

# Exercise-induced adaptations of rat soleus muscle grafts

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WHITE, TIMOTHY P., JOHN F. VILLANACCI, PEDRO G. MORALES, STEVEN S. SEGAL, AND DAVID A. ESSIG. *Exercise-induced adaptations of rat soleus muscle grafts*. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 56(5): 1325-1334, 1984.—In female Wistar rats ( $n = 316$ ) under pentobarbital sodium anesthesia, the soleus muscle was autografted with its nerve reimplanted. One purpose was to characterize the chronological development of graft innervation and recruitment during locomotion. Furthermore, we tested hypotheses regarding the efficacy of run conditioning of different intensities, durations, and postgrafting initiation times to alter mass and pyruvate-malate oxidation capacity of grafts. Choline acetyltransferase activity of grafts increased from 10% of control value at 7 days postgrafting to 55 and 100% at days 28 and 56, respectively. Running-induced glycogen depletion occurred in grafts; this is consistent with graft recruitment during locomotion. There was a threshold of conditioning intensity below which no improvements occurred and above which there were improvements. Sprint (50 m/min) and endurance (30 m/min) conditioning of a duration of at least 28 days that was initiated at 28 or 56 days postgrafting increased mass of grafts by 30% compared with grafts from nonconditioned rats. Easy conditioning (15 m/min) had no effect on graft mass. Changes in graft total protein content paralleled those of mass. Oxidation capacity of grafts increased significantly with some conditioning protocols, but not to the same extent as mass. The exercise-induced adaptations should improve graft function in the host organism.

muscle transplantation; oxidation capacity; muscle growth; myogenesis; physical conditioning; physical training; running; skeletal muscle

GRAFTING of whole skeletal muscles has been used in humans for the clinical treatment of partial facial paralysis, of anal and urinary incontinence, and in reconstruction after trauma or disease (14). Gross inspection and electromyographic assessment of grafts in humans indicate a significant improvement in function after the grafting of muscle into areas where function was previously lacking.

Animal studies have shown that after grafting skeletal muscle fibers degenerate and regenerate. The structural and functional characteristics of grafts change with time and reach a stable profile. These changes have been well documented in rat (6), cat (12, 25, 34), and monkey muscles (23). Many of the characteristics of the grafts remain below control values, including mass, functional cross-sectional area, and oxidation capacity. These variables potentially limit the usefulness of the graft to the

host organism in tasks requiring strength or endurance.

Regularly performed exercise can result in adaptive responses in normal control mammalian skeletal muscle. The nature of the exercise stimulus determines the rate, magnitude, and type of adaptation (10, 18). Electromyographical evidence from stable grafts in freely moving cats indicates that the grafts are recruited (16). The level of habitual use may influence the functional and structural characteristics of autografts.

One purpose of this study was to characterize the chronological development of soleus graft innervation and usage during running at selected times after grafting surgery. Additionally, we investigated the efficacy of physical conditioning of different intensities and durations to improve the mass and oxidation capacity of autografted soleus muscle. We hypothesized 1) that exercise-induced growth of grafts would be greatest when conditioning was initiated at the nadir of the growth curve of grafts from nonconditioned animals, 2) that growth of grafts would be greater in rats required to sprint condition than condition at slower speeds, and 3) that oxidation capacity of grafts would increase in response to exercise conditioning.

## METHODS

Female Wistar rats ( $n = 316$ ), pathogen-free, were obtained from Charles River Laboratories at 6 wk of age. Grafting operations were performed within 1 wk of arrival. All procedures were performed in accordance with the Principles in the Care and Use of Animals of the American Physiological Society. Rats were provided food and water ad libitum, and kept in a light-dark environment (LD12:12). Rats were anesthetized with pentobarbital sodium (initially 35 mg/kg ip; supplemented as required). To graft the soleus muscle, an incision was made through the skin and fascia on the lateral aspect of lower leg. The soleus muscle was isolated, and the nerve, blood vessels, and the proximal and distal tendons were cut. The muscle was replaced in its original orientation, and the tendons of the graft were sutured (7-0 silk) to the tendon stumps. The soleus nerve was implanted at the original motor end plate region and anchored with a purse-string suture (7-0 silk). The fascia and skin were closed separately with 5-0 monofilament nylon. Drinking water was supplemented with tetracycline for 4 days, starting the day before grafting. In 40 animals, grafting was performed bilaterally, whereas in others ( $n = 249$ ) one side was grafted and the other side

was a sham-operated control. We noted no differences in body masses and in all measured variables between grafts due to the bilateral or unilateral operations. There were no differences in any of the variables assessed between sham-operated control muscles and normal control soleus muscles of additional age-matched rats ( $n = 27$ ) that had no surgical interventions. Therefore data were pooled and no further distinction will be made regarding these methodological differences.

**Recruitment study.** An initial experiment was conducted to characterize the early time course of development of reinnervation in soleus grafts and to determine the time following grafting when the animals could use the grafts during locomotion. At 7, 14, 21, and 28 days after unilateral grafting, rats ( $n = 10, 5, 5,$  and  $5$ , respectively) were anesthetized, and graft and control muscles were analyzed for glycogen content and choline acetyltransferase (ChAT) activity. Control muscles and grafts from equal numbers of similar animals were used to obtain the same variables after a continuous 45-min run to exhaustion. The exhaustion protocol began with animals running on a motor-driven treadmill at 24 m/min at a 15% grade. After 15 min, speed and grade were increased every 5 min such that by 40 min they were running at 30 m/min at a 20% grade. The grade was held constant for the final 5 min, and the speed was varied between 24 and 30 m/min. By 45 min, the rats were exhausted as evidenced by lack of righting behavior.

