The contractile properties of standard and nerve-intact grafts of extensor digitorum longus muscles of rats were compared in vitro. Fourteen days after grafting, the time to peak tension and the half-relaxation times of nerve-intact grafts were shorter than those of standard grafts, but both were longer than control values. By 60 days, these variables attained normal values. At every sample period, the tetanic tensions of nerve-intact grafts were higher than those of standard grafts. Even at the early sampling periods, the twitch-tetanic tension ratios of nerve-intact grafts were close to normal values, whereas those of standard grafts were higher than normal. Stabilized nerve-intact grafts had a larger mass and greater maximum tetanic tension development than standard grafts, but were more fatigable. Compared with control EDL muscles, stable nerve-intact grafts show no differences except for lessened fatigability, whereas standard grafts demonstrate significant functional deficits.

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CONTRACTILE PROPERTIES OF STANDARD AND NERVE-INTACT MUSCLE GRAFTS IN THE RAT

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The nerve-intact model[7] is a useful tool in the analysis of factors that affect the mass and functional recovery of free muscle grafts. A nerve-intact graft is similar to a standard muscle graft in that both are completely removed from their beds and are completely devascularized, but the neural connections to the standard muscle graft are cut, whereas they are retained in a nerve-intact graft. In both types of grafts, all of the muscle fibers, except for a thin peripheral rim, degenerate from prolonged ischemia and subsequently regenerate.[1] Functional neuromuscular transmission begins in standard grafts at the end of the third or early in the fourth week, whereas it is restored early in the second week in nerve-intact grafts.[7] The number of skeletal muscle fibers in the standard and nerve-intact grafts of the rat extensor digitorum longus (EDL) muscle does not differ from values for control EDL muscles. In contrast to the standard grafts, which are typically restored to approximately half of normal mass and contractile force, nerve-intact grafts approach normal values for these characteristics. It has recently been shown that the greater mass and force development of nerve intact grafts cannot be attributed to the earlier restoration of the nerve supply, but is more likely due to the broader distribution of nerve fibers throughout the grafts.[2]

The present study was designed to document the development of contractile properties in nerve-intact grafts compared with standard grafts of the EDL muscles in rats. These results on the rat will then be compared with those obtained by Faulkner et al.[14] on standard and nerve-intact grafts of EDL muscles in cats.

MATERIALS AND METHODS

This experiment was carried out on 33 male Sprague-Dawley rats. At the time of muscle grafting, the rats weighed 175–200 g. The rats were anesthetized with ether. In one leg, the EDL was completely removed from its bed and then orthotopically replaced, with both tendons sutured to their respective stumps. No attempt was made to facilitate restoration of the neural or vascular supply (standard grafts). In the other leg, the EDL

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muscle was treated identically, except that the continuity of the motor nerve was not interrupted (nerve-intact grafts). In the nerve-intact grafts, ischemia during the immediate postgrafting period results in the degeneration of intramuscular portions of the nerves.[7] Grafts were evaluated at 14, 20, 30, and 60 days after grafting.

Animals were anesthetized and the EDL muscles or grafts were isolated and removed. The muscles or grafts were attached to a force transducer and immersed in a mammalian Krebs buffered bicarbonate solution.[9] Isometric contractile properties were measured in vitro at 37°C. The muscles and grafts were stimulated directly with two platinum field electrodes. Stimulation was by square-wave pulses of 0.2 msec duration and supramaximum voltage. The variables measured were: time to peak twitch tension (TPT) and half-relaxation time (RT 1/2), maximum twitch tension (P_t), maximum tetanic tension (P_o), and the optimal muscle length (L_o) for tension development.[15]

Muscle lengths were measured at L_o, and fiber lengths were estimated based on a fiber length/muscle length ratio of 0.4.[11] The cross-sectional area was calculated from mass, fiber length, and a muscle density of 1.06.[15] The fatigability of grafts was measured during a sustained contraction at 100 Hz lasting 10 sec. The tension at 10 sec, expressed as a percentage of the maximum tension, was used as an index of fatigue.

Significant differences between standard and nerve-intact grafts and between grafts and control EDL muscles in age-matched animals at 14 and 60 days after grafting were determined by *t* tests. The level of significance was set at 1%.

