5

Skeletal Muscle Adaptation to Decreased Use

CHAPTER OVERVIEW

Having laid the foundation in Chapter 4 of muscle response to increased use, this chapter will consider muscle response to decreased use. As in previous chapters, the response to the other extreme—decreased use—will be illustrated by considering various experimental models. This chapter will begin with the "simplest" model (simple at least from the point of view of creating it)—immobilization; it will then consider various spinal cord interruption models, followed by simulated weightlessness (such as that which occurs in spaceflight). In recent years, a tremendous boom in understanding the response of muscles to aging has been achieved, and this is presented as one of the final models. Finally, the denervation model is presented to demonstrate its uniqueness. Again, patterns emerge as each model adds to our understanding of muscle adaptation. The descriptions of many of these abnormal states also provide an understanding of many aspects of normal structure–function relationships in neuromuscular units. In this chapter, the model is presented first, followed by experimental data that describe the muscle changes resulting from the treatment.

EDUCATIONAL OBJECTIVES

- To be able to describe the basic experimental "models" of adaptation to decreased use
- To be able to explain the strengths and weaknesses of each model in its ability to provide an understanding of adaptation to decreased use
- To be able to define the factors that cause adaptation to decreased use
- To provide clinical examples that parallel the experimental models of decreased use
- To be able to predict changes that occur in muscles when subjected to experimental and clinical instances of decreased use

Our investigation of muscle adaptation continues with a detailed view of a muscle's response to decreased use. In contrast to the previous chapter that began by presenting a very "clean" model of *increased use* by chronic stimulation, there is no equivalently clean model of *decreased use* that results in uniform results across all fiber types. In other words, there is no chronic decreased use model because a "dramatic" decrease in use is difficult to elicit. Some muscle fibers are so rarely used that immobilization may not have much influence on them. As a result, investigators have developed various models that cause a decrease in muscle use, although in different ways. One of the models that is easiest to understand is immobilization. This one will be used as the starting point. However, for each model, as in the previous chapter, always consider the relevance of that model to your area of interest. Some models are very clean in terms of creating a reproducible response, but are not very relevant to real clinical problems. Others are very relevant, but so complex that they are difficult to decipher. Ideally, information from all types of models can be synthesized to permit a general understanding of neuromuscular plasticity to decreased use. I apologize in advance for the numerous models presented as well as the detailed response of the neuromuscular system to each model. Don't just read this as a list of treatments and changes. Rather, try to first predict the response of muscle to the treatment, and then try to predict the underlying muscular changes that could explain the functional responses measured. Use the discussion of these models to reinforce your understanding of neuromuscular structure and function.

ADAPTATION TO IMMOBILIZATION

Limb immobilization has been used since the turn of the 20th century to protect fractured bones and injured tissues from repeated injury. The most common complication of immobilization in clinical use is the muscle wasting that occurs secondary to decreased muscle use. However, you probably have some concerns about immobilization as a decreased use model since, as seen in the previous chapter, immobilization per se does not necessarily cause atrophy. Strictly speaking, atrophy-inducing models using immobilization implement immobilization when the muscle is in a shortened position.

The Immobilization Model

Immobilization models have long been used to study muscle adaptation. In addition to their obvious clinical relevance, immobilization models are relatively noninvasive—no surgery is necessary. In Chapter 4, you saw that a

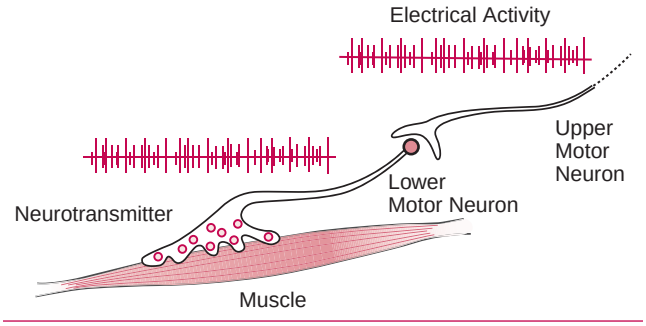

Figure 5-1 Schematic representation of the immobilization model. Each portion of the neuromuscular system is shown. Note that muscle electrical activity and even some degree of loading are present and, therefore, it is not appropriate to term this model "disuse," but rather, decreased use.

muscle responds to the tension level imposed upon it. (To date, muscle tension during immobilization has not been experimentally measured directly.) Immobilization should not be viewed as placing a muscle in a state of "suspended animation," since electrical activity, tension, and motion can still readily occur within the cast (Fig. 5-1). Do not consider immobilization as a "disuse" model either, in the sense that the muscle is completely unused. Rather, it is more appropriate to consider immobilization a "reduced use" model.

Electromyographic Changes during Immobilization

Since direct measurement of muscle tension within a cast is technically difficult, an indirect "measure" of muscle activity during immobilization was made by quantifying the electromyographic (EMG) activity. Experimentally, this was performed by implanting fine wire electrodes into muscles and routing the leads to an external connector. Then, at various time intervals after electrode implantation and immobilization, muscle EMG activity was recorded to determine the long-term activity changes resulting from immobilization.

Q&A

Why Isn't Immobilization the Same as *Disuse*?

The word *"disuse"* means no use and yet, when electrodes are implanted into immobilized muscles of animals, significant levels of muscle activity are still present. This is almost certainly true of humans as well. Thus, a more correct term for the atrophy that occurs during immobilization would be *"immobilization-induced atrophy."* In fact, as you will see, almost all disuse models are actually *decreased use* models, since some degree of electrical activity or mechanical loading is present.

Dr. Reggie Edgerton and colleagues implanted electrodes in both the fast-contracting medial gastrocnemius (MG) muscle and the slow-contracting soleus (SOL) muscle of the rat (Fournier, Roy, Perham, Simard, & Edgerton, 1983). Their purpose was to determine the extent to which immobilization resulted in muscle disuse. EMG activity was measured for several days after the electrodes were implanted, to determine whether the implantation procedure itself affected muscle activity (fortunately, it did not). Next, EMG activity was measured continuously for 15 minutes every hour for 24 hours on days 7, 17, and 28 postimmobilization. To produce varying degrees of atrophy, joints were immobilized such that the SOL and MG muscles were either shortened, at neutral length, or in a lengthened position. At these same time intervals, the muscle mass of the animals was measured as an index of "atrophy" (you will see below that wet muscle mass was probably not the best index of atrophy, but for the purposes of this study, it was probably adequate. Since skeletal muscle fibers may contain over 80% protein, protein content and muscle fiber size are the most common indices of muscle atrophy).

Fournier et al. demonstrated that the total EMG activity of both the SOL and MG muscles decreased markedly after only 1 week of immobilization, with the muscles in a shortened position. The SOL EMG activity decreased to a greater extent than did the MG EMG. EMG activity continued to remain low throughout the remainder of the experiment such that, after 28 days, SOL EMG had decreased by 77%, whereas MG EMG had decreased by 50%. With the muscle immobilized in the neutral position, no change was seen in MG EMG activity, whereas SOL EMG decreased by 50%.

The interesting aspect of the study was that the atrophic muscle response was not closely related to the magnitude of the EMG change (Fig. 5-2). For example, when the MG was immobilized in a neutral position, EMG did not change appreciably (Fig. 5-2A), but the muscle atrophied dramatically (black arrow, Fig. 5-2B). Also, in spite of the fact that the initial EMG responses of the MG in the neutral position and lengthened, were nearly identical, the magnitude of the atrophic response was different. The immobilization model decreased the level of MG "use" (by decreasing EMG and thus the electrical activity of the muscle fibers), but this change in use was not proportional to the magnitude of the resulting muscle atrophy. Similarly, for the SOL muscle, the EMG decreased to the same extent, whether the muscle was immobilized in the neutral or fully lengthened position (decreasing to about 50% of control values). However, the SOL muscle immobilized in the neutral position decreased in mass by about 50%, while the SOL muscle immobilized in the lengthened position showed no decrease at all (white arrow, Fig. 5-2B). Again, the change in the level of use as measured by EMG was not related to the magnitude of the atrophic response.

The take-home lesson of this study was that the change in muscle electrical activity was not the *cause* of

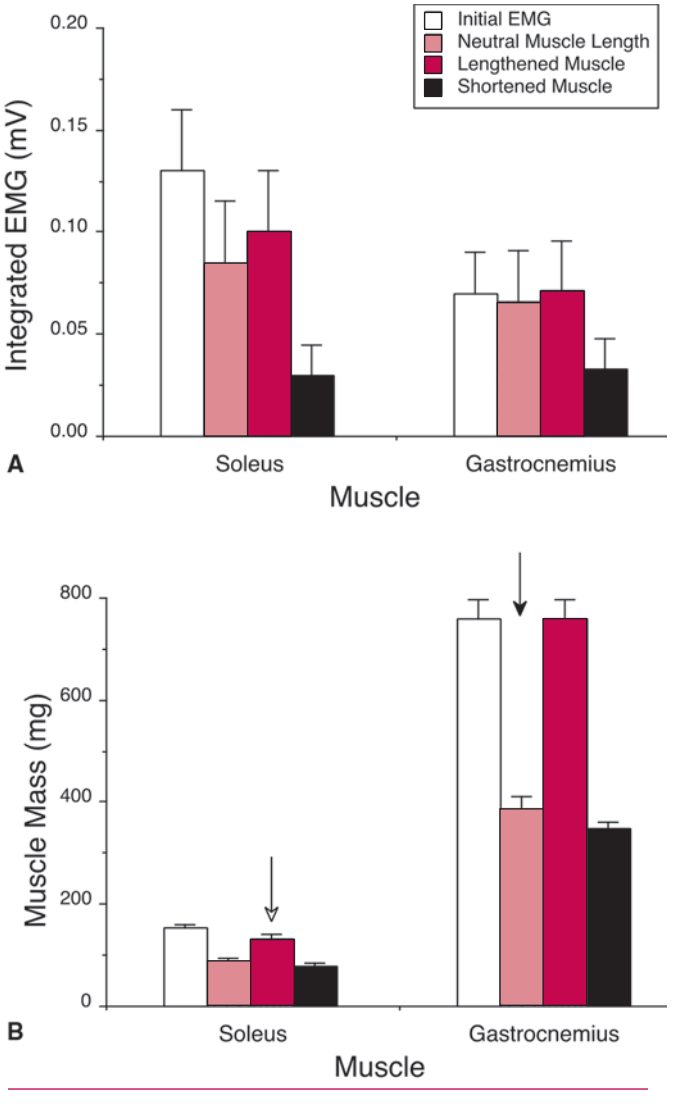

Figure 5-2 (A) Change in EMG activity from the rat soleus and medial gastrocnemius immobilized in the different positions denoted by different shading patterns. (B) Change in muscle mass from the rat soleus and medial gastrocnemius immobilized in different positions. Note that EMG changes are not paralleled by mass changes (*arrows*). (Data from Fournier, M., Roy, R. R., Perham, H., Simard, C. P., & Edgerton, V. R. [1983]. Is limb immobilization a model of muscle disuse? *Experimental Neurology, 80*, 147–156.)

muscle atrophy. It is also obviously inappropriate to refer to atrophy that occurs secondary to immobilization as disuse atrophy, since neural activity remained throughout the entire immobilization period, indicating that all the muscles were being used.

Dog Quadriceps Immobilization Model

To determine the nature of muscle response to immobilization, literally thousands of experiments have been performed using practically every conceivable muscle group and animal or human model. There is general agreement among studies that muscles composed mainly of slow fibers atrophy to a greater extent than muscles composed mainly of fast fibers. It also appears that antigravity muscles atrophy to a greater extent than their antagonists. Thus, the "fast" gastrocnemius atrophies to a greater extent than its fast tibialis anterior (TA) antagonist. However, there are numerous exceptions to these generalizations. One reason for this lack of agreement is the lack of control of muscle length during immobilization. If, for example, the ankle joint is immobilized with the ankle plantarflexed, the soleus will dramatically atrophy (due to the lack of tension), whereas the TA may actually hypertrophy (due to being stretched). Should it therefore be concluded that muscles composed primarily of slow fibers (such as the soleus) atrophy after immobilization, whereas muscles composed primarily of fast fibers (such as the TA) actually hypertrophy? No. It is important, therefore, to control for muscle length and other factors to properly generalize regarding the effects of immobilization on fast and slow skeletal muscles. In fact, as you review the literature on immobilization, be sure to define the relative degree of stretch placed upon a muscle, since this will affect its atrophic response greatly. For example, human plantarflexors immobilized with the ankle joint in a neutral position would be expected to atrophy less compared with plantarflexors immobilized with the ankle joint in a plantarflexed position.

A study of muscle immobilization was performed using, as our experimental model, three heads of the dog quadriceps muscles: the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) (Lieber, Fridén, Hargens, Danzig, & Gershuni, 1988). These three muscles contain nearly identical architectures and fiber lengths, but differ in fiber type percentage and the number of joints crossed. For example, the RF acts both as a knee extensor and hip flexor, and is composed of about 50% slow fibers. The VM and VL both function only as knee extensors, but the VL contains only about 20% slow fibers, whereas the VM contains about 50% slow fibers. This model thus allowed comparison between the VM and VL, which could be immobilized at precisely the same length but contain different percentages of slow and fast fibers. Similarly, comparisons between the RF and VM could be made since they have similar fiber type percentages but cross different joints. Thus, comparison of the vasti tests for the effects of fiber type, whereas comparison between the VM and RF tests for the effects of architecture.

Note that the dog muscle fibers have simply been referred to as fast and slow in spite of our relatively lengthy muscle fiber types discussion presented in Chapter 2. Why? Fortunately, dog muscles contain no fast glycolytic (FG)-type fibers (Armstrong, Saubert, Seeherman, & Taylor, 1982). Therefore, all fast fibers in dogs are of the fast oxidative glycolytic (FOG) type, and all slow fibers are of the SO type. Unequivocal fiber type identification can thus be made from a single histochemical stain for myofibrillar ATPase activity (remember that this type of statement is only valid having first experimentally determined that dog muscle contains no FG fibers).

Q&A

Don't All Muscles Atrophy to the Same Extent?

Absolutely not. Some muscles (primarily antigravity muscles) atrophy tremendously, while their antagonists do not atrophy much at all. Thus, different muscles are more vulnerable to immobilization-induced atrophy. In fact, even the same muscle fiber type within different muscles may atrophy to different extents, and the different types of the same fibers within the same muscles may atrophy to different extents. The extent of atrophy seems to be related to each muscle's, and perhaps even each fiber's, "set point" regarding the level of activity that will maintain its mass at a constant level.

Dog Quadriceps Immobilization Method

To ensure that muscle lengths were held constant during the immobilization period, a custom external skeletal fixator, designed by the orthopaedic surgeon Dr. David Gershuni, was used to fix the knee joint angle at 90° (Fig. 5-3). One leg was immobilized while the other leg was left as a control. However, note that contralateral legs from experimental animals are not truly "normal" for a variety of reasons. First, they probably bear more weight since the immobilized leg is raised. Second, systemic effects might affect all muscles differentially. In this particular study, control experiments were performed that demonstrated no differences between truly normal muscles, taken from untreated animals, and contralateral muscles from animals used in this study.

After 10 weeks of immobilization, small biopsies were taken from the VL, VM, and RF and prepared for histochemical analysis as described in Chapter 2 (Fig. 5-4). An added point that should be mentioned here is that the biopsies were always kept under some tension, since fiber shortening will cause an apparent increase in fiber cross-sectional area. After staining muscles for myofibrillar ATPase activity, and classifying each fiber as fast or slow, muscle fiber areas were measured using well-defined stereological methods (Weibel, 1980). It is important to sample muscle fibers across the entire section so that representative areas are obtained. As a result of this approach, the following simple experimental data set was obtained for each muscle:

1. Type 1 fiber area (μm^2)
2. Type 2 fiber area (μm^2)

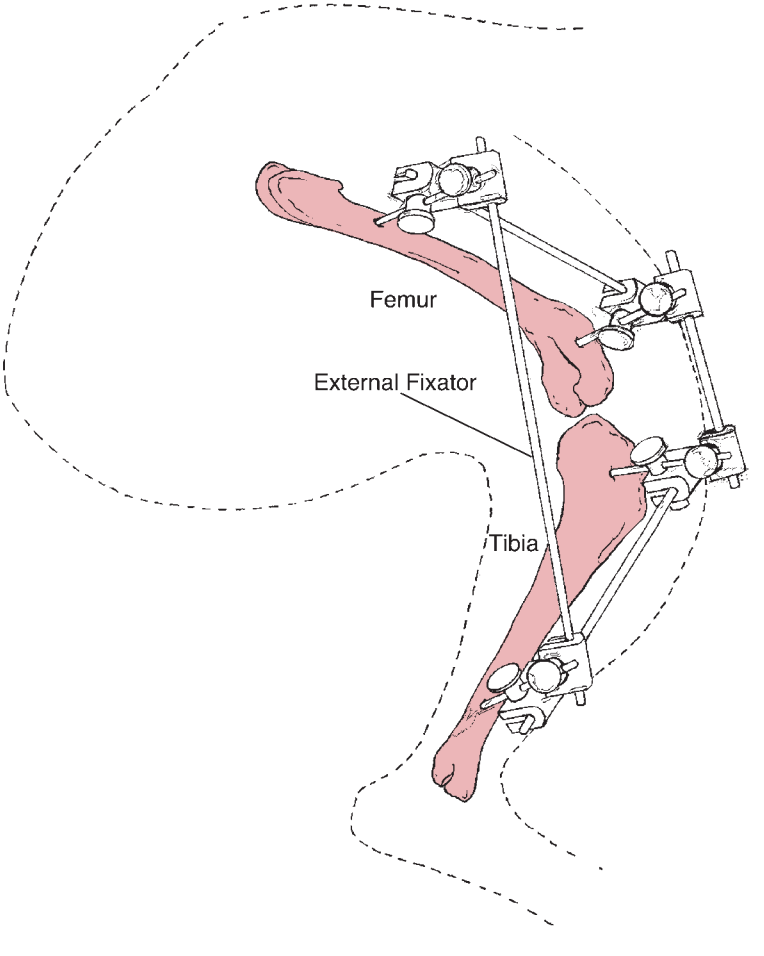

Figure 5-3 Experimental method for immobilization of the dog quadriceps muscles. An external skeletal fixator was used to maintain the knee angle at 90° of flexion. In this way, muscle length could be carefully controlled. (From Lieber, R. L., Fridén, J. O., Hargens, A. R., Danzig, L. A., & Gershuni, D. H. [1988]. Differential response of the dog quadriceps muscle to external skeletal fixation of the knee. *Muscle & Nerve, 11*, 193–201.)

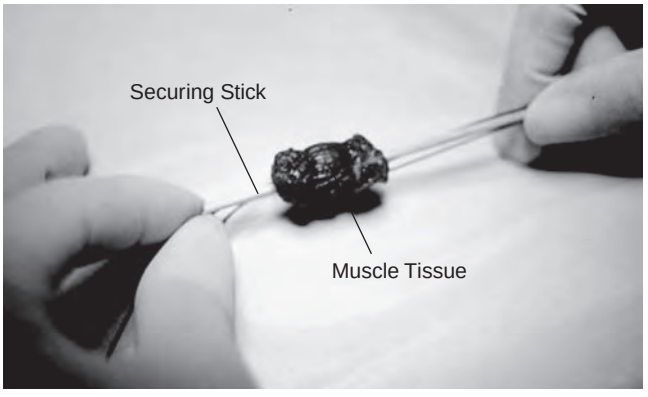

Figure 5-4 Photograph of a muscle biopsy obtained from a vastus lateralis muscle. Note that, to obtain excellent muscle fiber morphology, the biopsy is securely attached to a wooden stick with sutures. Since muscle fibers contract with a constant volume, muscle fiber shortening will erroneously be interpreted as fiber size increase, while muscle fiber lengthening will erroneously be interpreted as fiber size decrease. It is thus important to preserve muscle fiber length when obtaining a biopsy.

3. Percentage of each fiber type
4. Area fraction of endomysial and perimysial connective tissue (%)

Clearly, fiber area relates to the force-generating capacity of the fiber. If muscle fiber number does not change with immobilization, fiber area ought to be a good predictor of muscle force. The greater the fiber area, the greater the number of myofibrils arranged in parallel within the fiber. Thus, when muscle fiber atrophy is discussed, strictly speaking, myofibrillar number or a parameter that is closely related to it (such as fiber area) must be considered. As myofibrillar number decreases, the force generated by the fiber also decreases. Fiber area (as an index of myofibrillar number) is compared between control and immobilized muscles to determine their responses to immobilization. In the increased use models, fiber type percentage provided insights into the degree of use experienced by the muscle. Recall that in the chronic stimulation model the fast-to-slow fiber type conversion resulted from the dramatically increased level of fiber activity compared to normal activity. In the same way, a muscle's normal fiber type distribution is probably related to the amount of normal activity experienced by the muscle. In Chapter 1, during development, differentiation past the secondary myotube required innervation (Miller & Stockdale, 1987). It can be inferred from this that, during maturation, muscle fibers receiving a great deal of neural activity have a greater likelihood of becoming slow fibers. The take-home lesson is that a muscle's normal fiber-type distribution provides insights into its normal level of use and, as you will see, its response to decreased use.

Muscle Fiber Response to Immobilization

The most obvious response of the immobilized dog quadriceps was the sizable fast and slow muscle fiber atrophy (Fig. 5-5). However, although all micrographs were taken

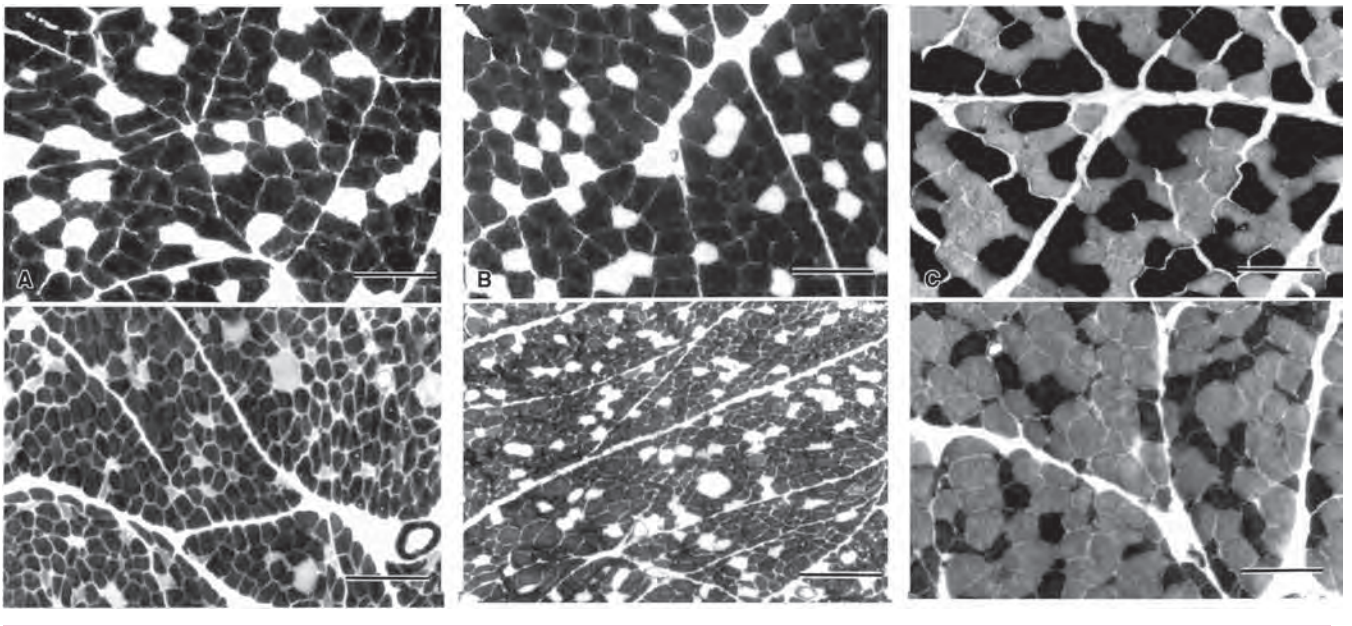

Figure 5-5 Light micrographs of the (A) vastus lateralis, (B) vastus medialis, and (C) rectus femoris muscles. The top panel represents micrographs from normal muscles, and the bottom panel represents micrographs from immobilized muscles. All micrographs were taken at the same magnification. Fast fibers appear dark, and slow fibers appear light. Calibration bars = 100 μm. (Reprinted with permission from Lieber, R. L., Fridén, J. O., Hargens, A. R., Danzig, L. A., & Gershuni, D. H. [1988]. Differential response of the dog quadriceps muscle to external skeletal fixation of the knee. *Muscle & Nerve, 11*, 193–201. Reprinted with permission of John Wiley & Sons, Inc.)

at the same magnification, it was obvious that the magnitude of the atrophy was different for the three muscles. Using stereometric techniques developed in Switzerland by Professor Weibel (1979), it was found that there was no difference in fast or slow muscle fiber area between any of the muscles on the control side. Yet, 10 weeks of immobilization caused significant decreases in both fast (Fig. 5-6) and slow (Fig. 5-7) muscle fiber area. Clearly this would result in decreased muscle force-generating capacity. However, the most interesting result was not that muscle fiber areas decreased in response to immobilization (a result that had already been demonstrated by numerous scientists), but that the amount of muscle fiber atrophy was different for each of the three muscles. Specifically, the fast fiber area of the immobilized VM and VL was less than the fast fiber area of the immobilized RF (Fig. 5-6). Similarly, in an even more dramatic differential response, the slow fiber area of the VM was much less than that of the VL, which, in turn, was much less than that of the RF (Fig. 5-7). The atrophic response for slow fibers was thus, in order from most to least atrophied: VM > VL > RF, while for fast fibers the corresponding order was VM = VL > RF. This difference was even more dramatic on inspection of the actual micrographs. Compare, for example, the fiber sizes in the micrographs of Figure 5-5, photographed at the same magnification. In addition to these changes in fiber area, a significant increase in fast fiber percentage following immobilization was observed in the VM. You may have already experienced firsthand the differential atrophy observed in human quadriceps after immobilization. One patient population in which this is common is the relatively young patients recovering from injury and reconstruction of their anterior cruciate ligament (ACL). The "caving in" of the distal portion of the VM (known as the VM obliques, or VMO, based on the oblique pennation angle of the fibers in this region of the VM) is easily appreciated in these patients and represents a challenge to the therapist who must treat this severe muscle atrophy.

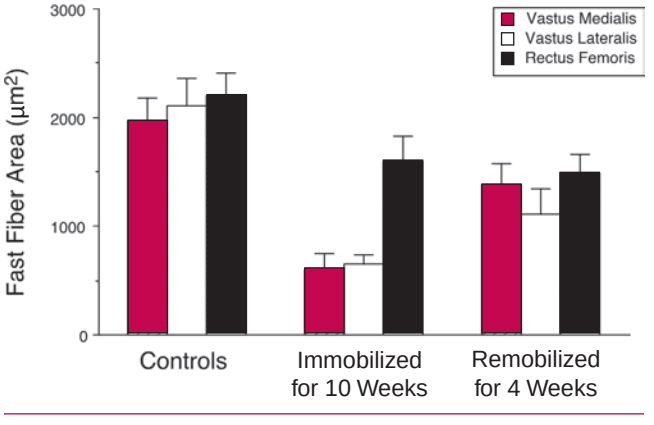

Figure 5-6 Graph of fast fiber area from control, immobilized, and remobilized dog quadriceps muscles. Note that the magnitude of atrophy was muscle specific. Upon remobilization, muscles returned to parity. (From Lieber, R. L., Fridén, J. O., Hargens, A. R., Danzig, L. A., & Gershuni, D. H. [1988]. Differential response of the dog quadriceps muscle to external skeletal fixation of the knee. *Muscle & Nerve, 11*, 193–201; Lieber, R. L., McKee-Woodburn, T., Fridén, J., & Gershuni, D. H. [1989]. Recovery of the dog quadriceps after ten weeks of immobilization followed by four weeks of remobilization. *Journal of Orthopaedic Research,* 7, 408–412.)

