Experimental

The Effect of Two Episodes of Denervation and Reinnervation on Skeletal Muscle Contractile Function

Kotaro Yoshimura, M.D., Hirotaka Asato, M.D., Sameer S. Jejurikar, M.D., Paul S. Cederna, M.D., Melanie G. Urbanchek, Ph.D., and William M. Kuzon, Jr., M.D., Ph.D.

Tokyo, Japan, and Ann Arbor, Mich.

Sensory or motor "baby-sitting" has been proposed as a clinical strategy to preserve muscle integrity if motion-specific axons must regenerate over a long distance to reach denervated target muscles. Denervated muscles are innervated temporarily by using axons from nearby sensory or motor nerves. After motion specific motor axons have reached the target, the baby-sitter nerve is severed and motion-specific axons are directed to the target. Although this strategy minimizes denervation time, the requisite second episode of denervation and reinnervation might be deleterious to muscle contractile function. This study was designed to test the hypothesis that two sequential episodes of skeletal muscle denervation and reinnervation result in greater force and power deficits than a single peripheral nerve injury and repair. Adult Lewis rats underwent either transection and epineurial repair or sham exposure of the left peroneal nerve. After a 4-month recovery period, the contractile properties of the extensor digitorum longus muscle of the sham exposure group (control, $n = 9$) and one of the nerve division and repair groups (repair group 1, $n = 9$) were evaluated with measurements of the maximum tetanic isometric force, peak power, and maximal sustained power. A third group of rats underwent a second cycle of nerve division and repair (repair group 2, $n = 9$) at this same time point. Four months postoperatively, contractile properties of the extensor digitorum longus muscles were evaluated. Maximum tetanic isometric force and peak power were significantly reduced in repair group 2 rats as compared with repair group 1 and control rats. Maximal sustained power was not significantly different between the groups. These data support our working hypothesis that skeletal muscle contractile function is adversely affected by two cycles of denervation and reinnervation as compared with a single episode of nerve division and repair. (*Plast. Reconstr. Surg.* 109: 212, 2002.)

Peripheral nerve injuries and repair can result in profound skeletal muscle atrophy and functional impairments.[1,2] Despite modern microsurgical techniques for nerve repair and technologic advances in equipment, clinicians have been unable to significantly affect this loss in muscle mass, force production, and power output. To this end, the "baby-sitter" procedure has been introduced to provide temporary motor innervation during periods of prolonged denervation.[3–6] Motor nerve baby-sitting was originally described in the treatment of unilateral facial paralysis; temporary innervation of the facial muscles was achieved and maintained through an ipsilateral nerve transfer until axons from a cross-facial nerve graft could be used to reinnervate the paralyzed face. Sensory nerve protection has also been utilized to preserve muscle mass. Transient innervation of muscles is preserved through sensory nerve axons until axons from the transected and repaired motor nerve reach the target muscle.[7–9] Unfortunately, the innervated muscle will not become reinnervated by the native motor nerve as long as it is innervated by the baby-sitter or the sensory nerve.[10–13] Thus, for both motor nerve baby-sitting and sensory protection, a second episode of denervation and reinnervation is required to permit successful reinnervation by the intended motor nerve. On the basis of the available data, it is exceedingly likely that two episodes of denervation and reinnervation are

From the Department of Plastic and Reconstructive Surgery, University of Tokyo; the Department of Surgery and The Institute of Gerontology, University of Michigan; and the Department of Veterans Affairs Medical Center. Received for publication December 18, 2000; revised February 22, 2001.

Presented in preliminary form at the Plastic Surgery Research Council 40th Annual Meeting, in New York, New York, from May 17 through 20, 1995.

deleterious to the recovery of skeletal muscle contractile function.