RESULTS

The rats were 47–50 days of age at the time of grafting. During the 60-day postgrafting period, the body mass of the rats increased from 267 to 453 g and the mass of the control EDL muscle from 137 to 221 mg. The RT 1/2 was stabilized at 9–10 msec by 50 days, but the TPT increased slightly from 10 msec to 12 msec between 50 and 160 days of postnatal development. The P_t/P_o decreased significantly. These results are in agreement with previous observations of developing EDL muscles in rats.[5,9]

Morphological Variables. During the 60-day postgrafting period, the mean value for body mass of the animals increased by 64% (Table 1). The mass of the nerve-intact grafts was greater than that of the standard grafts at each sample period. The difference ranged from 30% to 60%. Between 14 and 60 days after grafting, the mass of the grafts more than doubled, whereas the control muscle increased its mass by 70%. Sixty days after grafting, the mass of the standard grafts was 59% of the control value and that of the nerve-intact grafts was 87%.

The muscle length of age-matched control EDL muscles increased by 30% between days 14 and 60 after grafting (Table 1). On day 14, the standard and nerve-intact grafts had muscle lengths 80% and 87%, respectively, of the control value. On day 60, the muscle lengths of both types of grafts were 80% of the control EDL muscle. No significant differences were observed between the muscle lengths of the two types of grafts.

As with mass, the total cross-sectional area of the nerve-intact grafts was significantly greater than that of the standard grafts at each time point. Sixty days after grafting, the cross-sectional area of the standard grafts was 48% less than the control value. The 4% difference between the area of nerve-intact grafts and control EDL muscles was not statistically significant.

Contraction and Relaxation Times. During the early sample periods, the values for TPT of both standard and nerve-intact grafts were significantly prolonged in comparison with the values for TPT of control muscles (Table 2), but at 14 days, the TPT of nerve-intact grafts was already significantly shorter than that of standard grafts. Following the typical pattern of maturation for regenerating rat muscle, the TPTs rapidly became shorter during the first 30 days and returned to normal values by 60 days. The development of RT 1/2 followed a pattern similar to that of the TPT.

Twitch and Tetanic Tension (Tables 1 and 2). At 14 days, the P_t of standard grafts was greater than that of nerve-intact grafts, but with time, the P_t of the nerve-intact grafts increased to higher levels than did those of standard grafts. At 60 days, the value for P_t of standard grafts was 50% of the control value and that of the nerve-intact grafts was 73%. During the early postgrafting period, the P_o of the nerve-intact grafts rose more rapidly than the P_o of standard grafts (Fig. 1). At 60 days, the P_o of standard and nerve-intact grafts was 57% and 91% of the control value, respectively. During the first 30 days after grafting, the P_t of the standard grafts showed a greater increase relative to the control value than did P_o, whereas the values for the P_t and P_o of the nerve-intact grafts increased in

Table 1. The morphological characteristics of extensor digitorum longus (EDL) muscles, standard (STD) grafts, and nerve-intact (N-I) grafts.

	Animal mass (g)	Muscle mass (mg)	Muscle length (mm)	Muscle cross-sectional area (mm^2)
14-Day control				
EDL (N = 5)	267 ± 6	137.2 ± 6.6	29.4 ± 0.9	11.0 ± 0.6
14-Day grafts				
(N = 8) STD	276 ± 8	62.3 ± 6.4*	23.5 ± 0.7*	6.3 ± 0.7*
(N = 8) N-I	276 ± 8	80.6 ± 4.0*†	25.5 ± 1.7	7.7 ± 0.6*
20-Day grafts				
(N = 8) STD	295 ± 26	80.6 ± 4.0	27.8 ± 0.7	6.8 ± 0.4
(N = 8) N-I	295 ± 26	130.5 ± 6.0†	31.5 ± 0.5†	10.2 ± 0.4†
30-Day grafts				
(N = 8) STD	291 ± 8	72.5 ± 11.0	23.5 ± 1.0	6.6 ± 0.9
(N = 8) N-I	291 ± 8	113.2 ± 10.0†	25.0 ± 0.7	10.6 ± 1.0†
60-Day grafts				
(N = 8) STD	422 ± 14	131.1 ± 17.9*	30.8 ± 1.0	10.4 ± 1.4*
(N = 8) N-I	422 ± 14	192.4 ± 9.9†	30.8 ± 0.7	14.8 ± 0.8†
60-Day control				
EDL (N = 4)	453 ± 18	221.0 ± 17.5	38.5 ± 3.2	15.4 ± 1.3

Significant differences ($P < 0.05$) between grafts and control EDL muscles at 14 days and 60 days are indicated by an asterisk (*) and between nerve-intact and standard grafts at the same time period by a dagger (†). Comparisons were not made between grafts and control muscles 20 and 30 days after grafting. Data are for means ± 1 SEM.

concert. The result of these differences was significantly higher P_t/P_o ratios for the standard grafts than for the nerve-intact grafts during the early postgrafting period. By 60 days, the P_t/P_o ratios of both groups had reached control values.