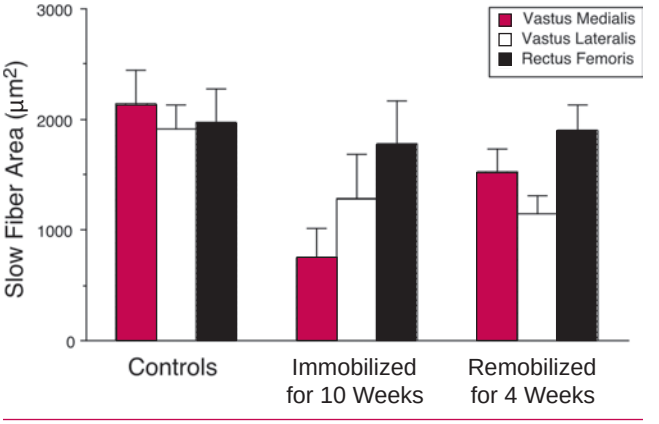

Figure 5-7 Graph of slow fiber area from control, immobilized, and remobilized dog quadriceps muscles. (From Lieber, R. L., Fridén, J. O., Hargens, A. R., Danzig, L. A., & Gershuni, D. H. [1988]. Differential response of the dog quadriceps muscle to external skeletal fixation of the knee. *Muscle & Nerve, 11*, 193–201; Lieber, R. L., McKee-Woodburn, T., Fridén J., & Gershuni D. H. [1989]. Recovery of the dog quadriceps after ten weeks of immobilization followed by four weeks of remobilization. *Journal of Orthopaedic Research,* 7, 408–412.)

Immobilization also caused proliferation of endomysial and perimysial connective tissue relative to the controls, with a significantly greater increase of these tissues in the immobilized VM and VL muscles compared to immobilized RF muscles.

Proposed Explanation for Differential Muscle Fiber Responses

How can the differences between these muscles be accounted for on the basis of our understanding of muscle plasticity? Why would slow fibers in one muscle dramatically atrophy, while slow fibers in another muscle atrophy only slightly?

Consider each muscle sequentially. The differences observed between the VM and VL could not simply be explained by differences in immobilization length. Immobilization length strongly influences the atrophic response as we have seen (Fig. 5-2), but the VL and VM were fixed at identical lengths. Using a similar argument, differences observed between the VM and RF could not be explained by differences in fiber type distribution, since they both began with about 50% fast and 50% slow fibers. The architecture of all three muscles is similar. The ratio of fiber length to muscle length has been studied in humans

and guinea pigs and approximates 0.2 for all three muscles. Thus, architectural differences cannot account for the differences observed. To explain the differential atrophy of the VM, VL, and RF, other factors must be considered.

The RF demonstrated the smallest degree of atrophy of the three muscles studied. Conversely, the most severe atrophy was observed in the VM. This is interesting in light of the fact that the two muscles initially contained nearly identical fiber type distributions. Therefore, initial fiber type distribution alone does not dictate the magnitude of the atrophic response. However, the RF crosses the hip and the knee, functioning both as a knee extensor and hip flexor, while the VM and VL cross just the knee, functioning as knee extensors. The RF is therefore less rigidly immobilized than either of the vasti, probably explaining the small atrophic response.

The slow fibers of the VM atrophied to a greater extent than those of the VL. Both the VM and VL were immobilized at the same length, since they both arise from the proximal femur and insert together with the rectus tendon onto the patella. It seems unlikely then that the small difference in anatomical location could account for the markedly different response. The VM initially contained a much larger proportion of slow fibers than the VL, which indicates that the VM was probably used more, since muscle fiber type distributions provide insights into muscle activation history. Therefore, after immobilization, the change in the amount of VM activation was probably greater than the change for the VL, even though the absolute levels following immobilization may have been similar. This provides support for the idea that immobilization represented a model of decreased use, but not disuse.

Fiber Type Transformation after Immobilization

Perhaps this large change in the VM's activation level could also account for the slow-to-fast transformation. This is our first exposure to the idea that, in contrast to increased use models that were shown to routinely cause a fast-to-slow transformation, decreased use models cause the opposite—a slow-to-fast transformation. This type of transformation has been observed clinically. For example, Haggmark and coworkers observed a significant increase in VL type 2 fiber percentage after surgical reconstruction of the ACL (Haggmark & Eriksson, 1979). Interestingly, the magnitude of the transformation seemed to be correlated with the change in use, since elite athletes (whose muscles were most "used" to high activity levels) demonstrated the greatest degree of slow-to-fast transformation. Similarly, Dr. Gunnar Grimby, a Swedish rehabilitation physician, observed dramatic slow-to-fast fiber type transformations in patients recovering from traumatic spinal cord lesions (Grimby, Broberg, Krotkiewska, & Krotkiewski, 1976). Muscle fiber type transformation after decreased use may have functional implications. First, recall that faster muscles only generate smooth tetanic contractions at the higher stimulation frequencies (Fig. 2-2). Thus, if contraction and relaxation speeds increase, higher neural drive frequencies will be required to maintain steady tension levels. This may result in decreased neuromotor control in these patients, many of whom retain significant residual function.

Generalizations Regarding Muscle Fiber Atrophy

This study established the relative influence of two factors that contribute to immobilization-induced atrophy. The most significant factor was the degree of immobilization (number of joints crossed), and next was the change in use relative to normal function. The initial percentage of slow muscle fibers was a fair indicator of the normal muscle use level and was a good predictor of the relative degree of atrophy. These data indicate that a blanket concept of "slow fiber atrophy" cannot apply to all muscles. Rather, it is a combination of factors that determines the muscular response to decreased use. Given the structure and fiber type distributions of the various human muscles, it is possible to predict those that are most vulnerable to immobilization-induced atrophy, that is, those that function as antigravity muscles, cross a single joint, and contain a relatively large proportion of slow fibers. This description fits the soleus, multifidus, VM, and vastus intermedius muscles. The next class of muscles tat are susceptible to immobilization-induced atrophy would be antigravity muscles, predominantly slow ones that cross multiple joints, namely the longissimus, transversospinalis (erector spinae), gastrocnemius, and RF. These muscle groups should be immobilized conservatively to avoid severe loss of strength. Conversely, phasically activated, predominantly fast muscles (e.g., TA, extensor digitorum longus (EDL), biceps) can be immobilized with lesser loss of strength.

THERAPIST'S COMMENTS

The muscle fiber changes associated with immobilization are surprising to many people. Most would assume that immobilization is associated with fast-to-slow fiber type changes; however, it has been pointed out that this is opposite from what is observed experimentally. If you understand that muscles adapt to their usage patterns, it is not surprising that reduced use is associated with faster, less oxidative muscle fibers. Given this understanding of physiology, it would be interesting to couple immobilization with low-level, chronic electrical stimulation. As long as the muscle activity did not disrupt the healing process of ligament, tendon, and bone, the recovery process may be abbreviated by this combination of therapies. Further research is needed to support this idea.

This hierarchy of susceptibility to immobilization is supported by the data of Dr. Edgerton and colleagues, who measured morphological, biochemical, and physiological properties of immobilized hindlimb muscles from *Galego senegalensis* (a small primate commonly known as the bush baby) and found that muscles atrophied in the order (most to least atrophy): soleus > plantaris > vastus intermedius = VL > gastrocnemius > TA = RF, agreeing well with the principles stated earlier (Edgerton, Barnard, Peter, Maier, & Simpson, 1975). Remember, increased atrophy is observed upon immobilization in muscles that are normally used a great deal. These tend to be muscles that contain a relatively high percentage of slow fibers.

Descriptors of Muscle Atrophy

Numerous immobilization studies have documented differing degrees of atrophy following different types of immobilization treatments. How should the magnitude of the atrophic response be calculated? Is the atrophy directly related to the change in muscle force? Is it related to the change in muscle mass? To describe appropriate measures of atrophy, return to the immobilization study performed in Edgerton's laboratory that was discussed in the Chapter 4.

Recall that muscles were immobilized at different lengths, after which architectural (Spector, Simard, Fournier, Sternlicht, & Edgerton, 1982) and contractile (Simard, Spector, & Edgerton, 1982) properties were measured. Compare the conclusions reached using muscle mass as a measure of atrophy to maximum tetanic tension as a measure of atrophy. The soleus and MG will be considered. The normal rat MG weighs about 800 mg. After immobilization in the lengthened position, the MG mass dropped to 635 mg, while in the shortened position, it decreased dramatically to 350 mg (Fig. 5-8A). In terms of mass, therefore, the stretched MG atrophied by only 15%, while the shortened MG atrophied by over 50%. These represent impressive and quite different changes. Unfortunately, these changes in mass had almost no relation to actual muscle performance following immobilization. While the shortened MG decreased in mass by 50%, MG P_o (maximum isometric tension) decreased by over 75% (Fig. 5-7B), which is quite a difference! The lengthened MG decreased in mass by 15%, while MG P_o decreased by over 40%—quite a disparity. Using a similar argument for the soleus, the results were quite impressive. After immobilization in the lengthened position, soleus mass dropped from 150 to 132 mg, while in the shortened position, mass decreased dramatically to 79 mg (Fig. 5-7A).

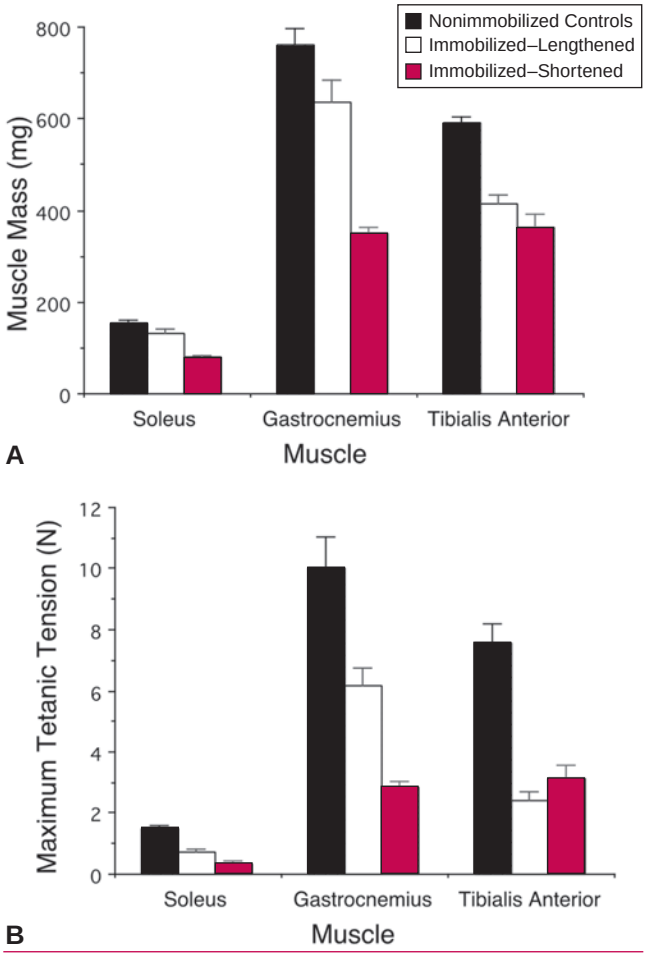

Figure 5-8 (**A**) Muscle mass and (**B**) P_o from normal and immobilized rat skeletal muscles. Note that mass and P_o changes are not uniquely correlated. Mass is thus a poor predictor of muscle tension. Note also that immobilization in a lengthened position spares the muscle of mass and tension losses, which accompany immobilization in the shortened position. (Data from Simard, C. P., Spector, S. A., & Edgerton, V. R. [1982]. Contractile properties of rat hindlimb muscles immobilized at different lengths. *Experimental Neurology,* 77, 467–482.)

THERAPIST'S COMMENTS

Another clinical example related to joint position is of the typical acute phase of rehabilitation after ankle sprain injury (Safran et al., 1999). This phase focuses on ice, compression, and immobilization. The immobilization can be of various forms, from a traditional plaster cast to an Aircast that allows some motion in plantarflexion and dorsiflexion. It is stated that the immobilization should be in as much dorsiflexion as possible to help lengthen the soleus. However, because of the swelling in the joint, the ankle will most likely be in a position of plantarflexion during the first week, which means that soleus atrophy and fiber type changes will occur to a greater degree. The therapist must be aware of this and progress to controlled movement as soon as possible to limit the detrimental effects of immobilization on the soleus that is shortened secondary to plantarflexion.

In terms of mass, therefore, the stretched soleus atrophied by only 12%, while the shortened soleus atrophied by 50%. In terms of contractile tension, the stretched soleus atrophied by 50%, and the shortened soleus atrophied by 80%. Again, very different results—12% mass versus 50% P_o and 50% mass versus 80% P_o. There is more atrophy if P_o is considered instead of mass. Why the disparity? Think about what you have already learned about the relationship between muscle force and muscle mass. This is a good test of your understanding of the relationship between muscle's physiological cross-sectional area (PCSA), as defined in Chapter 1 (Equation 1-1, p. 27), and muscle force generation.

The reason mass and performance were not closely related in these treatments is that performance is a direct function of architecture, whereas mass is simply proportional to the total amount of contractile material in the muscle. These immobilization treatments resulted in a change in muscle fiber length, as discussed in Chapter 4 (Fig. 4-8), which strongly affected PCSA calculations. The explanation for the disparity between the results using mass or P_o is that changes occurred in fiber length after immobilization. For example, immobilization in the shortened position resulted in decreased fiber length. This will show up as a decrease in mass, but fiber length changes alone will not affect contractile tension. The functional effects of fiber length change would be seen in measures of V_{max} or muscle excursion, neither of which were explicitly measured in the previous study. Therefore, the only conditions under which mass, or protein content, provides a reliable index of tension decrease are those in which no architectural adaptations are seen. To reiterate, knowledge of muscle mass change after immobilization provides little information regarding the functional consequences of immobilization without some knowledge of the concomitant architectural changes. Since mass is proportional to volume, the same error would be made from estimating atrophy from MRI reconstruction of muscle volume (Eng, Abrams, Smallwood, Lieber, & Ward, 2007).

Incidentally, Edgerton and his colleagues also demonstrated that the decrease in P_o was nearly proportional to the muscle fiber area decrease. It thus appears that the atrophy of muscle fiber cross-sections truly is the primary cause for decreased muscle force—the muscle fibers themselves generate less force. This relationship between muscle fiber size and torque was recently directly demonstrated using the dog immobilization model (Fig. 5-9) (Lieber et al., 1997). Small biopsies were removed from the muscles during a functional testing protocol, in which maximum quadriceps extension torque was also measured. Taken together, these animal studies provide strong support for the use of muscle fiber area from biopsies to infer functional properties of human muscles after immobilization. The biggest problem with such studies in humans is that the biopsies obtained represent a small area of the entire muscle and may not be representative, since all fibers within the muscle may not be equally represented in a muscle biopsy.

Q&A

Why Does Muscle Strength Not Decrease to the Same Extent As Muscle Mass?

Strictly speaking, muscle strength is proportional to the PCSA (Chapter 2). Since PCSA is a calculated value, it is almost never seen on any type of "scan" such as an MRI or a CT scan. Sometimes, immobilization results in muscle shortening that in turn results in a decrease in mass, but immobilization does not necessarily have the same effect on PCSA. Thus, this immobilization would have no effect on maximum strength. One must be careful to measure the correct parameter from a patient to describe muscle's response to atrophy.

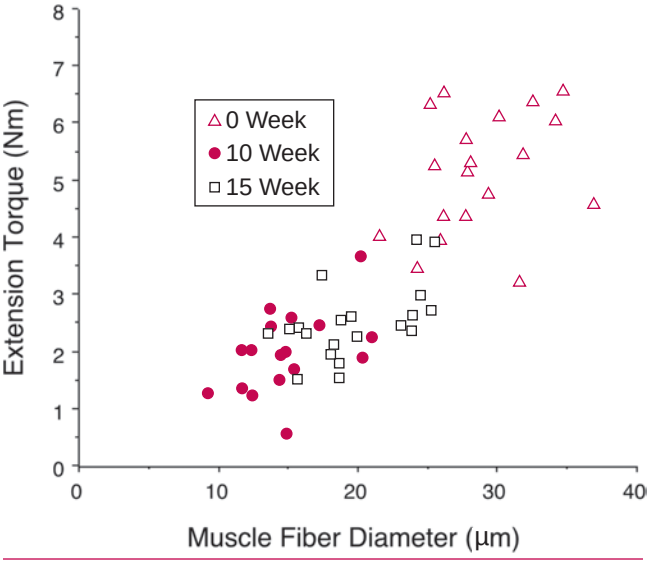

Figure 5-9 Relationship between fiber size and joint torque in the dog immobilization model. Each symbol is obtained at different times after immobilization from 0 *(open triangles)* to 10 weeks *(filled circles)* and after 5 weeks of remobilization *(open squares)*. The fact that the relationship between fiber area and torque is maintained during immobilization and remobilization suggests that torque changes are due to changes in muscle fiber size. (Data from Lieber, R. L., Jacks, T. M., Mohler, R. L., et al. [1997]. Growth hormone secretagogue increases muscle strength during remobilization after canine hindlimb immobilization. *Journal of Orthopaedic Research, 15*, 519–527.)

REMOBILIZATION AFTER IMMOBILIZATION

How long does it take to recover from immobilization-induced atrophy? Interestingly, very few remobilization studies have been performed. The few that have been performed suggest that it takes longer to recover from immobilization than to elicit the initial response. This is a similar finding to that seen in Figure 4-27, in which muscle "detraining" was about twice as fast as muscle "training" for endurance exercise. The dog study described above was essentially repeated by remobilizing canine quadriceps for 4 weeks, after 10 weeks of immobilization (Lieber, McKee-Woodburn, Fridén, & Gershuni, 1989). During this 4-week remobilization period, normal activity was permitted, and daily 1-hour walking/running outings were encouraged. Normal weight bearing resumed spontaneously within about 1 week.

As in the initial study, no difference in slow or fast fiber area was observed among any of the control muscles. However, 10 weeks of immobilization followed by 4 weeks of remobilization still resulted in about a 30% decrease (or not a full recovery) of both slow and fast fiber areas (Figs. 5-6 and 5-7) relative to the control. However, in contrast to the canine immobilization study just described, no difference between immobilized muscle fiber areas was seen. While immobilization-induced atrophy was muscle and fiber-type specific, recovery following immobilization was neither a function of muscle nor of fiber type. The fiber-type transformation that occurred with immobilization also returned to normal following remobilization.

A second difference between the immobilization and remobilization results was that, while a large increase in the amount of extracellular connective tissue was seen after 4 weeks of immobilization, no difference was seen between control and remobilized muscles in the amount of extracellular connective tissue. Thus, the previously elevated area fraction of connective tissue (about 20%), observed after immobilization, returned to control levels (about 10%) after the remobilization period. Since connective tissue may be associated with passive muscle stiffness, these data may imply that muscle stiffness, which had increased because of the immobilization process, returned to normal after remobilization. Joint stiffness after immobilization is a significant clinical problem. Most previous experimental models of such stiffness have focused on changes that occur in the affected joint capsules and ligaments (Akeson, Amiel, Abel, Garfin, & Woo, 1987). However, these studies show that the muscle itself may represent a source of increased joint stiffness after immobilization.

A muscle's response to remobilization may also be interpreted in light of the level of use experienced by each muscle after remobilization. Recall that the differential immobilization-induced atrophy based on the change in level of use relative to normal levels was explained. Now, during remobilization, it is presumed that normal use levels returned and brought fiber area back to parity. Detailed confirmation of this hypothesis awaits future experiments. However, quantifying use in terms of EMG, force, or movement is difficult technically, and excellent studies using these methods are lacking.

THERAPIST'S COMMENTS

Although these studies provide evidence for resuming physical activity after immobilization, we still need to understand how muscles change with aggressive physical activity (e.g., physical therapy). Does physical therapy speed muscle recovery after immobilization? Although current practice models attempt to balance the speed of recovery with protection of the original injury or surgical repair, we have not optimized the volume or intensity of exercise with specific injuries.

ADAPTATION TO SPINAL CORD TRANSECTION

Decreased use models have also been developed that induce either an upper or lower motor neuron lesion. As you will see, lower motor neuron disruption (denervation) induces numerous changes in muscle that are quite distinct from other decreased use models. (For example, a denervated muscle responds in a qualitatively different manner to chronic stimulation than a normal muscle.) This difficulty has been overcome by developing a model that interrupts the upper motor neuron pathway by transecting the spinal cord (i.e., cordotomy) (Fig. 5-10). This procedure has been performed in several animal models. Again, muscle contractile, histochemical, and biochemical alterations will be discussed after cordotomy.

Experimental Method of Rat Cordotomy

In a previous study, we were interested in the extent of muscle adaptation that occurred following long-term spinal cordotomy (Lieber, Johansson, Vahlsing, Hargens, & Feringa,

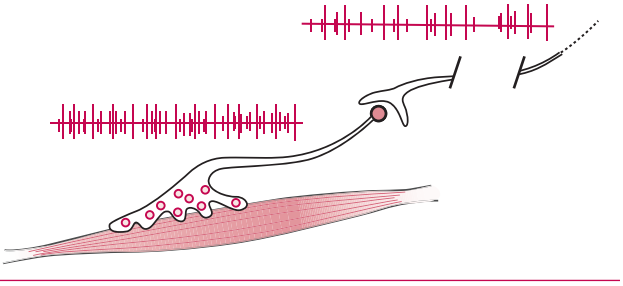

Figure 5-10 Schematic representation of the cordotomy model. The upper motor neuron pathway has been interrupted, but muscle EMG activity remains because of segmental influences. Muscle tension is low since the limbs are not used for locomotion.

1986a). The rat was chosen as the experimental model since rats live only 2–3 years, and 1 year of cordotomy would represent about half a lifetime of chronic disuse.

Two groups of rats were studied. Control rats were permitted normal growth for 1 year after entry into the study at age 6 weeks. At age 6 weeks (about 2 weeks after the muscle fiber types were differentiated), the experimental rats were anesthetized and, after laminectomy, the spinal cord and its coverings were completely transected.

Postoperative care of the cordotomized rats required special cage bedding to prevent pressure sores. Initially, the rats experienced flaccid paraplegia with their limbs dragging behind them as they crawled about in the cage. They were able to move using their forelimbs, and had no difficulty reaching food and water. At approximately 3–4 weeks, the paralyzed hindlimbs of the animals changed from flaccid to spastic. After spasticity developed, the limbs were almost always held in extension, and no recovery of voluntary activity was ever observed. At first, it was exciting in that we thought this model could mimic the spasticity that is often observed in patients after stroke or head injury. However, this spasticity was qualitatively different from that observed in humans. Unfortunately, we cannot explain fully why the rat spastic phase is transient and not reflective of the condition observed in humans. In fact, there is no adequate animal model for the type of spasticity seen in humans, which severely impairs our ability to understand and treat this debilitating condition.

Contractile Properties of Muscles after Long-term Cordotomy

Two muscles were chosen for study—the soleus and the EDL. The two muscles differ in fiber type percentage (80% slow fibers in the soleus and 5% in the EDL). In this way, it would be possible to determine whether any observed effects of cordotomy were fiber type or muscle specific. The contractile properties of the soleus and EDL muscles were tested in both groups of rats at age 58 weeks. The distal muscle insertion was carefully dissected free and attached to a force transducer. The peripheral nerve innervating the muscle was also carefully isolated and electrically activated using an artificial stimulator. Note that in spite of a year of cordotomy, the lower motor neuron was completely intact and able to be activated. Paralyzed muscle may still be quite functional. This is good news for therapists involved in electrical stimulation treatment of spinal cord–injured (SCI) patients (Popovic, Baker, & Loeb, 2007; Shields, Chang & Ross, 1998; Shields, Law, Reiling, Sass, & Wilwert, 1997).

The responses of the soleus from normal and transected rats stimulated at 5 and 10 Hz are shown in the upper and lower panels of Figure 5-11, respectively. Note that at 10 Hz, the transected soleus developed greater force and was less fused than the normal soleus, implying faster contraction and/or relaxation. Unfused tetani of the EDL stimulated at 10, 20, and 30 Hz are shown in Figure 5-12.

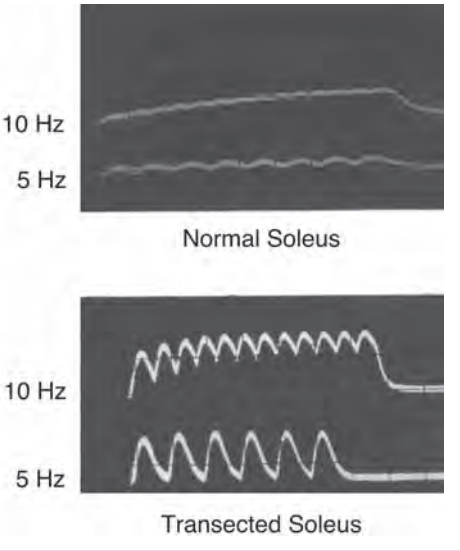

Figure 5-11 Unfused tetani from rat soleus muscles. **Upper panel**: records from control animals. **Lower panel**: records from transected animals. Note that the upper records demonstrate greater fusion than the lower records, implying increased calcium transport kinetics after cordotomy. (Adapted with permission from Lieber, R. L., Johansson, C. B., Vahlsing, H. L., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Experimental Neurology, 91*, 423–434.)

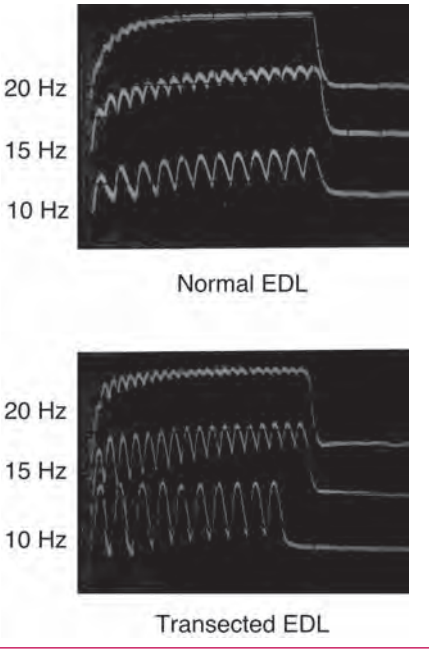

Figure 5-12 Unfused tetani from rat extensor digitorum longus muscles. **Upper panel**: records from control animals. **Lower panel**: records from transected animals. Very little difference is observed between records. (Adapted with permission from Lieber, R. L., Johansson, C. B., Vahlsing, H. L., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Experimental Neurology, 91*, 423–434.)

Q&A

How Does Spinal Cord Injury Change Muscle Properties?

Spinal cord injury, as a typical decreased use model, causes muscle to become weaker, because of muscle fiber atrophy, and faster, because of slow-to-fast fiber type conversion. In addition, there is evidence that, after chronic spinal cord injury, muscles become much more easily fatigued. Loss of strength, increased speed, and increased fatigability affect the patient's ability to perform normal activities of daily living. The good news is that all of these properties can be affected by the therapeutic interventions described in this book.

The differences between the normal EDL (upper panel) and transected EDL (lower panel) were clearly much less dramatic than those observed for the soleus.

