

Is Hypertrophy Limited in Elderly Muscle Fibers? A Comparison of Elderly and Young Strength-Trained Men

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Abstract

To investigate the capacity to hypertrophy and the satellite cell populations to change with age, the nucleo-cytoplasmic relationships and satellite cells were compared in skeletal muscles of young and elderly men before and after strength training. Vastus lateralis muscle biopsies were taken before and after 8 (young) or 16 weeks (elderly men) of strength training and compared to muscle from untrained men. The young men had more myonuclei in type II fibers than in type I, and the cytoplasm per nucleus was smaller. As muscle fibers hypertrophied with strength training, the larger cross-sectional area was matched by increasing nuclear numbers, maintaining a constant nucleus-to-cytoplasm ratio. The numbers of myonuclei were similar in elderly and young muscles, and strength training increased both, but not significantly. Myonuclear number was related to cross-sectional area in young untrained and trained muscles, but no relationship was found in untrained elderly men. With training, this relationship was restored in the elderly muscles. The relative increase in cross-sectional area in elderly muscles was similar to the young, but the elderly muscles began with such small fibers, that the hypertrophied fibers in the trained elderly muscles reached the size of untrained young muscle fibers. The results suggest that the increase in myonuclear number accompanies and is proportional to fiber hypertrophy in the young, but it is still unclear to what extent this occurs in the elderly. The percentage of satellite cells did not differ between young and elderly muscles. These satellite cells, responsible for contributing to myonuclear increase, did not decrease in numbers with aging.

Key words: muscle aging, muscle growth, strength training, nucleo-cytoplasmic relationships.

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Recent studies indicate that each nucleus within a skeletal muscle fiber controls a specific region around it, and that a muscle fiber consists of a series of overlapping nuclear domains. The nucleus controls the structural proteins within each domain [4, 9]. During development, the growing fiber requires the addition of new myonuclei. If this myonuclear addition is restricted by decreasing satellite cell division, then the fiber does not grow [8]. This suggests that the myonucleus has a limited region over which it might exert its control, and new nuclei must be added to the fiber in order for growth to occur, even under conditions to induce hypertrophy [10]. In addition to muscle growth, the nuclear composition of a muscle fiber appears to

become reduced when muscle atrophy occurs, as when animals are subjected to microgravity conditions [2, 5].

These interactions between the size of the muscle fibers and the nuclear composition led us to investigate whether the nucleus-to-cytoplasm relationship is constant and whether this relationship changes with age in human muscles. This is of major importance because of progressive inactivity with aging, which results in muscular atrophy. If this atrophy results in loss of myonuclei, and satellite cell activity is reduced in the elderly, then a resumption of activity to counteract the atrophy may not produce the benefits it might in young muscles.

To investigate this problem, the responses of young and elderly men to strength training were compared. The

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training was similar, although the elderly men underwent 16 weeks of training compared to 8 weeks for the young. Muscle biopsies taken from the vastus lateralis were examined to determine the changes in muscle fiber size before and after strength training, and the myonuclear and satellite cell compositions were determined.

Materials and Methods

Young (age 22.5 ± 5.8 , $n = 7$) and elderly (65.0 ± 6.0 , $n = 8$) males volunteered for a strength training study that was approved by the Ohio University Institutional Review Board. The volunteers were given both oral and written descriptions of the procedures prior to giving an informed consent. The vastus lateralis muscle was sampled by needle biopsy as described previously [17], and the samples were divided into pieces for electron microscopy and immunohistochemistry/histochemistry. The part for histochemistry was mounted, frozen in methyl butane cooled with liquid nitrogen, and stored at -70°C until sectioned.

The portion for electron microscopy was fixed overnight in 1.5% glutaraldehyde and 3% paraformaldehyde in 0.1M cacodylate buffer, pH 7.2, rinsed in buffered sucrose, post-fixed in 1% buffered osmium tetroxide, stained *en bloc* with 1% aqueous uranyl acetate, dehydrated, and embedded in an epon/araldite mixture. Sections were made with diamond knives, mounted on formvar-coated slot grids, and analyzed with the Zeiss EM 109 or JEOL 1010 electron microscope. The myonuclei and satellite cells associated with each fiber were counted in the electron microscope.

