaller muscle mass than men due to the smaller cross-sectional areas of all fiber types and apparently fewer fibers in the muscle [18]. Sexual differences also appear to exist in the hierarchy of fiber type sizes. In females the fiber sizes occur in the order: I > IIA > IIB, whereas in males the order is: IIA > IIB > I [29, 34, 35].

We have characterized the histochemical and physiological characteristics of women engaged in this high resistance training study, and have found an apparent fiber type conversion of the type II subtypes [36]. In addition, a significant hypertrophy of the muscle fiber types was observed. A number of questions arose from these observations. Do the mitochondrial and lipid volume densities become altered with training? Are there fiber type-specific changes in the distribution of these organelles? Since there is evidence for a IIB to IIA transformation, and these subtypes tend to differ in their oxidative capacities, do the trained IIA fibers (including former IIB) have a different mitochondrial density from the trained IIB?

The basis for the first question is that if a fiber enlarges without concomitant increase in its organelle contents, a "dilution" or decrease in organelle content would be manifested. If, in contrast, organelle distribution increases proportionally to fiber size increase, the content would be similar to the control. We can answer the remaining questions by using the cryostat-retrieval method [34] that we have used previously. With this method, we are able to examine the ultrastructure of muscle fibers that have been identified histochemically using the myofibrillar adenosine triphosphatase (ATPase) reaction to distinguish muscle fiber types.

The quantitative ultrastructural changes in endurance-trained human muscle have been well characterized [10], but reports on resistance-trained muscles are scare, and most have been made on men. The present investigation was therefore undertaken to examine the effects of heavy resistance training on the ultrastructure of histochemically identified skeletal muscle fibers in women.

Materials and methods

The original study, which was approved by the Ohio University Institutional Review Board, involved 24 untrained college-aged female subjects. The details of the training, subjects, biopsy procedures, and histochemical analysis have been presented elsewhere [35]. After all subjects had been informed of the procedures, risks, and benefits, each subject gave written consent of participation. Samples from 12 of the original 24 subjects were chosen because they showed the least artifactual freeze damage.

The 20-week training consisted of two phases: 2 weeks of orientation and preconditioning, and 18 weeks of heavy resistance training. The training period was divided into two phases of eight and ten weeks, with one week of rest between, when the students were on vacation. The resistance training involved the lower extremity, including full squats, leg presses, leg extensions, and leg curls. The resistance used on each subject was based on her 1 RM (repetition maximum) [20], which is equal to the maximal amount of weight the subject can lift for one repetition.

Subjects trained twice weekly at a high intensity for 18 weeks. Each workout consisted of two warm-up sets (ten repetitions per set using approximately 40% and 60% of the 1 RM value) and three sets of 6–8 RM for each exercise. The 6–8 RM is equal to the maximal amount of weight the subject can lift successfully for six to eight repetitions, and is approximately 80–85% of the 1 RM value. The weights were regularly adjusted to the increased performance capacity of each subject to maintain her 6–8 RM.

Biopsies (80–160 mg each) of the right vastus lateralis muscle were taken by the percutaneous needle biopsy method [3]. The first was taken prior to training and the second at the conclusion of the study using the original pre-training biopsy scar to establish proximity to the first biopsy. The samples were taken from similar areas to help prevent variability due to regional differences in fiber type distributions and possible differences between right and left limbs [14].

The samples were frozen in isopentane cooled with liquid nitrogen, then stored at −70° C. A cryostat-retrieval technique [9, 34], was used to study the ultrastructure of histochemically-identified muscle fiber types. The samples were thawed to −20° C, and serial thin (12 μm) sections and 40–50-μm-thick sections were cut with a cryostat. Thin sections were processed for histochemistry [35] and thick sections for electron microscopy.

To minimize thawing artifacts the sections for electron microscopy were treated with a fume fixation method [23] for 1 h in the cryostat. After further fixing in cold Karnovsky's fixative for 4 h, rinsing in sucrose and postfixing in 1% osmium tetroxide, all in 0.1 M cacodylate buffer, the sections were dehydrated and flat embedded in an Epon/Araldite mixture. Two to three regions free of damage from ice crystals and consisting of more than 50 fibers were matched with the adjacent histochemical preparations for myofibrillar ATPase activity, then thin-sectioned for electron microscopy as previously described [9]. The ATPase histochemical procedure was modified from Brooke and Kaiser [4] as described elsewhere [28]. The sections were preincubated in solutions at pH 4.3, 4.6, or 10.2 prior to the ATP incubation to differentiate fiber types [36]. Fiber type percentages of each muscle sample were determined by histochemical analysis [36].

