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Mass and fiber cross-sectional area of soleus muscle grafts following training

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ABSTRACT

CLARK, K. I., P. G. MORALES, and T. P. WHITE. Mass and fiber cross-sectional area of soleus muscle grafts following training. *Med. Sci. Sports Exerc.*, Vol. 21, No. 4, pp. 432–436, 1989. This study tested the hypothesis that endurance training initiated 28 d following grafting of the soleus muscle would increase fiber cross-sectional area concomitant with an increase in mass. Nerve-implant orthotopic grafting operations were performed on 6-wk-old rats anesthetized with pentobarbital sodium. A cohort of animals began running 28 d later. Control muscles were from age-matched, untrained rats. Mass and fiber cross-sectional area of grafts were 37 and 66% less than the respective control values at both 56 and 112 d post-grafting. Training increased graft mass by 49% over the non-run graft value of 82 ± 8 mg at 56 d post-grafting. Continued training did not increase mass further. The grafts of trained rats were 33% greater than untrained at day 112 due to growth of grafts in untrained rats. Running had no effect on fiber cross-sectional area of grafts through 56 d, but by 112 d the cross-sectional area of Type I fibers was 30% greater than the non-run graft value of 1,271 ± 81 μm^2. By 112 d fiber type profiles were not different between control muscle and grafts from trained and untrained rats. We conclude that there is a dissociation between mass and fiber cross-sectional area in grafts compared to control muscle, and training affects these variables by similar magnitudes but at different times.

EXERCISE, EXERTION, TRANSPLANTATION, MUSCLE DEGENERATION, MUSCLE REGENERATION, RAT, SKELETAL MUSCLE

Following free autografting operations in rats, approximately 95% of the muscle fibers degenerate and new fibers regenerate (4,5). Skeletal muscle grafts in rats stabilize with respect to mass at 56 d (5,27). In stable grafts, several morphological and physiological variables differ from control values (5,7,11,23). The sequence of events in regenerative growth recapitulate ontogenetic development (5); thus, the graft model allows for study of the impact of environmental factors, such as chronic exercise, on mechanisms of muscular growth. Endurance exercise training can lead to increased mass and protein content in grafts (27). The underlying morphological correlates of exercise-induced growth of soleus muscle grafts have not been reported.

The purpose of the present study was to characterize in rats the effects of endurance training on soleus muscle grafts with respect to mass, fiber cross-sectional area, and the histochemical demonstration of fiber type. The hypothesis was tested that training would increase fiber cross-sectional area in grafts concomitant with an increase in mass.

METHODS

Female Wistar rats (N = 29), specific pathogen free, were obtained from Charles River Laboratories (Kingston, NY) at 6 wk of age. In 19 rats, bilateral soleus muscles were grafted using a nerve-implant procedure (27). Rats were anesthetized with sodium pentobarbital (initially 35 $mg \cdot kg^{-1}$ i.p.; supplemented as required). Both soleus muscles were grafted orthotopically, with the soleus nerve implanted into the original motor endplate region and anchored with a purse-string suture (7-0 silk). Drinking water was supplemented with tetracycline 1 d prior to the grafting operation and for 2 d following the operation to prevent infection. All procedures were in accordance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society.

Rats with grafts were randomly assigned to non-trained (N = 10) or run-trained (N = 9) groups. Age-matched, unoperated rats (N = 10) provided control muscles. The training protocol was initiated 28 d following grafting. On the first day of training, rats ran for 15 min at 24 $m \cdot min^{-1}$ at a 15% grade. Duration and speed were increased daily such that by day 4 rats were running at 30 $m \cdot min^{-1}$ for 1 $h \cdot d^{-1}$, conditions which

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continued until the end of the experiment. Compared with other running protocols and initiation dates relative to the time of the grafting operation, this protocol induced the most significant adaptations (27). Following 28 or 84 d of running (i.e., 56 or 112 days following grafting operation), muscles were removed, trimmed of tendons and fat, and weighed. A sample was cut from the full cross-section of the muscle belly and frozen in isopentane cooled to −70°C by dry ice. Ten micrometer thick transverse sections were cut in a Damon IEC cryostat (Ames Company, Elkhart, IN). Serial sections were incubated for myofibrillar ATPase activity at pH 10.3 and 4.6 (3) and for succinate dehydrogenase (SDH) activity (19). Type I fibers appeared light at pH 10.3 and dark at 4.6 and were dark following SDH incubation (3). Fibers which were dark at alkaline pH were classified as Type II. These fibers were further classified as IIA if they appeared light at pH 4.6 and dark in SDH, or IIB if they were dark at pH 4.6 and light following SDH incubation. The histochemical sections which had been incubated in alkaline pH were projected at 600×, and approximately 500 cells were drawn in each muscle. Projections were planimetered to obtain fiber cross-sectional area, and cells were matched with serial sections from other incubations to obtain a fiber type profile.