Cross sections of frozen samples were cut at 12 μm thickness, mounted on plain (for histochemistry) or gelatinized (for antibody preparations) coverslips, and dried. For myofibrillar ATPase (ATPase) histochemistry, the procedure of Staron and Hikida [16] was used (Fig. 1), and analysis for this study was done using only the Type I and Type II fiber types because nucleocytoplasmic relationships have compared only Type I and II fibers in other studies. For myonuclear analysis, the procedure of Hikida et al. [5] was used. Briefly, unfixed sections were incubated in the dystrophin antibody (Mandys 8, Sigma Chemical Company, St. Louis) overnight, then processed for peroxidase conjugation using the Vector Laboratories (Burlingame, Ca) ABC Kit. Nuclei were stained with Delafield's hematoxylin. In this way the sarcolemma was stained, and myonuclei could be differentiated from nuclei of other cells (fibroblasts, satellite cells) closely adjacent to the fiber.

The dystrophin preparations were used to determine the myonuclear numbers and cross-sectional areas of the muscle fibers. Three to four fascicles were selected for analysis, and all fibers in each of these were measured for cross-sectional area using the NIH Image Program and a Cohu video camera attached to a Zeiss

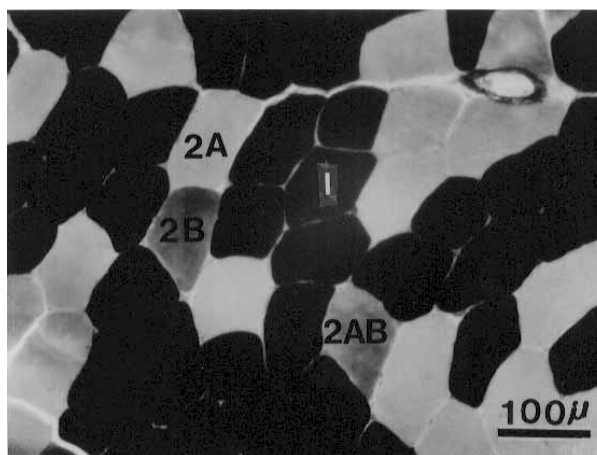


Figure 1. Cross section of an ATPase preparation after preincubation at pH 4.6. The major fiber types are labeled.

microscope. Approximately 250 fibers were analyzed from each biopsy.

Training

The young males were trained for eight weeks (20 sessions), using three types of training to strengthen the quadriceps femoris muscles (squat, double leg press and double leg extension). Each session was designed to evoke the maximum weight to allow 3 to 5 repetitions for four sets each time. As training progressed, the weights increased. The maximum performance was tested before and after the training period. The elderly underwent 16 weeks of strength training (32 sessions), using the same procedures, but using weights to achieve 6 to 8 repetitions, three sets per session. Testing for maximum weight lifted for each procedure was done prior to and after training.

Results

Strength gains

In the exercises designed to increase quadriceps strength, both the young and elderly increased in strength. Testing of the young for their quadriceps by using three procedures (double leg press, double leg extension, half squat) showed that the young men were initially stronger than the elderly (Table 1). Training caused both groups to increase strength significantly, and the difference in strength between the two groups was enhanced for two of the three tests, although the percentage increase in strength was similar for the two groups.

Fiber sizes

As with strength, mean muscle fiber cross-sectional area was 24% larger in young than the elderly men, and resistance training enhanced this difference to 30%. Both young and elderly muscle fibers were hypertrophied by training (25% and 18% respectively) in the dystrophin preparations. Comparison of

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dystrophin and ATPase preparations indicated shrinkage of the dystrophin preparations, especially in the elderly muscles (Table 2). The cross-sectional areas measured from ATPase preparations showed that the young muscle fibers were hypertrophied by 26% and the elderly by 37%.