Low-power electron micrographs were used to match fibers, measure fiber sizes, and analyze capillaries. The location of capillaries was verified with adjacent toluidine blue-stained, semi-thin sections with a light microscope. Capillaries were counted [2], with the capillary density (capillaries/mm^2) and capillaries per fiber ratio calculated for each section's area and for each fiber type. Between 5 and 18 fibers of type I, IIA, and IIB in each section were used for capillary investigation. Three to six fibers of each major type (I, IIA, IIB) in each section were chosen for the morphometric analysis of mitochondria, lipid droplets, myofibrils, and intermyofibrillar space, and subtypes (IC, IIC, IIAB) selected as available.

The Bioquant System IV measuring program (R & M Biometrics, Nashville, Tenn., USA) was used to measure cross-sectional area of the fibers and each subcellular component. The number of myofibrils and mitochondria in two electron micrographs of each fiber was counted and presented as number per unit area. The volume percentage of mitochondria, lipid droplets, myofibrils, and intermyofibrillar space for each fiber type in each section was calculated as the total area occupied by each component per area measured. The area ratio was used to estimate the volume ratio according to geometric probability theory [39]. The absolute volume of each subcellular component was obtained by multiplying the volume percentage of that component with the cross-sectional area of the fiber.

For enzyme activity, whole muscle homogenates were used for measurements of cytochrome c oxidase (EC 1.9.3.1, COX), citrate synthase (EC 4.1.3.7, CS), creatine kinase (EC 2.7.3.2), hexokinase (EC 2.7.1.1, HK), phosphofructokinase (EC 2.7.1.11), glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12), and B-hydroxyacyl CoA dehydrogenase (EC 1.1.1.35, HADH) enzyme activities (U/g muscle) at 30° C by spectrophotometry techniques [22].

All measurements were subjected to statistical analyses. Samples from each individual were compared pre- and post-training. The fiber type composition and diameter, and the absolute volume and volume percentage (log transformed) of each component for each fiber type were analyzed. One-way analysis of variance (ANOVA) was used to test the differences between individuals and between fiber types for volume percentages of each variable from control and trained samples. Differences between these were tested by the use of a two-way ANOVA. Significant differences between the means were further analyzed using Tukey's post-hoc analysis. Correlation analysis was used to test the re-