Analysis of the averaged contractile responses showed that no differences were observed between normal and transected EDLs (Fig. 5-13). In contrast, for the soleus muscle, dramatic changes were observed in all contractile properties measured. For example, time to peak tension decreased by about 50% (Fig. 5-13A), suggesting a change in the properties of the sarcoplasmic reticulum. The change was probably due to an increase in the calcium transporting ability of the SR. The soleus fusion frequency increased by 100% (Fig. 5-13B), which supports this hypothesis. Absolute maximum tetanic tension (in N) did not change significantly after transection. However, since the soleus muscle cross-sectional area decreased by about 50% (Fig. 5-13C), specific tension (in N/cm^2) of the soleus significantly increased by over 100% one year following transection. Thus, the soleus muscle was half the cross-sectional area, but still generating the same tension (Fig. 5-13D). How (see later in the chapter, p. 196)? To summarize, after transection, the soleus muscle increased in contractile speed and specific tension, while no significant contractile changes were seen in the EDL.

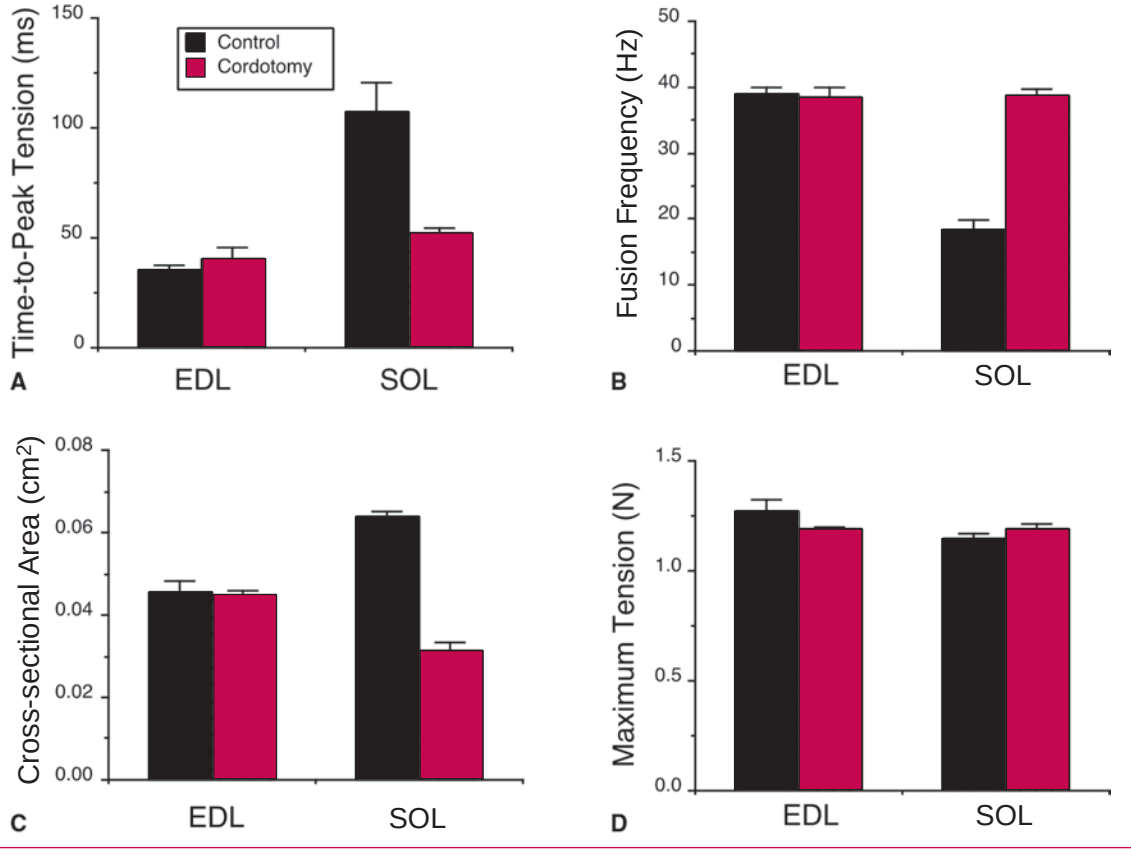

Figure 5-13 Contractile properties from normal *(black bars)* and cordotomized *(red bars)* rat skeletal muscles. (**A**) Time-to-peak twitch tension, (**B**) fusion frequency, (**C**) muscle cross-sectional area, and (**D**) maximum tetanic tension. Note that soleus contractile properties changed dramatically, while EDL properties show little change. (Adapted with permission from Lieber, R. L., Johansson, C. B., Vahlsing, H. L., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. I. Contractile properties. *Experimental Neurology, 91*, 423–434.)

Morphometric Properties of Muscles after Long-term Cordotomy

To try to understand the contractile results, the fiber type and fiber size distributions were measured within the soleus and EDL (Lieber, Fridén, Hargens, & Feringa, 1986).

Low-magnification cross sections of normal soleus and EDL muscles stained for myofibrillar ATPase are presented in the upper panels of Figures 5-14 and 5-15, respectively. Note that in the normal soleus, fast fibers are scattered throughout the muscle, while the transected soleus (lower panel of Fig. 5-14) is composed almost entirely of fast fibers. This represents a dramatic slow-to-fast muscle fiber type conversion (the opposite of what we observed with chronic stimulation). The SOL at higher magnification (Fig. 5-16) showed a decreased size of both the slow and fast fibers. While fiber type transformation occurred in the EDL also (lower panel of Fig. 5-15), the magnitude of the effect was small. The normal EDL contains only a few slow fibers (indicated by light staining) in the anterior superficial region of the muscle. After transection, even fewer slow fibers were visible.

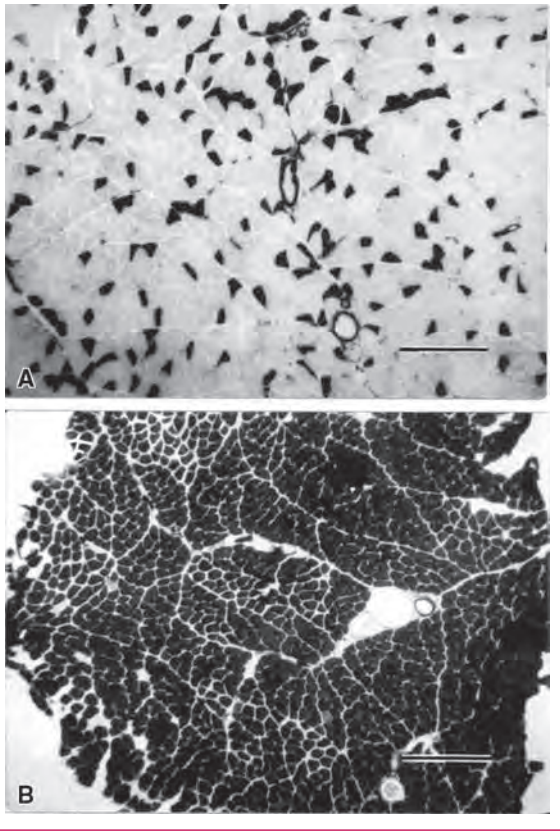

Figure 5-14 Low magnification micrographs from soleus muscle (**A**) before and (**B**) after cordotomy. Muscle sections stained for myofibrillar ATPase activity. Fast fibers are dark. Calibration bar represents 500 μm. Note the increase in fast fiber percentage following cordotomy. Figure 5-16 micrographs represent higher magnification views of same muscles. Note the modest fiber atrophy. Calibration bars represent 100 μm. (Adapted with permission from Lieber, R. L., Fridén, J. O., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection of fast and slow rat skeletal muscle. II. Morphometric properties. *Experimental Neurology, 91*, 435–448.)

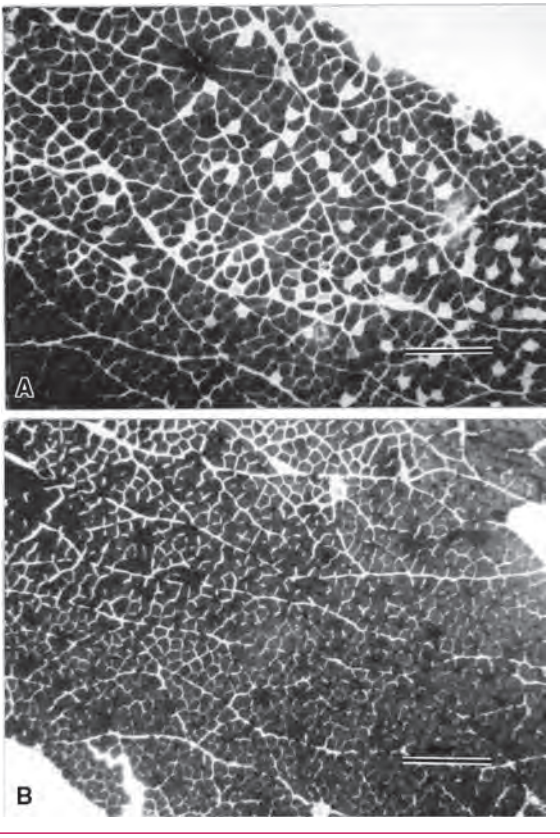

Figure 5-15 Low-magnification micrographs from EDL muscle (**A**) before and (**B**) after cordotomy. Muscle sections stained for myofibrillar ATPase activity. Fast fibers stain darkly. Calibration bar represents 500 μm. Note the increase in fast fiber percentage following cordotomy, which is more modest than that of the soleus. (Adapted with permission from Lieber, R. L., Fridén, J. O., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection of fast and slow rat skeletal muscle. II. Morphometric properties. *Experimental Neurology, 91*, 435–448)

Quantitative changes observed following transection were similar for both muscles, although the magnitude of the changes was greater for the SOL compared with the EDL. As can be appreciated from the micrographs, the percentage of slow muscle fibers decreased significantly for both muscles. The average slow fiber area decreased significantly by about 50% for the SOL but not for the EDL. The percentage of fast fibers increased significantly for both muscles. Again, the magnitude of the increase was greater for the SOL. Fast fiber area decreased by about 25% in both muscles. The percentage of extracellular connective tissue increased significantly for both muscles by about the same amount. Thus, both muscles demonstrated fiber atrophy, a slow-to-fast fiber type transformation, and a significant increase in connective tissue. However, since the EDL is

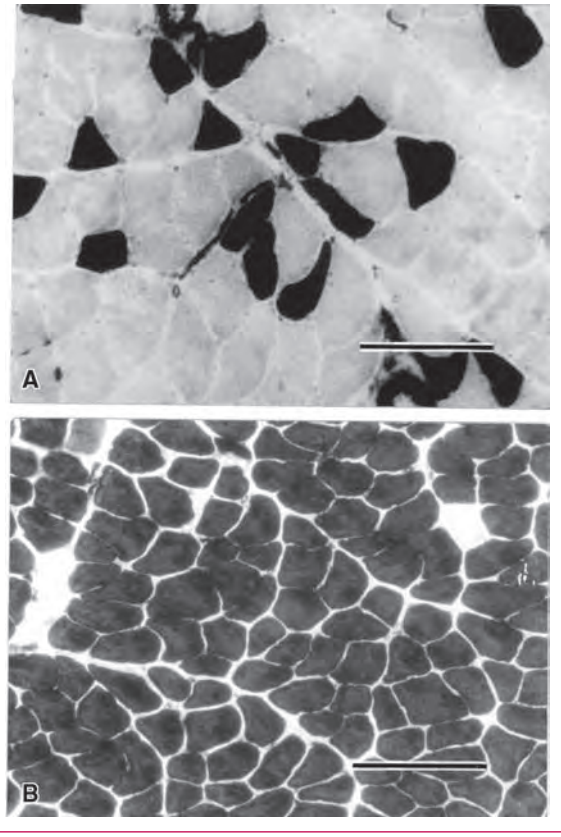

Figure 5-16 Higher magnification micrographs from soleus muscle (**A**) before and (**B**) after cordotomy. Muscle sections stained for myofibrillar ATPase activity. Fast fibers stain darkly. Note the modest fiber atrophy. Calibration bars represent 100 μm. (Adapted with permission from Lieber, R. L., Fridén, J. O., Hargens, A. R., & Feringa, E. R. [1986]. Long-term effects of spinal cord transection of fast and slow rat skeletal muscle. II. Morphometric properties. *Experimental Neurology, 91*, 435–448.)

normally composed of about 95% fast fibers, these changes had no effect on contractile properties. Clearly, such a transformation had much more profound consequences for the normally 80% slow soleus muscle. Finally, since the SOL muscle generated the same absolute contractile force in spite of its smaller muscle fibers, the force per unit area of muscle fiber (i.e., the specific tension) must have increased. Since most of the fibers in the SOL following cordotomy were fast, it was concluded that the fast fibers of the rat have a higher specific tension than the slow fibers.

The Importance of Tension in Determining Muscle Properties after Cordotomy

What factors contribute to the dramatic changes observed following cordotomy? What can be done to prevent these changes or to ameliorate recovery from such changes? In yet another interesting plasticity study, Dr. Reggie Edgerton and his colleagues exercised adult cats that had been spinalized (Roy et al., 1998). After transection at the mid-thoracic level and 1 month of postoperative recovery, animals were exercised on a treadmill for 30 min/day for 6 months. Measurement of EMG activity after spinalization had shown a reduction similar to that seen in the immobilization models. In addition, using implanted force transducers (such as those shown in Color Plate C-7A), direct measurement of muscle force during locomotion confirmed that significant weight bearing was induced by the exercise training procedure.

One of the goals of the study was to understand the role that muscle force played during rehabilitation of these severely atrophic muscles. To provide different muscle forces to the muscles, they compared training that involved standing alone with training that involved stepping along a treadmill with weight bearing (de Leon, Hodgson, Roy, & Edgerton, 1998, 1999; Roy et al., 1998). Standing involves lower loads that are relatively constant, while step training involves higher, more intermittent loading patterns to the muscles. In planning this study, it was not even clear that it would be possible for a cat, with a transected spinal cord to "walk" on a treadmill. Edgerton, Roy, and colleagues published a sequence of fantastic electromyograms from these spinalized animals, demonstrating tremendous and nearly normal rhythmic activity of the locomotory muscles (Fig. 5-17). This demonstrates that the spinal cord itself could be "trained" to recover the stepping pattern! This clearly indicated that the reciprocal activation of limbs, phasic muscular activity, and amplitude of muscle activation are all parameters that could be initiated and controlled by the spinal cord.

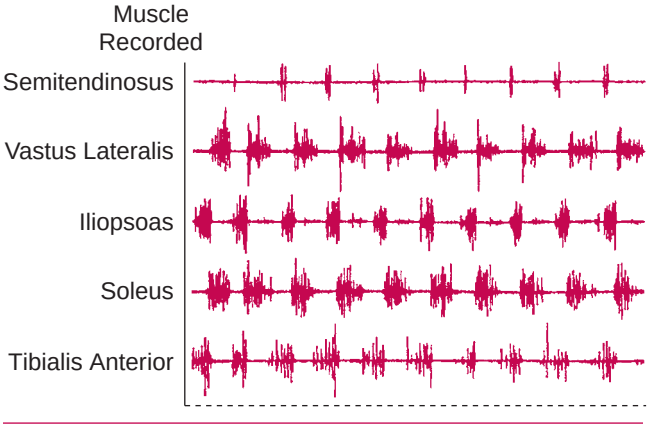

Figure 5-17 Raw electromyogram record from hindlimb muscles of a spinalized cat during treadmill walking at 0.4 m/s after step training had been performed. Note the rhythmic muscle activity and reciprocal activation of hip and ankle flexors and extensors. This indicates that the spinal cord can "learn" such properties in the absence of influence from higher centers. (From de Leon, R. D., Hodgson, J. A., Roy, R. R., & Edgerton, V. R. [1999]. Retention of hindlimb stepping ability in adult spinal cats after the cessation of step training. *Journal of Neurophysiology, 81*, 85–94.)

After the 6-month training period, soleus muscle fiber histochemical properties were measured and found to demonstrate the expected slow-to-fast fiber type transformation. Not only were the usual qualitative histochemical stains implemented, but quantitative measurements of myosin ATPase activity (MATPase), succinate dehydrogenase activity (SDH), and α-glycerophosphate dehydrogenase activity (α-GP) were also made, which enabled detailed description of the fiber metabolic properties themselves. Six months of exercise caused muscle fiber size to be somewhat larger compared with spinalization without exercise. For example, while the normal SOL muscle fiber area was about 4,200 μm^2, the spinalized slow fibers were about 2,700 μm^2, and the spinalized and exercised slow fibers were about 3,000 μm^2. The prevention of fiber atrophy by exercise was not complete, but the data suggest that the added muscle tension due to exercise improved muscle strength.

What about the functional changes that occurred in the muscles? The usual structural, functional, and biochemical properties were measured, revealing not only the influence of spinalization but also of training. First, compared with control subjects, the spinalized cats demonstrated significant loss in maximum tetanic tension (Fig. 5-18A), increase in V_{max} (Fig. 5-18B), and decrease in slow fiber type percentage (Fig. 5-18C). Thus, the muscle atrophy and velocity increase expected by decreased use is observed. Interestingly, step training was much more effective than training that involved standing alone in bringing muscle properties maximum force, muscle speed, and fiber-type percentage closer to "normal." The authors suggested that, not only was the load important in determining muscle properties, but the *rhythmic activity* of the motor nerves also caused muscle properties to be altered. These data may indicate that the muscle senses not only the amount of mechanical activity imposed upon it, but also the pattern of the activity. Although this is a highly speculative statement, it would not be surprising if muscles "tune" their structural and functional properties to fairly specific signals provided to them via the neuromuscular junction (NMJ).

As has been seen repeatedly, the soleus is a highly responsive muscle that often shows atrophic and hypertrophic changes that are not "typical" of all muscles. To determine whether more typical muscles demonstrate the same response to spinalization and exercise, Dr. Roland Roy and colleagues performed similar studies on the faster muscles of the hindlimb and showed trends that were in the same direction, but of a smaller magnitude (Roy et al., 1999). In fact, as you have already seen, a differential effect was demonstrated between the antigravity MG, a plantarflexor, and the TA, a dorsiflexor. As you might guess, the TA was much less responsive compared with the MG to spinalization and to the effects of exercise on the muscles. Again, all muscles are "not created equal" with regard to response to altered use.

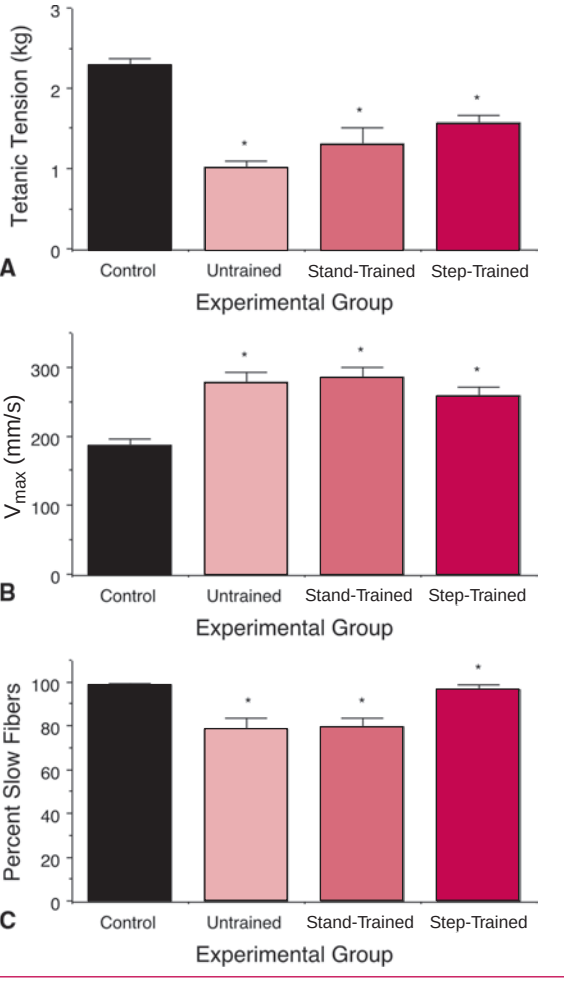

Figure 5-18 Functional and structural properties of soleus muscles from control and spinalized cats. **(A)** Maximum tetanic tension, **(B)** maximum contraction velocity, and **(C)** percentage of slow fibers. Note that in all cases, spinalization causes muscles to become weaker and faster, whereas training tends to reverse these effects toward normal. If anything, step training is more effective than stand training in this regard. (Data plotted from Roy, R. R., Talmadge, R. J., Hodgson, J. A., Zhong, H., Baldwin, K. M., & Edgerton, V. R. [1998]. Training effects on soleus of cats spinal cord transected (T12–13) as adults. *Muscle & Nerve, 21*, 63–71.)

Muscles that demonstrate the greatest response to changes in use are those that are most often used (e.g., antigravity muscles), such as the soleus, vastus intermedius, and, to a lesser extent, quadriceps and gastrocnemius muscles.

In terms of metabolic properties, muscle oxidative capacity, as indicated by SDH activity, did not change following spinalization and even increased with exercise. Because the SOL muscles also demonstrated a significant slow-to-fast fiber type conversion, this increase in oxidative capacity probably reflected the increased SDH activity of the FOG fibers relative to SO fibers. This study illustrates that, while increases in SDH activity can occur relatively rapidly and to a large extent following increased use,

the converse does not occur following decreased use. It appears that skeletal muscle fiber "baseline" oxidative capacity is not easily changed.

Application of Training to Humans

The obvious question one asks after reviewing the fantastic results reported by Edgerton, Roy, and colleagues in cats is, "Can these therapeutic approaches to rehabilitation of the SCI subject be applied to humans?" In the face of tremendous scientific and clinical disbelief, Edgerton and colleagues showed tremendous leadership by repeating their experiments on human SCI patients. In effect, they basically achieved the same fantastic results—demonstrations of rhythmic locomotor activity, manifestations of weight bearing, and presumably, improved muscle properties (Harkema et al., 1997). In fact, based largely on the pioneering work of Dr. Edgerton, a true "cottage" industry has been created, in which various types of treadmill-based locomotion training systems are sold and used (Fig. 5-19). One clear lesson here is not to be too quick to dismiss animal models while in search of answers to questions regarding human patients. A recent report from Dr. Edgerton's laboratory actually identified the neural pathways that were involved in this rehabilitative response. If you can believe it, all the surgical and rehabilitation procedures were performed in a mouse model, so don't write off these animal models too fast (Courtine et al., 2008)! In fact, it is difficult to name a result from animal muscle structure, function, or plasticity studies that, when repeated in humans, has not provided essentially the same result. This is good news for those interested in the physiological basis of therapy.

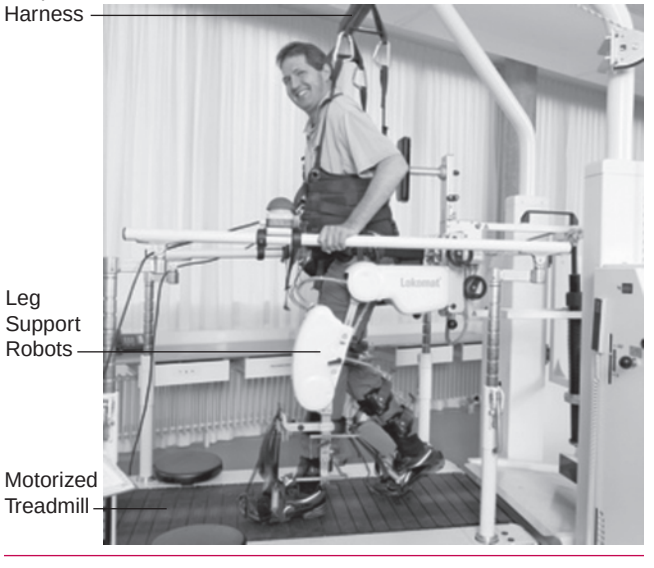

Figure 5-19 Spinal cord injured patient being "exercised" using a robotic training device. This patient is supported by a body harness, and simulated walking is imposed upon the legs by the leg support devices and the motorized treadmill. Using this device, peripheral movement and loading of the legs appear to train the spinal cord to activate leg muscles in a walking pattern. There is evidence that this improves recovery from spinal cord injury. (Photograph courtesy of Hocoma AG, Switzerland.)

Clinical Relevance

The main result of these studies is that, after spinal cord injury, a slow-to-fast fiber type transformation can occur. The consequences are that a dramatic change occurs in contractile properties of muscles that have a large proportion of slow fibers. This clearly happens in a number of animal models, as seen above and has been reported to occur in human patients as well (Grimby et al., 1976). As a result of this increased contraction and relaxation speed, these slow muscles become less able to generate low level, prolonged contractions, as is required of predominantly slow muscles (e.g., soleus and vastus intermedius). If the specific tension of fast and slow muscle fibers differs, slow-to-fast transformation may result in increased (or, at least not decreased) muscle strength, causing an imbalance of muscle forces about the joint. The joints would thus tend to remain extended as most "antigravity" muscles contain a larger proportion of slow fibers. This concept may explain the observation that, after transection, the rat hindlimbs remained fully extended. However, this observation, even in animal models, is not universal.

This study suggests that paraplegia is not necessarily associated with muscular weakness, especially after a relatively long period. While neuromuscular weakness follows injury, muscle strength is not necessarily compromised. This is not to say that muscle strength cannot decrease, only that it is not necessarily decreased.

These data also show that modalities that increase muscle tension are effective in reducing the atrophy seen with spinalization alone. Thus far, it has not been possible to completely reverse the effects of spinalization, but perhaps future treatments might include greater exercise duration or intense muscle contractions. (For more on eccentric contractions, see Chapter 6.)

Electrical Stimulation after Spinal Cord Injury in Humans

An obvious clinical intervention that could be used to prevent or at least minimize the effects of spinal cord injury is electrical stimulation. You have already seen that chronic electrical stimulation causes dramatic and persistent changes in mammalian skeletal muscle (Chapter 4, pp. 141–147) and, when used clinically, can assist in strength recovery after disuse or injury (Chapter 4, pp. 167–171). However, there are little physiological data available from muscles of SCI patients that would make the application of a therapeutic intervention more rational. As a result of this void, using a transcutaneous electrical stimulation model (similar to that shown in Fig. 4-24), Dr. Rick Shields and colleagues studied the human soleus muscle by determining its fatigue

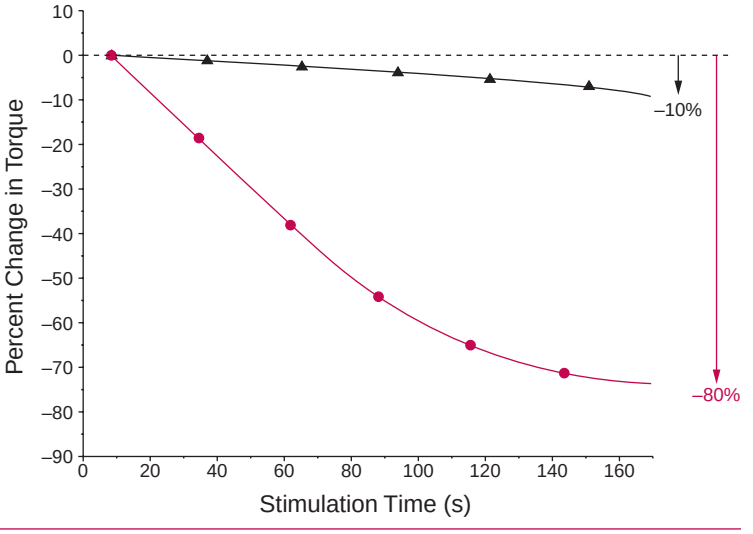

Figure 5-20 Increase in muscle fatigability after spinal cord injury. Quadriceps femoris muscles were electrically stimulated and torque measured as a function of time. Muscles of acutely injured patients *(black triangles)* showed a smaller drop in force compared to chronically injured (>2 years after injury) patients *(red circles)*. (Data from Shields, R. K., Law, L. F., Reiling, B., Sass, K., & Wilwert, J. [1997]. Effects of electrically induced fatigue on the twitch and tetanus of paralyzed soleus muscle in humans. *Journal of Applied Physiology, 82*, 1499–1507.)

properties in patients with complete spinal cord transection accompanied by complete sensory and motor paralysis. Two groups of patients were studied: those with acute paralysis (defined as less than 5 weeks from injury) and those with chronic paralysis (defined as more than 3 years since injury). Muscles were "fatigued" by activating the tibial nerve in the popliteal fossa using electrical stimulation. The pattern consisted of 20-Hz stimulation pulses delivered for 300 milliseconds every 1.5 seconds over a total period of 3 minutes (Shields, Law, Reiling, Sass, & Wilwert, 1997). By design, this protocol was similar to that used by Dr. Bob Burke and colleagues who defined the fatigability of motor units (Chapter 2, p. 77). (Burke, Levine, Tsairis, & Zajac, 1973). Shields et al. measured only about a 10% drop in torque generated by soleus muscles from the acute patients over the 3-minute stimulation period, but about an 80% drop in torque generated by the chronic patients (Fig. 5-20). Just as in the discussion of fatigue, part of the question regarding the mechanism of this change relies on an understanding of the site where the fatigue was measured. In a subsequent study, these authors demonstrated that, in response to electrical stimulation, the fatigued muscle actually generated near-normal EMG activity in spite of the large drop in torque. This means that the fibers themselves were still being activated, but the force they generated was severely compromised (Shields, Chang, & Ross, 1998). Future studies are necessary to define, more precisely, the muscle fiber defects that cause the decreased performance observed.