The dystrophin and ATPase preparations were taken from adjacent sections for the young muscles (fig. 2), but were not from the elderly. Therefore the fiber types based on ATPase activity could not be matched with the dystrophin measurements in the elderly. Because of this, the mean fiber size, irrespective of fiber type, was calculated based on cross-sectional areas of the ATPase preparations of young muscles (indicated as "Mean XSA" in Table 2).

Satellite cells (Table 3)

The electron microscope was used to count the number of myonuclei and satellite cells in cross sections of muscle fibers. Both myonuclei and satellite cells occur at the periphery of a muscle fiber, but the satellite cell lies between the sarcolemma and the muscle fiber's basal lamina. Satellite cells are presented as percentage

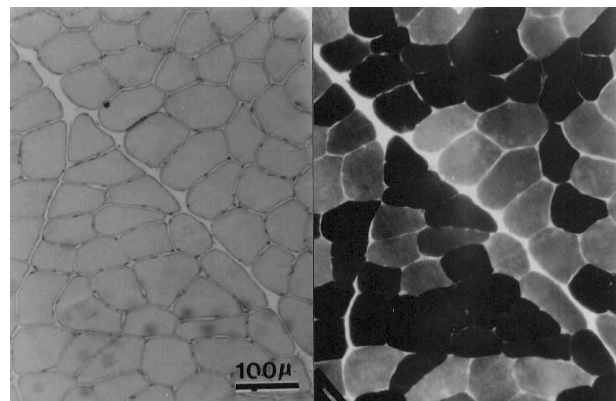


Figure 2. Serial sections stained for the dystrophin (left) antibody and ATPase (right) demonstrating the ease with which fibers can be matched.

of the total myonuclei plus satellite cells to allow comparison with other studies. The satellite cell percentages were no different in any of the young groups or the elderly groups, nor did they differ between the young and elderly.

Table 1. Strength changes^a.

	Leg Press			Leg Extension			Squat		
	Pre	Sign	Post	Pre	Sign	Post	Pre	Sign	Post
Young									
Trained Group	300	*	500 (67%)	95	*	155 (63%)	115	*	250 (117%)
Control Group	280	ns	300 (7%)	95	ns	100 (5%)	118	ns	140 (19%)
Elderly									
Trained Group	165	*	285 (73%)	62	*	94 (52%)	91	*	175 (92%)
Control Group	155	ns	170 (10%)	63	ns	67 (6%)	85	ns	89 (5%)

^aValues presented in kg, with percent increase over pre-training values in parentheses.

Pre: pre-training values; Sign: statistical significance (*, significantly different; ns, not significantly different); Post: post-training value.

Table 2. Cross-sectional areas of dystrophin preparations^a.

	YOUNG		ELDERLY	
	Control	Trained	Control	Trained
Pre-training	5158 ± 1130	4692 ± 1275	3550 ± 731	3667 ± 642
Significance	ns	*	ns	*
Post-training	5010 ± 491	5852 ± 1505	3488 ± 682	4985 ± 1522

^a Mean ± standard deviation, given in mm².

Significance listed as ns, not significantly different between pre- and post-training values, or *, indicating a significant difference between pre- and post-training values.

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Table 3. Cross-sectional areas and percentage fiber types based on ATPase preparations^a.

	Type I	Type IIA	Type IIB	XSA ^b
Young				
Control Pre	5024 ± 1892 (31)	6784 ± 1732 (35)	5251 ± 834 (34)	5717
Control Post	5837 ± 1520 (46)	7800 ± 1123 (30)	5282 ± 115 (24)	6293
Trained Pre	4194 ± 337 (36)*	4898 ± 664 (41)*	4465 ± 822 (23)*,*	4545*
Trained Post	5115 ± 624 (38)	6168 ± 1044 (45)	5331 ± 1142 (16)	5629
Elderly				
Control Pre	3724 ± 873 (47)	4254 ± 1342 (29)	3070 ± 1035 (17)*	3772
Control Post	3436 ± 598 (47)	4179 ± 1371 (28)	3337 ± 1043 (18.2)	3640
Trained Pre	3887 ± 1034 (52)*	4146 ± 834 (25)*,*	3418 ± 811 (17)*,*	3869*
Trained Post	5657 ± 1675 (55)	5529 ± 1350 (29)	5208 ± 1825 (8)	5577

^a Values presented as mean ± standard deviation, in mm² (percentage of the fiber type). Percentages do not total 100% because minor subtypes were not included. Each value between pre and post-training is not significantly different from one another except where indicated by an *. Where a second * is present, the fiber type percentage is significantly different.