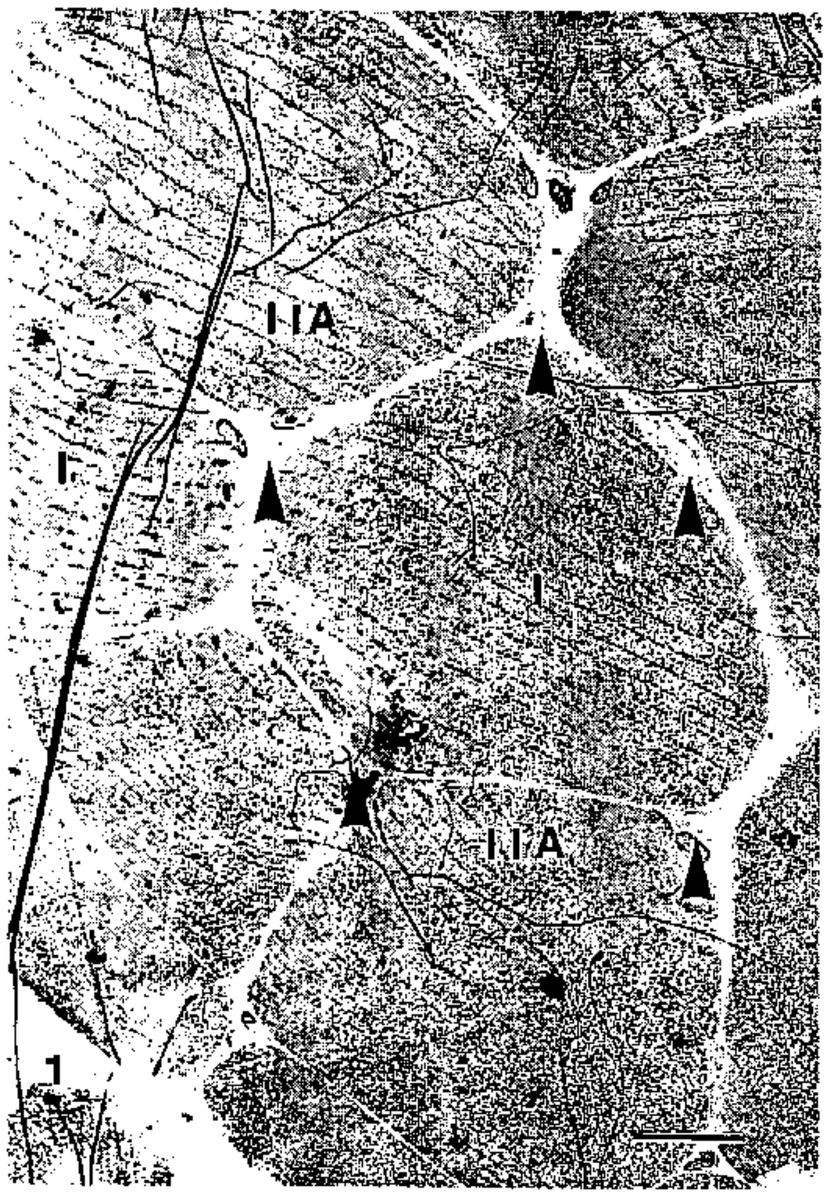

Fig. 1. Survey electron micrograph showing well-preserved areas. By matching this section with adjacent histochemical myosin adenosine triphosphatase preparations, the fiber types were identified as indicated. Capillaries (*arrowheads*) were verified by electron microscopy. ×525. *Scale bar* = 20 μm

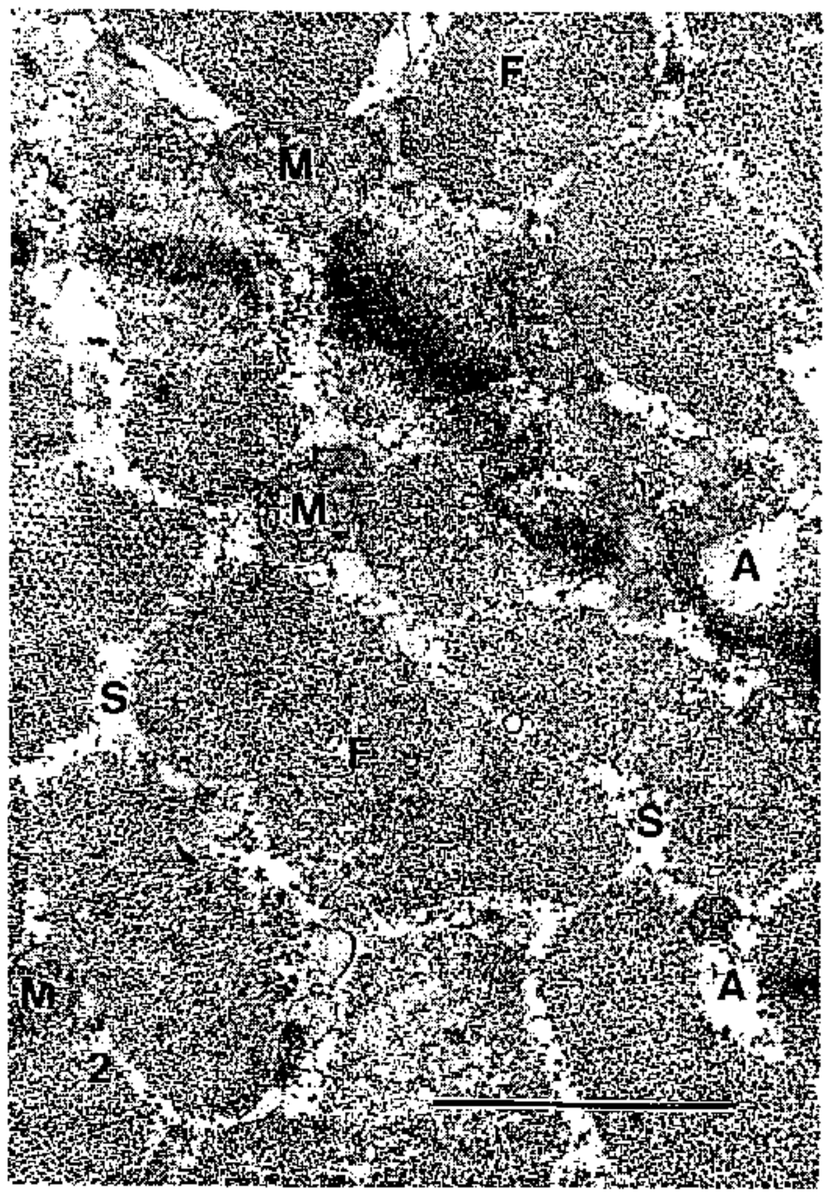

Fig. 2. A type I fiber from a subject before training. Mitochondria (*M*), artifacts (*A*), myofibrils (*F*), and interfibrillar spaces (*S*) are indicated. ×28,000. *Scale bar* = 1 μm

lationship between variables. Significance was accepted at the confidence level of 95% ($P < 0.05$), although significance at $P < 0.01$ was also noted.