Many studies have evaluated the contractile function of skeletal muscle following peripheral nerve injury and repair and have focused largely on isometric force measurement.[14–17] However, determination of whole muscle power production has great clinical relevance, particularly in regards to activities such as running, jumping, cycling, and rowing.[18] Power development is greatest during a single contraction, decreases rapidly with repeated maximal contractions, and then decreases more gradually with time because of a decreasing ability to maintain an adequate energy balance.[19,20] Previous experiments from this laboratory have demonstrated that both force production and power output during a single contraction are impaired in skeletal muscle subjected to one cycle of denervation and reinnervation.[21] It is not known whether additional cycles of denervation and reinnervation further harm the recovery of muscle contractile function. By using an animal model to create a peripheral nerve injury and repair, this experiment was designed to measure differences in whole muscle force and power output after denervation and reinnervation. Our working hypothesis was that skeletal muscle contractile function worsens in muscles that have undergone two cycles of denervation and reinnervation as compared with a single cycle.

Materials and Methods

Animal Model

Experiments were performed using 4-month-old Lewis rats (Charles River Laboratories, Wilmington, Mass.) with body masses ranging from 400 to 500 g. Rats were individually housed in a pathogen-free animal facility at the University of Michigan. For all surgical procedures, rats were anesthetized with an initial intraperitoneal injection of sodium pentobarbital (60 mg/kg); supplementary doses were administered as needed to maintain a deep state of anesthesia. Surgical procedures were conducted under aseptic conditions. All animal care and operative procedures were performed in accordance with the *United States Public Health Service Guide for the Care of Laboratory Animals* (National Institutes of Health Publication Number 85-23) and approved by the University Committee on the Use and Care of Animals.

Experimental Design

A total of 27 animals were randomly allocated into one of three experimental groups. Animals in the control group underwent exposure and isolation of the peroneal nerve with care taken to avoid injury to the nerve or extensor digitorum longus muscle. The animals then convalesced for 4 months before the measurement of contractile function. Animals in the other two experimental groups underwent sharp division of the left peroneal nerve 5 mm proximal to the extensor digitorum longus muscle and immediate microsurgical repair with interrupted 10-0 nylon epineurial sutures. The wound was closed with simple interrupted 4-0 chromic sutures. All animals recovered for 4 months postoperatively. Animals in repair group 1 then underwent measurement of contractile properties, whereas animals in repair group 2 underwent a second peroneal nerve division and then an immediate epineurial repair. Four months after the second episode of denervation, these animals underwent an operation to determine the contractile function of the extensor digitorum longus muscle.

Measurement of Muscle Contractile Properties

Contractile properties of the extensor digitorum longus muscle were measured in situ in a manner similar to that previously described.[19,22,23] After each rat was anesthetized, the left extensor digitorum longus muscle was isolated with careful preservation of the neurovascular pedicle. The extensor digitorum longus muscle was dissected free of surrounding tissue, and its distal tendons were identified, transected, and folded to create a tendon loop. The loop was secured at the musculotendinous junction with a 4-0 silk suture. To avoid motion artifact from adjacent muscle groups, the peroneus, gastrocnemius, soleus, plantaris, and tibialis anterior muscles were divided and reflected. The sciatic nerve was exposed throughout its entire course from the sciatic notch to the knee. The tibial and sural branches were divided proximal to the knee. The peroneal nerve to the extensor digitorum longus muscle was preserved, whereas all other visualized branches of the peroneal nerve were divided. The animal was placed on a platform maintained at 35°C with a temperature-controlled water circulator. The left femoral condyle and foot were fastened to the platform to stabilize the lower extremity during force and power

measurements. The extensor digitorum longus tendon loop was secured to a force transducer attached to a servomotor lever arm (Cambridge Technology Inc., Model 305B, Cambridge, Mass.). The extensor digitorum longus muscle and peroneal nerve were regularly bathed with warm mineral oil (36°C) throughout the testing. Muscle temperature was monitored regularly and maintained between 35°C and 36°C.