The fact that the fatigability of these muscles is altered after injury is actually a bit promising, in that it is a fairly straightforward task to change the endurance properties of skeletal muscle—presumably even paralyzed skeletal muscle. There is evidence that the endurance properties of muscles from SCI patients can be changed with electrical stimulation treatment. For example, increased VL muscle fiber size (23%) and increased numbers of capillaries (40%) were reported by Chilibeck et al., who trained six patients for 30 min/day × 3 days/week × 8 weeks (Chilibeck, Jeon, Weiss, Bell, & Burnham, 1999). Similarly, an increase in the percentage of type 2A myosin-heavy chain and decrease in percentage of type 2B myosin-heavy chain was reported by Anderson et al., who trained VL muscles for 30 min/day × 3 days/week for 6 and 12 months (Anderson, Mohr, Biering, Sorensen, Galbo, & Kjaer, 1996). There is thus no doubt that the muscles are able to adapt. Of course, the key issue is defining the mechanical conditions and "dose" of electrical stimulation necessary to elicit the appropriate functional changes (Baldi & Reiser, 1995).

THERAPIST'S COMMENTS

This is an important area of future work for physical therapists. As experimental therapies for spinal cord injury move into mainstream clinical practice, the incorporation of physical exercise (e.g., body weight supported treadmill training and electrical stimulation) will be more important and more common than it is today. There are certainly muscle level benefits to this type of exercise, but the interaction between exercise and neurophysiological recovery remains poorly characterized and is likely very important.

ADAPTATION TO HINDLIMB UNLOADING

A new model for studying muscle fiber atrophy has recently increased in popularity. In cooperation with the National Aeronautics and Space Administration (NASA), Dr. Emily Morey developed the hindlimb unloading model for simulating the weightless environment (Morey, Sabelman, Turner, & Baylink, 1979). It is well known that spaceflight results in muscle atrophy and loss of bone mineral content.

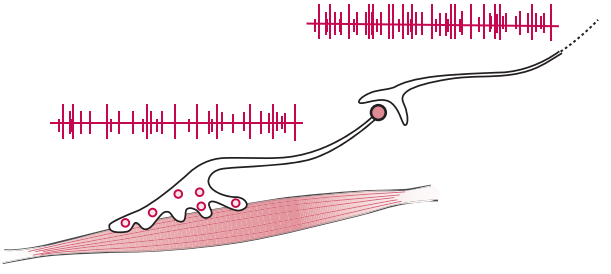

Figure 5-21 Schematic view of the hindlimb unloading model. Muscles experience little tension and decreased (but finite) EMG activity.

Astronauts who return from space are significantly weaker and more vulnerable to bone fracture. Such changes are similar to those observed with other decreased use models, and thus, there is great interest in discovering the factors that cause them. In addition, because there is a good deal of support from NASA to study the problem of muscle atrophy, many muscle physiologists have decided to "specialize" in muscle atrophy secondary to weightlessness (Edgerton & Roy, 2000). Because it is extremely costly and logistically difficult to perform scientific research in space, this "ground-based" experimental model, the so-called hindlimb unloading model, was developed (Fig. 5-21).

The Hindlimb Unloading Model

To mimic the effects of spaceflight, Morey and her colleagues removed the weight-bearing function of the rat hindlimbs. Connectors were secured to the base of the rat tail and attached to a revolving gimbal mount at the top of the cage. The rats could easily navigate about the cage, using their forelimbs to explore the cage and feed themselves. Early experimental results using this model were promising. After hindlimb unloading, rats demonstrated many of the physical changes that had been documented in spaceflight: muscle atrophy, bone mineral loss, interstitial fluid shifts, and decreased growth. From the point of view of studying muscle plasticity, the hindlimb unloading model also provided a unique opportunity to study a decreased use model in which the lower motor neuron was intact and muscle tension was extremely low. (Incidentally, it was shown that the model itself caused a small degree of transient stress in the rats, based on measurement of a small increase in adrenal gland mass and transiently increased plasma corticosterone levels. These levels were very similar to other treatments in which the animal was manipulated in some way, for example, applying a cast.)

Skeletal Muscle Activity during Hindlimb Unloading

In a manner similar to that described for their prior study of muscle immobilization, Alford et al. measured the chronic EMG activity from both the TA ankle flexor and the soleus and MG ankle extensors to better understand the level of muscle use that actually occurred during hindlimb unloading (Alford, Roy, Hodgson, & Edgerton, 1987). These investigators showed that the extensor activity of the soleus and gastrocnemius muscles decreased initially and then returned to control levels after about 4 weeks (Fig. 5-22). However, the TA, which was slightly loaded because of gravity-induced ankle extension, actually increased electrical activity by 2–4 times. Thus, as with most experimental models, the hindlimb unloading model did not cause complete disuse (since the muscles retained some EMG activity).

Q&A

What Does Sending Animals and Humans into Space Have to Do with Physical Therapy?

Spaceflight produces repeatable changes in muscle unloading patterns that result in muscle fiber atrophy, fiber type transformation, and neuromuscular weakness. These are all very similar to the problems that human patients experience after surgery, prolonged disuse, or injury. To the extent that we understand these phenomena in space and can develop ways to prevent atrophy or recover from it, we can assist our patients here on earth.

Changes in Muscle Contractile Properties following Hindlimb Unloading

Interestingly, in spite of the unremarkable changes in muscle EMG after hindlimb unloading, dramatic changes in muscle mass and muscle contractile forces have been reported by numerous investigators. The nature of these changes was as expected based on previous immobilization studies. For example, Dr. Don Thomason, working in Dr. Ken Baldwin's laboratory, showed that the mass of both the plantaris and soleus muscles decreased continually throughout the unloading period (Fig. 5-23). This provided a nice comparison of two extensor muscles, one primarily composed of slow fibers (the soleus) and the other primarily composed of fast fibers (the MG). Soon after unloading began, the mass of both muscles rapidly decreased. Mass decreased at an increasingly slower rate until after about 30 days, when mass stayed relatively constant. The properties of other muscles subjected to unloading had also been measured in other laboratories. Consistent with the change in mass, contractile tension of both muscle types decreased by about 50% during the same time period. Again, consistent with other decreased use models, the soleus muscle demonstrated an increased muscle speed, manifested in a decrease in both contraction and half relaxation times and a shift in fiber type percentage from slower to faster isoforms. Interestingly, Dr. Bob Fitts has reported

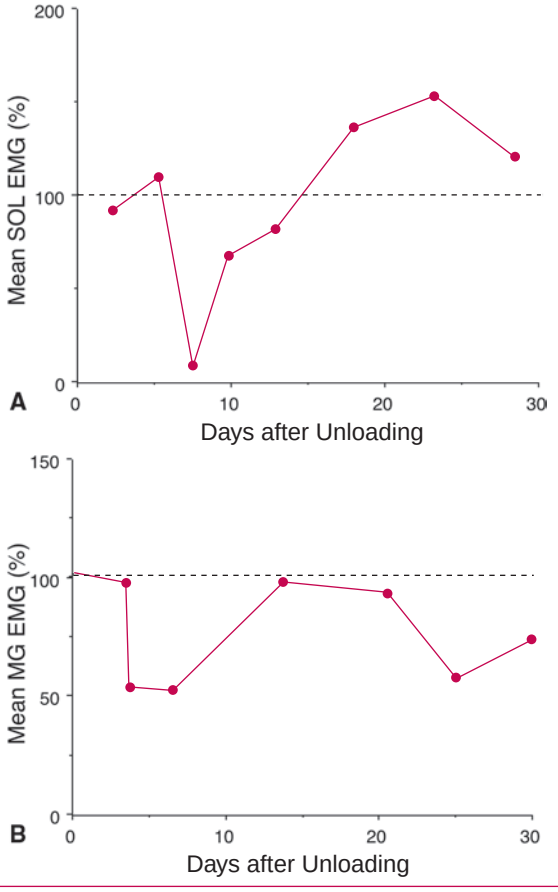

Figure 5-22 Skeletal muscle EMG after hindlimb unloading. Muscle EMG was measured continuously over 30 days after hindlimb unloading using indwelling electrodes. This graph shows that soleus (**A**) and medial gastrocnemius (**B**) EMG activity initially decreases and then returns to near normal levels after 7–10 days. Thus, hindlimb unloading is clearly not a disuse model. (Data from Alford, E. K., Roy, R. R., Hodgson, J. A., & Edgerton, V. A. [1987]. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hindlimb suspension. *Experimental Neurology, 96*, 635–649.)

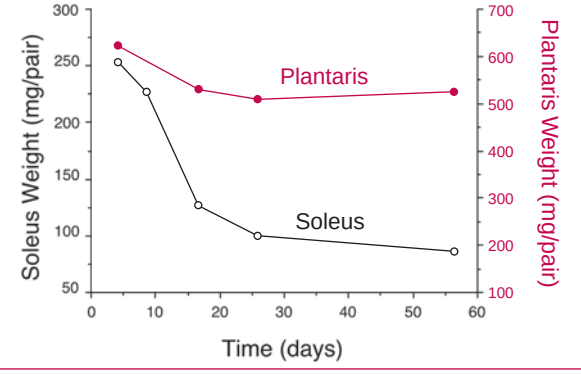

Figure 5-23 Change in muscle mass over an 8-week hindlimb unloading experiment. Muscle mass decreases quickly and then "levels off." The effect is more dramatic for the antigravity soleus muscle compared with the plantaris muscle, which has a greater percentage of fast fibers. (Data from Thomason, D. B., Herrick, R. E., Surdyka, D., & Baldwin, K. M. [1987]. Time course of soleus muscle myosin expression during hindlimb unloading and recovery. *Journal of Applied Physiology, 63*, 130–137.)

that rat soleus muscle single fibers, subjected to hindlimb unloading, have an increased V_{max} in the absence of a shift in the myosin-heavy chain. This indicates that V_{max} itself is determined by more than just the myosin isoform (McDonald, Blaser, & Fitts, 1994). These authors also demonstrated that not only do all the muscles not change properties to the same extent, but the fibers, even of the same type, isolated from different muscles do not adapt identically. For example, slow fibers from the soleus decreased size and specific tension, and increased their V_{max} values (Fig. 5-24A), whereas slow fibers from the MG muscle decreased size and specific tension, yet showed no change in V_{max} (Fig. 5-24B). Again, this is a situation in which not only muscles but also muscle fibers may have different susceptibilities to adaptation under altered use conditions.

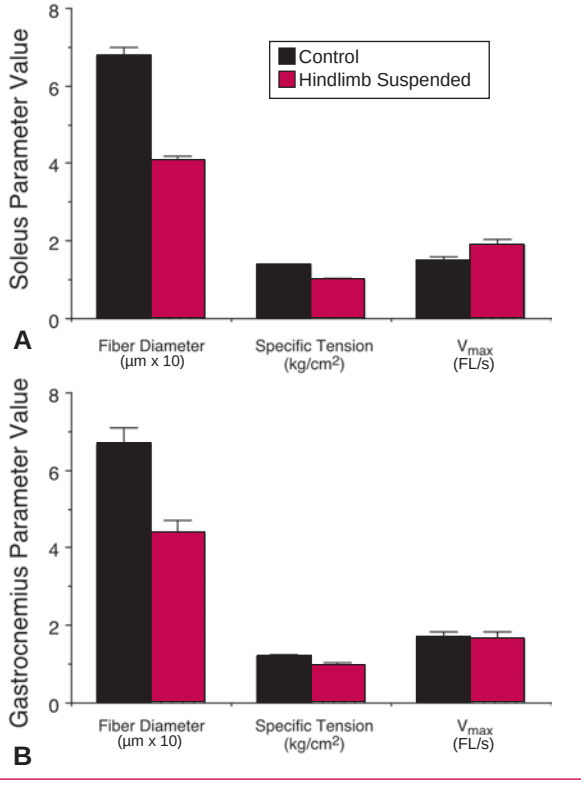

Figure 5-24 Change in fiber diameter, specific tension, and V_{max} in single fibers from (**A**) rat soleus single fibers or (**B**) rat medial gastrocnemius single fibers. Note that fiber diameters are divided by 10 for display on the same axes as the other parameters FL, fiber length. (Data from Gardetto, P. R., Schluter, J. M., & Fitts, R. H. [1989]. Contractile function of single muscle fibers after hindlimb suspension. *Journal of Applied Physiology, 66*, 2739–2749.)

Changes in Fast and Slow Muscle Fibers following Hindlimb Unloading

Using quantitative analysis of muscle fiber area and muscle fiber enzyme activity, Drs. Edgerton and Roy demonstrated that the lower limb plantarflexors and dorsiflexors adapted differently to the unloading (Hauschka, Roy, & Edgerton, 1987; Roy, Bello, Bouissou, & Edgerton, 1987). Whereas the fast and slow fibers within the gastrocnemius muscle atrophied by about 25%, the TA muscle fibers showed no atrophy whatsoever (Fig. 5-25). Part of the TA response was probably due to the slight stretch placed on the TA during unloading, but most of the change was probably attributable to the low responsiveness of the pretibial ankle flexor to decreased use. This is the same story that was seen with the immobilization and cordotomy models.

In terms of metabolic activity, the single fibers of both the MG and TA dramatically decreased in SDH activity, while only the MG dramatically increased in α-GP activity (Fig. 5-26). This actually represented a departure from the norm, in which decreased use models usually showed no change in muscle oxidative capacity. The increased α-GP activity was interpreted as indicating that glycolytic capacity appears to be matched to muscle fiber type. Thus, muscles that increase their proportion of fast fibers also increase their glycolytic capacity. (Incidentally, the same investigators demonstrated that 1.5 hours of exercise per day on a 30% grade at relatively high speeds could partially ameliorate these deleterious effects—further evidence that muscle tension can modulate the atrophic response.)

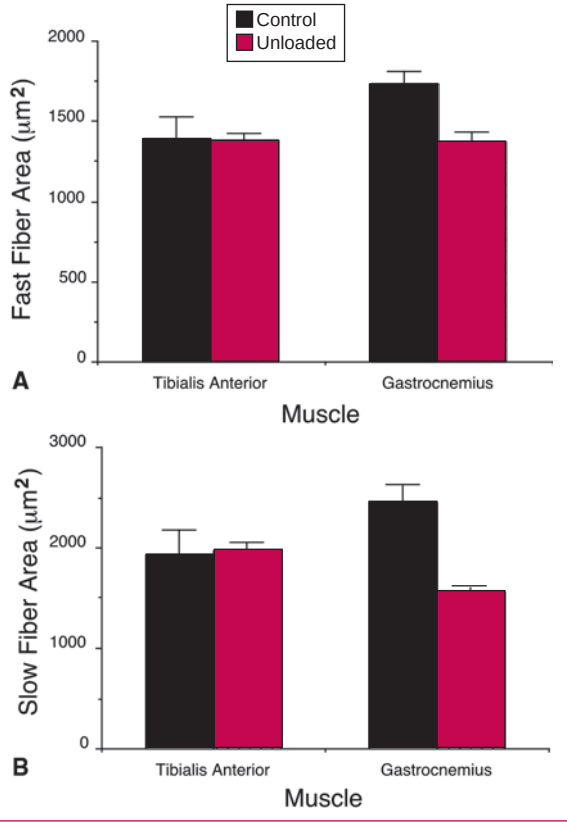

Figure 5-25 Muscle fiber area after hindlimb unloading. **(A)** Fast fiber area. **(B)** Slow fiber area. Note significant atrophy of the medial gastrocnemius but none of the tibialis anterior. These data indicate that not all muscles atrophy to the same extent. (Data from Roy, R. R., Bello, M., Bouissou, P., & Edgerton, V. R. [1987]. Size and metabolic properties of fibers in rat fast twitch muscles after hindlimb suspension. *Journal of Applied Physiology, 62*, 2348–2357.)

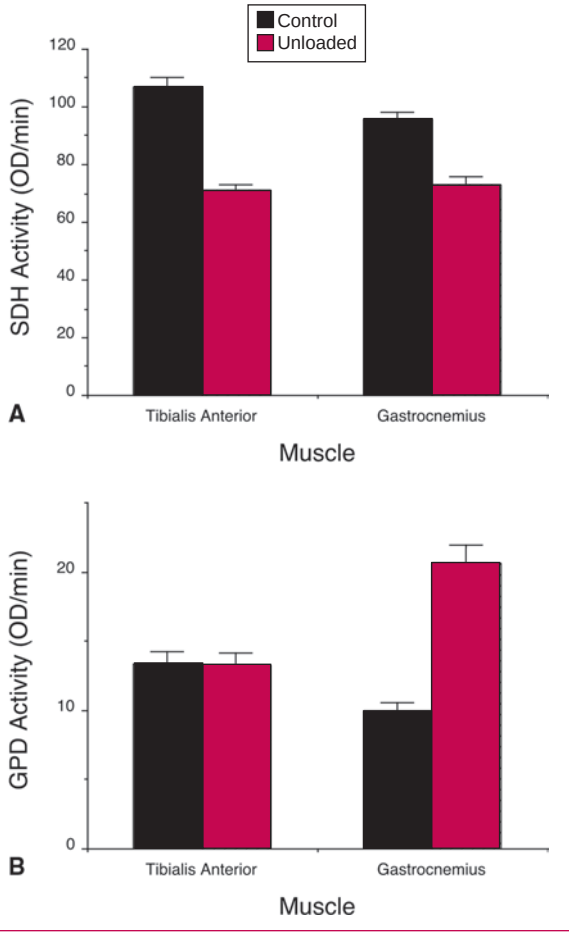

Figure 5-26 Muscle fiber enzyme activity following hindlimb unloading. **(A)** Muscle fiber oxidative capacity as indicated by succinate dehydrogenase (SDH) activity. **(B)** Muscle fiber glycolytic capacity as indicated by glycerol phosphate dehydrogenase (GPD) activity. Note the decrease in oxidative capacity is correlated with an increase in glycolytic capacity (Data from Roy, R. R., Bello, M., Bouissou, P., & Edgerton,V. R. [1987]. Size and metabolic properties of fibers in rat fast-twitch muscles after hindlimb suspension. *Journal of Applied Physiology, 62*, 2348–2357.)

MECHANISM OF MUSCLE FIBER ATROPHY

Introduction to Muscle Protein Turnover

You know already that the muscle fiber size is related to the number of myofibrils that are arranged in parallel within the fiber, and that myofibrils comprise 70–80% of the total protein within the muscle cell. Thus, it follows that muscle force would be related to the total amount of myofibrillar protein within the fiber.

It may not be obvious, but all proteins (indeed, all cellular components) eventually "wear out" and die. The rate at which these different components are replaced depends on their size, location, and function. The control center of the cell (the nucleus) is responsible for synthesizing new muscle proteins and repairing the portions of the cell that have been damaged or that have aged. If protein synthesis is completely blocked, the cell cannot repair itself and eventually dies (this is the basis for use of some chemotherapeutic drugs).

Protein synthesis and protein degradation are always occurring within the muscle cell, or any cell for that matter. In this way, cells retain the ability to adapt to a new environment. If proteins were permanently in place after synthesis, there would be no way to change them. Thus, proteins are constantly "turning over" to yield to the current cellular demands. It is this study of protein turnover in atrophying muscle that has provided tremendous new insights into the atrophy mechanism. To study these phenomena, it is not enough to simply know how much contractile material is present (e.g., by measuring muscle mass) since the instantaneous amount of tissue mass present represents a balance between the amount of protein being made (synthesis) compared with the amount of protein being lost (degradation) (Fig. 5-27).

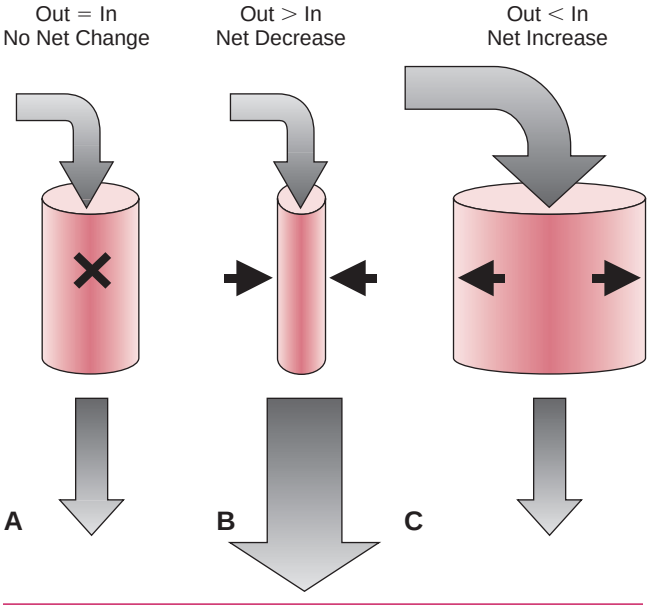

Figure 5-27 Schematic figure illustrating the balance between synthesis and degradation and its effect on mass. (**A**) Equal inflow to and outflow from the tank so that the net volume of water (analogous to mass) remains the same. (**B**) Inflow to tank is less than outflow from tank so that net volume decreases. This is analogous to muscle fiber atrophy. (**C**) Inflow to tank is greater than outflow from tank so that net volume increases. This is analogous to muscle fiber hypertrophy.

Protein Turnover during Hindlimb Unloading

Studies of protein turnover in muscle were pioneered in the muscle research laboratories of Drs. Ken Baldwin and Frank Booth. These investigators measured rates of synthesis and degradation in muscles following various "altered use" models and have "painted a picture" of muscle mass regulation following altered use. Specifically, hindlimb unloading was studied by Dr. Don Thomason working in both laboratories (Thomason, Herrick, Surdyka, & Baldwin, 1987). Recall the data demonstrating that both plantaris and soleus muscle mass decreased continually throughout the unloading period. For about the first 30 days of unloading, mass decreased at an increasingly slower rate (Fig. 5-23). After about 30 days, mass stayed relatively constant. Muscle mass (or change in mass) simply represents the net balance between protein synthesis and degradation. This means that mass could decrease even if degradation rate decreased, so long as synthesis rate decreased more. Similarly, mass could increase even if synthesis rate decreased, as long as degradation rate decreased even more. What was the explanation for muscle mass changes following hindlimb unloading?

Changes in Synthesis and Degradation

Thomason et al. showed that, soon after hindlimb unloading (within a day), soleus muscles decreased their protein synthetic rate by about 50%, and this rate remained relatively constant for the remainder of the unloading period (up to 7 days have been measured) (Thomason, Biggs, & Booth, 1989). The protein synthesis rate decrease occurred in spite of the fact that soleus EMG activity continued to increase (another lesson demonstrating that increased electrical "activity" does not translate into increased strength). Since muscle mass continued to decrease during this rate of constant protein synthesis, the data predicted that protein degradation rate must have increased from the 4th to 14th day of unloading, then decreased to a value less than that of the control, at which time degradation must have become relatively constant (Fig. 5-28). All these calculations are based on the fact that total muscle mass always represents the net balance between synthesis and degradation.

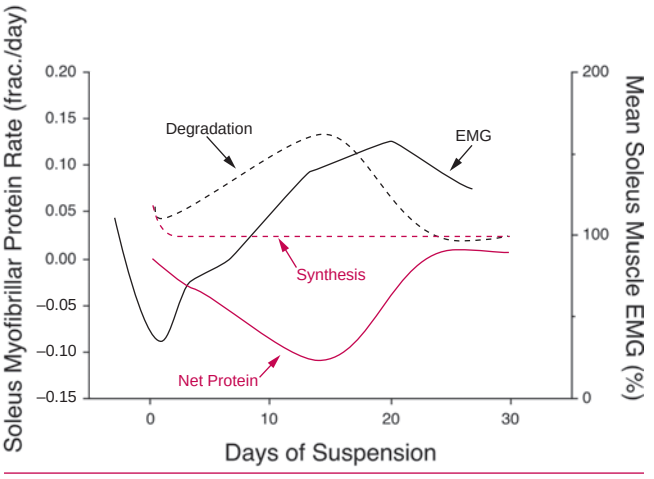

Figure 5-28 Time course of muscle mass and protein synthesis and degradation during rat hindlimb unloading. Initially, the electromyogram (EMG) activity decreases, resulting in an increased protein degradation and decreased protein synthesis rate. Subsequently, EMG increases. (From Thomason, D. B., Herrick, R. E., Surdyka, D., & Baldwin, K. M. [1987]. Time course of soleus muscle myosin expression during hindlimb suspension and recovery. *Journal of Applied Physiology, 63*, 130–137.)

The take-home lesson from these experiments is that, in hindlimb unloading, it took about 30 days for the muscle to reach a new state of homeostasis, even under constant unloading conditions. The phenomenon must be even more complicated under conditions when muscle state changes continually (e.g., exercise, immobilization, continuous passive motion).

Transcriptional Control of Synthesis and Degradation after Unloading

What are the factors that caused the synthetic and degradation rates observed? Clearly, electrical activity was not the cause of either the synthetic or degradation rates (compare Fig. 5-22 with Fig. 5-28). Take a step back from measuring the rates of protein synthesis and look at the earlier events that precede protein synthesis, namely, mRNA formation. This is the trend of many adaptation studies being performed today. Instead of measuring protein levels, most investigators are studying mRNA levels, because, by looking at earlier events, it is possible to look at the immediate effects of the treatment rather than events that happen much later.

Recall that the central dogma of molecular biology is that DNA is produced by replication, DNA single strands are encoded into mRNA by transcription, and mRNA single strands are used to make protein by the process of translation. Thus, potentially, replication, transcription, or translation can all be used to change the level of any cellular protein. Booth and his colleagues have looked at several different mRNAs and have found very small decreases in mRNA coding for either α-actin–(measured after 1 day of unloading) or the β-myosin–heavy chain (measured after 7 days of unloading) (Babij & Booth, 1988a). This small change was measured even though the synthesis rates of both proteins had decreased precipitously during these time periods. Specifically, after 7 days, α-actin mRNA concentration decreased by only 30%, but its synthesis rate decreased by about 60%. Finally, administration of clenbuterol abolished the 30% decrease in α-actin mRNA, but had no effect on protein synthesis rate.