After removal of muscles, visible fat and tendons were dissected free. The remaining tissue was weighed and homogenized in 9 vol 0.5% Triton X-100 in 10 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4. ChAT activity was used as a marker of reinnervation (7, 8, 24) and was assayed from the homogenate according to the method of Fonnum (13) and modified for changes in choline specificity (24). ChAT was determined on control muscles and grafts at 56 days after grafting in other animals ( $n = 5$ ). Glycogen depletion during the run was used as a qualitative marker of muscle or graft use (2, 3). To ensure consistency between ChAT and glycogen data, a 500- $\mu$ l sample of the ChAT homogenate was aspirated, frozen, and lyophilized. Five to ten milligrams of dry mass was acid hydrolyzed in 0.5 ml 2.0 N HCl at 95–100°C for 2 h. Samples were neutralized with 667 mM NaOH, brought to 2.0-ml final volume, and the free glucose residues were assayed enzymatically (28) in a fluorometer.

**Conditioning study.** In a second series of experiments, other rats were randomly assigned to nonconditioned or run-conditioned groups. The latter were characterized by the time between grafting surgery and the initiation of a run-conditioning protocol (14, 28, and 56 days), the duration of the conditioning protocol (7, 14, 28, 56, and 84 days), and the intensity of running (easy, endurance, and sprint). Easy running was defined as 15 m/min for 1 h/day, endurance running as 30 m/min for 1 h/day, and sprint running as 50 m/min for 10 min/day. On the first day of easy and endurance conditioning rats ran for 15 min at 80% of the final speed. Duration and speed were increased daily, such that they were running at the final exercise level by day 4. Sprint-conditioning rats

increased from  $2 \times 1.25$  min sprints on day 1 to the maintained exercise level of  $4 \times 2.50$  min by day 4. The running was performed in a 10-stall motor-driven small animal treadmill at 15% grade. Performance was enforced when necessary with mild electrical shock and forced air.

At death, graft and control soleus muscles were removed, trimmed of tendons and fat, and weighed. A sample was cut for histology from the full cross section of the muscle belly and quick-frozen in isopentane cooled to  $-70^\circ\text{C}$  by dry ice. Transverse sections 10  $\mu$ m thick were cut in a cryostat ( $-20^\circ\text{C}$ ) and stained with hematoxylin and eosin. The remainder of the muscle was trimmed of fascia and homogenized in 9 vol of ice-cold 175 mM KCl and 2 mM EDTA, pH 7.4, preparatory to quantifying oxidation capacity with pyruvate-malate as the substrate (19). Pyruvate-malate oxidation was measured at  $30^\circ\text{C}$  in an oxygen electrode system. A 1.0-ml reaction medium contained the following (in mM):  $\text{MgCl}_2$  5; malate sodium 1; KCl 87.5; potassium phosphate buffer 40; EDTA 1.6; pyruvate sodium 2.5; cytochrome *c* 0.078; sucrose 50; adenosine 5'-diphosphate 2. A volume of homogenate corresponding to 15 mg tissue was added. Protein concentration of the homogenates was assayed by the procedure of Lowry et al. (21).

Data on tissue mass are based on all animals in the study. Biochemical measurements of oxidation capacity and total protein were not determined for every tissue. Control muscles and grafts not used for biochemical measurements were used for parallel studies of protein synthesis (11) and functional recruitment (31). For biochemical measurements, each data point represents between 4 and 14 muscles [mode 5, median 5;  $6.9 \pm 2.9$  (mean  $\pm$  SD)]. In a very few instances, the number of independent measurements made were less than the number of muscles represented. In these instances, it was because grafts were small and two had to be combined to provide enough tissue for analysis. Means  $\pm$  SE were calculated for grouped data. Differences between groups were statistically evaluated with analysis of variance and least-squares regression analysis. Post hoc analyses were made at the 95% confidence level. These analyses followed the Welch-Aspin convention and did not require equal sample size and assumed variances to be unknown and not necessarily equal (22).

## RESULTS

Histological observations of our nerve-implant soleus grafts during the early time course after grafting indicated that 95% of the fibers degenerate. Approximately 5% of the fibers located in a thin peripheral rim survive the ischemic period. After the degenerative phase, new muscle cells regenerate. These processes of degeneration and regeneration have been described in detail by Carlson and co-workers (6) for free grafts of the extensor digitorum longus muscle of the rat.

The ChAT assay was variable, as evidenced by group values for control soleus muscle ranging from  $236 \pm 18.7$  to  $328 \pm 19$  pM Ach formed per hour per milligram protein. Because of this variability, we found it impera-

tive to simultaneously measure ChAT activity of graft and control muscles and express our graft data as a percentage of control muscle value. In the experiment characterizing the reinnervation and running-induced glycogen depletion of soleus grafts, ChAT activity of control muscles or grafts was not different between rats that ran to exhaustion and those which did not run. Therefore, data for this variable were pooled. ChAT-specific activity in grafts increased significantly from 10% of control value at 7 days after grafting to 25% at day 14, 42% at day 21, 52% at day 28, and 100% at day 56.