Results

Artifacts

Freeze damage, created by freeze-thawing during preparation, is a major artifact that affects morphological analysis in this type of study. Although the least-damaged fibers in well-preserved regions were selected (Fig. 1), there was still evidence of minor damage. These were seen as empty irregular spaces, about 0.2 μm² in size and occupying about 4% of the total fiber volume (Fig. 2). The volume percentage of artifacts varied between individuals, but pre- and post-training values were similar so the results were not affected.

Fiber type composition and size

The samples used in this study were a subset of the biopsies reported in a published histochemical study [35]. The cross-sectional areas (Table 1) were smaller than those reported in the original study. This was most likely due to the shrinkage brought on by the preparation procedures for electron microscopy compared to histochemistry.

The hierarchy of sizes of fiber types before training was I > IIA > IIAB = IIB, but the differences were not significant here, although they were significantly different in the histochemical study. Resistance training for 20 weeks significantly increased the fiber sizes of I, IIA, IIAB, and IIB fibers (Table 1). The fiber sizes of type IIA and IIAB increased more than that of the type I and IIB, as shown by 2-way ANOVA, and altered the hierarchy of fiber sizes to I = IIA > IIAB > IIB.

Table 1. Fiber cross-sectional area

Fiber type	Control (μm²)	Percentage	Trained (μm²)	Percentage
I	3293 ± 135 (54)	48.00	3942 ± 164 (50)*	31.25
IC	3317 ± 572 (5)	2.94	5091 ± 518 (5)	3.13
IIC	2746 ± 496 (4)	2.35	4050 ± 366 (10)	6.25
IIA	3079 ± 134 (55)	32.35	3972 ± 159 (53)*	33.13
IIAB	2880 ± 240 (17)	10.00	3721 ± 223 (27)*	16.88
IIB	2833 ± 168 (35)	20.59	3453 ± 366 (15)*	9.38*

Values are means ± SE (n); * control significantly different from trained ($P < 0.05$

Table 2A. Absolute volume and volume percentages of subcellular components

Fiber type	Absolute volume		Volume percentage	
	Control	Trained	Control	Trained
Myofibrils				
I	2688 ± 125	3222 ± 148*[b]	81.89 ± 0.56	81.99 ± 0.66
IC	2849 ± 261	3756 ± 499	84.89 ± 2.49	80.55 ± 1.82
IIC	3204 ± 323	3347 ± 400	83.39 ± 2.49	81.87 ± 1.41
IIA	2597 ± 120	3271 ± 125*[b]	83.47 ± 0.71	83.54 ± 0.62
IIAB	2381 ± 190	3213 ± 163*[b]	83.84 ± 1.15	83.77 ± 1.02
IIB	2405 ± 133	2554 ± 169	84.74 ± 0.87	83.12 ± 1.28
Interfibrillar space				
I	305.6 ± 17.5	386.2 ± 34.3*[b]	9.84 ± 0.62	9.69 ± 0.60
IC	296.1 ± 86.5	671.4 ± 199.0*[b]	9.18 ± 2.31	12.58 ± 1.70
IIC	363.7 ± 66.4	437.9 ± 95.5	10.06 ± 0.65	10.46 ± 1.25
IIA	305.2 ± 25.2	392.6 ± 31.9*[b]	10.04 ± 0.86	9.95 ± 0.59
IIAB	283.8 ± 37.7	392.0 ± 50.7*[b]	9.79 ± 1.06	10.01 ± 0.95
IIB	279.0 ± 15.1	352.8 ± 42.9	9.47 ± 0.79	11.11 ± 1.15
Lipid droplets	*[a]			
I	22.80 ± 2.75	26.16 ± 2.39	0.70 ± 0.06	0.63 ± 0.06
IC	24.80 ± 8.88	30.99 ± 6.24	0.74 ± 0.22	0.63 ± 0.15
IIC	27.11 ± 6.74	22.75 ± 4.61	0.75 ± 0.22	0.54 ± 0.12
IIA	19.29 ± 2.38	24.87 ± 2.21*[b]	0.58 ± 0.06	0.65 ± 0.05
IIAB	14.53 ± 2.76	22.00 ± 2.72*[b]	0.49 ± 0.10	0.58 ± 0.09
IIB	12.81 ± 1.62	16.48 ± 2.67	0.44 ± 0.08	0.54 ± 0.11
Mitochondria	*[a]	*[a]	*[a]	*[a]
I	249.5 ± 14.9	295.4 ± 13.0*[b]	7.77 ± 0.27	7.67 ± 0.22
IC	175.6 ± 27.9	280.7 ± 34.8*[b]	5.22 ± 1.01	6.27 ± 0.60
IIC	211.3 ± 25.5	284.0 ± 34.4	5.79 ± 1.01	7.12 ± 0.47
IIA	184.1 ± 10.6	229.5 ± 13.1*[b]	5.89 ± 0.29	5.79 ± 0.21
IIAB	153.7 ± 11.7	220.0 ± 12.7*[b]	5.62 ± 0.46	5.75 ± 0.34
IIB	153.0 ± 13.0	162.9 ± 15.3	5.35 ± 0.35	5.23 ± 0.43