Data are presented as mean ± 1 SEM. Group data were evaluated statistically with an analysis of variance and Tukey's *post hoc* procedure (10), and $P \leq 0.05$ was the criterion used for statistical significance.

RESULTS

At 56 and 112 d post-grafting, the mass of grafts from nontrained rats was 38 and 36% less than the control values ($P \leq 0.05$; Table 1). The effect of endurance running was most pronounced during the first 28 d of training and increased the graft mass by 49% over the non-run value ($P \leq 0.05$; Table 1 and Fig. 1). Graft mass in the trained group was not significantly different from control muscle at 56 and 112 d.

The fiber cross-sectional area irrespective of fiber type of soleus grafts from nontrained rats was 65 and 67% less than the control values at 56 and 112 d, respectively ($P \leq 0.05$; Table 1). There was no effect of endurance running on fiber cross-sectional area during the first 28 d of training. After 84 d of training (i.e., 112 d post-grafting), the 26% increase in area irrespective of type was significant only at $P = 0.08$ (Table 1 and Fig. 1). For Type I fibers at 112 d, the cross-sectional area was 30% greater than the non-run graft value ($P \leq 0.05$; Table 2). Running had no effect on the cross-sectional area of Type IIA or IIB fibers.

There was a larger percentage of Type I fibers with small cross-sectional areas in grafts compared to control muscles as the frequency distribution of graft areas was skewed to the left of the distribution of control muscles (Fig. 2). At 56 d, there were fewer Type I fibers ≤500 μm^2 in grafts from run-trained than in grafts from nontrained rats ($P \leq 0.05$). At 112 d, the decreased percentage of the smallest fibers (≤500 μm^2) with training was accompanied by an increased percentage of fibers ≈3500 μm^2 ($P \leq 0.05$; Fig. 2).

At 56 d, the Type IIA fibers accounted for a greater percentage of the total number of fibers in grafts from

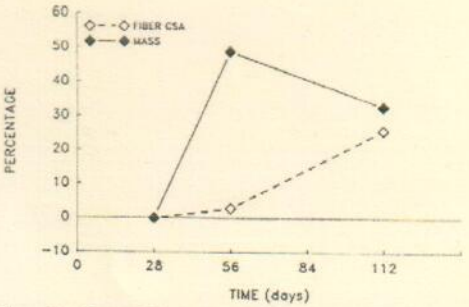

Figure 1—Mass and fiber cross-sectional area irrespective of fiber type of grafts from run-trained rats expressed as a percentage of the value for grafts from nontrained rats. Grafting operations occurred at day 0, and training began at day 28.

TABLE 1. Mass and fiber cross-sectional area of control soleus muscles and soleus grafts from untrained and trained rats.

	Muscle Mass (mg)			Fiber Cross-Sectional Area (μm^2)		
Group	28 d	56 d	112 d	28 d	56 d	112 d
Control	91 ± 1.1 (4)	134 ± 5.1 (10)	148 ± 6.4 (8)	N/A	3,573 ± 152 (10)	3,709 ± 80 (8)
Graft, untrained	63 ± 10.0* (7)	82 ± 7.8* (9)	95 ± 5.3* (8)	843 ± 67 (7)	1,235 ± 137* (9)	1,239 ± 127* (8)
Graft, trained	N/A	122 ± 5.9† (9)	126 ± 10.8† (6)	N/A	1,271 ± 81* (9)	1,560 ± 173‡ (6)

Values are mean ± SE. Numbers in parentheses represent the number of muscles in each group.
* Different from control value ($P \leq 0.05$).
† Different from untrained graft value ($P \leq 0.05$).
‡ Untrained graft value vs trained graft value: $P = 0.08$.

TABLE 2. Histochemical profiles of control soleus muscles and soleus muscle grafts from untrained and trained rats.