The run to exhaustion reduced the concentration of glycogen in control soleus muscle by 60% (Fig. 1). After grafting, resting glycogen concentration increased 16% with time but was always significantly less than values for resting control muscle. The 45-min exhaustive run significantly decreased glycogen concentration in grafts at all times after grafting. The magnitude of the change increased with time; at 7 and 28 days glycogen use was 25 and 55% of the nonrun value, respectively.

The changes in body and muscle mass after grafting were documented for this model and increased over the 112 days after grafting (Tables 1-4;  $P < 0.05$ ). The 45% increase in body mass and control soleus muscle mass with time in these growing animals precluded comparison between groups of absolute mass across time. When control muscle mass (in milligrams) was expressed relative to body mass (in grams), the ratio ( $0.497 \pm 0.009$ ) did not change with time. Consequently, to address the issue of exercise-induced growth of grafts, we chose to analyze graft mass expressed relative to body mass to minimize potential interpretive difficulties attributable to ontogenic growth.

After grafting and without exercise intervention, the muscle mass-to-body mass ratio increased to 170% of control value at day 4 and decreased to a nadir of 45% between days 21 and 28 (Table 1, Fig. 2). Thereafter, the muscle mass-to-body mass ratio increased gradually and

reached 60% of control by day 56 and 65% of control by day 112.

To test the hypothesis regarding the effects on graft mass of initiating run conditioning at different times postgrafting, we compared grafts in which endurance conditioning started at 14, 28, and 56 days after surgery. With conditioning starting at 14 days (Table 2), mass of the grafts through day 56 was not significantly different from grafts of nonconditioned rats (Table 1). When conditioning started 28 days postsurgery (Table 3, Fig. 2), the mass of grafts was not different from that of nonrunners until day 56 when they were 30% greater ( $0.39 \pm 0.02$  mg/g) than the value for unconditioned animals ( $0.31 \pm 0.01$  mg/g) ( $P < 0.05$ ). With continued conditioning, graft mass increased slowly and by day 112 values were not different from control soleus muscles ( $P > 0.05$ ). After 28 and 56 days of run conditioning, which commenced 56 days postgrafting (i.e., at 84 and 112 days

TABLE 1. Descriptive data for nonconditioned animals at selected days after grafting

Postgrafting Conditioning	Days			
	14	21	28	35
	0	0	0	0
	Mass			
Body, g	207 ± 17 (8)	247 ± 10 (11)	234 ± 5 (31)	230 ± 5 (22)
SOL <sub>c</sub> , mg	103 ± 5 (9)	111 ± 3 (9)	115 ± 3 (26)	106 ± 3 (26)
SOL <sub>g</sub> , mg	60 ± 8 (7)	60 ± 4 (12)	56 ± 3 (32)	67 ± 4 (16)
	Days			
Postgrafting Conditioning	42	56	84	112
	0	0	0	0
	Mass			
Body, g	256 ± 3 (22)	270 ± 3 (41)	279 ± 6 (7)	299 ± 12 (10)
SOL <sub>c</sub> , mg	129 ± 3 (22)	129 ± 3 (43)	134 ± 6 (9)	148 ± 6 (10)
SOL <sub>g</sub> , mg	71 ± 3 (20)	82 ± 3 (37)	84 ± 7 (5)	95 ± 5 (8)

Values are means ± SE; nos. in parentheses are no. of rats or muscles. SOL<sub>c</sub>, SOL<sub>g</sub>, control and grafted muscle mass, respectively.

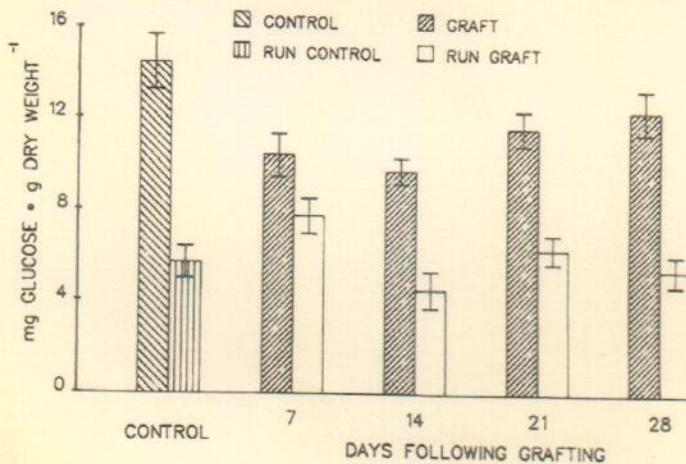


FIG. 1. Glycogen concentration of grafts and control soleus muscles for nonrun rats and rats following a 45-min run to exhaustion. Control histograms are each based on 25 muscles. Graft histograms are based on 10 grafts each at day 7 and 5 grafts each at days 14, 21, and 28. Data are means ± SE.