The specific tensions (tension in Newtons normalized per square centimeter of cross-sectional area) of the nerve-intact grafts were greater than those of the standard grafts at each sample period. The specific tensions for both types of grafts increased significantly with time after grafting. At 60 days, the specific tension of the nerve-intact grafts was not significantly different from the control value.

Frequency-Force Relationship. The frequency-force curve of the standard grafts was shifted upwards and to the left to a considerably greater degree

Table 2. Contractile properties of extensor digitorum longus (EDL) muscles, standard (STD) grafts, and nerve-intact (N-I) grafts

	TPT (msec)	RT 1/2 (msec)	P_t (mN)	P_o (mN)	P_o (N/cm^2)	P_t/P_o
Control EDL						
(N = 5)	10.3 ± 0.5	11.1 ± 1.0	424 ± 26	1958 ± 122	18.0 ± 1.0	0.22 ± 0.01
14-Day grafts						
(N = 8) STD	22.3 ± 0.4*	17.8 ± 0.8*	142 ± 10*	276 ± 23*	4.8 ± 0.6*	0.53 ± 0.03*
(N = 8) N-I	15.9 ± 0.7*†	16.3 ± 1.0*	120 ± 10*	541 ± 48*†	7.2 ± 0.9*†	0.22 ± 0.01†
20-Day grafts						
(N = 8) STD	22.1 ± 1.0	20.1 ± 0.7	133 ± 27	331 ± 88	5.7 ± 0.9	0.35 ± 0.04
(N = 8) N-I	14.9 ± 0.5†	14.7 ± 1.0†	180 ± 32	1010 ± 117†	10.0 ± 1.0†	0.18 ± 0.02†
30-Day grafts						
(N = 8) STD	13.7 ± 1.3	16.6 ± 2.0	118 ± 21	386 ± 100	6.2 ± 0.8	0.39 ± 0.05
(N = 8) N-I	9.5 ± 0.2†	8.1 ± 1.0†	221 ± 22†	1285 ± 145†	11.4 ± 0.8†	0.18 ± 0.01†
60-Day grafts						
(N = 8) STD	12.2 ± 0.4	11.2 ± 0.5	202 ± 51*	1355 ± 261*	10.4 ± 1.7*	0.15 ± 0.02
(N = 8) N-I	12.1 ± 0.2	10.1 ± 0.3	291 ± 17*	2151 ± 89†	13.6 ± 0.9	0.14 ± 0.01
Control EDL						
(N = 4)	12.0 ± 0.3	9.9 ± 0.5	400 ± 31	2367 ± 128	15.5 ± 0.9	0.17 ± 0.01

Significant differences ($P < 0.05$) between grafts and control EDL muscles at 14 days and 60 days are indicated by an asterisk (*) and between nerve-intact and standard grafts at the same time period by a dagger (†). Comparisons were not made between grafts and control muscles 20 and 30 days after grafting. Data are for means ± 1 SEM.

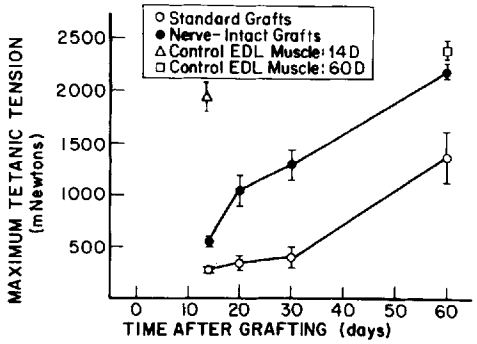

FIGURE 1. The maximum isometric tetanic tension of control EDL muscles, standard grafts, and nerve-intact grafts at time periods from 14 to 60 days after grafting. Means ± 1 SEM are indicated.

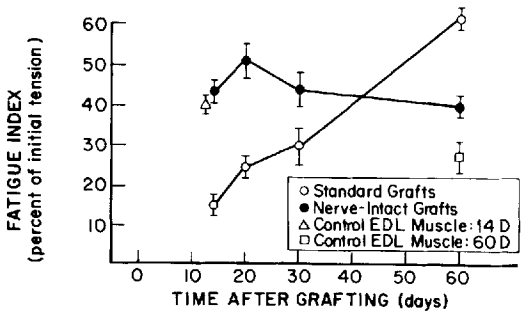

FIGURE 3. The fatigue indices of control EDL muscles, standard grafts, and nerve-intact grafts at time periods from 14 to 60 days after grafting. Means ± 1 SEM are indicated.

than the frequency-force curve of the nerve-intact grafts (Fig. 2). The frequency-force curve of the nerve-intact grafts returned to the control relationship by 30 days after grafting. The frequency-force curve of the standard grafts did not achieve the control relationship until 60 days after grafting.