^b Mean cross-sectional area is given as the area multiplied by the percentage of the fiber type. This allows the ATPase data to be compared to the dystrophin measurements.

Myonuclei

Myonuclear numbers obtained by light microscopy using the dystrophin preparations (fig. 3) were compared with the numbers obtained by electron microscopy. In both young and elderly muscles, there was a significant underestimation of the myonuclear numbers with light microscopy. About 40% more myonuclei were counted by electron microscopy. With either method, no significant difference in myonuclei per fiber cross section occurred between the pre- and post-training groups, although trained groups had a consistently higher number of myonuclei.

Nucleo-cytoplasmic relationship

Although significant hypertrophy occurred after 8 weeks of strength training **in the young**, the area per nucleus was unchanged (Table 5). This suggests that the nuclear content was increased, but the increase in nuclei

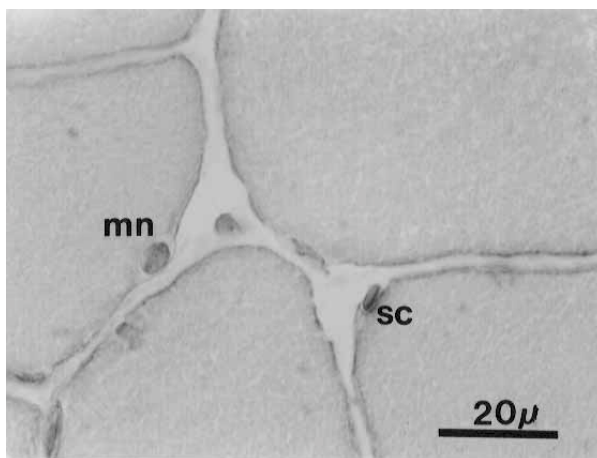


Figure 3. Higher magnification micrograph of a dystrophin preparation demonstrating the myonucleus (mn) and satellite cell (sc).

per fiber was not significant ($p = 0.18$). In the **elderly men**, resistance training again caused significant hypertrophy, but the number of nuclei per fiber was unchanged. The cytoplasm to nucleus ratio increased, but not significantly ($p = 0.12$).

In order to compare young versus elderly muscles, the pre-training samples of the control and trained groups of both age groups were combined and compared. The fiber sizes of the elderly were significantly smaller than in the young (3609 versus 4891 mm²). However, the myonuclei per fiber were similar, and the cytoplasm-to-nucleus ratio was identical, although the variances of the latter were not equal. Because of the latter, the analysis was by a Kruskal-Wallis one-way ANOVA on ranks.

Because of the great variability between individuals, especially in the cross-sectional area, the average cross-sectional area for each subject was plotted against the mean number of myonuclei per fiber. The small number of subjects in the training group made the analysis weak, but a distinct difference was observed between the elderly and young. The young had a correlation coefficient (r) of 0.55 ($p = 0.098$) (fig. 4) for the pre-training regression, and $r = 0.52$ ($p = 0.10$) for the post-training regression (Fig. 5). In contrast to this, the elderly showed no relationship between fiber size and myonuclear content for the pre-training ($r = 0.15$, $p = 0.60$) (fig. 6), or for the post-training preparations ($r = 0.04$, $p = 0.93$) (Fig. 7). One elderly subject was an outlier in terms of myonuclear content, both in the pre-training and post-training samples. When this subject was removed from the analysis, the pre-training samples remained non-significant. However, the post-training samples were identical to the post-training young samples ($r = 0.67$, $p = 0.098$). No increase in myonuclei with fiber hypertrophy was noted in the elderly trained muscles.