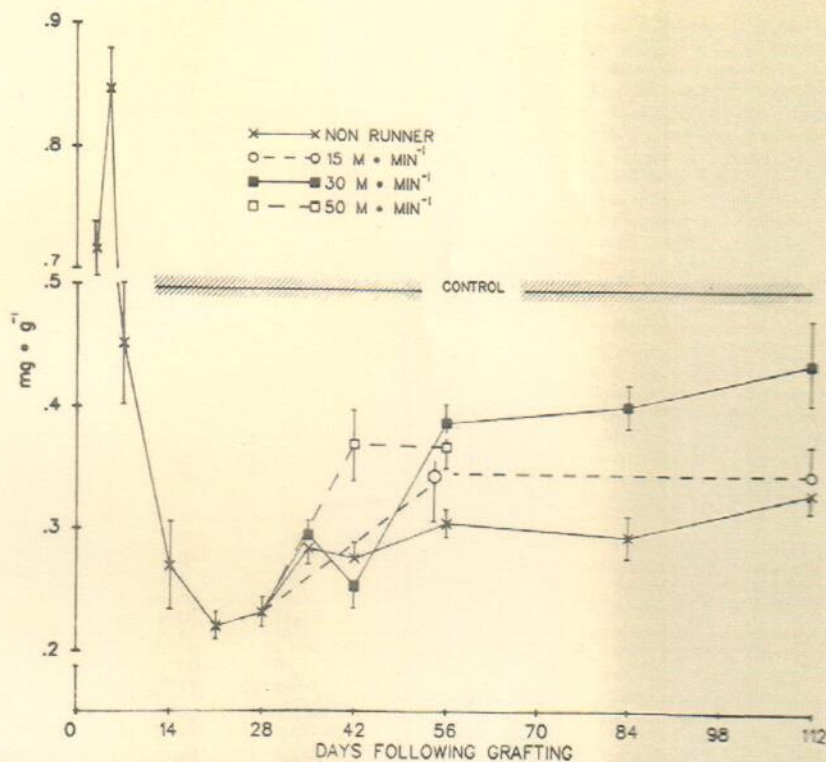


FIG. 2. Comparison of intensity of exercise conditioning that began 28 days postgrafting on mass of soleus grafts, expressed relative to body mass. Graft muscle:  $n = 6, 8,$  and  $13$  at  $2, 4,$  and  $7$  days postgrafting.

TABLE 2. Descriptive data for animals conditioned at  $30$  m/min commencing 14 days postgrafting

Postgrafting Conditioning	Days			
	21	28	42	56
	7	14	28	42
	Mass			
Body, g	$206 \pm 4^*$ ( $n = 5$ )	$229 \pm 4$ ( $n = 5$ )	$255 \pm 4$ ( $n = 10$ )	$250 \pm 5^*$ ( $n = 5$ )
SOL <sub>c</sub> , mg	$104 \pm 4^*$ ( $n = 4$ )	$134 \pm 8$ ( $n = 5$ )	$131 \pm 3$ ( $n = 10$ )	$125 \pm 3$ ( $n = 5$ )
SOL <sub>g</sub> , mg	$42 \pm 1^*$ ( $n = 5$ )	$63 \pm 5$ ( $n = 5$ )	$76 \pm 3$ ( $n = 8$ )	$88 \pm 9$ ( $n = 5$ )

Values are means  $\pm$  SE;  $n =$  no. of rats or muscles. SOL<sub>c</sub>, SOL<sub>g</sub>, control and grafted muscle mass, respectively. \* Significantly different from nonconditioned group (Table 1) at same day postgrafting ( $P < 0.05$ ).

after grafting; Table 4), mass of grafts was not different from values for animals at the same number of days after grafting that started running at 28 days. However, the mass of grafts in these animals remained statistically

less than that of control soleus muscles ( $P < 0.05$ ). respectively; for other postgrafting times see Tables 1 and 3 for no. of muscles comprising each point. Data are means  $\pm$  SE.

less than that of control soleus muscles ( $P < 0.05$ ).

To test the hypothesis regarding the intensity of exercise, comparisons were made between groups that started conditioning 28 days after grafting and ran at either 15, 30, or 50 m/min (Table 3). In addition, we probed differences between sprint (50 m/min) and endurance (30 m/min) conditioning that commenced 56 days after grafting (Table 4). With 14 days conditioning initiated at day 28, the mass of grafts from sprint-conditioned animals was 45% greater than grafts from endurance-conditioned animals. After 14 days of conditioning that started at day 56, grafts from sprinters were 20% larger than from endurance rats. After 28 days of conditioning, this difference between running intensities disappeared. The greatest and most sustained growth was evident in the grafts of rats that ran at 30 m/min. Grafts from rats that ran at 15 m/min were not significantly larger than those from nonrunners at any time ( $P > 0.05$ ).

Total muscle protein content was calculated as the product of protein concentration of whole-tissue homogenates and muscle wet mass. A positive correlation between total protein content and wet mass was not ap-

parent during the initial 7 days after grafting when total protein content declined immediately (Fig. 3) despite the increase in wet mass (Fig. 2). Thereafter, total protein content followed the same qualitative time course as the wet mass responses. For example, the greatest and most sustained increase in total protein content was evident

TABLE 3. Descriptive data for animals conditioned at 15, 30, or 50 m/min commencing 28 days postgrafting