*[a] Significantly different between fiber types, as shown in Table 2B; *[b] Significantly different between the control and trained values. Sample sizes are the same as in Table 1

Table 2B. Significxant differences between fiber types

Lipid droplet absolute volume:	IIC IC I IIA IIAB IIB
Mitochondrial absolute volume:	
before training:	I IIC IIA IC IIAB IIB
after training:	I IIC IC IIA IIAB IIB
Mitochondrial volume percentage:	
before training:	I IIA IIC IIAB IIB IC
after training:	I IIC IC IIA IIAB IIB

Fiber types on the same line are not significantly different from each other, as indicated by Tukey's post-hoc analysis. Based on means shown in Table 2A

Myofibrils

Training significantly increased the absolute volume of myofibrils in type I and IIA but not IIB, fibers (Table 2). The volume percentage of myofibrils, however, did not change. There was no difference in myofibril cross-sectional area between fiber types before training (Table 3). With training, the area of myofibrils underwent fiber

Table 3. Size and number of myofibrils and mitochondria

Fiber type	Size (μm^2)		Number/μm^2	
	Control	Trained	Control	Trained
Myofibrils		*[a]		
I	0.696 ± 0.028	0.705 ± 0.032	1.06 ± 0.38	1.02 ± 0.03
IC	0.695 ± 0.104	0.574 ± 0.090	0.99 ± 0.11	1.19 ± 0.07
IIC	0.830 ± 0.104	0.563 ± 0.070*[b]	0.88 ± 0.04	1.23 ± 0.07
IIA	0.704 ± 0.029	0.731 ± 0.031	1.06 ± 0.04	1.04 ± 0.04
IIAB	0.722 ± 0.052	0.634 ± 0.056	0.99 ± 0.05	1.15 ± 0.05
IIB	0.713 ± 0.034	0.602 ± 0.064*[b]	1.03 ± 0.03	1.12 ± 0.07
Mitochondria	*[a]		*[a]	*[a]
I	0.133 ± 0.003	0.117 ± 0.003*[b]	0.50 ± 0.02	0.55 ± 0.02*[b]
IC	0.119 ± 0.013	0.104 ± 0.010	0.36 ± 0.05	0.49 ± 0.03*[b]
IIC	0.128 ± 0.013	0.123 ± 0.007	0.38 ± 0.04	0.48 ± 0.03
IIA	0.126 ± 0.004	0.115 ± 0.003*[b]	0.40 ± 0.01	0.43 ± 0.02
IIAB	0.116 ± 0.007	0.116 ± 0.005	0.41 ± 0.03	0.42 ± 0.02
IIB	0.119 ± 0.004	0.120 ± 0.007	0.38 ± 0.02	0.37 ± 0.02

Significant differences

Myofibril size after training:
IIA I IIAB IIB IC IIC

Mitochondrial number/μm^2 before training:
I IIAB IIA IIC IIB IC

Mitochondrial number/μ^2 after training:
I IC IIC IIA IIAB IIB

Mitochondrial size before training:
I IIC IIA IC IIB IIAB

*[a] Significantly different between fiber types, which were grouped by Tukey tests as shown below; *[b] Significantly different between control and trained samples. Value are means ± SE, sample sizes same as in Table 1. Significant differences: those fiber types underlined by the same line are not significantly different from each other based on Tukey's post-hoc analysis

type-specific changes. No change occurred in type I and IIA fibers, but IIB fibers were significantly smaller; this caused the IIA and I fibers to be significantly larger than IIB fibrils after training. Type IIAB myofibrils were significantly smaller than IIA, but overlapped with I and IIB. Myofibril number per unit area was similar between fiber types and did not change with training.

The absolute volume of intermyofibrillar space increased with training. This increase was significant for all fiber types except IIB. No fiber type differences were noted. The volume percentage of this intermyofibrillar space did not change with training, and was again similar between fiber types.

Mitochondria

Absolute volume of mitochondria was different between fiber types. In controls, mitochondrial volume was larger in type I than type II fibers. Training increased the absolute volume in type I and IIA fibers, and type I remained significantly greater than all of the type II fibers (Table 2). The volume percentage of mitochondria was significantly larger in type I than IIAB and IIB fibers before training. After training, the volume percentage remained the same as before training for all fiber types, and was again larger in type I than all type II subtypes.

Mitochondrial size in type I fibres (Table 3) was larger than in types IIB and IIAB control muscles. After training, the mitochondrial size decreased significantly in type I (12%) and IIA (8%) fibers. Due to this differential response of mitochondria of different fiber types to training, the mitochondrial size differences between fiber types were lost after training.