Group	Fiber Type:	56 d I	56 d IIA	56 d IIB	112 d I	112 d IIA	112 d IIB
Cross-Sectional Area							
Control		3,633 ± 155 (10)	2,670 ± 144 (9)	1,593 ± 642 (3)	3,786 ± 99 (8)	2,769 ± 243 (4)	1,580 ± 1,338 (2)
Graft, untrained		1,287 ± 135* (9)	794 ± 103* (9)	553 ± 186 (6)	1,271 ± 134* (8)	678 ± 224* (7)	1,000 ± 363 (4)
Graft, trained		1,451 ± 93* (9)	663 ± 69* (9)	797 ± 310 (7)	1,647 ± 201*† (6)	642 ± 81* (5)	743 ± 169 (4)
Percentage by Number							
Control		93 ± 2	6 ± 1	1 ± 1	94 ± 3	5 ± 3	1 ± 1
Graft, untrained		83 ± 4	12 ± 4*	5 ± 1*	94 ± 3	4 ± 1	3 ± 2
Graft, trained		79 ± 5*	16 ± 4*	5 ± 2	93 ± 3	6 ± 3	1 ± 1
Percentage by Area							
Control		95 ± 1	5 ± 1	0	98 ± 2	5 ± 2	0
Graft, untrained		88 ± 5	10 ± 4	2 ± 1	96 ± 2	2 ± 1	2 ± 1
Graft, trained		89 ± 3	9 ± 8	2 ± 1	97 ± 1	3 ± 1	1 ± 1

Values are mean ± SE. Numbers in parentheses represent the number of muscles containing fibers of that type.
* Different from control value ($P \leq 0.05$).
† Different from untrained graft value ($P \leq 0.05$).

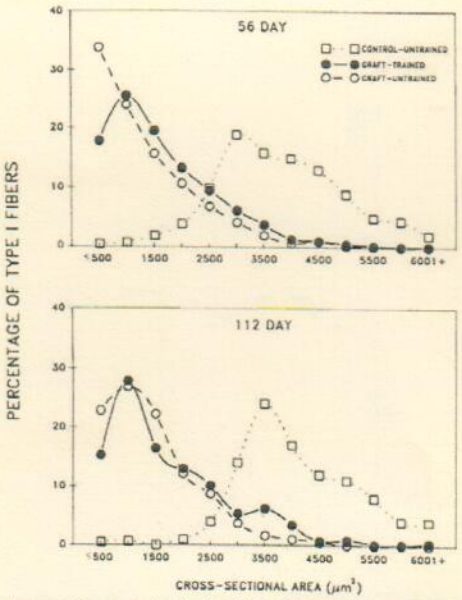

Figure 2—Frequency distribution of Type I fiber cross-sectional area in control and grafted muscles 56 and 112 d following grafting. Fiber cross-sectional area is presented on the *ordinate* in increments of 500 μm^2, with the percentage of fibers in a given range on the *abscissa*.

both trained and nontrained rats than in control muscles ($P \leq 0.05$; Table 2). Compared to control muscles, there were fewer Type I fibers in grafts from run-trained rats ($P \leq 0.05$), but this was not evident in grafts from nontrained rats. There were no differences between grafts and control muscles when profile data are expressed as a percentage by area. This results from the fact that the cross-sectional area of Type I fibers is larger relative to Type II fibers, and this relative difference is more pronounced in grafts than in control muscle. At 112 d, fiber type profiles of grafts were unaffected by training and did not differ from control muscles when expressed as percentage by number and by area.

DISCUSSION

After the first 7 d following grafting, changes in the mass of soleus grafts are paralleled by changes in protein content (27). Thus, mass values reported herein at 56 and 112 d following grafting are not inflated by edema. A dissociation between mass and fiber cross-sectional area of grafts compared to control muscle was evident at both time points as mass was 37% less than control value while area was 66% less. This dissociation has been documented previously in grafts of rats but appears to be specific to muscle type, gender, and operative procedure (7,8). A marked dissociation occurred in nerve-intact soleus grafts of male rats in which fiber cross-sectional area attained 40% of control value while mass was not different (7). In female rats with nerve-intact grafting operations, no dissociation was observed in soleus muscle, whereas a small dissociation was evident in extensor digitorum longus muscle (7). The smaller dissociation in nerve-intact grafts compared to nerve-implant or free grafts is the result of facilitated reinnervation and increased fiber cross-sectional area with the nerve-intact procedure (6). These observations lend support to the hypothesis that the mechanism for the dissociation is related to innervation.

Connective tissue protein concentration and inulin space of 56 d nerve-implant soleus grafts are 77 and 35% greater than control values, respectively (23). Since these variables are small fractions of the total mass, even large relative changes explain only a portion of the dissociation between mass and fiber cross-sectional area. Muscle mass can also be affected by changes in fiber architecture (12). While muscle length decreased slightly (9%) in grafts, fiber length increased 23% above control values (23), but this too does not fully account for the dissociation between mass and area. While the aforementioned changes in architecture are minor with respect to the mass of entire muscle, the dissociation between mass and fiber cross-sectional area likely results from the aggregate sum of many small differences rather than a large change in any one variable.