	Days				
Postgrafting	35	42	56	84	112
Conditioning	7	14	28	56	84
Running at 15 m·min <sup>-1</sup>					
Body mass, g	NA	NA	268 ± 13 (5)	NA	284 ± 16 (4)
SOL <sub>c</sub> , mg	NA	NA	NA	NA	NA
SOL <sub>g</sub> , mg	NA	NA	91 ± 9 (9)	NA	96 ± 10 (6)
Running at 30 m·min <sup>-1</sup>					
Body mass, g	242 ± 4 (19)	255 ± 5 (10)	273 ± 4 (30)	268 ± 4 (5)	286 ± 6 (4)
SOL <sub>c</sub> , mg	121 ± 4* (19)	126 ± 2 (10)	137 ± 2 (25)	144 ± 5 (5)	NA
SOL <sub>g</sub> , mg	72 ± 3 (18)	65 ± 5 (8)	106 ± 4* (34)	108 ± 5* (5)	126 ± 11* (7)
Running at 50 m·min <sup>-1</sup>					
Body mass, g	NA	247 ± 8 (9)	274 ± 8 (10)	NA	NA
SOL <sub>c</sub> , mg	NA	132 ± 8 (9)	139 ± 8 (10)	NA	NA
SOL <sub>g</sub> , mg	NA	91 ± 7*† (9)	100 ± 5* (8)	NA	NA

Values are means ± SE; nos. in parentheses are no. of rats or muscles. SOL<sub>c</sub>, SOL<sub>g</sub>, control and grafted muscle mass, respectively; NA, not available. \* Significantly different from nonconditioned group (Table 1) at same day postgrafting ( $P < 0.05$ ). † Significantly different from group conditioned at 30 m·min<sup>-1</sup> at same day postgrafting ( $P < 0.05$ ).

in grafts of rats that ran at 30 m/min compared with 15 and 50 m/min. Inspection of hematoxylin and eosin transverse sections of grafts at 56 days after grafting indicated that grafts were composed primarily of viable muscle fibers (Fig. 4, A-C). The fibers in grafts were smaller than control and were characterized as mature with respect to peripheral nuclei and intact membranes. We conclude that the increased mass of soleus grafts from run-conditioned rats is composed of viable muscle fibers.

Fourteen days after grafting, the in vitro oxidation capacity of graft homogenates ( $2.9 \pm 0.4 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  protein) was 20% of the value for control soleus muscle ( $15.7 \pm 0.4 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  protein; Fig. 5). In the non-conditioned groups, this increased to 50% of control value by day 42 and did not change thereafter through

TABLE 4. Descriptive data for animals conditioning at 30 or 50 m/min commencing 56 days postgrafting

	Days		
Postgrafting	70	84	112
Conditioning	14	28	56
Running at 30 m·min <sup>-1</sup>			
Body mass, g	257 ± 4 (9)	286 ± 5 (5)	299 ± 8 (10)
SOL <sub>c</sub> , mg	128 ± 3 (9)	153 ± 9 (5)	153 ± 7 (10)
SOL <sub>g</sub> , mg	81 ± 5 (9)	115 ± 12 (5)*	112 ± 6 (8)*
Running at 50 m·min <sup>-1</sup>			
Body mass, g	271 ± 10 (10)	291 ± 5 (10)	NA
SOL <sub>c</sub> , mg	142 ± 5 (10)†	144 ± 5 (10)	NA
SOL <sub>g</sub> , mg	100 ± 6 (10)†	103 ± 7 (9)	NA

Values are means ± SE; nos. in parentheses are no. of rats or muscles. SOL<sub>c</sub>, SOL<sub>g</sub>, control and grafted muscle mass, respectively; NA, not available. \* Significantly different from nonconditioned group (Table 1) at same day postgrafting ( $P < 0.05$ ). † Significantly different from group conditioned at 30 m·min<sup>-1</sup> at same day postgrafting ( $P < 0.05$ ).

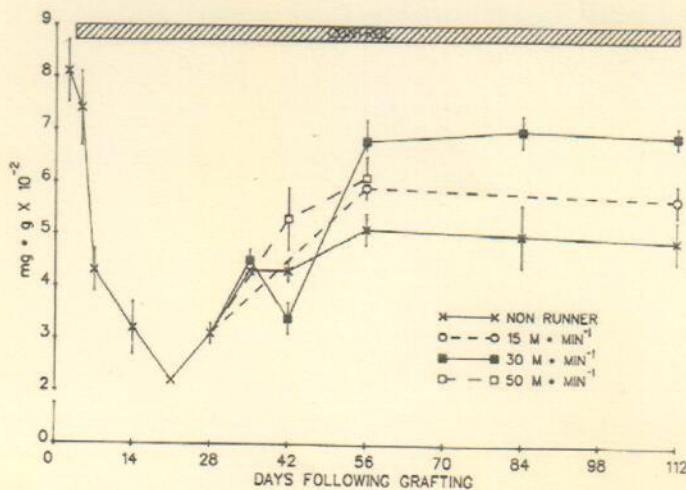


FIG. 3. Comparison of running intensity on total protein content expressed relative to body mass with conditioning starting at 28 days following grafting. See METHODS for no. of muscles comprising each point. Data are means ± SE.