Force Measurements

Isometric contractions of the extensor digitorum longus muscle were obtained with supramaximal pulses (0.2-msec pulse duration, 2 to 6 volts) generated by a Grass S88 Stimulator (Grass Instrument Co., Quincy, Mass.) and delivered with a shielded bipolar silver-wire electrode (Harvard Apparatus, South Natick, Mass.) to the proximal peroneal nerve. Force production of the extensor digitorum longus muscle was transduced using the servomotor and displayed on a storage oscilloscope (Gould Inc., Romulus, Mich.). A microcomputer (Dell Computer Corp., Austin, Texas) equipped with a digital-to-analogue converter (Data Translation, Marlboro, Mass.) and custom software (Asyst Software Technologies, Inc., Rochester, N.Y.) controlled the position of the servomotor lever arm. During maximum tetanic isometric force measurements, the lever arm remained stationary. The microcomputer sampled force data during contractions by means of analogue-to-digital channels on the Data Translation board. Muscle length was adjusted to give maximum twitch force. The optimal muscle length for force development (L_o) was held constant for all subsequent isometric force measurements. L_o was measured using a caliper as the total muscle length, not including the tendons of origin and insertion. Peak twitch force (F_t) was measured during single-twitch contractions. Maximum isometric tetanic force (F_o) was measured by stimulating the extensor digitorum longus muscle for 250 msec at incrementally increasing frequencies from 30 to 350 Hz until a force plateau was reached. Two minutes elapsed between tetanic contractions to permit muscle recovery.

Power Measurements during a Single Contraction

Measurements of power during a single contraction, or peak power, were made in a manner previously described.[19,22,24] Force and power output were determined during isovelocity-shortening contractions. An extensive investigation of the Lewis rat extensor digitorum longus muscle fiber length and architecture has been previously described.[21] On the basis of this previous work, the ratio of average fiber length to muscle belly length (L_f/L_m ratio) for the extensor digitorum longus muscles of adult Lewis rats is known to be 0.35 ± 0.03.[21] To permit muscle shortening as close to L_o as possible, the servomotor lever arm was programmed to displace the muscle through 12 percent of L_f. The muscles were prestretched to 106 percent of L_f and then shortened to 94 percent of L_f. The peroneal nerve was stimulated simultaneously with the initiation of the shortening ramp and throughout its entire duration. The average force (F_c) generated during a single concentric contraction was determined through calculations of the area underneath the force curve. The power during a single contraction was the product of F_c and the velocity of shortening. The velocity of shortening was increased incrementally until peak power (P_{max}) was achieved; further velocity increases resulted in lower power. The optimal shortening velocity (V_{opt}) for the generation of power was determined for a range of stimulation frequencies up to 500 Hz. The peak power during a single contraction was plotted against the stimulation frequency to construct a power-frequency curve; P_{max} was defined as the maximum power measured at V_{opt}.

Power Measurements during Repeated Contractions

Sustained power (P_s), the power generated during repeated contractions, was evaluated using a series of isovelocity-shortening contractions with increasing duty cycles (C_d). The duty cycle is the segment of time during the work-rest cycle in which the muscle performs work.[25,26] The stimulation frequency (range, 120 to 150 Hz) that produced approximately 85 percent F_o was used to determine P_s. The velocity of shortening selected was that which produced maximal power at the previously defined stimulation frequency. The time of each contraction remained constant, but the train rate was raised, thereby increasing the duty cycle. Muscles were stimulated repetitively at increasing duty cycles until the power output during each stimulation train had stabilized, usually lasting 3 to 5 minutes. The final contraction of each stimulation interval was used

for the calculation of sustained power, which was as follows:

$$P_s = (F_c)(C_d)(V_{opt})$$

Where P_s is the sustained power, F_c is the average force generated during a single concentric contraction, V_{opt} is the optimal velocity of shortening for the generation of power, and C_d is the duty cycle.

Beginning at a work-rest ratio of 0.01, the duty cycle was increased at predetermined increments until the maximal sustained power was determined. After the completion of force and power measurements, the animals were killed with a lethal injection of sodium pentobarbital.

Data Analysis

The mean and SD of each variable measured were computed for every group. To test for statistically significant differences, data were assessed by means of a single factor analysis of variance. Post hoc comparison of individual group means was performed only if the F ratio for the overall analysis of variance was significant; appropriate Bonferroni corrections were applied to all post hoc comparisons. Statistical computations were performed using a microcomputer and appropriate statistical software (Statistical Analysis System, SAS, Inc., Cary, N.C.; Sigma Stat, SPSS Science, Chicago, Ill.). The level of significance was set at $p < 0.05$.