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Table 4. Myonuclei and satellite cell content by electron microscopy^a.

	YOUNG		ELDERLY	
	Pre	Post	Pre	Post
Myonuclei per fiber				
Control	2.42 ± 0.36 (6)	2.54 ± 0.70 (6)	2.01 ± 0.33 (9)	2.12 ± 0.35 (9)
Trained	2.08 ± 0.48 (8)	2.27 ± 0.50 (8)	2.23 ± 0.46 (8)	2.38 ± 0.25 (8)
Satellite cells as percentage of total myonuclei and satellite cells				
Control	2.35 ± 0.11 (6)	2.40 ± 1.53 (6)	2.30 ± 0.89 (9)	2.43 ± 0.91 (9)
Trained	1.61 ± 1.22 (8)	2.06 ± 1.64 (8)	2.45 ± 0.87 (8)	2.21 ± 0.82(8)

^a results presented as mean ± standard deviation

Pre: indicates the pre-training, or initial biopsy results; Post: indicates the post-training, or second biopsy results. No statistical difference occurred between any of these values.

Table 5. Myonuclear analysis by light microscopy^a.

CONTROL	YOUNG		ELDERLY	
	PRE	POST	PRE	POST
XSA	5158 ± 1130	5010 ± 491	3550 ± 731	3488 ± 682
NUCLEI/FIB	1.21 ± 0.38	1.41 ± 0.48	1.03 ± 0.56	1.07 ± 0.37
XSA/NUCL.	3546 ± 857	3804 ± 832	4374 ± 2166	3529 ± 1094
TRAINED				
XSA	4700 ± 1164	*5852 ± 1505	3667 ± 642	*4985 ± 1522
NUCLEI/FIB	1.14 ± 0.21	1.36 ± 0.36	1.48 ± 0.84	1.70 ± 1.53
XSA/NUCL.	3863 ± 941	3910 ± 581	3517 ± 1197	4371 ± 1366
COMBINED				
XSA	4891 ± 1122		*3609 ± 667	
NUCLEI/FIB	1.17 ± 0.28		1.25 ± 0.73	
XSA/NUCL.	3731 ± 881		3946 ± 1748	

^a Mean ± standard deviation. PRE: first biopsy, pre-training. POST: second biopsy (controls) or post-training. XSA: mean cross-sectional area, in mm². NUCLEI/FIB: mean number of nuclei per cross-sectional profile. XSA/NUCL.: cytoplasm per nucleus ratio, in mm². * This value is significantly different from the preceding value.

When many young untrained muscles were plotted for myonuclear number per fiber against mean cross-sectional area, there was a highly significant relationship between these (fig. 8).

Fiber type-specific characteristics in the young (Table 6)

Because the myofibrillar ATPase and nuclear comparisons were studied in adjacent sections only in the young, a fiber type analysis was done only for that group. The type II fibers were larger and had more myonuclei per cross-sectional profile than the type I. The sarcoplasm to nucleus ratio was significantly larger in the type I fibers when a large group (n = 22) of young muscles was compared. However, the subset (n = 7) used for this study showed a similar percentage difference, but this was not significant. This ratio was also not significantly different in the trained samples.

Discussion

Myonuclei and satellite cells

The results demonstrate that a linear relationship exists between myonuclear number per cross section and the mean cross-sectional area of the muscle fibers. As muscle fibers enlarge, this relationship is maintained by adding more nuclei to the fiber. The addition of myonuclei has been shown to be due to satellite cell activation, proliferation, and incorporation [3]. The fact that satellite cell proliferation is required for hypertrophy to occur (presumably by adding nuclei to the fiber) has been demonstrated for growing muscles and compensatory hypertrophy of growing muscles [10, 12]. Information about additional myonuclei to accommodate exercise-induced hypertrophy in fully adult mammals and any study with normal human muscles have not been studied.