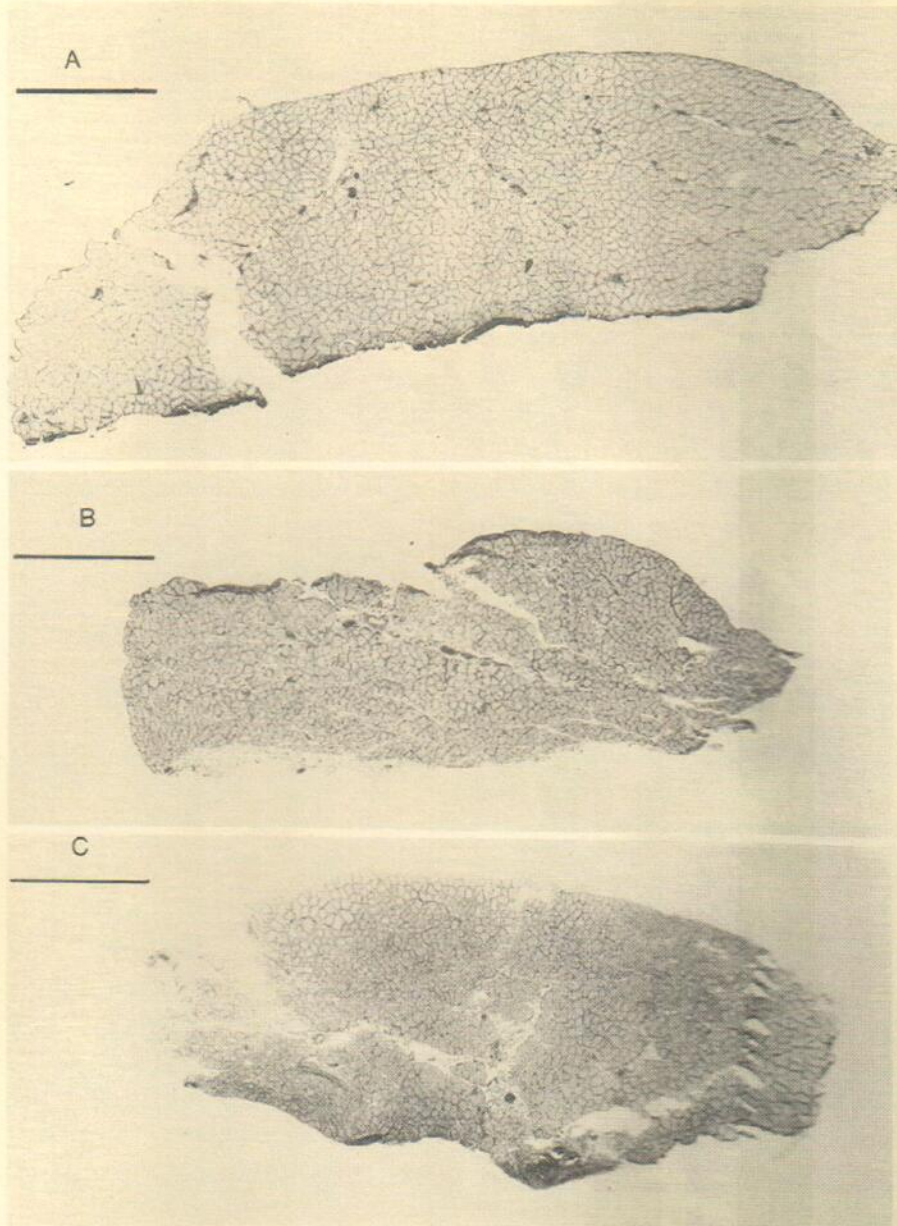


FIG. 4. Transverse hematoxylin and eosin section of control soleus (A), nonconditioned 56-day graft (B), and sprint-conditioned 56-day graft (C). Conditioning started at 28 days postgrafting. Bar, 1.0 mm.

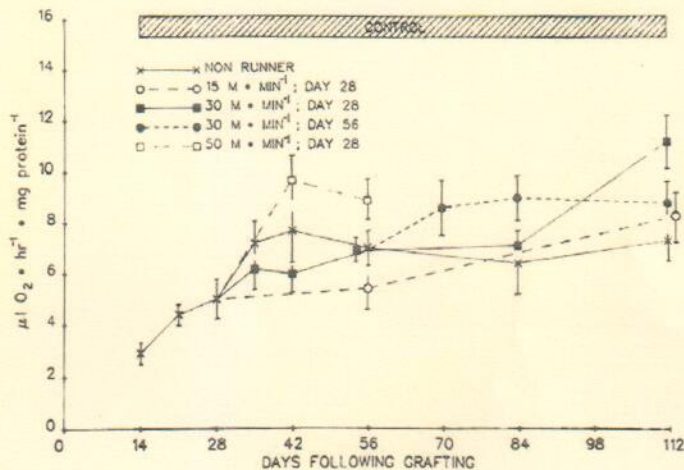


FIG. 5. Comparison of date of initiation of exercise conditioning and intensity of conditioning on oxidation capacity of graft whole muscle homogenates with pyruvate-malate as substrate. See METHODS for no. of muscles comprising each point. Data are means  $\pm$  SE.

112 days. At all times, oxidation capacity of grafts from conditioned or unconditioned animals was lower than unconditioned control soleus muscle (Fig. 5). Easy running initiated 28 days postgrafting did not influence oxidation capacity of grafts even when running continued for 84 days (i.e., through day 112 postgrafting). The effect of endurance conditioning on oxidation capacity was a function of the time conditioning was initiated. Endurance conditioning initiated at 14 days and continued for 42 days did not induce significant changes in oxidation capacity ( $6.35 \pm 0.72 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ) compared with nonrun grafts ( $7.0 \pm 0.7 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ). Endurance conditioning that started at 28 days postgrafting resulted in a 55% increase in oxidation capacity only after 84 days of conditioning (Fig. 5). When endurance running started at day 56, oxidation capacity increased 30% ( $P < 0.05$ ) by 28 days of running and did not increase further, despite continued running.