RESULTS

A total of 27 animals were included in the study ($n = 9$ for each group). One animal in the control group failed to complete the protocol, leaving 26 animals for analysis. All remaining animals tolerated the surgical procedures well and demonstrated no significant abnormalities. No statistically significant differences were observed among any of the groups in measurements of body mass, muscle mass, and muscle fiber length (Table I).

Force Measurements

Results are summarized in Table I. Maximum isometric tetanic force was significantly reduced after a single episode of denervation and reinnervation (repair group 1) as compared with the control. A second episode of denervation and reinnervation (repair group 2) resulted in even greater force deficits compared with repair group 1 and control animals.

TABLE I

Isometric Force Measurements: A Comparison of Control, Repair Group 1, and Repair Group 2[a]

	Control ($n = 8$)	Repair Group 1 ($n = 9$)	Repair Group 2 ($n = 9$)
Body mass (g)	447 ± 23	466 ± 27	476 ± 34
Muscle mass (g)	191 ± 12	189 ± 23	177 ± 27
L_f (mm)	14.9 ± 0.5	14.6 ± 0.6	15.0 ± 1.0
P_t (mN)	684 ± 73	517 ± 130*	497 ± 49*
F_o (mN)	4080 ± 260	3430 ± 750*	2780 ± 580*†

[a] Data are displayed as mean ± SD of mean. Muscle mass, given in wet muscle mass; L_f, muscle fiber length; P_t, maximum isometric twitch force; F_o, maximum isometric tetanic force.

* Statistically significant versus control group at $p < 0.05$.

† Statistically significant versus repair group 1 at $p < 0.05$.

Power Measurements

All power measurements are summarized in Table II. P_{max} during a single isovelocity-shortening contraction increased as the stimulation frequency was incrementally raised from 100 to approximately 350 Hz. As the frequency was increased beyond this, P_{max} diminished. For all groups, the power-frequency curves were parallel. P_{max} was significantly decreased in both repair group 1 and repair group 2 compared with the control. Furthermore, P_{max} was significantly decreased in repair group 2 as compared with repair group 1. This reduction in P_{max} (39.1 percent) exceeds the change observed in F_o (18.9 percent) and can be explained further by a reduction in the optimal velocity of shortening (V_{opt}). No statistically significant differences in duty cycle (work-rest ratio) in which P_{max} occurred were identified in any of the experimental groups. Although a reduction in V_{opt} was identified in muscles undergoing two episodes of denervation and reinnervation, these changes did not translate into changes in sustained power (P_s).

TABLE II

Power Measurements: A Comparison of Control, Repair Group 1, and Repair Group 2[a]

	Control ($n = 8$)	Repair Group 1 ($n = 9$)	Repair Group 2 ($n = 9$)
V_{opt} (L_f/sec)	2.0 ± 0.1	1.7 ± 0.3	1.6 ± 0.3*
P_{max} (mW)	43.2 ± 4.6	32.2 ± 9.9*	19.6 ± 9.7*†
C_d	0.10 ± 0.08	0.07 ± 0.05	0.11 ± 0.08
P_s (mW)	0.70 ± 0.3	1.06 ± 0.6	0.78 ± 0.5

[a] Data are displayed as mean ± SD of mean. V_{opt}, optimum velocity for maximum power; P_{max}, maximum power during isovelocity shortening contractions through 10 percent of L_f at optimum velocity; C_d, duty cycle (ratio of work to rest); P_s, maximum power sustained during repeated isovelocity-shortening contractions.

* Statistically significant versus control group at $p < 0.05$.

† Statistically significant versus repair group 1 at $p < 0.05$.

Discussion

These data support our working hypothesis. Two cycles of denervation and reinnervation result in significant deficits in maximal tetanic isometric force production and peak power output as compared with one cycle. Measurements of maximal sustained power did not match these findings, with no differences observed among the three groups. These findings indicate that two episodes of denervation and reinnervation are deleterious to the recovery of muscle contractile function and suggest that treatment modalities utilizing strategies of motor nerve baby-sitting or sensory nerve protection should be used with caution.