They concluded that while hindlimb unloading resulted in downregulation of both transcription and translation, the dramatic synthesis rate decrease was primarily due to the downregulation of translation. Apparently, there was more than enough mRNA in the muscle cell that α-actin mRNA concentrations did not limit the synthetic rate. Recently, Booth reviewed other plasticity models and showed that regulation can occur (and does occur) at many different levels within the cell. The unifying principles that explain why regulation should occur at one point or another remain to be elucidated (Booth & Thomason, 1991).

In an attempt to address the molecular basis of muscle adaptation to unloading, Dr. Baldwin and his colleagues developed a model in which they directly transferred DNA fragments into rat soleus muscles and used "reporter genes" (i.e., genes that code for a visible or measurable protein) to determine which portions of the DNA were active. In most genes, the portion of the DNA that precedes the actual coding region determines the conditions under which the gene is transcribed and is termed the "promoter." To identify the specific region of the DNA sequence that was responsible for activating the soleus muscle myosin-heavy chain gene, they inserted different "constructs" (pieces of DNA created with specific regions of the upstream region inserted into a plasmid) into the muscle and measured reporter gene activity. Three constructs were investigated—those containing the 3500, 914, or 408 base pairs (bp) of DNA that were normally observed before the coding region (expressed as negative numbers since the DNA is "upstream" of the coding region) (Fig. 5-29). They found that, for the 3500, 914, and 408 bp constructs, reporter gene activity decreased by about 40% with hindlimb unloading, indicating that the constructs still contained the "load-sensitive" portion of the gene, while with the 215 bp construct, there was no significant change in reporter gene activity. Thus, the muscle's ability to "sense" unloading was contained in the DNA sequence between 215 and 408 bp upstream of the myosin heavy chain gene (Fig. 5-29). You can probably anticipate that the next series of experiments will be to identify the specific DNA-binding regions and even DNA sequences that affect the mechanical and biological response to unloading. You have already seen that there are many DNA-binding proteins that affect muscle development (the so-called myogenic regulatory factors or MRFs). It is highly likely that similar transcription factors are at work in regulating muscle response to load. Since

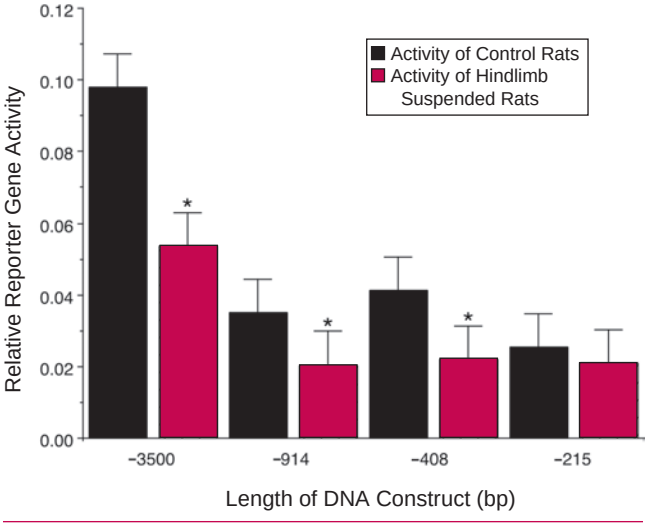

Figure 5-29 Reporter gene activity measured in rat soleus muscles after 1 week of hindlimb unloading. Black bars represent activity of control rats, while red bars represent activity of hindlimb-suspended rats. Note that for the −3,500, −914, and −408 bp constructs, activity decreases by about 40% with hindlimb unloading *(asterisks)*, whereas with the −215-bp construct, there is no significant change in activity. This suggests that the portion of the promoter region that is sensitive to hindlimb unloading lies between 215 and 408 bp upstream of the myosin-heavy chain gene. (Data from Giger, J. M., Haddad, F., Qin, A. X., & Baldwin, K. M. [2000]. In vivo regulation of the beta myosin heavy chain gene in soleus muscle of suspended and weight bearing rats. *American Journal of Physiology, 278*, C1153–C1161.)

muscle atrophy is the "problem" that is addressed by these studies, there is the intriguing possibility that, in the future, gene therapy could be used to cause muscle hypertrophy in postoperative or injured patients, or that it could be used to prevent atrophy in the first place. A small piece of DNA is delivered to the muscle in a patient with severe muscle atrophy. The gene contains the promoter region to "turn on" the muscle hypertrophy program. After delivery of the gene to the muscle, it is turned on using a simple antibiotic such as tetracycline because the piece of DNA contained a tetracycline-sensitive region. The patient could cause muscle hypertrophy as long as necessary to recover their function. A bit far-fetched to be sure, but not out of question. Another molecular mechanism of protein regulation has been discovered that relies on selective protein degradation by the cell. Most proteins have a fairly well-defined "lifetime" or, more scientifically, a well-defined half-life. This may vary from a few minutes for regulatory proteins to weeks or months for muscle contractile proteins. How is half-life "encoded" on a protein? How can one alter the half-life of a protein when it is no longer needed? This fascinating area of cell biology focuses on the cellular organelle known as the proteasome. As its name implies, the proteasome is a structure that hydrolyzes proteins. It is basically a cylindrical structure formed from several protein subunits, some of which are proteases (inside the cylinder) and some of which recognize proteins to be destroyed (Etlinger & Goldberg, 1977; Etlinger & Goldberg, 1980; Lecker, Goldberg & Mitch, 2006; Sacheck et al., 2007). Typically, the proteasome acts on proteins that are "marked" for destruction in the same way that a tree might be "flagged" to be cut down in a forest. The "marker" for proteins that are to be destroyed is a protein known as ubiquitin. Thus, the ubiquitin–proteasome pathway is a powerful complex that regulates protein degradation across a wide range of conditions such as cachexia, sepsis, burn, cirrhosis, starvation, and in specific muscle atrophy–causing conditions, such as denervation, hindlimb suspension, and immobilization-induced atrophy (Bodine et al., 2001).

One of the landmark studies in this area was performed outside of academia, which underscores the "commercial" importance of understanding skeletal muscle atrophy. Dr. David Glass and his colleagues at Regeneron, Inc. in New York, studied three models that cause muscle atrophy: hindlimb suspension, immobilization, and denervation. Since all three caused muscle mass to decrease to about the same extent (Fig. 5-30A), they surmised that there might be common molecular mechanisms leading to this atrophy.

To attempt to discover novel genes involved in this process, they used a fantastic method known as "gene tagging," in which all of the expressed mRNA transcripts in a tissue are converted to complementary DNA strands (cDNA) and analyzed and sorted (Wong et al., 2003). This approach yielded 74 transcripts that were differentially regulated. Importantly, they went on to search for "universality," since most of the genes that were regulated by immobilization were similarly regulated during denervation, but were not similarly regulated by hindlimb suspension. In fact, in the end, there were only two genes found to be universally regulated by all three models: Muscle Ring Finger 1 (*MuRF1*) and Muscle Atrophy F-box (*MAFbx*). These genes are selectively expressed only in heart and skeletal muscles, and are members of the family known as E3 ubiquitin ligases. As the name implies, these enzymes are involved in marking proteins for degradation by placing strings of ubiquitin molecules on them. After discovering these novel-acting genes, the investigators went on to prove that not only were they expressed in atrophying tissues, but they could be manipulated to either cause or prevent atrophy.

In the first experiment, both *MuRF1* and *MAFbx* knockout mouse models were generated (denoted $MuRF1^{-/-}$ and $MAFbx^{-/-}$, respectively). Then, the knockout mice of both models were "challenged" to atrophy by denervation and, as hoped, they atrophied significantly less compared to their wild-type counterparts (Fig. 5-30B). This was measured both in terms of muscle mass and muscle fiber size, so that it was clear that mass differences

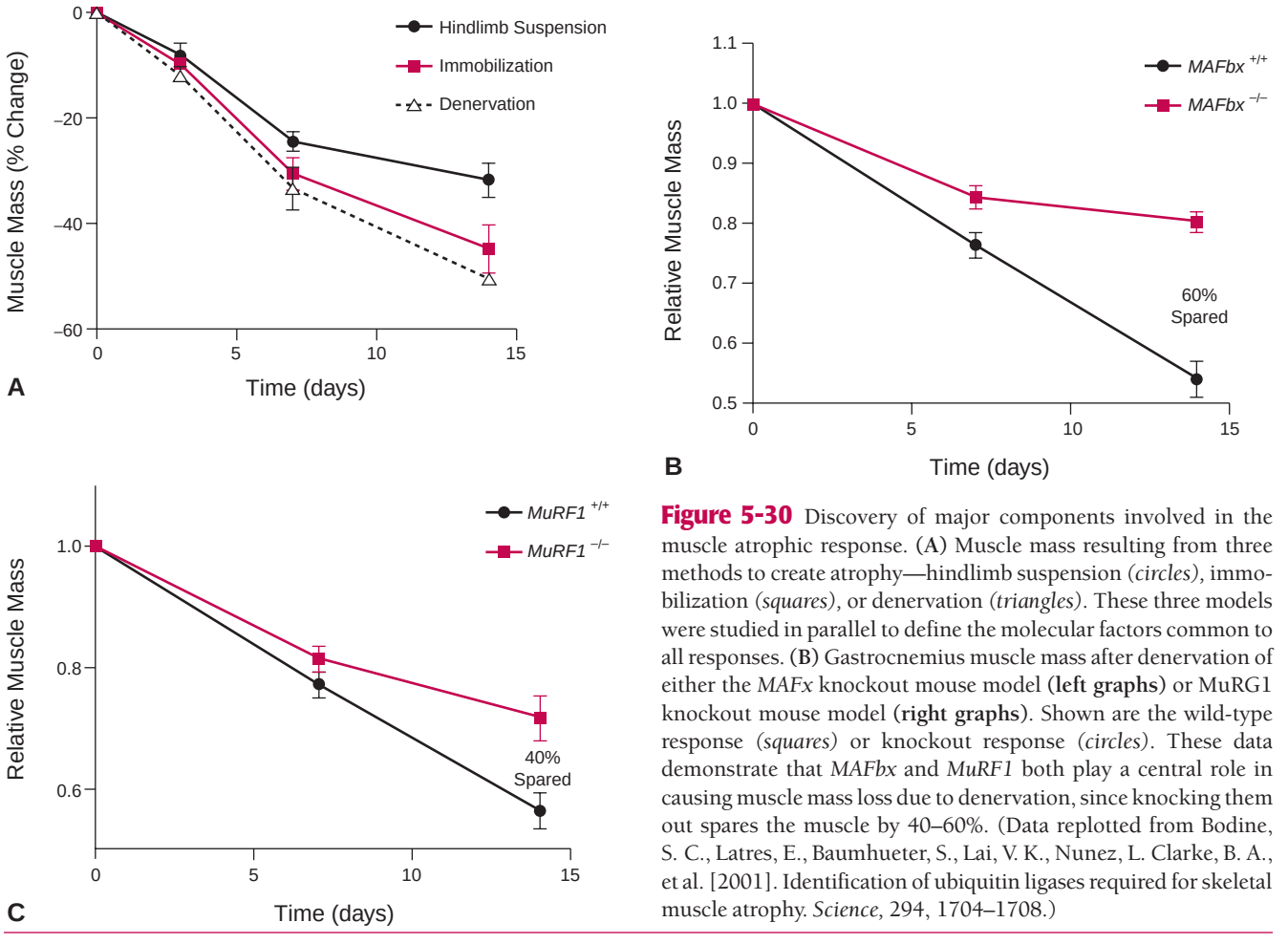

Figure 5-30 Discovery of major components involved in the muscle atrophic response. **(A)** Muscle mass resulting from three methods to create atrophy—hindlimb suspension *(circles)*, immobilization *(squares)*, or denervation *(triangles)*. These three models were studied in parallel to define the molecular factors common to all responses. **(B)** Gastrocnemius muscle mass after denervation of either the *MAFx* knockout mouse model **(left graphs)** or MuRG1 knockout mouse model **(right graphs)**. Shown are the wild-type response *(squares)* or knockout response *(circles)*. These data demonstrate that *MAFbx* and *MuRF1* both play a central role in causing muscle mass loss due to denervation, since knocking them out spares the muscle by 40–60%. (Data replotted from Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K., Nunez, L. Clarke, B. A., et al. [2001]. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science,* 294, 1704–1708.)

were not a simple result of altered water content, inflammation, or architectural alterations.

Of course, as soon as the MuRF1 and MAFbx proteins were identified as related to the atrophic response, the next obvious question was, what regulates or controls MuRF1 and MAFbx? In a series of studies by Dr. Alfred Goldberg and colleagues in Boston, the pathway has systematically been dissected and is now fairly clearly understood. At this point, I am a bit worried about creating an "alphabet soup" of receptors, signaling molecules, transcription factors, and proteins to describe the molecular mechanism. However, since many of these factors represent potential therapeutic targets for patients that can be used to prevent muscle atrophy, it would be nice for you to at least be familiar with their names.

The most important "player" in this pathway is the transcription factor known as Foxo. Foxo is a member of the important family of transcription factors known as "forkhead box" proteins, or Fox proteins, for short. (The name "Fox" refers to a sequence of 80 to 100 amino acids that are part of the protein, have sort of a "forked" structure, and bind to DNA.) As with many transcription factors, they were originally discovered in fruit flies and are small proteins that bind DNA to cause transcription of a gene or, in this case, several genes. What was most surprising about the Foxo transcription factor was that it could participate in both the atrophy and the hypertrophy pathway (Sandri et al., 2004). The current thinking is that factors that cause atrophy (discussed in this chapter—immobilization, denervation, hindlimb suspension, spinal cord injury) activate the Foxo transcription factor, which causes it to enter the nucleus and upregulate transcription of the ubiquitin ligases MAFbx and MuRF-1 (Fig. 5-31). Conversely, factors that cause hypertrophy (discussed in the previous chapter—exercise, electrical stimulation, chronic overload) cause activation of another kinase known as akt, which inhibits the Foxo transcription factor and, at the same time, activates the hypertrophy program (Fig. 5-32). Thus, you can see an intimate molecular arrangement that allows the cell to transduce mechanical use pattern via titin (see below) and then initiate a specific molecular response to induce the appropriate gene program—protein synthesis, resulting in hypertrophy, or protein degradation, resulting in atrophy.

Since this is a muscle book and we have discussed titin (Chapter 2, pp. 49–52) in a variety of contexts, let me discuss it here again. It turns out that MuRF-2 (closely related to MuRF-1) binds to titin in a specific domain

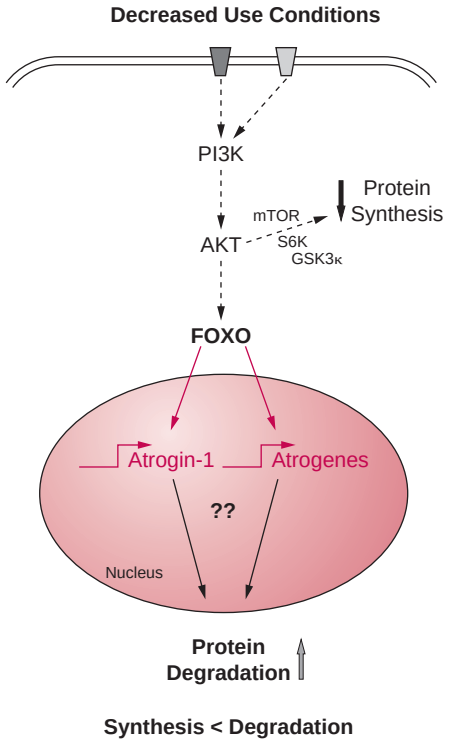

Figure 5-31 Role of various receptors, signaling molecules, and transcription factors in creating the atrophy response. Note that a focal role is played by the FOXO transcription factor, which enters the nucleus and activates the "atrogenes," i.e., those genes involved in the atrophic response. Contrast this response to the hypertrophy response (Fig. 5-32), which involves the same subcellular components but produces the opposite functional response. Red factors represent activated proteins or genes. (Adapted with permission from Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., et al. [2004]. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell, 117*, 399–412.)

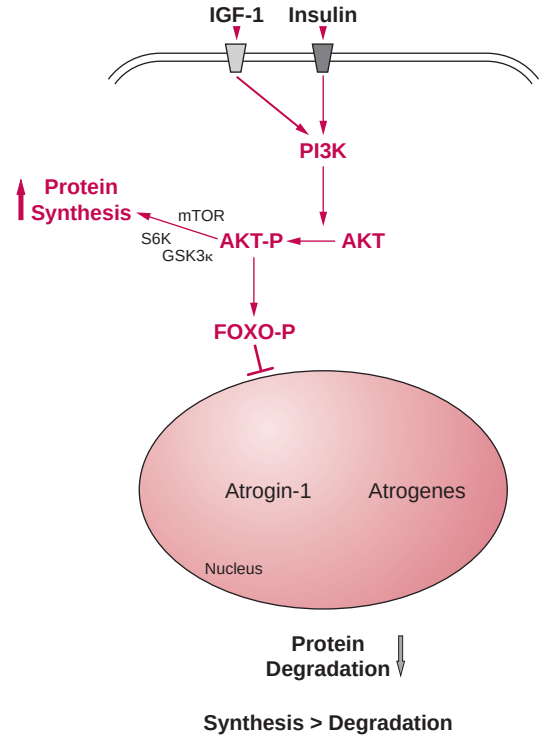

Figure 5-32 Role of various receptors, signaling molecules, and transcription factors in creating the hypertrophy response. Note that a focal role is played by the FOXO transcription factor, which is inhibited by AKT activation and is thus kept out of the nucleus. Thus, the "atrogenes" are not activated. Contrast this response to the atrophy response (Fig. 5-31) that involves the same subcellular components but produces the opposite functional response. Red factors represent activated proteins or genes. (Adapted with permission from Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., et al. [2004]. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell, 117*, 399–412.)

known as the ubiquitin-binding domain (UBD) and is also translocated to the nucleus following muscle inactivity (Lange et al., 2005). Since titin is strategically placed in the sarcomere to transduce mechanical state, these findings imply that titin can couple mechanical inactivity to gene regulation.

ADAPTATION TO AGING

One could argue that aging represents a chronic increased use model since the changes that occur in aged muscle occur after many decades of activity. However, current experimental evidence suggests that the changes observed are actually due to the low level of use experienced by muscles as an individual ages. The literature in this area is fairly new and extremely relevant, since the aged population is the fastest growing subgroup in the world (NIA Aging and Genetic Epidemiology Working Group, 2000). Experimental data obtained from the aging model in both humans and animals offer the National Institute of Aging working group the unique opportunity to test much of the muscle knowledge gained elsewhere in this textbook. As the data are presented, think about the underlying structural bases that could explain the observations and see if you can come up with the next experiment.

Strength Changes with Aging

Studies of muscle strength in humans across a range of ages have demonstrated that strength loss occurs in a fairly similar fashion in different muscle groups . The small intrinsic muscles of the hand seem to get about as weak as muscles within the proximal and distal leg segments. Thus, to a first approximation, when discussing muscle aging, the muscle group studied does not need to be extremely specified. Primarily for practical reasons, the most studied muscle group in humans is the VL. You have already seen this muscle studied extensively in trying to understand muscle

Q&A

Why Is Older Muscle Weaker?

With aging, muscle becomes weaker for several reasons. First, muscle fiber size decreases, which results in a muscle that has a smaller cross-sectional area. Second, the number of fast muscle fibers decreases, which itself causes strength loss. In addition, there is evidence that ability to activate motor units decreases with age, so that even the fibers that remain in the muscle are not used. The good news is that exercise, even performed by patients older than 90, is effective at reversing many of these changes.

adaptation to exercise. Many of the tools developed to study quadriceps in general and the VL in particular have now been applied to studies of the aged individual.

A review of the quadriceps strength data available suggests that a pronounced decline in strength does not occur until well into the sixth decade of life. At this point, across many studies and using a variety of methods, strength begins to decrease precipitously (Fig. 5-33). Of course, the underlying question is, "Why?" "Strength" is a very complex phenomenon that has underlying muscular, tendinous, nervous, and skeletal components. One of the first questions to be addressed is, "Is the strength loss associated with aging primarily muscular or neural in nature?" This is important if rational therapeutic measures are to be developed to combat the aging process and to understand the effects of age on each system independently. The tools applied to the study of neural drive in aged muscle are similar to those used by individuals trying to identify the site of muscle fatigue (Chapter 2, pp. 84–88). An individual is asked to maximally activate their muscle, and an electrically generated muscle "twitch" is superimposed upon the voluntarily contracting muscle. Although there are some technical limitations to this so-called "twitch interpolation method" (Enoka & Fuglevand, 1993), there are several reliable reports demonstrating that neural drive does not limit strength in aging (Vandervoort & McComas, 1986). Of course, the tasks measured were very simple; this does not imply that aging might be accompanied by more subtle neurological changes (see below).

Muscle Fiber Size and Number Changes with Aging

If strength is affected by muscular changes, what types of changes have been measured? What do you think would be the most definitive demonstration that muscle changes cause muscle weakness? As you might expect, many studies report the fiber type and fiber size distribution within a relatively small, but hopefully representative, VL muscle biopsy (Essen-Gustavsson & Borges, 1986; Larsson, Sjodin, & Karlsson, 1978; Lexell, Taylor, & Sjöström, 1988). These authors generally agree that aging results in an increase in the relative percentage of type 1 muscle fibers and that the fiber atrophy observed is most pronounced for type 2 fibers. The magnitude of such changes ranges from about 5% to 15%, certainly not enough to explain the dramatic strength losses. Even so, how can the change in fiber type percentage be explained? The most obvious explanation invokes a type 2 to type 1 fiber type transformation secondary to chronic increased use. While this is attractive, it is a difficult proposition to test. It would require serial testing of muscles at very long time intervals. It has not yet been presented in this text, but another obvious explanation for an increase in the percentage of type 1 muscle fibers would be a selective loss of type 2 muscle fibers, which might die as a result of aging. Again, such a hypothesis would be extremely difficult to test. However, a series of excellent

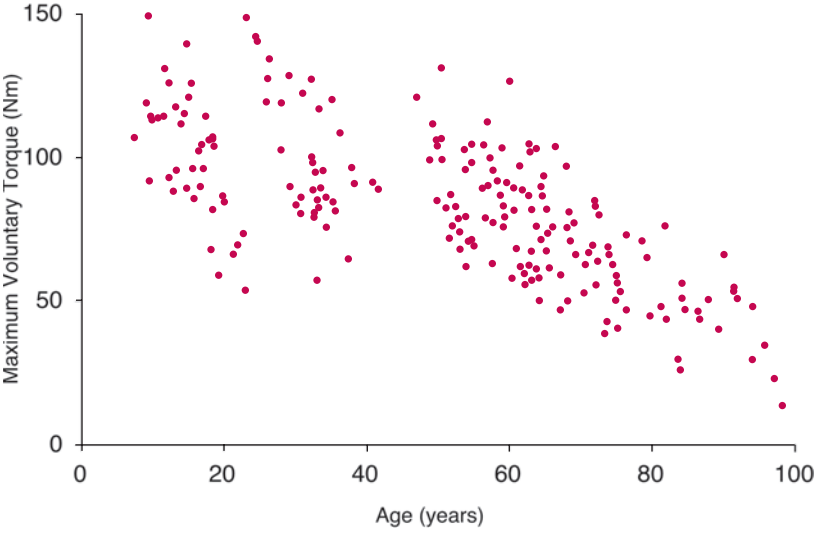

Figure 5-33 Maximum quadriceps extension torque measured in 111 healthy subjects ranging from 20 to 100 years old. Note that maximum strength declines near the sixth decade of life. (Data from Vandervoort, A. A., & McComas, A. J. [1986]. Contractile changes in opposing muscles of the human ankle joint with aging. *Journal of Applied Physiology, 61*, 361–367.)

studies by the Swedish neurologist Dr. Jan Lexell and his colleagues have provided such a test. Lexell et al. developed a novel method for freezing, sectioning, staining, and sampling the *entire* VL muscle from humans who had suffered a sudden accidental death (Lexell, Downham, & Sjöström, 1984). Muscles were obtained within 3 days postmortem, and the histochemical methods described in Chapter 2 were used to create an entire profile of fiber type and fiber size across the muscle. This was performed in relatively young, healthy men as well as an aged population ranging from 19 to 84 years (Lexell & Downham, 1992; Lexell, Henrikkson-Larsen,Winblad, & Sjöström, 1983). These authors confirmed the increase in type 1 fiber type percentage from about 50% in the "20-year-olds" to about 60% in the "70-year-olds" (Fig. 5-34A). However, this effect was weak and not statistically significant. What was more dramatic was the large atrophy in type 2 fiber area (almost 50%, Fig. 5-34B) and, unexpectedly, the large decrease in type 2 fiber numbers. Using these two parameters, they calculated the relative area of the muscle occupied by type 2 fibers and found that it decreased from about 55% of the muscle in the younger age groups to only about 30% of the muscle in the older age groups (Fig. 5-34C). This then provided the explanation for the dramatic loss in strength and the relatively modest increase in the percentage of type 1 muscle fibers. More recently, the detailed properties of aged human muscle fibers have been studied with high-resolution mechanical and biochemical experimentation on single fibers obtained from human muscle, and even on myosin molecules obtained from single fibers of human muscle (Degens, Yu, Li, & Larsson, 1998; Hook, Li, Sleep, Hughes, & Larsson, 1999; Olsson et al., 2006).