Fatigability. The fatigability of nerve-intact grafts was quite different from that of the standard grafts at all sampling periods after grafting (Fig. 3). The nerve-intact grafts maintained a fairly constant fatigue index of from 41% to 51% throughout the 60-day postgrafting period. At 14 days postgrafting, the standard grafts had a fatigue index of 15%, which increased to 62% at 60 days (Fig. 3). At 14 days postgrafting, the fatigue index of the standard grafts was significantly less than the control value, whereas that of nerve-intact grafts was not different from that of the control EDL muscles. At 60 days, both standard and nerve-intact grafts were less fatigable than the control muscles. The P_o provides an effective estimate of the viable cross-sectional area of muscle fibers in muscles, and particularly in grafts that have large amounts of connective tissue.[14,15] The fatigue indices of small and large EDL muscles and grafts were plotted against maximum tetanic tension to evaluate the role viable cross-sectional area of muscle fibers plays in the development of fatigue (Fig. 4). No clear overall relationship was apparent. The poorly developed 14-, 20-, and 30-day standard grafts and large mature control muscles had short fatigue indices, but with low and high values for P_o, respectively. The 60-day standard grafts, all nerve-intact grafts, and small immature control muscles had prolonged fatigue indices in spite of a wide range of P_os.

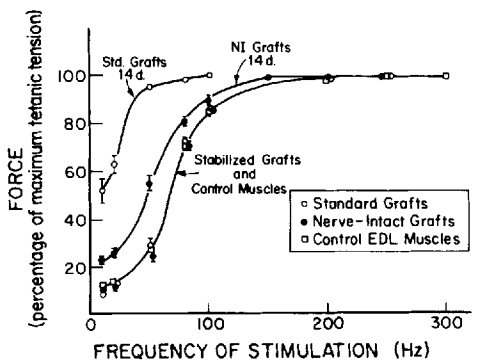

FIGURE 2. The frequency-force curves for control EDL muscles and for standard and nerve-intact grafts at selected time periods after grafting. The standard grafts (○) and nerve-intact grafts (●) are shown at 14 days after grafting (upper left plots) and after return to control values by 30 days for the nerve-intact grafts and by 60 days for the standard grafts (lower right plots). The means ± 1 SEM are indicated. Some error bars are within symbols.

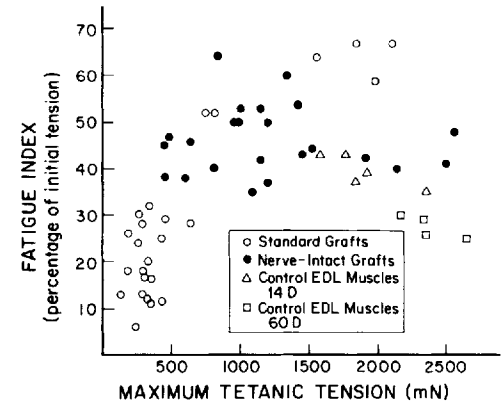

FIGURE 4. The fatigue indices of individual control EDL muscles, standard grafts, and nerve-intact grafts plotted against maximum isometric tetanic tension.

DISCUSSION

A comparison of the development of standard with nerve-intact muscle grafts leads to three major generalizations. The first is that the contraction and relaxation times and frequency-force relationships of both types of grafts ultimately return to control values. The second generalization is that, in keeping with their greater mass and cross-sectional area, nerve-intact grafts develop greater maximum tension than do standard grafts. Lastly, at 60 days after grafting, both types of grafts demonstrate a greater resistance to fatigue than control EDL muscles.

Previous work[3,5] has shown that immature regenerating muscles in rats have a prolonged TPT, but that by about 40 days, control values are reached. During the first 3–4 weeks after grafting, the TPT of the regenerating muscle fibers shortens considerably in the presence or absence of a functional innervation. This myogenic maturation of muscle function occurs even in permanently denervated regenerating muscle.[6] This pattern of maturation is reflected in the present data for standard grafts.