Disruption of neuromuscular integrity leads to dramatic alterations in muscle structure and function.[27–32] Previous work from this laboratory has demonstrated a reduction in force production and power output in denervated and reinnervated muscle.[21] The mechanisms responsible for these changes are not completely understood, but may include denervation atrophy, reduced axonal numbers, altered axonal spatial organization, diminished muscle oxidative capacity, motor unit remodeling, and intrinsic alterations in skeletal muscle fibers themselves.[14,21,22,33–37] This study demonstrates that two episodes of skeletal muscle denervation and reinnervation intensify the observed deficit in whole-muscle isometric force production. Muscle and body mass did not significantly differ among the groups, suggesting that denervation atrophy does not play a primary role in the reduction of isometric tension.

Measurements of optimal velocity of shortening and peak power output have distinct physiologic meaning. Activities such as lifting, jumping, and sprinting require maximization of power output, not of isometric force production.[38] In skeletal muscle with heterogeneous fiber types, measurements of the maximal velocity of shortening, rather than that of the velocity that promotes optimal power output, do not accurately reflect whole muscle function at higher loads and have a large margin of error at lower loads.[39,40] In this experiment, the maximal power deficit in repair group 2 compared with both repair group 1 and the sham control group (39.0 percent and 54.6 percent, respectively) is, in part, explained by a reduced optimal velocity of shortening. Thus, the reduction in power output seen after two cycles of denervation and reinnervation is caused by mechanisms altering both the velocity of shortening and force production. Despite the reduction in peak power output seen in both experimental groups compared with the control group, no significant differences were seen among any of the groups in measurements of sustained power. The ability to sustain power is greatest in muscles having a large percentage of fast-oxidative glycolytic muscle fibers, followed by slow-oxidative, and lastly by fast-glycolytic fibers.[24] Differences in the capacity to sustain power therefore should be seen if reinnervated muscle experiences a reduction in peak power output or a shift in fiber-type composition.[19,35] Previous work has demonstrated that, in this model, no significant changes in fiber-type composition are observed after a single episode of denervation and reinnervation.[21] Considering that, in this study, sustained power production did not diminish despite a fall in both the optimal velocity of shortening and force production, it is possible that two episodes of denervation could result in an increase in the relative numbers of fast-oxidative glycolytic or slow-oxidative fibers in the extensor digitorum longus muscle. The differences in isometric force and peak power observed among all the groups, in contrast with the lack of significant differences in sustained power measurements, reinforces that the mechanisms responsible for functional deficits in skeletal muscle following denervation-reinnervation injury are heterogeneous. Although sustained power output is preserved with two episodes of denervation and reinnervation, the resulting deficit in isometric force and peak power production could result in a significant clinical disability.

This experimental model was chosen to specifically address the effects of two cycles of denervation and reinnervation on skeletal muscle contractile function. The peroneal nerve was sharply transected and immediately repaired in close proximity to the neuromuscular junction of the extensor digitorum longus muscle. This experimental model was chosen specifically to allow for extremely rapid reinnervation of denervated extensor digitorum longus muscle fibers. In a similar experimental paradigm, muscle fibers have been observed to generate action potentials indicative of reinnervation by day 11 after nerve injury.[41] In addition, in the rabbit rectus femoris muscle, 60 days has been reported to be sufficient to allow stabilization of muscle contractile

properties after neurovascular muscle transfer.[15,42] Previous data from our laboratory indicate that, in our hands, peroneal nerve transection and repair is rapidly followed by reinnervation and functional recovery in the extensor digitorum longus muscle.[21,22] Therefore, after each episode of nerve transection and repair, muscle fiber reinnervation time was likely very short, and functional recovery was stabilized before measurement of contractile properties or the second cycle of denervation and reinnervation was enacted. These data make it very unlikely that the diminished contractile function observed in repair group 2 was caused by prolonged denervation as a result of the first nerve transection. Despite optimizing experimental conditions to ensure maximal recovery, a substantial functional deficit exists with two cycles of denervation and reinnervation that is significantly greater than deficits observed after a single episode.