Lipids

In control groups, absolute volume of lipid was greater in type I than IIB fibers. Lipid content was increased in trained groups, but significantly only in IIA and IIAB fibers (Table 2). Volume percentages of lipid did not differ between fiber types. Training did not change the volume percentages of lipid.

Capillaries

The mean number of capillaries per fiber associated with each fiber type varied among individuals and differed

Table 4. Enzyme activities

Enzyme	Control	Trained	% difference	F ratio	*P*-value
CK	483 ± 62	469 ± 56	− 2.9	0.49	0.50
PFK	59 ± 10	62 ± 12	5.1	0.65	0.44
GAPDH	521 ± 108	583 ± 91	11.9	3.35	0.10
COX	4.45 ± 1.19	5.69 ± 1.86	27.9	7.75	0.02*
CS	7.41 ± 1.42	8.21 ± 1.66	10.8	1.87	0.20
HADH	13.79 ± 3.43	13.70 ± 2.90	− 1.0	0.01	0.92
HK	1.02 ± 0.16	1.28 ± 0.27	25.5	32.17	0.001*

* Significantly different between control and trained samples at $P < 0.05$ (analyses of variance were performed with repeated measurements). Data are expressed as U/g wet weight, mean ± SD ($n = 11$). CK, Creatine kinase; PFK, phosphofructokinase; GAPDH, glyceraldehyde phosphate dehydrogenase; COX, cytochrome oxidase; CS, citrate synthase; HADH, 3-hydroxyacyl CoA dehydrogenase; HK, hexokinase

between fiber types before training. The type I fibers had 4.95 caps/fiber, which was significantly larger than for type IIB fibers (4.16), and the IIA fibers had an intermediate number (4.60). Although the capillaries per fiber of the trained group was increased for all fiber types, the increase was significant only in type IIB fibers (4.84, increase of 16%). The small, non-significant increases in type I (3.4%) and IIA (9.5%) fibers resulted in the cap/fiber for all three fiber types being similar after training.

The capillary/fiber ratio and capillary density were not influenced by training. The capillary density (number/mm^2) did not change (from 522 in the control to 492 in the trained).

Enzyme activities (Table 4)

Oxidative and glycolytic enzyme activities were measured from whole muscle homogenates and expressed in U/g wet weight. The COX and HK increased significantly ($P < 0.05$), while the other enzymes were not influenced significantly by the training.

Relationship between variables

The muscle fiber cross-sectional area, regardless of fiber type, had a positive linear relationship with the absolute volume of mitochondria ($r = 0.75$), myofibrils ($r = 0.98$), interfibrillar space ($r = 0.58$), and the number of capillaries per fiber ($r = 0.55$), but a negative relationship with the capillary density ($r = -0.73$).

The absolute volume of mitochondria was positively related to the absolute volume of myofibrils ($r = 0.7$). The volume percentage of mitochondria was not correlated with the number of capillaries per fiber ($r = 0.29$), nor with the capillary density ($r = 0.27$). The absolute volume of lipid droplets was not related to any other variables.

Discussion

The cryostat-retrieval technique provides a reliable method of morphometric evaluation of specific muscle fiber types. However, this method does produce artifacts. Although several methods were used to minimize freezing artifacts they were not entirely eliminated. The sites of ice crystal formation were in the myofibrillar regions, and the immediate area surrounding these showed myofilaments more closely packed than in other regions. This indicates that the freezing artifacts produced only very local changes. Furthermore, because the volume percentage of artifacts was similar between pre- and post-trained samples, the morphometric results were not affected.

Fiber type composition and fiber sizes

Fiber type composition is sex independent [25, 26]. The vastus lateralis of both females and males contains about an equal percentage of type I and II fibers. Within type II subgroups, there are approximately twice as many IIA fibers as IIB fibers in untrained muscle. In the present study the fiber type percentages correspond well with data from the literature and from the histochemical analysis of the same biopsies [35]. After 20 weeks of resistance training the type IIA fiber percentages increase and IIB fibers decreased, suggesting a conversion of type IIB to IIA, which has been shown histochemically [35] and biochemically (R. S. Staron and C. Allemeier, unpublished observations, myosin heavy chain analysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE). In addition, detraining causes transformation in the reverse direction (IIA to IIB) [36].

This conversion of type IIB to IIA is normally associated with endurance training [2]. However, it is likely that high-intensity resistance training causes the recruitment of motor units not normally active during daily activities (type IIB) [38]. If the type IIB fibers are recruited often enough for a long enough period of time, it appears they will begin switching off the gene for myosin heavy chain (MHC) IIb and switching on the gene for MHC IIa.