Nerve-intact grafts are innervated by 7–14 days,[7] and the contractile data for the nerve-intact grafts reflect the influence of a functional innervation superimposed upon the intrinsic maturation of the regenerating muscle fibers. This is shown in the shorter time for contraction throughout the periods studied, but an even stronger indication is seen in the P_t/P_o. Immature muscle, whether in normal ontogenesis or during regeneration, is characterized by a P_t/P_o close to 0.5.[9] As the muscle matures, the ratio approaches 0.15–0.25, depending on the muscle and the species.[9,10] The pronounced difference in P_t/P_o between standard and nerve-intact grafts at 14–30 days postgrafting is probably the best indicator of the maturation-enhancing effect of the nerve supply on regenerating muscle. By the time grafts in rats or cats[14] are stable, no differences are observed in contraction or relaxation times or in frequency-force relationships. We conclude that the beneficial effect of the nerve-intact procedure for grafting lies elsewhere than in the contractile machinery of mature regenerated muscle fibers.

A major difference between small 100-mg grafts in rats and large 3-g grafts in cats is in the functional mass and number of muscle fibers that regenerate in grafts of rats and cats. In the rat, the mass and numbers of muscle fibers in nerve-intact grafts are restored to essentially normal values, and the P_o is close to 90% of control value.[7] Conversely, in the cat, both mass and number of muscle fibers are below normal values, and the P_o attains only 67% of the normal value.[14]

The specific tension of a variety of control muscles from different species range from 15 to 30 N/cm^2.[10] Our values for control EDL muscles are in the lower range of these values. Muscles that have contracted vigorously over a period of hours have a mass approximately 20% greater than rested muscles.[15,16] Furthermore, EDL muscles larger than 50 mg do not sustain P_o over time when tested at 37°C in vitro.[17] We have reported values for specific tension of 28 N/cm^2 for both EDL grafts and EDL muscles in cats.[14,15] The low value for specific tension of control muscles results from a decline in maximum tension with time at 37°C in vitro and the increased fluid volume.

Lower values for specific tensions of grafts when compared to control EDL muscles have been reported previously in rats[11] and cats.[14,15] The low specific tensions of grafts have been accounted for previously by an increased content of noncontractile tissue in grafts.[15] The higher specific tensions for the nerve-intact grafts indicate relatively more contractile tissue and less connective tissue in nerve-intact than in standard grafts.

The second generalization concerns fatigability. The fatigability of the small EDL grafts in rats is complex.[12] The standard grafts in rats are initially more fatigable than control EDL muscles. The mechanism for the rapid fatigue of the small immature standard grafts is not a function of mass. A significant factor may be the relative tension development at 100 Hz. The 14-day standard grafts are developing maximum tetanic tension, whereas control muscles and other grafts range from 75% to 90% of maximum (Fig. 2). By 60 days, both types of grafts have longer fatigue indices than control EDL muscles. Stabilized standard and nerve-intact EDL grafts of rats have demonstrated a greater resistance to fatigue than control EDL muscles with various in situ tests of fatigability.[8,11] Compared to control EDL muscles, the grafts have the same number, but smaller, fibers,[1,7] higher capillary density,[8] and a higher concentration of cytochrome-c.[8] These characteristics of the small EDL grafts in rats might explain the high resistance to fatigue measured in situ, but they do not account for an increased resistance to fatigue in vitro.

The increased resistance to fatigue of mature grafts in rats contrasts with the data on large, standard and nerve-intact grafts in cats that are more fatigable than control EDL muscles at all time pe-

riods up to a year after grafting.[14,15,18] The difference in the fatigability of small grafts in rats and large grafts in cats may result from the differences in the number, cross-sectional area, and type of fibers that regenerate. During the 10-sec in vitro fatigue protocol, the resistance to fatigue should be a function of the total available energy, the rate of energy utilization, the build-up of metabolic end-products, or some combination of these factors.[13] The exact mechanism of fatigue remains unknown.[12] The major differences in the fatigability of standard grafts at different time periods after grafting and between the fatigability of small 100-mg and large 3-g grafts offers a unique opportunity to study the factors responsible for fatigue.

Lastly, the main difference between the degree of recovery achieved by nerve-intact compared to the standard grafts is the larger total functional mass of the former. Evaluation of the two primary factors limiting the functional mass attained by muscle grafts indicates that interruption of the vascular supply is not critical, but interruption of the nerve supply to grafts of 100–150-mg EDL muscles in the rat results in a significant functional deficit. Although the return of the blood supply does not appear to play a significant role in limiting the mass of muscle that regenerates in these small grafts, more complete innervation may indirectly influence revascularization. In contrast, maintaining the nerve intact was not sufficient for the recovery of full functional mass in the larger EDL grafts in the cat. Apparently, some aspect of the return of the vasculature, either the timing or extent of revascularization, plays a more important role in the larger grafts. By standardizing the degree of reinnervation, the nerve-intact procedure allows one to investigate other factors, such as revascularization or the mechanical environment, as variables that could influence the restoration of structure and function of a muscle graft.

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