Of course, in the clinical setting, motor nerve injury and repair rarely occur under such ideal conditions, and extended atrophy and fibrosis of the muscle, and crushing injuries to the motor nerve, possibly even requiring nerve grafting, certainly contribute to even greater functional deficits. Procedures intended to prevent denervation atrophy, including those utilizing the principles of motor nerve baby-sitting and sensory protection, may subject the target skeletal muscle to two episodes of denervation and reinnervation. In the true baby-sitter paradigm, the second episode of muscle reinnervation presumably comes from a nerve that has only been axotomized once, differing from the model used in this study. Although providing optimal conditions for rapid reinnervation, this study is unable to separate the effects of two episodes of denervation and reinnervation from those of two cycles of axotomy and axon regeneration. Prolonged denervation, independent from prolonged axotomy, leads to poor functional recovery of muscle secondary to the progressive deterioration of intramuscular nerve sheaths.[43] In this instance, the primary cause of poor muscle contractile function is a profound reduction in the number of axons that successfully regenerate through the deteriorating intramuscular nerve sheaths, which is also seen after prolonged axotomy. Muscle force capacity after denervation, regardless of the duration of axotomy, is further compromised by the incomplete recovery of muscle fibers from denervation atrophy.[43] This suggests that multiple axotomy is not the critical element behind the diminished function seen in repair group 2. The data from this experiment indicate that two cycles of denervation and reinnervation, even accounting for all other factors, are, by themselves, harmful to skeletal muscle contractile function.

The experimental model clearly has limitations in comparison with the clinical setting. Motor nerve baby-sitting and sensory nerve protection both are performed with the intent of preventing denervation atrophy and the accompanying decrease in muscle contractile function. In many instances, when the site and mechanism of nerve injury are unfavorable, these clinical interventions must be performed on skeletal muscle that has been denervated for several weeks to months. In this setting, the efficacy of multiple attempts at reinnervation is not known. In addition, the passage of time after denervation injury worsens muscle atrophy and contractile function.[1,44–47] It is not certain whether two cycles of denervation and reinnervation actually lead to a worse outcome than prolonged denervation followed by a solitary attempt at reinnervation. This is a matter that warrants further investigation, as it will dramatically affect the manner in which physicians address motor nerve injuries.

In summary, skeletal muscle undergoing two cycles of denervation and reinnervation demonstrates significant deficits of force and power production in comparison to muscle undergoing just one such episode. These findings have direct clinical relevance in patients with nerve injury, particularly when considering the use of surgical procedures that rely on the principles of motor nerve baby-sitting and sensory nerve protection.

William M. Kuzon, Jr., M.D., Ph.D.
University of Michigan Medical Center
2130 Taubman Center
1500 East Medical Center Drive
Ann Arbor, Mich. 48109-0340
wkuzon@umich.edu

ACKNOWLEDGMENTS

This work was supported by grant NS34380 from the National Institute of Neurologic Disorders and Stroke. Dr. Cederna was supported by grant T32 AG00114 from the National Institute on Aging.