Such a transformation may be easily underestimated using routine myofibrillar ATPase histochemistry. Recent single-fiber data indicate that many fibers classified histochemically as type IIB (especially in trained

muscle) may contain, in addition to MHC IIb, a small amount of MHC IIa [30, 32]. These hybrid fibers are termed type IIAB and appear to represent a transition state between the "pure" type IIB and type IIA fibers. Since only those fast type IIAB fibers that contain a significant amount of MHC IIa (i. e., 50% or more MHC IIa) are easily detectable, many fibers in transition from type IIB to IIA may be misclassified as pure type IIB fibers. These misclassifications may explain why no increase in the type IIAB population was observed after training in the present study. Data from MHC analysis give a much better representation of the amount of transformation occurring in trained muscle (R. S. Staron and C. Allemeier, unpublished observations, 31).

Quantitative changes in type IIB fibers

Most of the variables measured were more similar between pre- and post-training type IIB than for the other fiber types. One reason for this may be due to the conversion of many type IIB fibers to IIA fibers which would mask the total changes occurring in the transforming fibers and also within the post-training IIA group. If we assume that most of the IIB fibers converted to IIA, the control IIB fibers can be compared to trained IIA fibers to determine the magnitude of the change. The absolute volumes of all components (myofibrils, space, mitochondria, and lipids) were significantly larger in trained IIA fibers than the control IIB fibers. The transformation of type IIB to IIA occurred not only in terms of altered myosin expression and increased fiber size, but also increased volume of myofibrils, mitochondria, intermyofibrillar space, lipid droplets, and capillaries. Thus, the significant changes in type IIB fibers, from pre-training type IIB to post-training type IIA, would eventually increase the power output and the oxidative capacity of the trained muscle.

Cellular components

A dilution effect, i. e., decreased volume percentage of mitochondria and lipids due to fiber hypertrophy, has been reported with heavy resistance training [15, 17]. In the present study, no evidence of dilution was found for any cellular component.

Myofibrils

The mean volume percentage of myofibrils (about 83%), similar for all fiber types, corresponded with measurements by others for both control females and males [11]. The mean volume percentage of interfibrillar space (9.6%), similar for all fiber types, also agreed with prior studies.

In trained subjects, a high positive correlation between absolute volume of myofibrils and fiber size ($r = 0.98$) suggests that fiber hypertrophy was accompanied by a corresponding increase in myofibrils. The increase in absolute volume of myofibrils has been attributed to the increase in number and size of myofibrils [16]. In the present study, the increase in absolute volume of myofibrils, while maintaining a constant volume percentage and size, indicated an increase in the total number of myofibrils and is probably due to myofibrillar splitting.

Mitochondria

A slightly higher mitochondrial volume percentage has been reported for males than females [12]. However, the volume percentage of mitochondria remains largest in type I, smallest in IIB, and intermediate in IIA fibers, although the overlapping values and large variances cause the lack of significant differences between the measurements.

After training, the increase in absolute volume of mitochondria was positively related to the increase in fiber size ($r = 0.75$) and of myofibrils ($r = 0.7$), resulting in the maintenance of the same relative volume percentage of mitochondria in all fiber types. The absolute volume increased significantly in type I, IIA and IIAB fibers. However, in type I and IIA fibers a significant decrease in the mean size of mitochondria was accompanied by an increase in the number of mitochondria per unit area, suggesting the possibility of mitochondrial proliferation in the hypertrophied type I and IIA fibers.

The 20 weeks of resistance training significantly increased COX activities, suggesting that this training regimen produced an increase in the oxidative capacity of the muscle as a whole. However, the increased activity of COX did not correlate with the volume percentage of intermyofibrillar mitochondria measured in this study. These remained unchanged in all fiber types. This apparent discrepancy may be due to the total amount of change being small, especially when compared to the adaptation reported for endurance-trained muscle. Another reason may be that the fibers with the lowest oxidative capacity (type IIB) would respond the greatest to increased usage while transforming into IIA fibers [33]. Therefore, a significant increase in the volume percentage of mitochondria may not be detectable in the type IIA fiber group which after training includes transformed IIB fibers. Another explanation is that the volume percentage of subsarcolemmal mitochondria, which was not measured here, might increase to produce the increased activity of COX. The subsarcolemmal mitochondria from various human skeletal muscles have been found to have about six times higher cytochrome oxidase activity than do interfibrillar mitochondria [7]. The cytochrome oxidase activity increases more in subsarcolemmal than interfibrillar mitochondria with both endurance and interval training in rat skeletal muscle [19].