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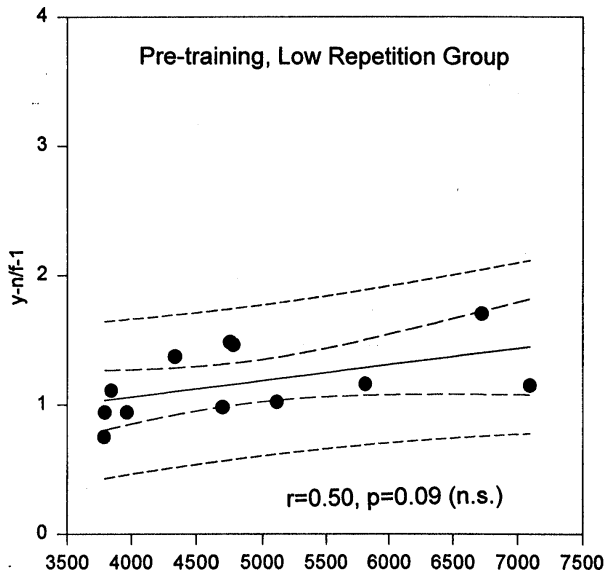


Figure 4. Plot of the number of nuclei per fiber against cross-sectional area for the young untrained subjects.

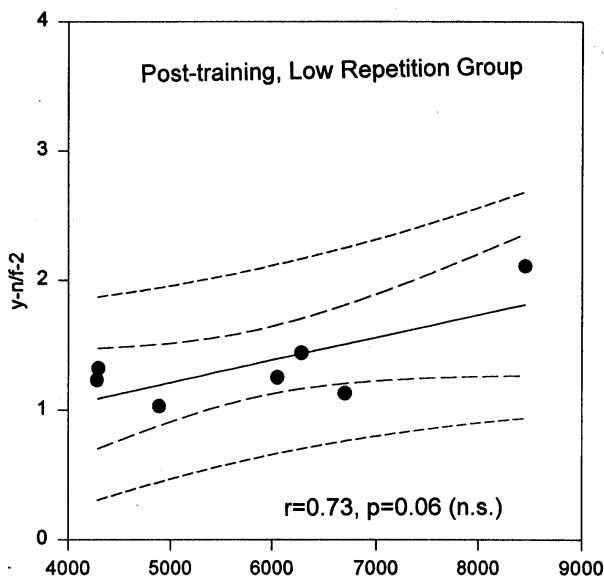


Figure 5. Same as Figure 4, but after training.

In contrast to the myonuclear increase in hypertrophy, the myonuclear numbers appear to decline with inactivity in rats [2, 5, 21]. Along with this decrease in myonuclei, satellite cell proliferative activity declines with muscular inactivity in growing rat muscles [13]. Since satellite cells are required to maintain growth in immature muscles, the decline in proliferative activity demonstrates that growth is arrested [12]. This is expected, but in mature animals, the reduction in myonuclear numbers suggests that atrophy results in apoptotic loss of nuclei in addition to inhibition of satellite cell activity.

Satellite cell numbers decrease with age in mouse soleus muscles [15], based on sampling of myonuclei and

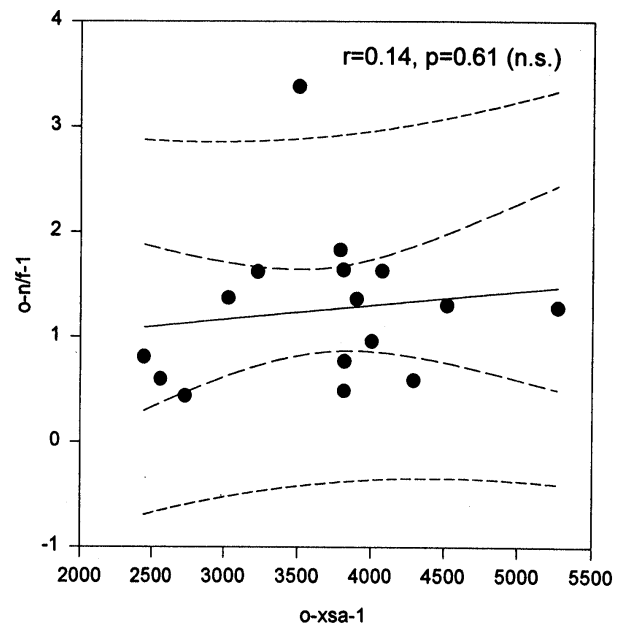


Figure 6. Plot of nuclei per fiber against cross-sectional area for all of the elderly subjects (control and trained groups together) prior to training. No relationship exists.