Motor Unit Changes with Aging

If muscle fiber number decreases with age, what happens to the number of muscle fibers within each motor unit? If motor neurons and muscle fibers are lost at the same relative rate, innervation ratio will stay constant. If relatively more muscle fibers than neurons are lost, innervation ratio will decrease, whereas if relatively more neurons than fibers are lost, innervation ratio will increase. Using the spike-triggered averaging method presented in Chapter 2 (p. 84), it has been demonstrated that the number of motor units decreases and average motor unit innervation ratio increases after about age 60 (Brown, Strong, & Snow, 1988; McComas, 1995; Stalberg & Fawcett, 1982). In other words, there are more muscle fibers per motor unit, and there are even some electrophysiological and morphological manifestations of denervation and reinnervation (giant electrical potentials and fiber type grouping). These observations suggest that neurons may be lost, and the denervated muscle fibers remaining are reinnervated by nearby sprouting axons from existing motor units. There is evidence of a loss in α-motor neurons within the spinal cord with subsequent degeneration of their axons (Kawamura, Okazaki, O'Brien, & Dych, 1977a, 1997b). It

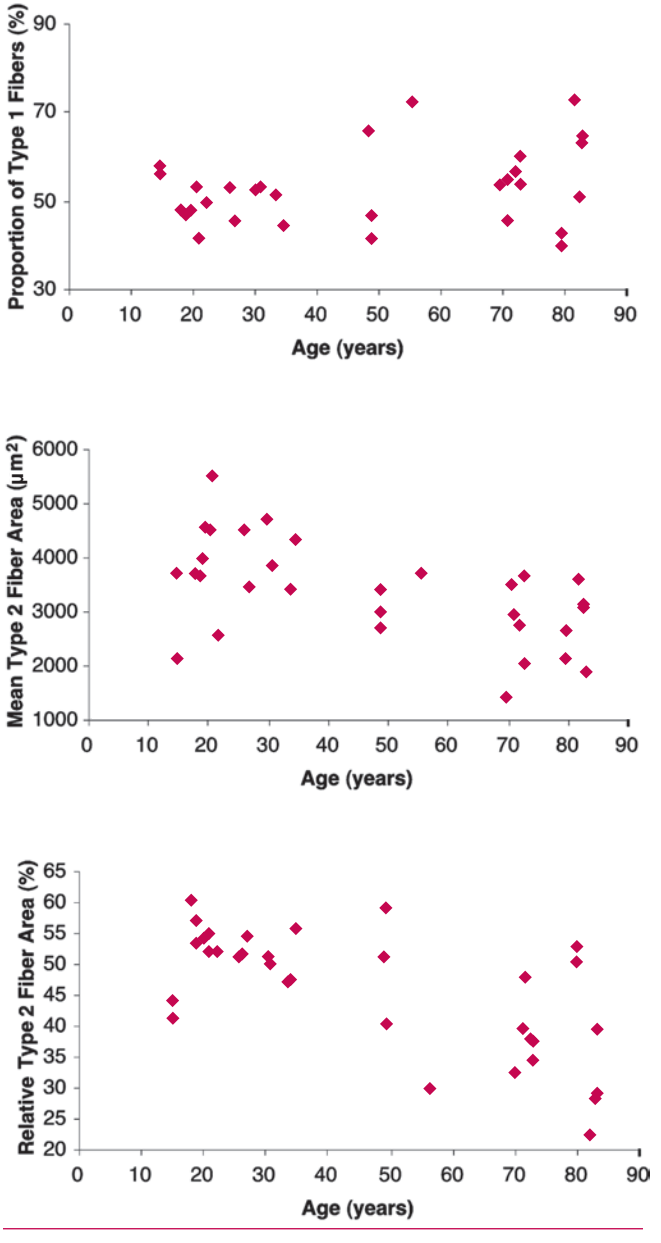

Figure 5-34 Muscle fiber characteristics change with age. (**A**) Proportion of slow (type 1) fibers, (**B**) mean fast fiber area, and (**C**) relative type 2 fiber area. The relative area of fast fibers across the muscle appears to decrease with age because of their decreased size. (Data from Lexell, J., Henriksson-Larsen, K., Winblad, B., & Sjöström, M. [1983]. Distribution of different fiber types in human skeletal muscles: Effects of aging studied in whole muscle cross sections. *Muscle & Nerve, 6*, 588–595.)

is no wonder that many of the current "antiaging" therapies focus on strategies to increase the quality and quantity of nervous tissue available. It is exciting to see such studies. Stay "tuned in" to the fantastic studies currently underway in this field throughout the world.

A final word about treating the aged muscle—there is conclusive evidence that even very old muscle (from individuals older than 90 years) can be strengthened using

exercise therapy (Fiatarone et al., 1990; Hagberg et al., 1989; Pyka, Lindenberger, Charette, & Marcus, 1994). It was once thought that exercise in the elderly, which involved high exertion levels, might be dangerous from the point of view of excessive blood pressure that could lead to stroke. However, several individuals have shown that this is not the case and, in fact, there are tremendous physical and psychological benefits to strength training in the elderly.

Mechanisms of Cellular Aging

The Oxidative Stress Hypothesis

Currently, there is not complete agreement on what defines aging at the cellular level. However, one idea that has been gaining momentum, and for which a good deal of experimental support exists, is the idea that free radicals generated by the normal oxidative processes might initiate damage and lead to degenerative changes (Sohal & Weindruch, 1996). Free radical scavengers exist in nature, and their quantity decreases with age. Thus, the free radicals produced by, for example, oxidative phosphorylation may cause damage to cellular components, most importantly nuclear DNA, that could alter the lifespan of the cell. A fascinating experiment was performed by the gerontologists, Drs. Raj Sohal, Rick Weindruch, and colleagues, in which the lifespan of insects was altered simply by adjusting the level of free radicals within their tissues. The experimental animal of choice was the fruit fly because it has such a short lifespan (~50 days) that literally hundreds of generations can be studied within a relatively short period of time. In an early experiment, these authors created a genetically altered line of flies that overexpressed the free radical scavenger molecules superoxide dismutase (SOD) and catalase (Orr & Sohal, 1994; Sohal & Weindruch, 1996). Both these molecules "absorb" free radicals that are generated by the tissue, presumably eliminating the damage that they would cause. Amazingly, transgenic flies overexpressing SOD and catalase had a lifespan that was nearly one third longer than their nontransgenic controls, and biochemical evidence of either protein damage or DNA damage was much attenuated (Fig. 5-35A). The flies with the longer lifespan (open circles, Fig. 5-35A) also had a lower fraction of oxidized proteins in the tissue. These flies also walked faster and consumed more oxygen over their lifespan (per unit body weight). Subsequently, the authors demonstrated that the ability of mice to swim through a maze was correlated with oxidative damage to the cerebral cortex (the portion of the brain that controls movement), and coordination during movement was correlated with oxidative damage to the cerebellum (the portion of the brain that controls coordination) (Forster et al., 1996). It thus appears that oxidative damage to tissue may be a general phenomenon, and not just limited to muscle. Perhaps it even relates to differences in longevity among different populations around the world (Trichopoulou & Vasilopoulou, 2000).

Caloric Restriction and Aging

A second method that these investigators used to minimize cellular oxidative stress was to limit the caloric intake of experimental animals. Obviously, food must be provided at some minimal level to maintain life itself, but there is conclusive evidence in laboratory rodents and supportive evidence in primates that food consumption above an optimal level progressively shortens lifespan. The authors have suggested that the "excess" calories (i.e., those in excess of basic metabolic requirements) serve as fuel for the production of free radicals. This effect of caloric intake on lifespan was clearly seen in mice that were permitted to eat ad libitum. They weighed about 50 g and lived about 30 months (Fig. 5-35B). In contrast, mice that had their caloric intake restricted to about 40 kcal/week (less than half of the calories eaten by the other mice) only weighed about 20 g and lived almost 50 months! These are clearly effects that are dramatic and deserve serious consideration for future aging studies. The data provide support for the consumption of enough calories to maintain normal physiological processes, but not so many that they serve as a source of free radicals that can themselves cause tissue damage.

A more recent caloric restriction study was performed by these same investigators using the modern molecular method of "gene profiling." This powerful method measures expression levels of literally the entire genome within an animal after a particular treatment and expresses those changes relative to some control animal (Lockhart et al., 1996; Marshall, 1999). The power of this method is that it provides a global indication of the large players in any treatment, whether it be aging, exercise, disease, or surgery. This approach is more general in that the investigator, who may have a bias toward a particular gene or protein, can still obtain a "novel" result from a less familiar gene or protein. In addition, by investigating thousands of genes, it is possible to understand global changes to entire "classes" of genes (e.g., genes encoding contractile proteins,

Q&A

Can You Really Eat Less and Live Longer?

It seems so. Fairly conclusive evidence in insect and rodent models demonstrates that caloric restriction results in greatly increased life spans. Increased lifespan also results from experimental treatments, in which free radical scavengers such as vitamin E and superoxide dismutase (SOD) are given to the animals. Caloric restriction and antioxidants, at the molecular level, seem to prevent change in about 85% of the genes that normally change with age.

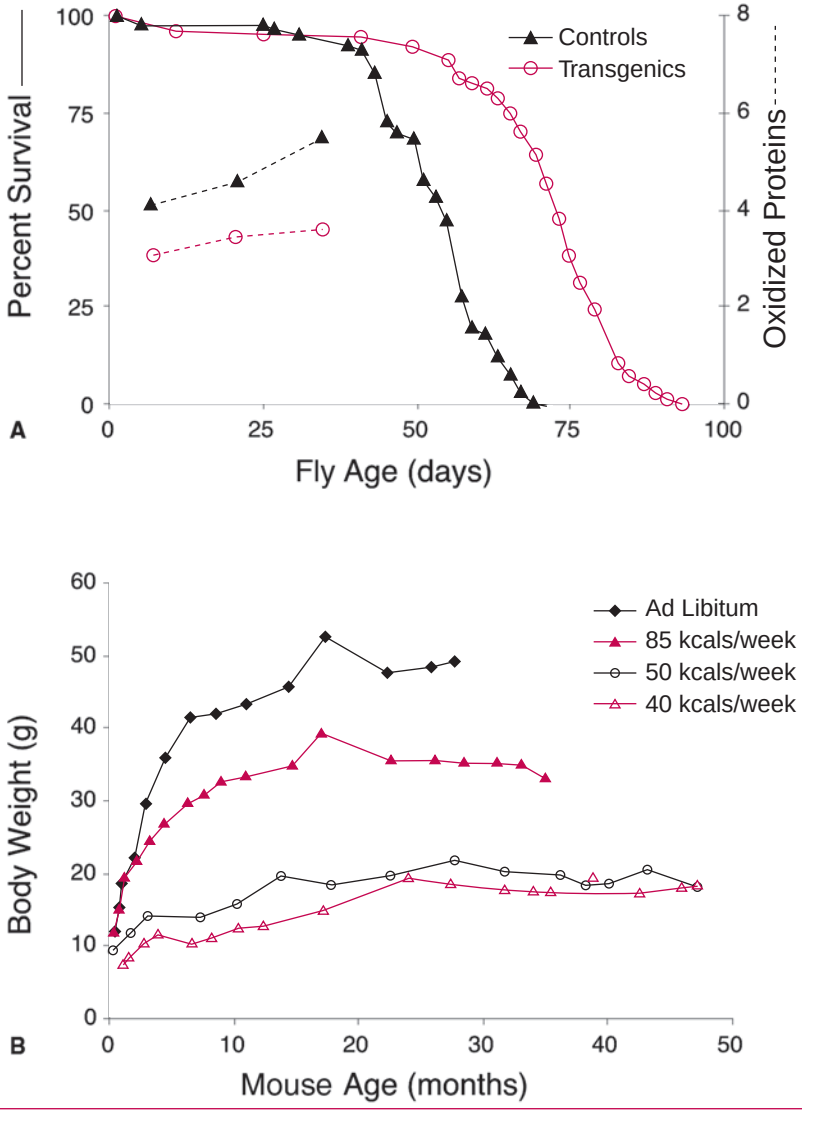

Figure 5-35 (**A**) Left symbols: Amount of DNA demonstrating "injury" in normal (*filled symbols*) and genetically altered fruit flies (*open symbols*) as indicated by the relative frequency of oxidized proteins. Right symbols: Difference in lifespan in fruit flies that overexpress a free-radical scavenger compared with normal fruit flies. Expression of a free radical scavenger decreases DNA damage and increases lifespan. (**B**) Body weight of mice permitted to eat different amounts of food ranging from 40 kcal/wk to unlimited amounts (ad libitum) (Data from Sohal, R. S., & Weindruch, R. [1996]. Oxidative stress, caloric restriction, and aging. *Science, 215*, 1415–1418.)

metabolic enzymes, DNA repair enzymes, etc.). There is no doubt that you will see an increasing number of these types of studies.

Weindruch and colleagues compared 6,347 genes between adult (aged 5 months) and old (aged 30 months) mice (representing 5–10% of the mouse genome) (Fig. 5-36) (Lee, Klopp, Weindruch, & Prolla, 1999). Initially, you might think that the aged mice would show that all genes merely "slow down" with time. In fact, this was clearly not the case. Specifically, only about 0.9% of the genes displayed a greater than twofold increase in expression, while only about 0.9% displayed a greater than twofold decrease with age. Clearly aging was not synonymous with "running down." Most of the genes that increased with age (16% of those that changed) were mediators of the stress response, such as proteins that "chaperone" other injured proteins for destruction or repair, DNA repair proteins, or other proteases (solid bars, Fig. 5-36). Consistent with changes that have already been discussed in aging—the inability of motor nerves to reinnervate muscle fibers—9% of the upregulated genes were involved in neuronal growth. Of the genes that showed decreased expression with age, most (13%) were involved with energy metabolism including glycolysis, ATP synthesis, and glycogen metabolism (red bars, Fig. 5-36). Other enzymes involved in protein and fatty acid synthesis decreased as well. The authors then used the same experimental approach applied to old mice who also had experienced a 24% caloric restriction similar to that described above and compared those results to aging alone (in this latter experiment, the aged mice became the "control" group). Recall that caloric restriction in fruit flies and mice increased their lifespan significantly (Lee et al., 1999; Sohal & Weindruch, 1996). What was the molecular basis for such an effect? The

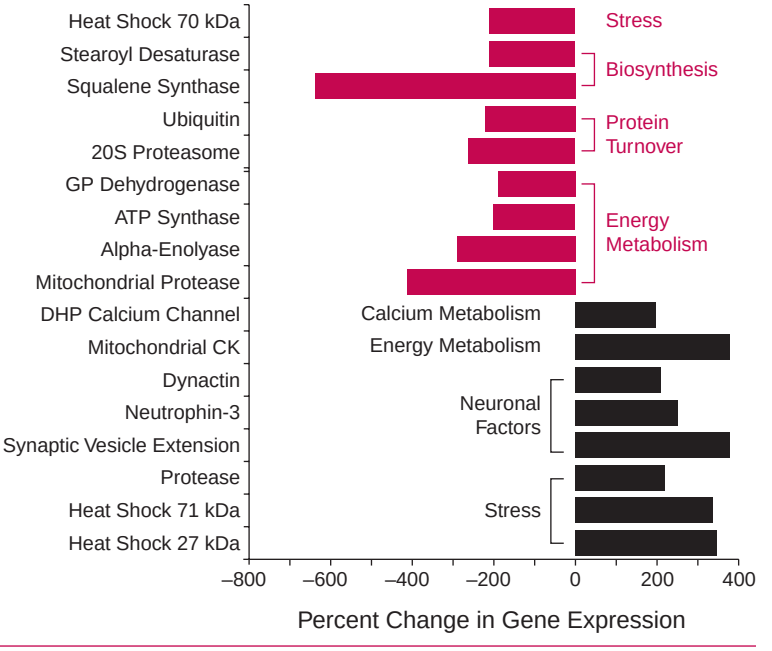

Figure 5-36 Summary of genes that are upregulated and downregulated with aging as determined by gene array screening. Black bars represent upregulated genes, while red bars represent downregulated genes. In spite of the large number of genes listed here, less than 1% of all the genes studied were affected. (Data from Lee, C. K., Klopp, R. G., Weindruch, R., & Prolla, T.A. [1999]. Gene expression profile of aging and its retardation by caloric restriction. *Science, 285*, 1390–1393.)

authors found that caloric restriction completely or partially prevented the age-related changes in 84% of the genes that had shown significant changes with aging. Additionally, caloric restriction caused upregulation of other metabolic genes and synthetic genes and downregulation of DNA repair and stress genes. This implies an overall ability of the calorically restricted animals to resist oxidative stress, and a decreased necessity to repair damaged cellular components—all good news.

Aged Muscle Susceptibility to Eccentric Injury

The next chapter will provide a comprehensive discussion of mechanical injury to muscle that is forced to lengthen while activated (so-called eccentric contractions). For now, it suffices to say that forcing a muscle to lengthen while it is activated can cause injury, and the greater the stretch, the greater the injury. To investigate the susceptibility of aged muscle to resist injury, Drs. Susan Brooks and John Faulkner compared the magnitude of force deficit after a single eccentric contraction between adult mice (aged 9–12 months) and aged mice (aged 27–34 months). They found that eccentric contractions caused injury in both adult and aged muscles. However, the magnitude of the force deficit was much greater in the aged muscles compared with the adult muscles (Fig. 5-37) (Brooks & Faulkner, 1990). This differential injury and limited recovery of aged muscles subjected to chronic eccentric exercise was even suggested as the explanation for the age-induced loss in muscle force production (Faulkner, Brooks, & Zerba, 1995). Whether injured, aged muscles lose force-producing capability and are less able to mount the hypertrophic response has never been tested. This is, of course, a critical gap in our knowledge because

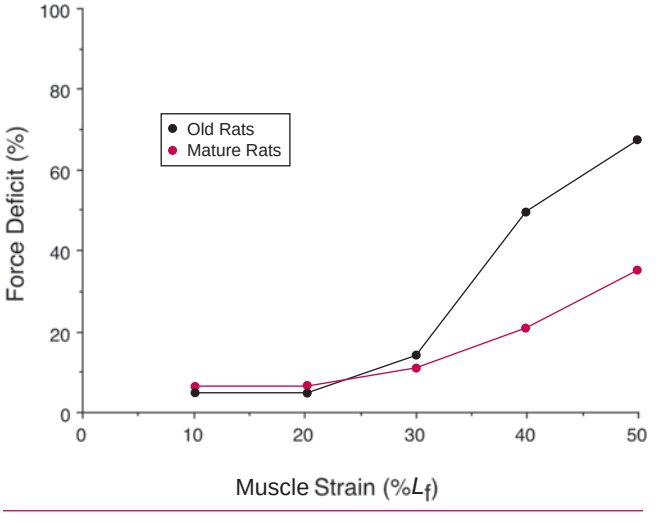

Figure 5-37 Relationship between muscle fiber strain (%L_f) and force deficit (percentage decline from initial force level) for mature *(red circles)* and old *(black circles)* extensor digitorum longus muscles. Force deficit increases with increasing strain and to a greater extent in the aged muscles. Aged muscles appear to be more sensitive to fiber strain compared with adult muscles. (Data from Brooks, S. V., & Faulkner, J. A. [1990]. Contraction induced injury: Recovery of skeletal muscles in young and old mice. *American Journal of Physiology, 258*, C436–C442.)

if aged muscle is more easily injured and unable to adapt, then such injury should be judiciously avoided. If, however, injured aged muscle produces a vigorous strengthening response, the eccentric contractions would be expected to provide a potent strengthening stimulus to aged muscle. Again, future studies must resolve these unknown issues.

THERAPIST'S COMMENTS

This is an important section for geriatric physical therapy. As noted above, muscles from older individuals adapt appropriately, although the magnitude and timing of these changes may be different compared to younger muscle. Well-controlled studies identifying optimal strength and endurance programs for older individuals will likely have high impact on our society as the average age of our population increases.

ADAPTATION TO TENOTOMY

Yet another model of decreased use is created when the insertion site of a tendon is surgically released or traumatically ruptures. This is known as tenotomy. In the early days of muscle research, this was used as a model to create "disuse atrophy" of the muscle. However, in the same way that a muscle without its associated motor nerve is neither normal nor "happy," a muscle under no load is also not happy. As with denervation, (see below) there are vast clinical implications of the changes that occur both in muscles and tendons after tenotomy because its occurrence is so common. There are numerous examples of tendon injuries that lead to severe debilitation. These include rotator cuff rupture, Achilles tendon injury, flexor and extensor tendon laceration in the hand, and patellar tendon rupture. Surgeons also intentionally cut tendons in corrective surgery. For example, surgical tenotomy is used in ophthalmology for realignment of the optic axis (Saunders, Bluestein, Wilson, & Berland, 1994). Tenotomy is commonly performed in foot and ankle surgery for treatment of hallux valgus, in hand surgery for tendon transfers and treatment of Mallet finger (Brzezienski & Schneider, 1995), in sports medicine for treatment of tendinitis (Leadbetter, Mooar, Lane, & Lee, 1992), in the treatment of rheumatologic diseases such as hamstring tenotomy for hemophiliac arthropathy of the knee, in pediatric orthopaedics for correction of deformities in cerebral palsy (Simon & Ryan, 1992), and in orthopedic traumatology for the management of compartment syndrome contractures (Hargens et al., 1989). Thus, it is important to understand the effects of tenotomy on skeletal muscle, both in the short term, immediately after surgery, and in the long term, after the muscle has had the chance to adapt to its new environment.

The immediate effects of tenotomy or tendon rupture on muscle are straightforward. Because most normal muscles are under resting tension, there is typically an immediate decrease in the tension across the muscle as well as muscle shortening after the muscle loses one of its attachments. Additionally, the specialized neural sensors are affected with respect to the length and tension information that they receive (see below).

Tenotomy Decreases Muscle Mass and Force Generation Capacity

Tenotomy, studied across a wide range of species, results in decreased muscle mass. The specific effect of tenotomy depends both on the species and the muscle studied. For example, in the rat soleus muscle, 12 days after tenotomy, muscle mass decreases by approximately 50% compared to a normal muscle (Buller & Lewis, 1965; Jakubiec Puka, Catani, & Carraro; 1992). As with the other models that have been presented, the antigravity muscles (composed primarily of slow fibers) atrophy to a greater extent than their antagonists (composed primarily of fast fibers). In many animal models, unintended reformation of a distal attachment of the muscle to either the tendon stump or surrounding connective tissue with subsequent application of tension may be a confounding factor in decreasing the true level of muscle atrophy after tenotomy. Since you have seen many examples in which muscle forces play a large role in causing the particular adaptive response, it is important to characterize the mechanical response to tenotomy to permit accurate interpretation.

Tenotomy, with intact innervation, leads to several changes in muscle contractile function. The eminent neurophysiologists Buller and Lewis performed pioneering studies in the 1960s (Buller & Lewis, 1965). They completely tenotomized all muscles around the rabbit ankle and showed significantly decreased twitch tensions in the tenotomized group to approximately 50% of the control values within only 3 weeks. Additionally, they noted an increase in the speed of muscle contraction, which may have indicated conversion of slow fibers to fast fibers, as occurs with other decreased use models. Of course, fiber type was not explicitly determined because, at that time, routine histochemistry was not available.

Tenotomy Causes Alterations in Sarcomeres and Supporting Structures

Tenotomy in general leads to an increase in the muscle connective tissue and microcirculation (Józsa et al., 1990). Within 1 week of tenotomy in the rat soleus and gastrocnemius muscles, there was a qualitative increase in both the epimysial and perimysial connective tissue layers. By 3 weeks after tenotomy, the volume density of connective tissue had increased by a factor of 10, with a greater increase in the soleus compared to the gastrocnemius. In parallel with the increase in connective tissue, there was a loss in the number of capillaries. By 3 weeks posttenotomy, only 47% of the soleus capillaries remained. The capillary effect was more profound in the soleus compared to the gastrocnemius.

Tenotomy also plays a role in the organization of sarcomeres in series within a muscle. It was demonstrated that tenotomy of the proximal and distal tendons of rat soleus muscle not only leads to muscle belly shortening as one would expect, but that this shortening was distributed to the sarcomeres in such a way that they shortened

THERAPIST'S COMMENTS

Tenotomy is an important model for physical therapists to understand. Although muscle atrophy (PCSA reduction) is most commonly expected, changes in muscle fiber length and connective tissue content are equally important. A repaired rotator cuff tendon tear, for example, may demonstrate weakness not only because of atrophy but also because the muscle fibers are forced to change length more often than a muscle with normal-length fibers. This too, will impair muscle force generation. The degree to which muscles regain muscle fiber area and length after this type of injury is unknown. Therefore, a repaired rotator cuff muscle may be mechanically different than it was prior to injury.

proportionally, from an average length of 2.6 to 1.8 μm (Baker & Hall-Craggs, 1978). These authors showed that over the course of the following 4 weeks sarcomere length returned to normal by reducing the sarcomere number, suggesting that the reduction of sarcomeres in series was made to optimize the contractile function of each sarcomere. If this were true, it would be similar to studies of immobilization presented in Chapter 4, in which sarcomere length returns to optimal by changing the sarcomere number. However, it should be noted that, as in the sarcomere number changes described in Chapter 4, tenotomy itself is not a model that isolates a single variable for study.

Relationship between Tenotomy and Innervation

The effect of tenotomy on neural function is complex, involving both the efferent and afferent pathways. Tenotomy has been used as a method for simulating immobilization of muscle (Herbison, Jaweed, & Ditunno, 1979), but this is probably not a clean treatment, since the magnitude and the duration of muscle unloading are not explicitly known. EMG activity has been reported to be decreased or absent in animal tenotomy models (Vrbova, 1963), which is analogous to what is observed with hindlimb unloading (Alford et al., 1987); it may represent an inability of the muscle to be reflexively activated, or it may truly represent decreased neural drive to the muscle itself.

Differential Response to Tenotomy of an Activated Muscle Compared to a Relaxed Muscle

In light of the strong influence of muscle mechanical properties on muscle plasticity, we were interested in comparing the response of skeletal muscle to what we termed "active" and "passive" tenotomy. Since tenotomy during muscle activation is a violent event, associated with rapid muscle shortening and even perforation of the Z-disk by myosin filaments (Ramsey & Street, 1940), it was reasoned that it would be much more injurious to muscle compared with tenotomy of a muscle that was in a relaxed state. When relaxed muscle tendons are cut, there is a relatively modest retraction of the tendon. Distinguishing between the effects of these tenotomy types is clinically important because, as mentioned above, tenotomy occurs under both types of conditions, and their treatments may not be identical.

To make this comparison, the effects of active tenotomy were compared with the effects of passive tenotomy, over the 3-week period after the tenotomy was performed (Abrams, Tsai, Watson, Jamali, & Lieber, 2000). It was discovered that after 1 week the active tenotomy group produced lower maximum tetanic tensions than the passive tenotomy, which was interpreted as indicating greater damage to the contractile elements (Fig. 5-38). By 21 days, however, the active tenotomy group had recovered to a significantly greater degree than the passive tenotomy group. One possible explanation of these findings was that the active tenotomy initially caused a greater muscle injury, leading to a greater regenerative response, allowing more complete restoration of the contractile function. However, a number of important structural features of the muscle were measured, none of which supported this idea. Surprisingly, differences between group P_o values were not explainable based upon careful quantitative analysis of muscle architecture, muscle fiber size, or magnitude of regeneration as indicated by positive immunostaining with antibodies against developmental myosin (Tables 5-1 and 5-2).

On the basis of the inability to explain contractile changes in terms of alterations in the contractile apparatus, it was hypothesized that the force changes resulted from disruption of the excitation contraction (EC) coupling system within the muscle. As described in Chapter 2, the EC coupling system includes electrical and subcellular events such as muscle fiber action potential conduction, transverse tubule conduction, "activation" of the dihydropyridine (DHP) receptors, and calcium release in the vicinity of the myofilaments (Ebashi, 1976). Precedent for selective disruption of EC coupling after muscle injury that already exists in the literature will be discussed in Chapter 6 (Warren et al., 1993). Perhaps tenotomy could lead to a similar effect. It was further hypothesized that, should EC coupling be affected by tenotomy, upregulation of proteins associated with the EC coupling apparatus should be observed, such as the postsynaptic acetylcholine receptor or neural cell adhesion molecule (NCAM). During development, NCAM is expressed, presumably to attract motor nerves to innervate muscles. In adult muscle, NCAM is expressed after denervation (see p. 218), presumably to "reattract" a motor nerve to reinnervate the muscle and restore function.

NCAM Expression after Tenotomy

Bupivicaine was used as a positive control for NCAM expression. It is markedly toxic to muscle fibers and led to disruption of muscle fibers and infiltration of leukocytes, as well as a subsequent regenerative response characterized by formation of myotubes and immature myofibers that were observed on hematoxylin and eosin staining.