REFERENCES

1. Sunderland, S., and Ray, L. J. Denervation changes in mammalian striated muscle. *J. Neurol. Neurosurg. Psych.* 13: 159, 1950.
2. Tower, S. S. The reaction of muscle to denervation. *Physiol. Rev.* 19: 1, 1939.
3. Endo, T., Hata, J., and Nakayama, Y. Variations on the "baby-sitter" procedure for reconstruction of facial paralysis. *J. Reconstr. Microsurg.* 16: 37, 2000.
4. Kalantarian, B., Rice, D. C., Tiangco, D. A., and Terzis, J. K. Gains and losses of the XII-VII component of the "baby-sitter" procedure: A morphometric analysis. *J. Reconstr. Microsurg.* 14: 459, 1998.
5. Mersa, B., Tiangco, D. A., and Terzis, J. K. Efficacy of the "baby-sitter" procedure after prolonged denervation. *J. Reconstr. Microsurg.* 16: 27, 2000.
6. Terzis, J. K. "Babysitters": An exciting new concept in facial reanimation. In D. Castro (Ed.), *Proceedings of the Sixth International Symposium on the Facial Nerve.* Rio de Janeiro: Kulger and Ghedini, 1988.
7. Hynes, N. M., Bain, J. R., Thoma, A., Veltri, K., and Maguire, J. A. Preservation of denervated muscle by sensory protection in rats. *J. Reconstr. Microsurg.* 13: 337, 1997.
8. Ochi, M., Kwong, W. H., Kimori, K., Takemoto, S., Chow, S. P., and Ikuta, Y. Delay of the denervation process in skeletal muscle by sensory ganglion graft and its clinical application. *Plast. Reconstr. Surg.* 97: 577, 1996.
9. Zhang, F., Lineaweaver, W. C., Ustuner, T., et al. Comparison of muscle mass preservation in denervated muscle and transplanted muscle flaps after motor and sensory reinnervation and neurotization. *Plast. Reconstr. Surg.* 99: 803, 1997.
10. Elsberg, C. A. Experiments on motor nerve regeneration and the direct neurotization of paralysed muscles by their own and foreign nerves. *Science* 45: 318, 1917.
11. Bennett, M. R. Development of neuromuscular synapses. *Physiol. Rev.* 63: 915, 1983.
12. Covault, J., and Sanes, J. R. Neural cell adhesion molecule (N-CAM) accumulates in denervated and paralyzed skeletal muscles. *Proc. Natl. Acad. Sci. U. S. A.* 82: 4544, 1985.
13. Lieber, R. L. *Skeletal Muscle Structure and Function.* Baltimore: Williams & Wilkins, 1992. Pp. 248–257.
14. Frey, M., Gruber, H., Havel, M., Steiner, E., and Freilinger, G. Experimental free-muscle transplantation with microneurovascular anastomoses. *Plast. Reconstr. Surg.* 71: 689, 1983.
15. Guelinckx, P. J., Faulkner, J. A., and Essig, D. A. Neurovascular-anastomosed muscle grafts in rabbits: Functional deficits result from tendon repair. *Muscle Nerve* 11: 745, 1988.
16. Lewis, D. M., Parry, D. J., and Rowlerson, A. Isometric contractions of motor units and immunohistochemistry of mouse soleus muscle. *J. Physiol.* 325: 393, 1982.
17. Terzis, J. K., Sweet, R. C., Dykes, R. W., and Williams, H. B. Recovery of function in free muscle transplants using microneurovascular anastomoses. *J. Hand Surg. (Am.)* 3: 37, 1978.
18. McComas, A. J. *Skeletal Muscle Form and Function.* Champaign, Ill.: Human Kinetics, 1996. Pp. 178–179.
19. Brooks, S. V., and Faulkner, J. A. Maximum and sustained power of extensor digitorum longus muscles from young, adult, and old mice. *J. Gerontol.* 46: B28, 1991.
20. Dudley, G. A., and Fleck, S. J. Metabolite changes in aged muscle during stimulation. *J. Gerontol.* 39: 183, 1984.
21. Yoshimura, K., Asato, H., Cederna, P. S., Urbanchek, M. G., and Kuzon, W. M. The effect of reinnervation on force production and power output in skeletal muscle. *J. Surg. Res.* 81: 201, 1999.
22. Cederna, P. S., Youssef, M. K., Asato, H., Urbanchek, M. G., and Kuzon, W. M., Jr. Skeletal muscle reinnervation by reduced axonal numbers results in whole muscle force deficits. *Plast. Reconstr. Surg.* 105: 2003, 2000.
23. Brooks, S. V., Faulkner, J. A., and McCubbrey, D. A. Power outputs of slow and fast skeletal muscles of mice. *J. Appl. Physiol.* 68: 1282, 1990.
24. Brooks, S. V., and Faulkner, J. A. Forces and powers of slow and fast skeletal muscles in mice during repeated contractions. *J. Physiol.* 436: 701, 1991.
25. Clark, B. J., III, Acker, M. A., McCully, K., et al. In vivo 31P-NMR spectroscopy of chronically stimulated canine skeletal muscle. *Am. J. Physiol.* 254: C258, 1988.
26. Dillon, P. F., and Murphy, R. A. High force development and crossbridge attachment in smooth muscle from swine carotid arteries. *Circ. Res.* 50: 799, 1982.
27. Fansa, H., Plogmeier, K., Feistner, H., and Schneider, W. J. Plasticity and function—The fate of a free, neurovascular muscle graft ten years post-reconstruction. *J. Reconstr. Microsurg.* 13: 551, 1997.
28. Eisenberg, H. A., and Hood, D. A. Blood flow, mitochondria, and performance in skeletal muscle after denervation and reinnervation. *J. Appl. Physiol.* 76: 859, 1994.
29. Marquez, A., and Finol, H. J. Effects of neurotomy on human skeletal muscle ultrastructure. *Acta Cient. Venez.* 42: 319, 1991.
30. Connor, E. A., Sugarman, H., and Rotshenker, S. Molecular alterations in the perijunctional region of frog skeletal muscle fibres following denervation. *J. Neurocytol.* 20: 323, 1991.
31. Oldfors, A., Mair, W. G., and Fogdestam, I. The morphological sequences in man of de- and reinnervation in free muscle transfer with microneurovascular anastomoses. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 23: 35, 1989.
32. Pulliam, D. L., and April, E. W. Degenerative changes at the neuromuscular junctions of red, white, and intermediate muscle fibers: Part 1. Response to short stump nerve section. *J. Neurolog. Sci.* 43: 205, 1979.
33. Cheng, N., Li, X., and Huang, A. Experimental comparison of muscle contractility after three methods of reinnervation. *Ann. Plast. Surg.* 33: 166, 1994.
34. Irintchev, A., Draguhn, A., and Wernig, A. Reinnervation and recovery of mouse soleus muscle after long-term denervation. *Neuroscience* 39: 231, 1990.
35. Larkin, L. M., Faulkner, J. A., Hinkle, R. T., Hassett, C. A., Supiano, M. A., and Halter, J. B. Functional deficits in medial gastrocnemius grafts in rats: Relation to muscle metabolism and beta-AR regulation. *J. Appl. Physiol.* 83: 67, 1997.
36. Nemeth, P. M. Electrical stimulation of denervated muscle prevents decreases in oxidative enzymes. *Muscle Nerve* 5: 134, 1982.
37. Rafuse, V. F., and Gordon, T. Incomplete rematching of nerve and muscle properties in motor units after extensive nerve injuries in cat hindlimb muscle. *J. Physiol.* 509: 909, 1998.