At given time points of initiation of run conditioning, sprinting had different effects on oxidation capacity compared with endurance running. Fourteen days of sprinting initiated 28 days postgrafting resulted in significantly greater oxidation capacity than an equivalent amount of endurance conditioning ( $P < 0.05$ ). With continued conditioning, the oxidation capacity of grafts from sprint-conditioned rats held constant, whereas endurance-conditioned values increased slightly (Fig. 5). After 28 days of conditioning that commenced at day 56, oxidation capacity of grafts from sprint-conditioned rats ( $8.1 \pm 0.9 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ) was not different from endurance-conditioned rats ( $9.0 \pm 0.9 \mu\text{l O}_2 \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ).

#### DISCUSSION

The establishment of reinnervation after grafting of the soleus muscle is implied by the increase in ChAT-specific activity. During ontogenic myogenesis, the appearance of ChAT activity coincides with the arrival of

the first exploratory nerve fibers (32). Therefore, it may be possible to measure ChAT activity before the establishment of stable and functional nerve-muscle connections. However, in rat extensor digitorum longus (EDL) grafts, Carlson et al. (8) reported an increase in ChAT activity coincident with establishment of motor end plates as visualized with silver staining. This stage of reinnervation occurred between 14 and 21 days postgrafting. Mong et al. (26) demonstrated a sharp drop in extrajunctional acetylcholine receptors in rat EDL grafts between 14 and 21 days following grafting, which is indicative of the formation of functional motor end plates. Our data are consistent with the time course of observations in both of these studies.

The small size of the grafts during the first 28 days after grafting made it technically difficult to use procedures that could quantitatively assess graft use during locomotion. Therefore, glycogen depletion was used as a qualitative marker of muscle recruitment (2, 3). The 45-min run to exhaustion was of sufficient intensity and duration to significantly reduce muscle glycogen in control soleus muscle. The magnitude of the decrease is similar to that reported previously (3). The significant decrease in glycogen content of grafts is consistent with active recruitment of grafts during the run to exhaustion. At 7 days after grafting, the glycogen decrease was the smallest and may reflect glycogen depletion in the thin peripheral rim of surviving fibers. This small decrease in glycogen may reflect the effects of passive stretch during locomotion or result from elevated plasma catecholamine levels during exercise. The progressive increase in glycogen use after grafting is consistent with an enhanced recruitment during locomotion.

Regenerating myofibers are dependent on reinnervation for muscle fiber type differentiation (5). In grafts that are kept denervated the fibers regenerate, undergo secondary denervation atrophy, and differentiation does

not occur. If reinnervation of grafts is facilitated by leaving the nerve intact during grafting surgery, the graft regenerates more rapidly and stabilizes at a greater mass (7). Others who have grafted the soleus muscle in the soleus site have been unsuccessful in producing a viable graft that persisted with time (9, 29). Our surgical procedures differ from those of Coan and Tomanek (9) and Schmalbruch (29) in that we surgically implanted the nerve in the original motor end plate region. Although the graft is denervated, the physical placement of the soleus nerve at the time of grafting provides a scaffold necessary for nerve regeneration and the establishment of functional neuromuscular junctions. We conclude that our grafts are functionally reinnervated based on the ChAT activity, the persistence of mass with time, increased electromyogram activity during locomotion (31), and the differentiation of fiber types (unpublished observations). Furthermore, a contractile response was evoked in grafts when the nerve was mechanically manipulated at the kill time. This contractile response was noted infrequently in kills during the first 21 days after grafting and in over 98% of grafts after that time.

After grafting surgery, the initial increase in graft mass (Fig. 2) was in response to edema caused by the osmotic pressures within the degenerating tissues (6). The immediate decline in total protein (Fig. 3) confirms that the transient increase in wet mass was of nonprotein origin. The temporal changes in graft mass are in agreement with those reported for EDL-free muscle grafts in the rat (6). The decline in skeletal muscle protein due to surgical intervention is not unique to grafting. Jakubiec-Puka and co-workers (20) have indicated a preferential loss of thick filaments relative to other contractile proteins as a result of denervation.

By initiating run conditioning at 14, 28, and 56 days postgrafting, we were able to assess the effects of exercise on a graft during the regenerative phase and on grafts in which mass had stabilized. It is interesting to note that grafts from rats that started endurance conditioning at 14 days postgrafting and continued running for 42 days were not significantly larger than grafts from unconditioned rats. Yet grafts in rats that started conditioning at 28 days postgrafting for only 28 days duration were 30% greater in mass than nonconditioned grafts. It is possible that running during the early phase of reinnervation and regeneration inhibited subsequent growth. In experiments with reinnervating (but not regenerating) skeletal muscle, Herbison et al. (17) indicated that increased "activity" early in the reinnervation process inhibited reinnervation and retarded subsequent muscle development. Our results indicate that acute and chronic running had no deleterious effect on the amount of reinnervation in the graft, as indicated by ChAT activity. A more likely explanation is that in rats which started conditioning at 14 days postgrafting, there must have been a compensatory increase in recruitment of synergistic muscles for the animal to run successfully. As reinnervation and regeneration of fibers in the graft progressed with time, either the greater recruitment of synergists was not attenuated or the progression of motor unit recruitment in grafts occurred in such a gradual

fashion that the stress of running did not exceed a required threshold for adaptive growth. In contrast, when conditioning started 28 days postgrafting, the graft had greater innervation. These grafts must have been recruited sufficiently at the onset of conditioning such that a threshold was exceeded and exercise-induced growth was elicited.