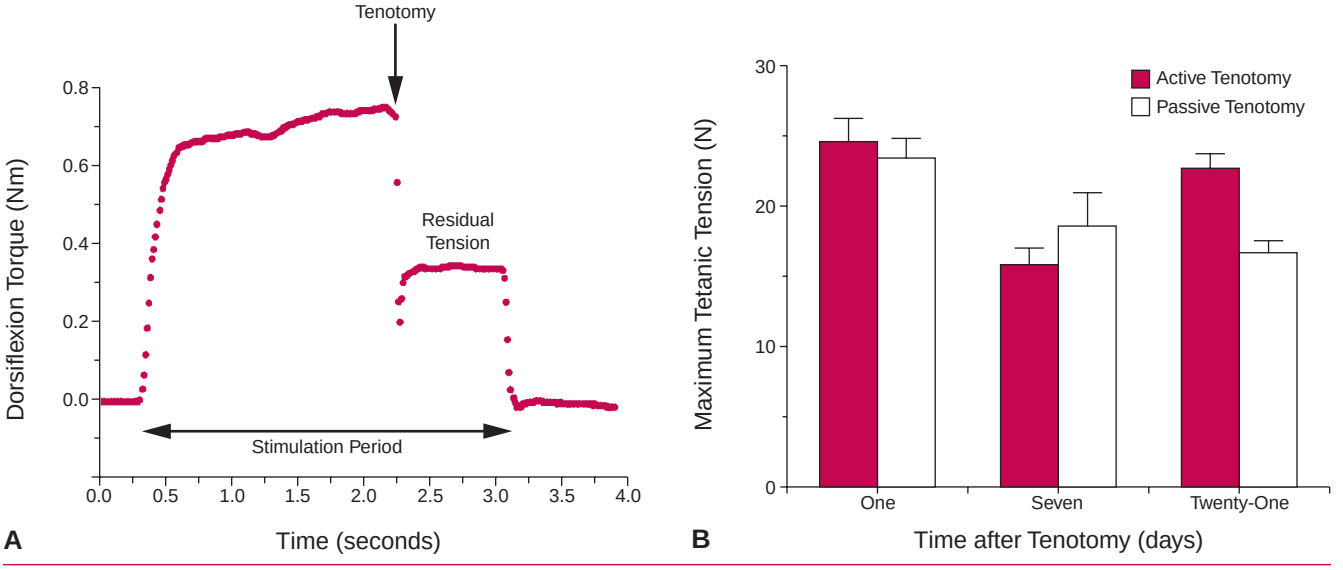

Figure 5-38 (A) Contractle record measured during active tenotomy of the rabbit EDL muscles. Note that peak torque is reached and then tendons are cut *(arrow)*. Torque then drops to a residual level, which is the torque generated only by the remaining tibialis anterior muscle. (B) Maximum isometric tension generated by EDL muscles after active tenotomy *(red bars)* or passive tenotomy *(open bars)*. Active tenotomy represents tenotomy during muscle contraction, while passive tenotomy represents tenotomy with the muscle at rest. (Data from Abrams, R. A., Tsai, A. M., Watson, B., Jamali, A., & Lieber, R. L. [2000]. Skeletal muscle recovery after tenotomy and 7 day delayed muscle length restoration. *Muscle & Nerve, 23*, 707–714.)

Bupivicaine-injected muscles also demonstrated pronounced expression of embryonic myosin in the regions of maximum myofiber disruption and, in many of those same fibers, positive staining for NCAM was seen. Interestingly, NCAM was also expressed in a surrounding population of morphologically normal muscle fibers that did not express embryonic myosin. This indicates that regeneration is not required for NCAM expression. Control micrographs of normal muscles did not express NCAM (Fig. 5-39A). NCAM was then expressed in tenotomized rabbit EDL muscle at significantly higher levels (Józsa, Kannu, Thöring, Reffy, Järvinen & Kvist, 1990). Qualitatively, NCAM levels increased from barely detectable levels 1 day after tenotomy (Fig. 5-39B) to

Table 5-1

EDL Architectural Properties

Experimental Group	Muscle Mass (g)	Muscle PCSA (cm^2)	Serial Sarcomere Number
Active Tenotomy (n = 9–12/group)			
1 day	3.68 ± 0.24	2.54 ± 0.26	6,233 ± 302
7 days	3.32 ± 0.24	2.05 ± 0.14	6,193 ± 190
21 days	3.89 ± 0.13	2.23 ± 0.50	5,323 ± 287
Passive Tenotomy (n = 9–11/group)			
1 day	2.96 ± 0.06	1.84 ± 0.05	6,170 ± 159
7 days	3.40 ± 0.31	2.44 ± 0.28	5,939 ± 217
21 days	2.85 ± 0.07	1.92 ± 0.12	5,844 ± 185
Control Muscles (n = 6)[a]			
3.56 ± 0.21	1.92 ± 0.20	5,844 ± 185	

[a]Control data from Lieber, R. L. & Blevins, F. T. (1989). Skeletal muscle architecture of the rabbit hindlimb. Functional implications of muscle design. *Journal of Morphology, 199,* 93–101.

Abbreviations: EDL, extensor digitorum longus; PCSA, physiological cross-sectional area.

Source: Abrams, R. A., Tsai, A. M., Watson, B., Jamali, A., & Lieber, R. L. (2000). Skeletal muscle recovery after tenotomy and 7 day delayed muscle length restoration. *Muscle & Nerve, 23*, 707–714.

Table 5-2

EDP Morphometric Properties

Experimental Group	Fast Fiber Area (μm^2)	Number of Fast Fibers	Slow Fiber Area (μm^2)	Number of Slow Fibers
Active Tenotomy (n = 9–12/group)				
1 day	2715/62	499	1598/58	95
7 days	2520/90	222	1429/30	66
21 days	2215/60	271	1451/31	65
Passive Tenotomy (n = 9–11/group)				
1 day	2444/60	378	1506/36	117
7 days	2971/62	159 1	544/41	53
21 days	2384/53	495	1393/31	134

Source: Abrams, R. A., Tsai, A. M., Watson, B., Jamali, A., & Lieber, R. L. (2000). Skeletal muscle recovery after tenotomy and 7-day delayed muscle length restoration. *Muscle & Nerve, 23*, 707–714.

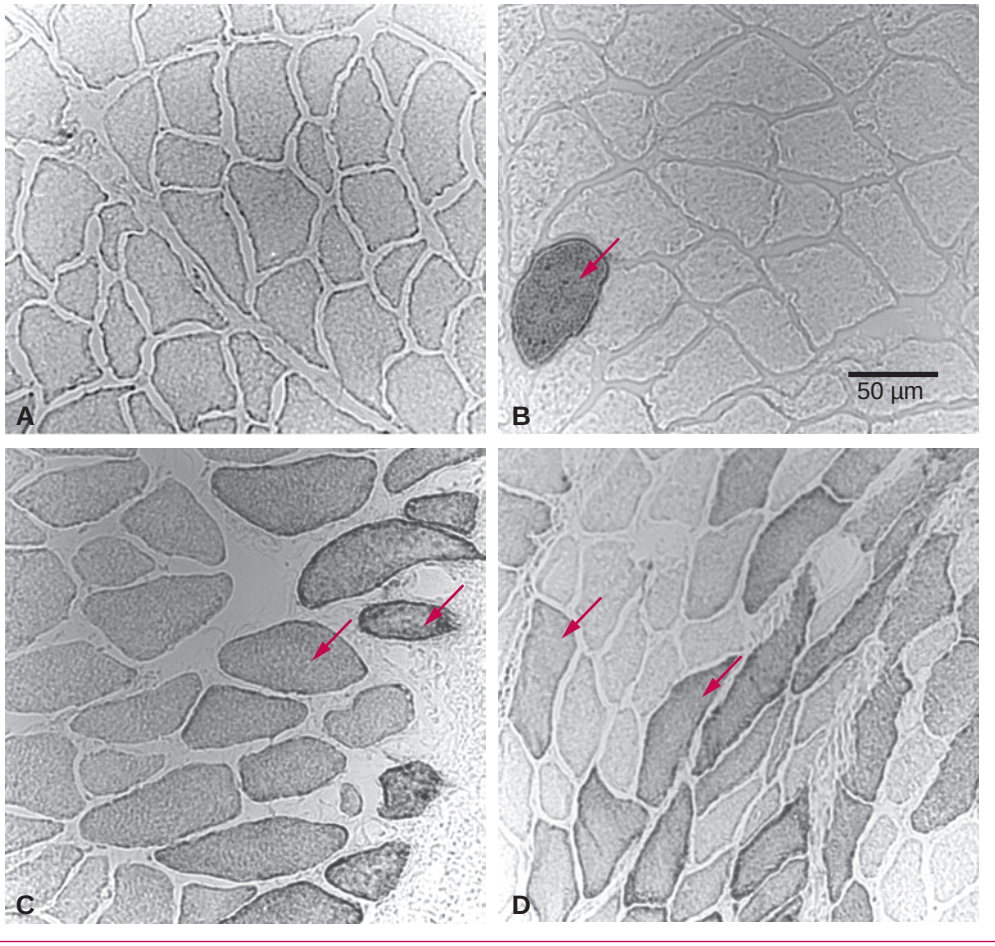

Figure 5-39 Representative micrographs of rabbit EDL muscles immunoassayed for NCAM. Quantitative results from stereological analysis shown in Table 5-2 above. (**A**) Control EDL muscle that received no treatment. (**B**) Tenotomized muscle, 1 day after tenotomy, (**C**) tenotomized muscle, 7 days after tenotomy, and (**D**) tenotomized muscle, 21 days after tenotomy. Darkly stained fibers are positive for the NCAM protein although the range of staining intensity varies among fibers. Arrows point to selected fibers expressing NCAM. (Reprinted with permission from Jamali, A., Afshar, P., Abrams, R., & Lieber, R. L. [2002]. Expression of neural cell adhesion molecule (NCAM) in tenotomized skeletal muscle. *Journal of Orthopaedic Research*, *20*, 364–369. Reprinted with permission of John Wiley & Sons, Inc.)

relatively high levels 7 and 21 days after tenotomy (Figs. 5-39C and 5-39D). Quantitatively, NCAM labeled 2–5% of fibers 1 day after tenotomy. These data demonstrate a time-dependent effect of tenotomy type, reminiscent of, but certainly not proving, cause for the observed functional data (Fig. 5-38).

Probably the key question here is, what was the muscle "thinking" after tenotomy? Why would it be advantageous to interrupt neuromuscular communication? Clearly the answers to these questions are speculative at this point, but one possibility is that the muscle, somehow, "sensed" the loss of load and "turned off" the NMJ to prevent muscle activation and subsequent injury. This would represent a type of "circuit breaker" to protect the muscle from activation while unloaded. A second possibility is that there is a load-sensing mechanism within the muscle that is related to its interpretation of the state of innervation. Perhaps when the muscle senses no load, it "thinks" that there is no motor nerve innervating it and expresses NCAM to reattract the motor nerve. Many future studies are clearly required to understand both the muscle's response to tenotomy as well as methods to restore function after tenotomy. It should be noted that, in the study above, after a 7-day delay period, in a separate group of animal subjects, the distal tendons were reattached to the retinaculum to simulate a repair, and the muscle properties recovered fully (Abrams, Tsai, Watson, Jamali, & Lieber, 2000). Thus this "sensing" effect is clearly reversible.

ADAPTATION TO DENERVATION

Now consider a "completely different" type of decreased use model—denervation (Fig. 5-40). Denervation was used in early muscle plasticity studies as a model of disuse, because it was easy to perform and was viewed as a way to instantly and permanently decrease the use placed upon the muscle. However, as has been seen repeatedly, a denervated muscle is a different kind of muscle. For example, the denervated soleus muscle responded quite differently to electrical stimulation patterns than a normally innervated muscle does. As such, consider denervation, not so much as a typical model of decreased use, but rather as a model that illustrates the intimate relationship between muscles and nerves. The unique aspects of denervation that have significant clinical implications will also be considered. Certainly, denervation is a relevant plasticity model and represents an important clinical treatment problem. How does one "treat" a denervated muscle? Are the adaptive responses to tension and stretch similar? How do denervated muscle fibers become reinnervated? Can muscle properties return to normal following reinnervation?

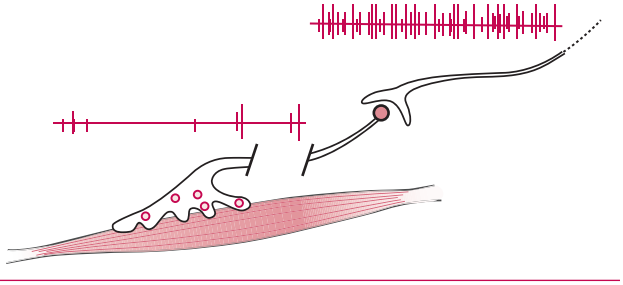

Figure 5-40 Schematic diagram of denervation model. Lower motor neuron is transected.

Muscle Strength Changes after Denervation

The most obvious muscular change that occurs after denervation is muscle atrophy. This atrophy affects both fast and slow muscle fibers and results in decreased fiber diameter and decreased muscle force. Analogous to previously mentioned decreased use models, denervated muscles increase their contractile speed because of fiber type conversion in the slow-to-fast direction. In spite of this conversion, the muscles still generate much lower forces than normal.

Muscle Fiber Atrophy after Denervation

While the mechanism of muscle fiber atrophy is not fully understood in any experimental model, recent experiments have implicated some intramuscular proteolytic enzymes in the atrophic process. An interesting denervation/reinnervation experiment was performed by Dr. Marie Badalmente, who applied the protease inhibitor leupeptin to primate thenar muscles after median nerve denervation and surgical repair (Badalmente, Hurst, & Stracher, 1989). Muscle fibers normally contain enzymes within them that are capable of digesting the cellular constituents. This is another way in which the muscle can alter properties in response to a change in environment (these enzymes will be discussed in more detail in Chapter 6 during the discussion of muscle injury and muscular dystrophy). Muscle fibers thus have the capability to "self-destruct" if these proteolytic enzymes are activated. One family of enzymes is activated automatically when the intracellular calcium levels rise too high for a prolonged period of time (the calcium-activated neutral proteases, Calpains) (Croall & Demartino, 1991). Since intracellular calcium levels are not normally high for a long time, increased free calcium within a muscle fiber signifies that muscle fiber integrity has been lost (the cell membrane has been broken) and subsequent regeneration should occur. (See a further discussion of this mechanism in Chapter 6.) Badalmente and her colleagues injected monkeys with the Calpain inhibitor leupeptin, to attenuate Calpain's effect. They confirmed that Calpain was suppressed by leupeptin, and they then showed that the fiber diameter of leupeptin-treated abductor pollicis and opponens pollicis muscles were greater than the nontreated denervated muscles, suggesting less atrophy (Fig. 5-41). Additionally, a greater number of axons survived when muscles were treated with leupeptin. These data may suggest

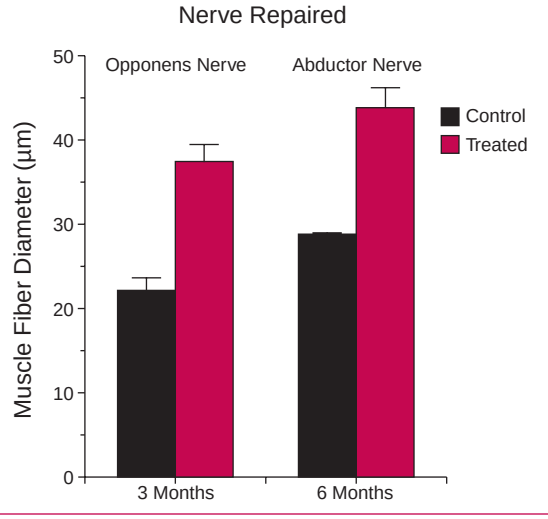

Figure 5-41 Muscle fiber diameter of primate muscles after application of leupeptin to muscles whose nerves were transected and then repaired. Treated muscles received application of leupeptin, the calcium protease inhibitor *(red bars)*. Control muscles were simply denervated *(black bars)*. (Data from Badalmente, M. A., Hurst, L. C., & Stracher, A. [1989]. Neuromuscular recovery using calcium protease inhibition after median nerve repair in primates. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 5983–5987.)

that maintenance of muscle properties following denervation may have a beneficial effect on the reinnervating nerve. Alternatively, Calpain may have a direct axon-sparing role. Again, here is a situation in which a muscle change may affect, in a retrograde fashion, changes within the nerve.

Muscle Fiber Changes after Denervation

Perhaps the most interesting muscle responses to denervation are the subtle changes around the fiber that "signal" to the outside world that the nerve and muscle are no longer in communication. This may not be surprising since there is not really anything more important to a muscle than maintaining an intact neural connection. For example, recall in-muscle development, that the myotube was literally covered with acetylcholine receptors (AChRs) until the nerve "arrived." After innervation, the density and number of extrajunctional AChRs decreased dramatically, and the only remaining receptors were those at NMJ (see Chapter 1, p. 9). After denervation, a reversal of the developmental process is observed, in that a proliferation of extrajunctional AChR occurs (Fig. 5-42). Some view this response as a sort of signal that causes nerves to sprout new axons and prepare to form a new NMJ. The fact that denervation mimics part of the development of the NMJ is one reason why developmental biologists use denervation as an experimental model to study the process of nerve–muscle connection. (See experiments described in Chapter 1). What other signals are available to guide incoming nerves? As mentioned above, the extracellular matrix molecule, NCAM, is implicated in the denervation–reinnervation process. Recall that during development, NCAM was expressed on the surface of the

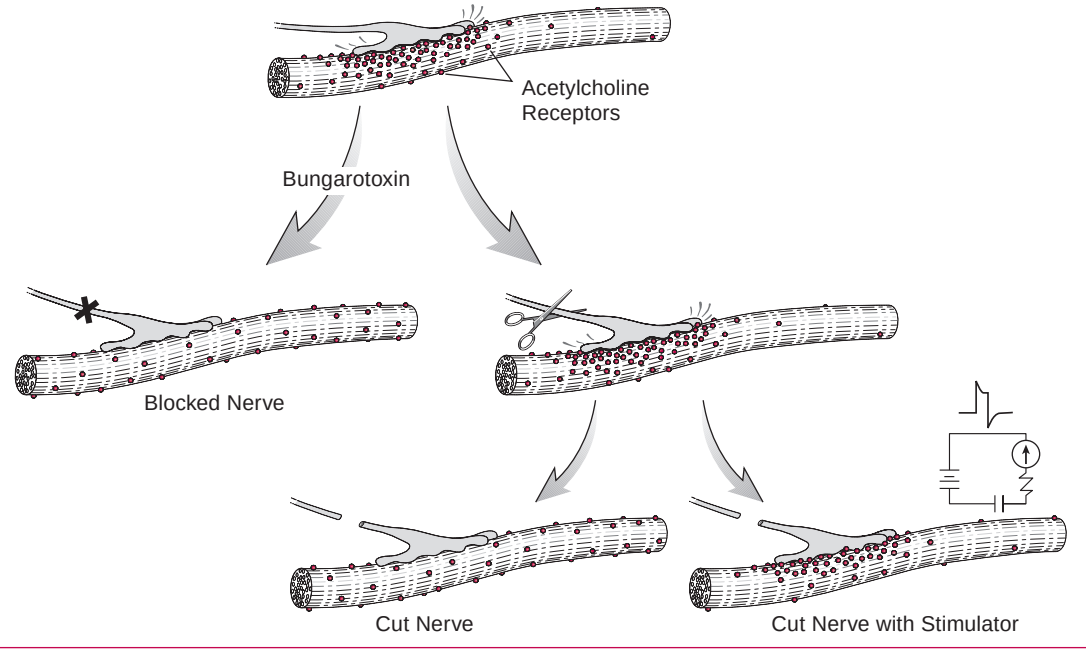

Figure 5-42 Relationship between the distribution of ACh receptors and the presence or absence of the nerve. If the nerve is blocked with bungarotoxin, ACh receptors are expressed along the fiber length similar to what happens in denervation (**left path**). However, if the nerve is cut and then stimulation is superimposed on denervation (**right path**), ACh receptor expression is suppressed.

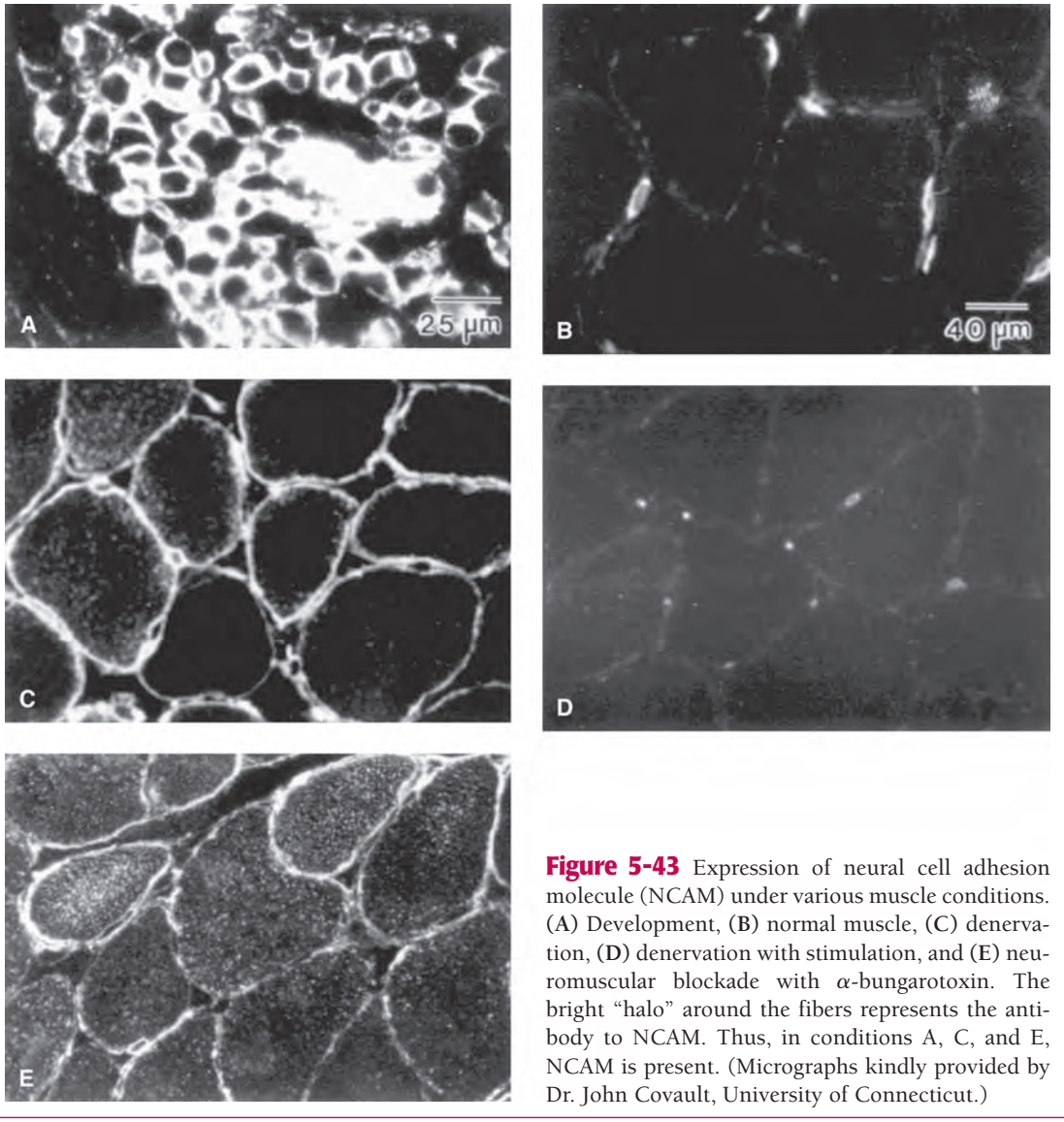

Figure 5-43 Expression of neural cell adhesion molecule (NCAM) under various muscle conditions. (**A**) Development, (**B**) normal muscle, (**C**) denervation, (**D**) denervation with stimulation, and (**E**) neuromuscular blockade with α-bungarotoxin. The bright "halo" around the fibers represents the antibody to NCAM. Thus, in conditions A, C, and E, NCAM is present. (Micrographs kindly provided by Dr. John Covault, University of Connecticut.)

primitive myotubes to perhaps "guide" incoming nerves to the muscle fiber. In denervation, NCAM is again expressed by the muscle fiber (Fig. 5-43). In fact, the NCAM molecule appears to be expressed any time that the muscle fiber is "receptive" to incoming nervous innervation. In the next section, some of the experimental demonstrations of muscle fiber innervation by foreign nerves are described.

Denervated Muscle Fibers Increase "Receptivity" to Nerves

A nerve can be surgically implanted into a normal skeletal muscle and will often continue to grow (Fig. 5-44). It can sprout small neuronal processes, but these processes will not form new synapses with the muscle fiber. Why not? Apparently, the muscle fiber signals the nerve that it is normally innervated and thus, remains refractory to further innervation. If, however, the muscle's normal nerve is cut, the nerve that is surgically implanted into the muscle will now form functional synapses (Fig. 5-44). In fact, if the muscle is only partially denervated (only a fraction of the nerve is cut), the implanted nerve will innervate only those fibers whose nerve branches were cut. Quite an effective signaling method is working here.

Experimentally, other treatments can cause muscle fibers to change their receptivity to innervation by foreign nerves. As you might predict, these are treatments that change the activity level of the muscle fibers. For example, a muscle can be paralyzed with botulinum toxin (a toxin that enters the presynaptic nerve terminal and inhibits ACh release), and the fibers will become receptive to innervation. Conversely, if a denervated muscle is electrically activated, it will not permit innervation by the incoming nerve in spite of the fact that it has no normal nerve of its own (Fig. 5-44). Why not?

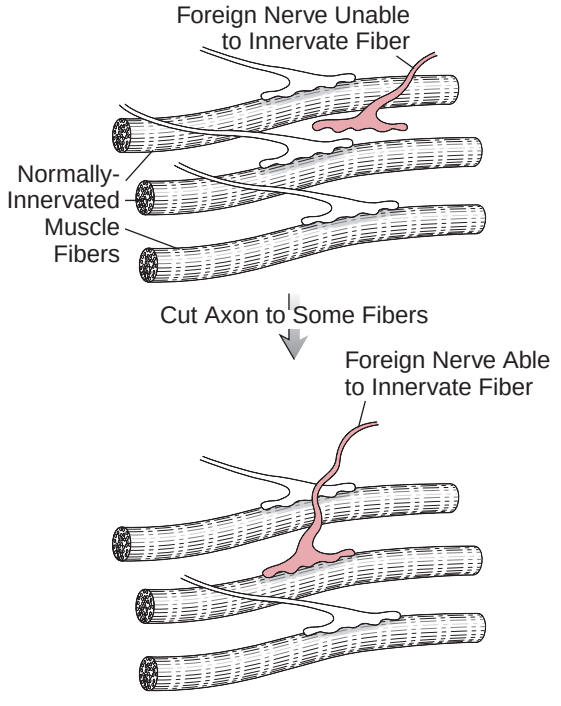

Figure 5-44 Synapse formation does not occur in an already-innervated muscle fiber **(upper panel)**. Note that a foreign nerve *(thick wiggly line)* growing in a muscle composed of innervated fibers does not form an additional synapse. However, if the motor nerve to a muscle fiber is cut **(lower panel)**, a new synapse will form.

Dr. Joshua Sanes and his colleagues showed that all of these conditions are consistent with the timing of a muscle fiber's expression of NCAM (Fig. 5-43). Thus, under conditions of high activity (normally innervated muscle or electrically stimulated, denervated muscle), NCAM is not expressed and synapse formation does not occur. However, when muscle fiber activity is low (in denervation, pharmacological paralysis, or development), NCAM is expressed, and nerves synapse with the fiber (Sanes & Covault, 1985).

Q&A

Why Is Reinnervated Muscle Weak?

The main cause for weakness after reinnervation is a decrease in the number of functional neurons that actually grow back into the correct muscle. These nerve fibers must grow from the site of the lesion back into the correct muscle. Even if they grow back, if they synapse with the wrong muscle, they will be dysfunctional. Thus, nerve injuries that occur very close to a muscle have a better prognosis of functional recovery compared with nerve injuries that occur far away from the muscle.