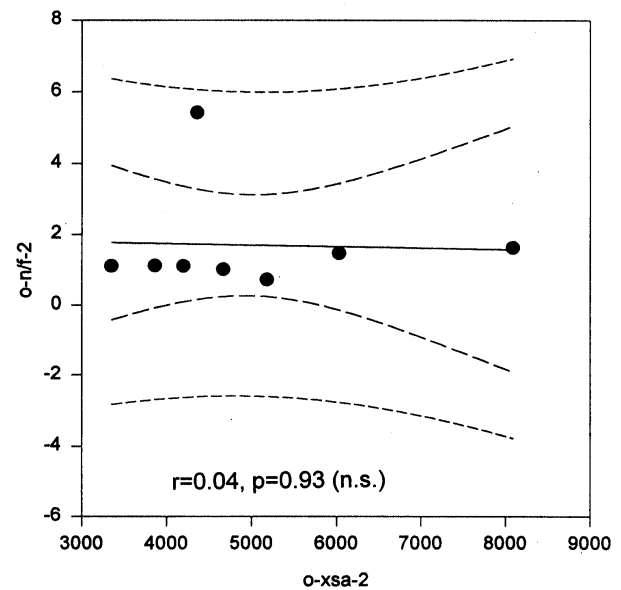


Figure 7. Nuclei per fiber plotted against fiber size for the trained elderly men. No relationship exists, but if the single outlier is removed from analysis, the correlation coefficient, r , becomes 0.67, with $p = 0.098$.

satellite cells in sections examined by electron microscopy. In addition to a decreased percentage of satellite cells, the ability to proliferate decreases in aged rat muscles [14].

The present study suggests that human muscles are not similar to those of rat and mice. Satellite cell percentages do not change between young and elderly adult men.

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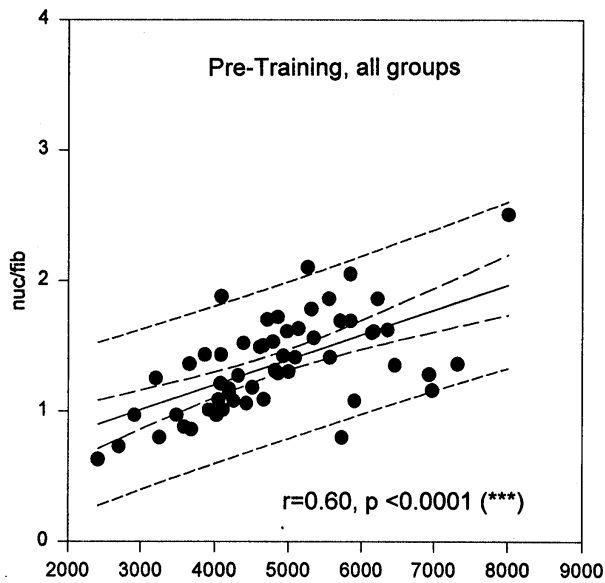


Figure 8. All of the young (control and training subjects) plotted before the training, showing the relationship between nuclei per fiber and fiber size.

Furthermore, the myonuclear population does not appear to decline with atrophy associated with aging.

Fiber type differences

Most studies have shown that in muscles of rats and cats, the slow twitch type I fibers have more myonuclei than the type II fast twitch fibers [1, 5, 7, 21]. Because of this, it was surprising to us that in human vastus lateralis muscle, type II fibers had significantly more myonuclei per cross-sectional profile than the type I in the pre-trained biopsies. Not only were there more myonuclei

per fiber profile in pre-training young muscle samples, but the cytoplasm-to-myonucleus ratio was significantly smaller in type II fibers than type I (Table 6). Clearly then, more myonuclei were present in the type II fibers, and this differs from the rat muscles.