Since a whole muscle must equal the sum of the constituent parts, an increased number of fibers could be a viable explanation for part of the dissociation between mass and fiber cross-sectional area in nerve-implant soleus grafts. Theoretically, a 40% increase in fiber number appearing in cross-section, in concert with the known changes in fiber length, inulin space, and connective tissue concentration (23), would resolve the observed dissociation between mass and fiber cross-sectional area in nerve-implant soleus grafts. Fibers counted in cross-section might lead to erroneous interpretations unless all fibers in the muscle are represented (13,17). Due to the nature of the measurements made herein, we do not have direct fiber counts, and because of the aforementioned concerns we chose not to count fibers in cross-section.

No difference in fiber number has been observed between nerve-intact or free extensor digitorum longus grafts and control muscle (4,6). There is no reason to expect group comparisons not to be valid, but it should be noted that the fiber number was obtained from cross-sections and the values are ≈25% less than control values obtained with direct counts (9,13). Soleus grafts could differ considerably from extensor digitorum longus grafts with respect to cellular proliferation because of the higher recruitment and weight-bearing function of the soleus muscle during standing and locomotion compared to ankle dorsiflexors (1,25). Additionally, there are more satellite cells in both control and regenerated soleus muscles compared to EDL muscles (22). Satellite cells are the precursor stem cells for skeletal muscle regeneration (5,24). A 60% proliferation of fibers was found in soleus grafts compared to control muscle (21), and a two-fold difference in fiber number was observed in cross-section when soleus grafts were loaded further by ablation of synergistic muscle (8). The degree of hyperplasia induced by increased functional load in nongrafted muscle is not resolved. A small increase in fiber number has been observed in muscles that were subjected to force loads induced by weightlifting in cats (14,15), and nascent fibers appeared in hypertrophied chicken anterior latissimus dorsi muscles (16). However, cell number did not change in muscles of rats when hypertrophy was induced by ablation of synergistic muscles (13).

Another possible mechanism for increased muscle mass in the absence of increased fiber cross-sectional area is branching of the fibers which are present. Preliminary studies in our laboratory have indicated that fiber branching is prevalent in soleus muscle grafts. The notion of fiber branching and formation of multiple fibers within one basal lamina in grafts has been raised previously (20,21). Ontell (20) showed that, in mice, grafting resulted in significant branching which persisted even after 100 d.

The observation that mass and fiber cross-sectional area increase at different times with training may reflect alterations in recruitment and loading patterns in grafts. During locomotion at selected velocities and grades, electromyographic activity of 56 d soleus grafts does not differ from control muscles (26). Since nerve-implant soleus grafts have capacities for isometric force development (P_O) which are 42% of control value (23), the power developed during running is likely a greater percentage of maximum in grafts compared to control muscles. Furthermore, the lower percentage of Type I fibers in 56 d grafts compared to control muscles is additional evidence that some fibers in grafts may not be innervated, as differentiation to Type I requires innervation in control muscle (2) and grafts (5,6).

A population of denervated fibers coupled with the lower P_O would increase the loading of the viable innervated fibers in grafts during locomotion. The increased loading may result in injury to these fibers as high force production relative to P_O induces fiber injury (18). Coan and Tomanek (8) found a two-fold increase in cross-sectional fiber number in soleus grafts subjected to an increased load due to ablation of synergist muscle. Many of the fibers in the grafts were very small, and the majority of grafts were resorbed, indicative of an overload coupled with inadequate reinnervation (8). Obtaining unequivocal evidence for hyperplasia in grafts due to training *per se* will be difficult, for it is likely not possible to distinguish small fibers that may result from branching due to training from the large pool of such fibers (20) which are normally present in grafts.

The increased fiber cross-sectional area in the absence of changes in mass during the latter portion of training could be accounted for by cellular hypertrophy coupled with decreased connective tissue, inulin space, and/or fiber number. Hypoplasia due to lack of reinnervation of some fibers in grafted muscle has been reported previously (5,6,8). The fiber type profile observed at 112 d in grafts did not differ from control value and is consistent with this interpretation, and it likely results

from differentiation of innervated fibers to Type I and the resorption of Type II fibers which were not reinnervated.

In summary, there is a dissociation between mass and fiber cross-sectional area in nerve-implant soleus grafts compared to control muscles. It is possible that soleus grafts contain more fibers than control muscles, but this possibility requires experimental confirmation. Run training affects these variables by similar magnitudes, but adaptations occur at different times. The different temporal sequences of adaptations of mass and fiber cross-sectional area to training likely reflect alterations in recruitment and fiber loading patterns in grafts.

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