Several studies have reported that the volume percentage of mitochondria and myofibrils decrease in hypertrophied muscle fibers following resistance training in human males [13, 17]. In contrast, the present study with females showed that the volume percentage of all

subcellular components remained unchanged as a result of an increase in the absolute volume of all components. Those prior studies [13, 17] did not differentiate fiber types, and because of differential hypertrophy of fiber types, a discrepancy in interpretation of mitochondrial volume changes within compartments might occur. Furthermore, the specific mitochondrial population (intermyofibrillar or subsarcolemmal) was not reported.

Lipids

Lipid volume percentage is higher in type I and IIA fibers than in IIB fibers of untrained females [12]. Intracellular lipid droplets have been reported to be greater in muscles of endurance-trained individuals than control or resistance-trained subjects [11, 21]. In the present study, the absolute volume of lipid droplets increased with training in all major fiber types, but significantly only in type IIA and IIAB fibers. In contrast, the volume percentage of lipid droplets in world-class power lifters has been reported to be lower than in untrained subjects [21]. This may be due to different training methods. Power lifting consists of high load with few repetitions and long rest periods between sets and exercises, requiring high anaerobic capacities. The training used in the present study is similar to a body-building regimen and consisted of "moderately" high loads and high repetitions with short rest periods, activating both anaerobic and aerobic metabolic pathways.

Capillary supply

Capillary density is not significantly different between fiber types of untrained subjects. Generally, the values for capillary numbers per fiber type are larger in males than females, corresponding to the larger cross-sectional area of all fiber types in males [1]. Despite the large differences in capillaries per fiber and capillaries per cross-sectional area in different studies, the hierarchy of capillarity for both males and females is in good agreement [1]. It is significantly higher in type I than IIB fibers, and the IIA fibers are intermediate. The number of capillaries per area of fibers was similar between fiber types.

The number of capillaries per fiber was positively correlated with increased fiber size, suggesting that capillaries had proliferated to compensate for the fiber hypertrophy. This capillary per fiber ratio increased slightly for all fiber types, but significantly only in type IIB fibers.

These results agree with the results from male body builders [28], but differ from male weight lifters [37]. Capillary proliferation in the vastus lateralis has been observed in body builders, but not weight lifters. The discrepancy is again believed to be related to the training regimens. Body builders use moderately high loads and more repetitions, while weight lifters emphasize high loads and fewer repetitions.

Enzymes

The absence of changes in CS and HADH activities and the increase in COX activity suggest that the Krebs cycle and β-oxidation metabolic pathways were not significantly altered in response to resistance training, while the oxidative phosphorylation potential of the muscle was improved. It is usually assumed that a constant proportion exists in the enzyme activity level of metabolic pathways involved in the skeletal muscle aerobic-oxidative metabolism, but this may not always occur. For instance, Howald et al. [13] have shown that the correlation between mitochondrial volume density and CS activity of human skeletal muscle is not the same as between this ultrastructural parameter and COX activity, suggesting that the activity levels of CS and COX were not proportionally maintained. On the other hand, COX enzyme activity can remain unchanged in the vastus lateralis muscle of competitive cyclists exposed to long-term endurance training notwithstanding the fact that CS activity is increased by about 25% (J. A. Simoneau et al., unpublished results).

These examples suggest that different mechanisms may contribute to the biosynthesis of proteins involved in different metabolic pathways such as the Krebs cycle, electron transport, or β-oxidation. Among the potential mechanisms that can be involved in the disproportional enzymatic changes in CS and COX is the fact that the level of CS activity is regulated by a nuclear gene whereas the level of COX activity requires the regulation of both the mitochondrial and nuclear COX genes. It cannot be excluded that the signalling mechanisms triggered by repeated maximal exercise of short duration and rest periods may have different consequences on the nuclear and mitochondrial gene expressions. Perhaps an increase in COX activity, that appears to be not coordinated with other enzyme markers for the Krebs cycle or β-oxidation, may be linked to the reduced nicotinamide adenine dinucleotide (NADH) oxidation processes that are requested by the increase in the intramitochondrial content of NADH [24], or to the oxygen-dependent processes of the creatine phosphate repletion following exhausting exercise [25, 40]. Increase in activity level of COX, i. e., a regulatory complex of oxidative phosphorylation, would favor these metabolic processes.

In conclusion progressive, heavy, resistance training increases muscle force production as indicated by muscle hypertrophy of all major types. Type IIB fibers were hypertrophied to the greatest extent and transformed to type IIA fibers, which consequently altered the fiber type composition. Fiber hypertrophy and the conversion from type IIB to IIA were accompanied by a corresponding increase in the amount of myofibrils, mitochondria, lipid droplets and capillaries. Increases in both the subcellular components and fiber size resulted in a constant volume percentage of these components with this type of resistance training. The mechanism linking heavy resistance training with these adaptive changes is not clear but apparently is related to the amount of force developed by the muscle as well as the duration of activity. Changes in the hormones and their