When conditioning started at 28 days postgrafting, the rate of growth induced by running decreased markedly after 28 days of conditioning. We cannot distinguish whether this plateau of growth indicates the exercise stimulus was no longer of sufficient intensity, frequency, and duration to stimulate growth or that the graft had reached its maximum capacity to adapt. Grafts older than 56 days were also capable of exercise-induced growth. The relative improvements in graft size are similar at the two times of conditioning initiation, suggesting that the intrinsic magnitude of the response is independent of the time of initiation.

Compared with endurance conditioning, 14 days of sprint conditioning initiated at either 28 or 56 days postgrafting significantly increased the mass of grafts. There were no persistent differences between the effects of endurance and sprint conditioning, since by the 28th day of conditioning there were no differences in the mass of grafts elicited by these two protocols. It is possible that the energetic demands of 60 min of running per day in the endurance-conditioned groups limited the amount of substrate available for protein biosynthesis compared with the sprint-conditioned group (10 min/day). Additionally, recruitment of muscles synergistic to the soleus is enhanced with sprinting compared with endurance running (15, 31, 33). This may have decreased the functional load on the soleus graft during each step cycle and resulted in an activation-tension state more compatible with inducing growth.

Our study is the first to address the influence of running exercise on enhancing growth of whole free muscle grafts. Two previous studies have attempted to address the influence of increased activity on the growth of regenerating muscle (9, 30). Coan and Tomanek (9) grafted soleus muscles without attaching the nerve and, at 7 days postgrafting, ablated the synergistic gastrocnemius muscle. Ablation attenuated the decline in mass of grafts during this time. However, a large fraction of the larger graft mass was fluid, which was likely attributable to an acute response to the surgical trauma of synergist ablation (1). With continued time following grafting and synergist ablation, the grafts were resorbed, indicative of lack of adequate functional reinnervation. Van Handel and co-workers (30) characterized the effects of exercise on minced muscle grafts. Minced grafts are useful experimentally to demonstrate the extent to which regenerating muscle fibers and their connective tissue stroma can reconstitute a grossly identifiable muscle. However, mincing is not relevant for clinical practice (whereas free whole muscle grafting directly applies) because of the high concentration of connective tissue in the graft and the numerous adhesions of the graft to surrounding tissues (4). Approximately 75% of the gastrocnemius muscle was placed into the site of the entire



triceps surae muscle group in one hindlimb of young rats (30). At 4 days postsurgery, an exercise group began easy treadmill running that continued through 45 days postsurgery. Graft wet mass increased in exercised rats compared with the sedentary group, and in all cases the grafts were smaller than control muscle. Interpretation of these results is compromised, as the animals were studied during a rapid body growth period and neither graft nor control mass were normalized for body size. No data were provided on biochemical or morphological markers of reinnervation.

The improvement in oxidation capacity was related to the intensity of exercise. No change was observed in grafts of rats that ran at the slow speed. The marked increase in oxidation capacity of grafts from sprint-conditioned animals is consistent with data on control soleus muscle in which short-duration sprints increased cytochrome *c* concentration by amounts similar to endurance conditioning of longer duration (10).

We have no data on the linkages between the running exercise and the adaptive increase in mass. In part, it appears related to running intensity. Whether frequency of training is a primary variable remains for further study. A threshold of running intensity, above which an improvement in mass of grafts occurs and below which there is insufficient stimulus to elicit an adaptive response, appears to exist. Easy running (15 m/min) was below this threshold, and both endurance (30 m/min) and sprint (50 m/min) conditioning were suprathreshold.

The percent increase in mass exceeded the percent improvement in oxidation capacity due to either sprint or endurance conditioning. For example, at 112 days postgrafting in the endurance group that started conditioning at 28 days, mass was not different from control, whereas oxidation capacity was 75% of control value. These running protocols had no effect on the mass of control soleus muscle (Tables 1-4). Others have shown that conditioning programs of similar intensity but longer duration elicit a twofold increase in oxidative

enzymes in control soleus muscle without effecting a change in muscle mass (10, 18). It is tempting to conclude that the mass of muscle grafts is more adaptable to exercise conditioning than control muscles. This interpretation may not be warranted because compared with control muscle the conditioning stimulus imposed on the regenerating soleus may have resulted in an afterload at a greater percentage of the maximum capacity to develop tension, thereby eliciting a growth response. Indeed, 28 days of isometric training of control muscles in rat hindlimbs leads to significant fiber growth (27). Compared with endurance running, isometric training undoubtedly invokes a greater afterload on control muscle during contraction. These results illustrate that the conditioning stimuli for skeletal muscle grafts need to be evaluated in specific terms relative to the capacity of the graft, rather than in absolute terms. This may be especially important for future applications of exercise conditioning in clinical rehabilitation.

The increased mass and oxidation capacity of grafts due to run conditioning should have physiological benefits to the host organism. The increase in oxidation capacity presumably reflects an enhanced ability to generate ATP by aerobic metabolism. If this is the case, autografts with improved oxidation capacity due to physical conditioning would likely demonstrate a greater resistance to fatigue. Furthermore, since the larger mass of conditioned grafts is composed of viable muscle fibers, exercise-conditioned grafts should be capable of generating greater maximal tension.

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