38. Jones, N. L., McCartney, N., and McComas, A. J. *Human Muscle Power.* Champaign, Ill.: HumanKinetics, 1986.
39. Claflin, D. R., and Faulkner, J. A. The force-velocity relationship at high shortening velocities in the soleus muscle of the rat. *J. Physiol.* 411: 627, 1989.
40. Podolin, R. A., and Ford, L. E. Influence of partial activation on force-velocity properties of frog skinned muscle fibers in millimolar magnesium ion. *J. Gen. Physiol.* 87: 607, 1986.
41. Muchnik, S., and Kotsias, B. A. Characteristics of reinnervation of skeletal muscle in the rat. *Acta Physiol. Latinoam.* 26: 481, 1976.
42. Guelinckx, P. J., Carlson, B. M., and Faulkner, J. A. Morphologic characteristics of muscles grafted in rabbits with neurovascular repair. *J. Reconstr. Microsurg.* 8: 481, 1992.
43. Cabaud, H. E., Rodkey, W. G., McCarroll, H. R., Jr., Mutz, S. B., and Niebauer, J. J. Epineurial and perineurial fascicular nerve repairs: A critical comparison. *J. Hand Surg. (Am.)* 1: 131, 1976.
44. Fu, S. Y., and Gordon, T. Contributing factors to poor functional recovery after delayed nerve repair: Prolonged denervation. *J. Neurosci.* 15: 3886, 1995.
45. al-Amood, W. S., Lewis, D. M., and Schmalbruch, H. Effects of chronic electrical stimulation on contractile properties of long-term denervated rat skeletal muscle. *J. Physiol.* 441: 243, 1991.
46. Miledi, R., and Slater, C. R. On the degeneration of rat neuromuscular junctions after nerve section. *J. Physiol.* 207: 507, 1970.
47. Schmalbruch, H., al-Amood, W. S., and Lewis, D. M. Morphology of long-term denervated rat soleus muscle and the effect of chronic electrical stimulation. *J. Physiol.* 441: 233, 1991.