The post-training samples similarly had a significant difference in myonuclei per cross-sectional profile. But in this case, the type II fibers hypertrophied more than the type I fibers, as has also been observed in other studies [6, 18-20]. This type II hypertrophy resulted in an identical nucleo-cytoplasmic ratio in the two fiber types. Therefore the number of nuclei were identical when compared as nuclei per cross-sectional area. The explanation for this may be that with the additional recruitment of type II fibers induced by training, the muscle fibers hypertrophied to the full extent of the area each myonucleus was capable of directing. Since type I fibers are usually first to be recruited in normal activities [11], they are assumed to have begun as being close to their maximum potential (maximum cytoplasm per nucleus) because of their constant recruitment. Because of the difference in recruitment with training, one would expect the type II fibers to be most influenced by resistance training; this has been demonstrated by several studies. It is expected that once the maximum cytoplasm per nucleus ratio has been achieved, any further hypertrophy would require additional nuclei.

For the above hypothesis to be true, there should be a linear relationship between nuclear number and sarcoplasm. There can be two reasons for this relationship to be absent: (1) The myonuclear number remains constant and does not change with changing fiber size. (2) The myonuclear number changes with the

Table 6. Myonuclear analysis, young men, fiber-typed^a.

Fiber type	Cross-Sectional Area	Myonuclei/fiber	Cross-Sectional Area per Nucleus
Control Muscles (n = 22)			
Type I	4046 ± 1068	1.12 ± 0.27	3696 ± 835
Significance	*	*	*
Type II	4786 ± 1032	1.54 ± 0.36	3305 ± 885
Training, Pre-trained Muscles (n = 7)			
Type I	4488 ± 1140	1.14 ± 0.21	4016 ± 1137
Significance	*	*	ns
Type II	4987 ± 1208	1.35 ± 0.17	3773 ± 1047
Training, Post-trained Muscles (n = 7)			
Type I	5194 ± 1473	1.43 ± 0.34	3933 ± 684
Significance	*	*	ns
Type II	6033 ± 1702	1.65 ± 0.28	3639 ± 721

^a Mean ± standard deviation of ATPase matched with dystrophin preparations. * significant difference between type I and type II fibers. ns: no significant difference between type I and type II fibers.

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fiber size, but this adaptability decreases in the elderly. The results suggest that the first possibility is not likely, but the results neither support nor preclude the second possibility.

Hypertrophy

The muscle fibers of the young and elderly hypertrophied to a similar relative amount in response to strength training. However, because the elderly muscles began so much smaller, the amount of hypertrophy increased the size of the trained elderly muscle fibers to that of the pre-trained fiber size of the young men. One of the purposes of this study was to determine whether hypertrophy is limited in the elderly because of loss of myonuclear or satellite cell activity. This study can not make a definitive case for limitation of hypertrophy in the elderly. Our studies (data not shown) show that even after 18 weeks of training, the strength continued to increase in the elderly. If they had not yet peaked, perhaps further hypertrophy would have occurred and this would have necessitated a significant increase in myonuclei, which had not occurred by 18 weeks.

Evaluation of the methods

The over-riding advantages of the method used in this study are that many fibers can be assayed, the fiber types can be obtained easily, and the cross-sectional area can be matched with the nuclear counts. Two major disadvantages exist. The first is that the nuclei tended to be undercounted by the investigators in this study. A second is that since the fiber is a long cylinder with nuclei distributed at intervals along its periphery, the sampling of a cross section is not very efficient. However, the latter can be compensated for by sampling many fibers. A problem associated with sampling cross sections is that any changes in nuclear content tend to be minimized. Therefore in spite of consistent increases in nuclear numbers with training, these increases were not statistically significant. This lack of significance is likely due to the poor resolution of this method.

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