Formation of New Muscle Synapses

Sanes and his colleagues, as well as others, have implanted nerves into denervated muscles to determine where along the fiber length the synapse is formed. If nerves are implanted close to the original NMJ, the synapse forms at exactly the original location. If, however, a site remote from the original NMJ is chosen, a new, ectopic synapse will form. In an effort to determine what was special about the NMJ, Glicksman and Sanes experimentally "killed" muscle fibers by mechanically crushing them (Glicksman & Sanes, 1983). Normally, the muscle fibers would regenerate (Chapter 6) to form a new muscle. However, these investigators prevented muscle regeneration by repeated exposure to intense X-rays, leaving only muscle-free "ghosts"—essentially only the basal lamina filled with muscle fiber debris. Recall that the basal lamina is the structure that ensheathes muscle fibers during development (pp. 1–3). It is outside of the sarcolemma and is thus an extracellular structure. Interestingly, reinnervation of these muscle-free ghosts proceeded in a relatively normal fashion. Relatively normal NMJs were formed in precisely the location of the original NMJ, and the nerve presynaptic terminals were "loaded" with normal-appearing ACh vesicles. These data provide strong evidence that, at least in the early stages of synapse formation, the basal lamina provides a sufficient molecular signal to the nerve for synaptogenesis. But what about the expression of NCAM? Didn't that cause the reinnervation? No. This experiment reinforces an important lesson: Just because one event is associated with another event does not mean that it causes it. Clearly, NCAM expression was associated with reinnervation, but it alone did not cause reinnervation.

MUSCLE FIBER SPECIFICITY TO REINNERVATION

The conditions surrounding reinnervation of a muscle fiber have been discussed, but take this one step further: How does a nerve select which muscle fiber type to innervate? This is a "hot" research area, and definitive answers are not yet available. The reason it is so important is that, as discussed in Chapter 2, muscle fiber recruitment proceeds in an orderly fashion according to the size principle (pp. 82–84). This is one of the important bases for motor control and coordination. If that sophisticated relationship between nerve and muscles is lost during reinnervation, it will have profound functional consequences. Therefore, there is great interest in understanding the factors that control reinnervation in an effort to improve functional results after reinnervation. A number of clever experiments have been devised to study this problem. You can imagine that determining experimentally which muscle fiber is attached to which axon would be technically difficult. However, in spite of these difficulties, several experiments

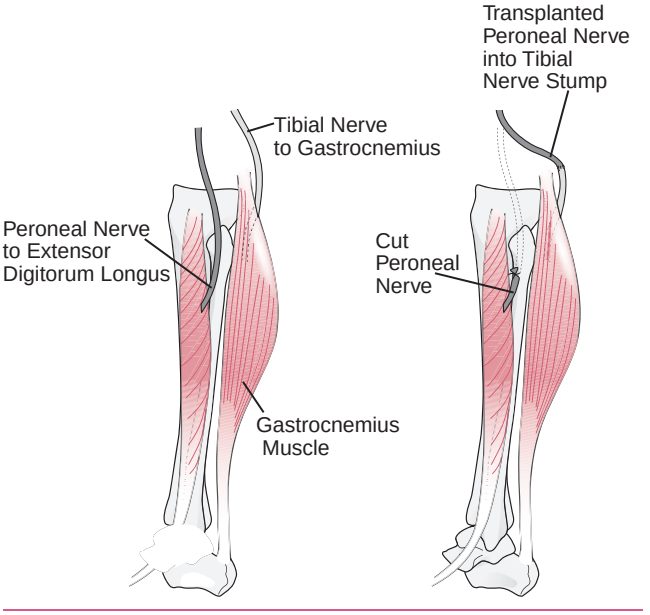

Figure 5-45 Schematic diagram of the cross-reinnervation experiment. The peroneal nerve, which normally innervates the anterior compartment muscles, is surgically reattached to the distal stump of the tibial nerve, which normally innervates the plantarflexors.

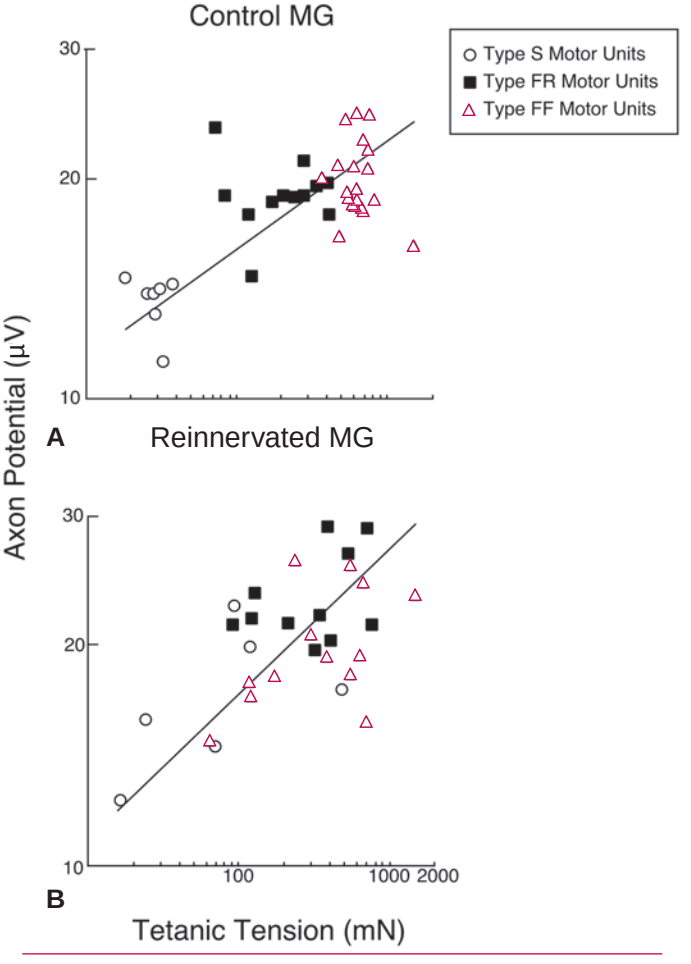

Figure 5-46 Relationship between motor unit tension and action potential amplitude for (**A**) normal and (**B**) cross-reinnervated cat medial gastrocnemius. Each symbol represents a different motor unit type. Normally, S units are associated with the smallest axons, represented by low axon potentials, and FR and FF units have increasing tension and axon potential amplitude. However, after reinnervation, this relationship is dramatically altered. (Data from Gordon, T., Thomas, C. K., Stein, R. B., & Erdebil, S. [1988]. Comparison of physiological and histochemical properties of motor units after cross-reinnervation of antagonistic muscles in the cat hindlimb. *Journal of Neurophysiology, 60*, 365–378.)

have provided clues that lead us to believe that nerves generally innervate the nearest receptive fiber, regardless of type. But the story is not that simple.

Motor Unit Properties Following Cross-Reinnervation

To study the specificity of reinnervation, a nerve that normally innervates slow fibers is given the opportunity to innervate slow or fast fibers (or vice versa). This can be done by surgically cross-reinnervating the triceps surae muscles with the antagonistic motor axons from the common peroneal nerve. In this case flexor neurons are forced to innervate extensor muscles. Such an experiment was performed by Drs. Tessa Gordon, Richard Stein, and their colleagues, using the cat hindlimb model (Fig. 5-45). After performing the surgical reanastomosis (microreconnection) of the proximal flexor nerve with the distal extensor nerve stump (and vice versa), they waited 1–2 years, and again measured motor unit properties (as discussed in Chapter 2, pp. 76–81) (Gordon, Thomas, Stein, & Erdebil, 1988).

Motor Unit Tension after Reinnervation

These investigators had already documented the relationship between motor unit tension and axon size as a function of unit type (Fig. 5-46A). As expected, a wide range of normal MG motor unit tensions were observed, ranging from only 10–20 to over 1000 mN (1 mN equals 1.0×10^3 N or 9.8 g). Also, as expected, on the basis of the size principle, the normal MG units that generated the smallest tensions had the smallest axon action potentials, and therefore, the smallest axon size. Units that developed the highest tensions had the largest axon sizes. Finally, low-tension units were identified as S units, the intermediate tension units as FR units, and the high-tension units as FF units. This was all consistent with the idea of the "size principle" previously presented (Henneman, Somjen, & Carpenter, 1965).

After reinnervation was induced by cutting the nerve and then surgically repairing it to the antagonistic nerve, a number of changes occurred. Before you read the results, try to predict what could have happened. Remember that the MG normally contains all three unit types that generate over 2–3 times the average force of comparable units in the flexor

muscles that are normally supplied by the peroneal nerve. What tensions do you think were generated by the reinnervated MG? It turns out that the reinnervated MG motor unit tensions were roughly the same as the normal MG motor units, suggesting that each peroneal nerve axon innervated more fibers in the MG than it had in the dorsiflexors. This might have been because fewer axons grew out of the peroneal nerve to innervate the denervated MG fibers. Another explanation might have been that the peroneal nerve axons had the intrinsic capability of innervating more fibers and simply seized the opportunity. Axon action potential was still directly correlated with motor unit tension, implying that the size principle still held for the reinnervated muscle. In fact the regression relationship between motor unit tension and axon action potential was almost identical before and after reinnervation (Fig. 5-46B).

Motor Unit Specificity following Reinnervation

What about the specific muscle fiber types innervated by the reinnervating axons? Did the axons of the appropriate size find the appropriate fiber types? The answer was clearly negative. Whereas in the normal MG, the low-tension units were of the S type, following reinnervation, motor unit tension was not related to motor unit type (Fig. 5-47). Thus, some S units developed high tensions and some FF units developed very low tensions. Reinnervation was not perfectly fiber type specific.

Other Consequences of Reinnervation

A few notable results were observed with the soleus muscle. The normal cat soleus is composed of 100% S units, and 100% SO fibers. However, after reinnervation, the motor unit percentage was 39% S units, 22% FR units, and 39% FF units. The fiber type percentage was not explicitly measured, but it was noted that there were absolutely no FG-type muscle fibers in the reinnervated soleus. No FG fibers, but many FF units? Right. The data demonstrate two things: First, it was not possible to completely transform the soleus muscle fibers into each and every fiber type, and second, motor unit classification and muscle fiber type classification were not necessarily interchangeable. Recall that normal muscle FF units are composed of FG fibers, but this is obviously not necessarily the case, since FF units were identified in the absence of any FG fibers. This result highlights the complex nature of motor unit performance and suggests caution in going from fiber-type distribution to speculating on performance.

Using the indirect computational methods of Burke et al. (1973) (see Chapter 2 for discussion), Gordon and her colleagues calculated that the innervation ratio of the S and FR units increased, while the innervation ratio of the FF units decreased (Fig. 5-48). They also measured the fiber areas of the various muscle fiber types and found that, generally, the SO and FOG fibers increased in size, while the FG fibers decreased in size. Can you explain

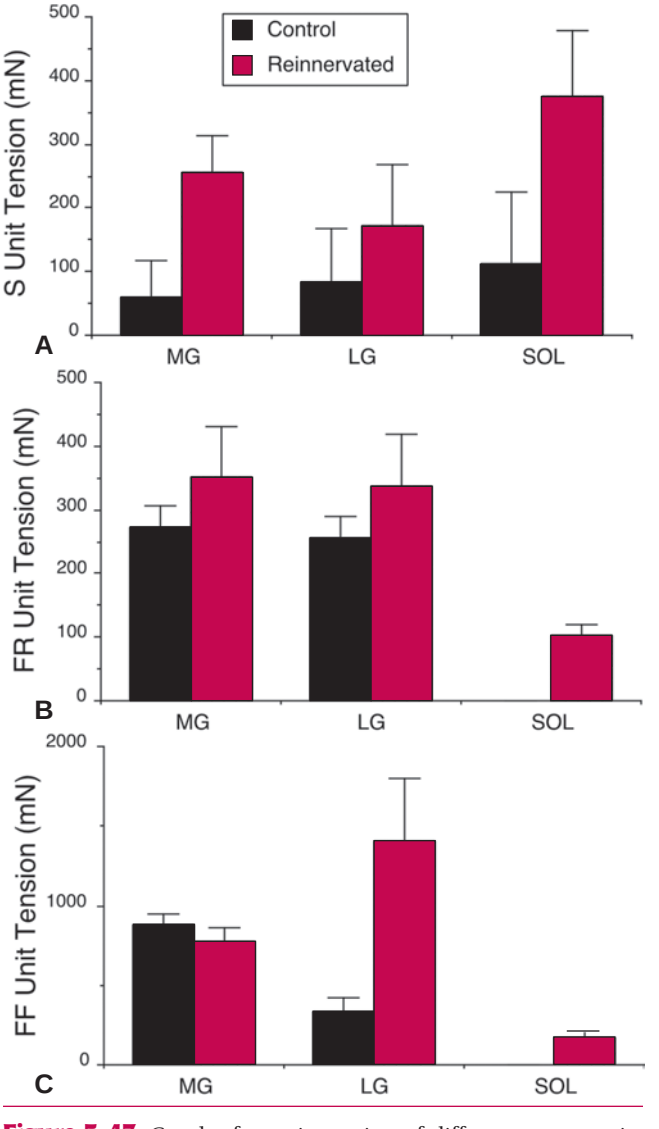

Figure 5-47 Graph of tetanic tension of different motor units from control *(black bars)* or cross-reinnervated *(red bars)* cat skeletal muscles. Note that the normal motor units generate tension according to the size principle. However, following reinnervation, this relationship no longer holds. (Data from Gordon, T., Thomas, C. K., Stein, R. B., & Erdebil, S. [1988]. Comparison of physiological and histochemical properties of motor units after cross-reinnervation of antagonistic muscles in the cat hindlimb. *Journal of Neurophysiology, 60*, 365–378.)

how these changes could result in the motor unit tension values measured? Consider each unit type separately. S unit innervation ratio increased by 18% and SO fiber size increased by 47%, but S unit tension increased disproportionately, by 23%. The only plausible explanation for this result was that the specific tension of the S units increased (since tension increased more than what would be expected simply on the basis of an increased innervation ratio and the measured fiber size). Analogously, FR unit innervation ratio increased by 60%, and FOG fiber size

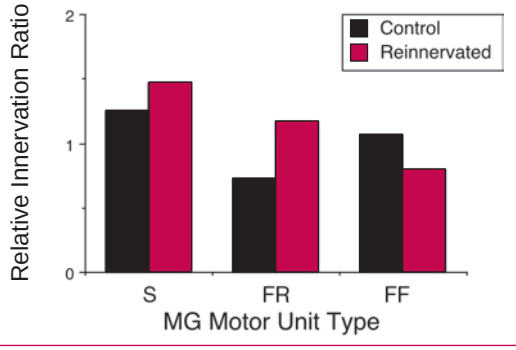

Figure 5-48 Innervation ratios calculated for control *(black bars)* or cross-reinnervated *(red bars)* cat skeletal muscles. (Data from Gordon, T., Thomas, C. K., Stein, R. B., & Erdebil, S. [1988]. Comparison of physiological and histochemical properties of motor units after cross-reinnervation of antagonistic muscles in the cat hindlimb. *Journal of Neurophysiology, 60*, 365–378.)

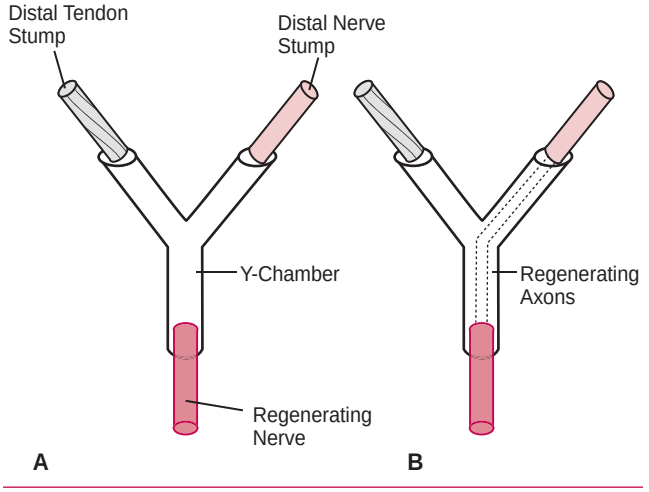

Figure 5-49 **(A)** Representation of the experiment in which a severed nerve was given the option of growing through a Y-tube into either the original nerve or a tendon. **(B)** In all cases, the nerve selectively chose to grow toward the distal nerve stump. This experiment suggests some type of attraction for incoming axons. (From Lundborg, G. [1988]. *Nerve injury and repair* (p. 18). New York: Churchill Livingstone.)

increased by 35%, but FR unit tension increased only by 25%. Again, a reasonable explanation was that specific tension of the FR units decreased (since the tension increased less than what was expected on the basis of the increase in innervation ratio and the measured fiber size). Can you explain the FF units result? (Hint: specific tension was predicted to increase, but for different reasons than the S units. This is a good exercise to see if you understand the contributing factors to motor unit tension). Since the MG motor unit–specific tensions were "correct" after reinnervation (in the sense that they were ordered correctly, although their absolute values were different), but innervation ratio and fiber area were not, the conclusion was that specific tension was the only parameter that was uniquely determined by the innervating axon. The main limitation of this interpretation is that calculation of innervation ratios and specific tensions using this model are very indirect. "Specific tension" is always the term that is "left over" to balance the motor unit tension equation. Future experiments are required to clarify these issues.

To summarize, denervated muscle represents a "different beast," since loss of neuromuscular communication leads to dramatic alterations in muscle structure, function, and adaptive ability. In this exciting research area, you are beginning to understand the factors that regulate nerve muscle interaction. It is critical to make advances in this area to improve muscle function after traumatic nerve injury.

Evidence for Specificity of Reinnervation

The previous denervation-reinnervation discussion might lead one to the conclusion that reinnervation is completely random. However, this is far from the truth. Many studies have demonstrated that axons that grow out from a denervated stump are able to "navigate" to the correct distal stump and reinnervate the appropriate muscle. This was shown in a most dramatic fashion early last century by Cajál, who cut the motor nerve to a fast muscle and then constructed a Y-chamber to give this reinnervating nerve a choice of either the original fast muscle or a different slow muscle (Fig. 5-49). If reinnervation were completely random, an equal number of axons would have chosen each "branch" of the Y-chamber (Cajál, 1928). However, almost 80% of the reinnervating axons "chose" to innervate the correct distal stump, providing evidence that some type of signal permitted specific reinnervation of nerves. More recent experiments have implicated various chemicals such as growth factors and specific extracellular proteins as the chemical signals for providing navigational aid to incoming axons.

A more recent demonstration of reinnervation specificity was provided by Rende et al. who used the "double-labeling" technique to trace the connections between axons and motoneuron cell bodies in the spinal cord (Fig. 5-50) (Rende, Granato, Monaco, Zelano, & Toesca, 1991). The experimental model was the rat TA. Most of the motoneuron cell bodies to the TA are located at spinal cord level L3. Rende et al. used two separate tracer dyes to follow the connections between the TA and spinal cord motoneurons before and after denervation-reinnervation. They started by injecting the blue tracer known as "fast blue" into the TA muscle. Fast blue (FB) is taken up at the NMJ and moved via axonal transport into the motoneuron cell bodies (Fig. 5-50A). After 8 days (enough time for the axons to transport FB to the motoneuron cell bodies), they cut the sciatic nerve (which contains the motor nerves to the TA, other dorsiflexors and plantarflexors, as well as many sensory nerves) and placed both cut ends of the nerve in a small plastic tube, leaving a long 10-mm gap between ends (Figs. 5-50B and 5-50C). This configuration

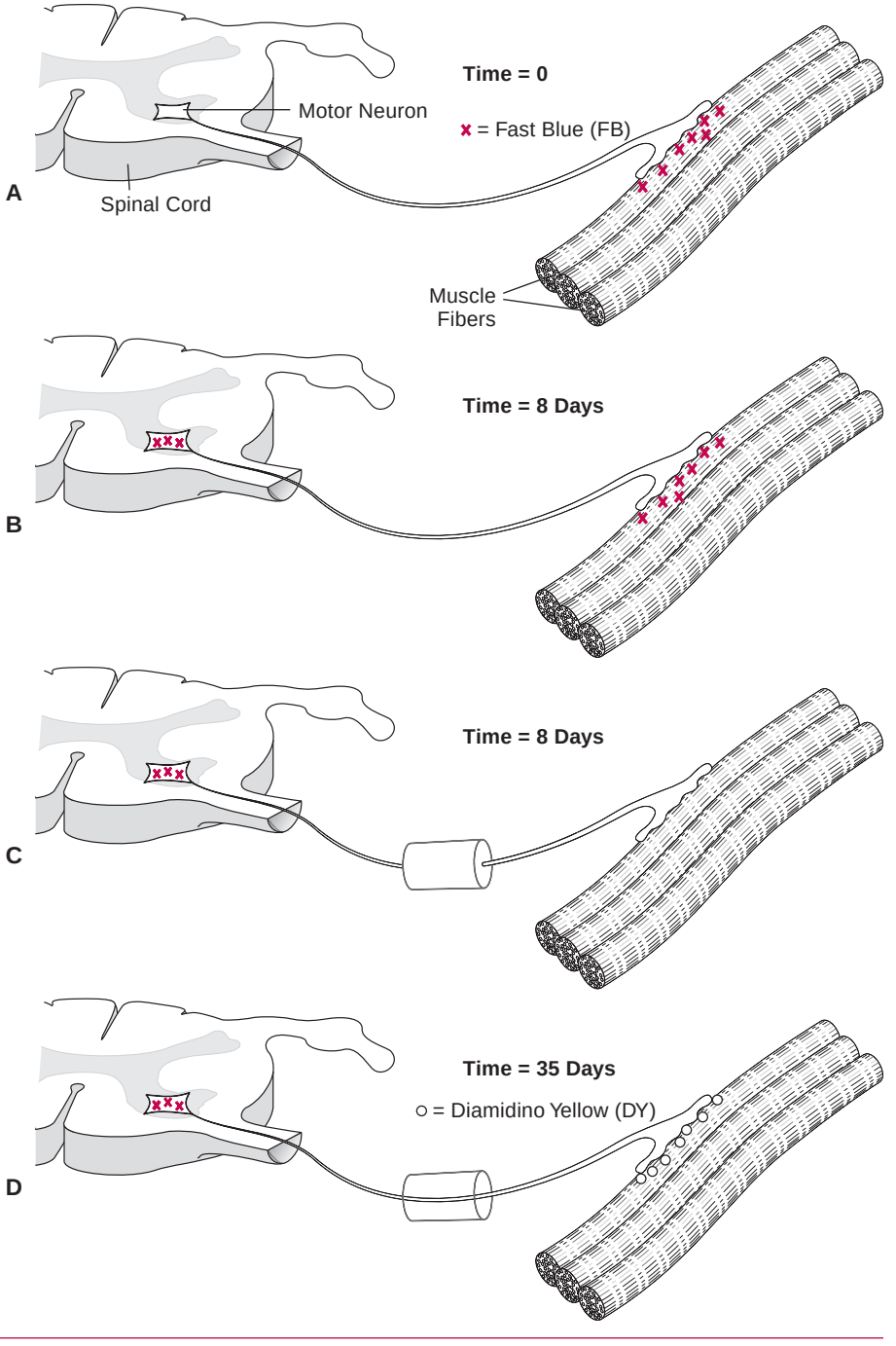

Figure 5-50 Schematic representation of the double tracer method used to identify specificity of connections between motoneurons in the spinal cord and peripheral muscle targets. **(A)** Original connections between nerves innervating tibialis anterior (TA) labeled using fast blue (FB). **(B)** After 8 days, FB is transported retrogradely to cell bodies in the spinal cord. **(C)** Nerve cut and allowed to grow back through a 10-mm gap in a plastic tube. **(D)** After TA reinnervation, connections between TA and spinal cord labeled using diamidine yellow (DY).

allowed the outgrowing axons to make a choice between several nerve fascicles—some correct, some incorrect. After 30–35 days (enough time for many outgrowing axons to arrive at the muscles), they reinjected the TA muscles with a different tracer known as "diamidino yellow dihydrochloride" or, more simply, DY (Fig. 5-50D). The DY acts similar to the FB in that it is transported back to the motoneurons where it can be observed microscopically. Before reading on, try to predict the appearance of motoneuron cell bodies, which were correctly innervated. Which tracers would be present? How would motoneurons appear that had originally projected to the TA but were not correctly innervated?

The results, shown schematically in Figure 5-51, provided convincing evidence that 30% of the reinnervating axons found the TA muscle. Motoneurons that showed both the FB and DY tracers were those that had originally been labeled with FB, and then subsequently labeled with DY after correct reinnervation. However, many other motoneurons at the L3 level showed only FB, which

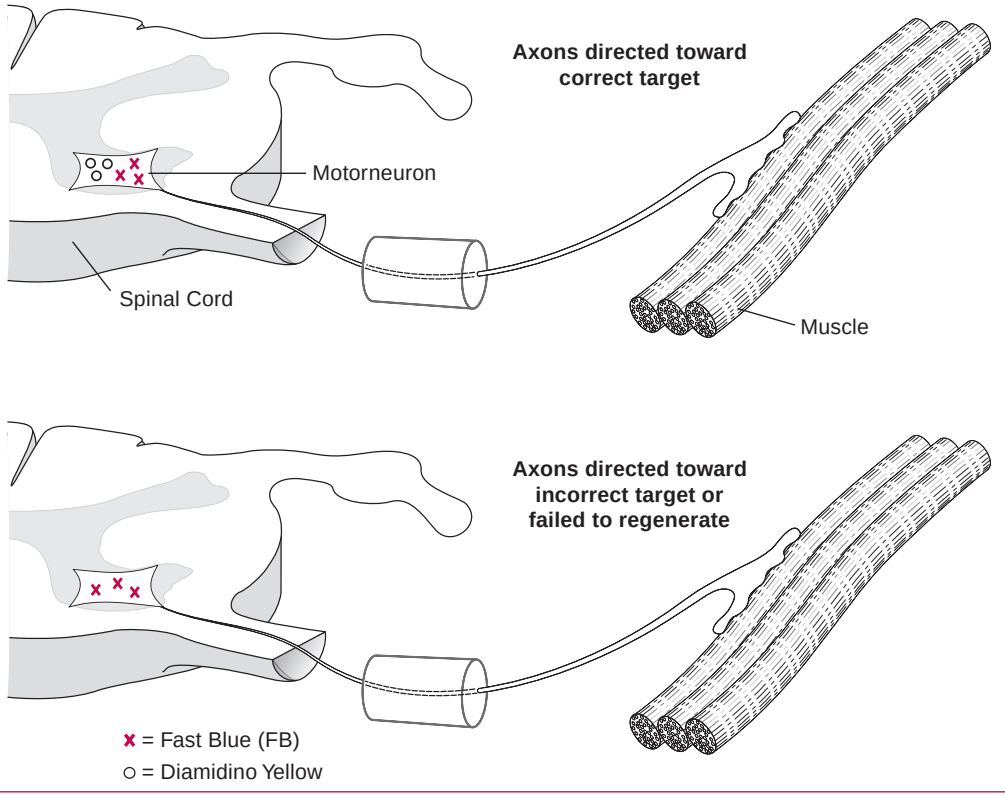

Figure 5-51 Schematic representation of innervation specificity following denervation of the rat sciatic nerve and permitting regrowth across a 10-mm gap within a plastic tube. Symbols refer to the tracers described in Figure 5-49. Note that if the reinnervating axon is directed toward one of the originally innervated fibers, both tracers are in the cell body. However, if axons are directed toward the wrong target (where no diamidine yellow was applied) or if the axon failed to regenerate, only fast blue is found in the cell body.

suggested that either they were originally labeled and failed to reinnervate anything or they were originally labeled and reinnervated the wrong muscle (Fig. 5-51). Current research in this area will use this type of model to investigate the signals (chemical, mechanical, hormonal) that control specificity of reinnervation. Ultimately, improved reinnervation specificity will improve functional recovery of patients following nerve injury. It looks like the main hope is not so much in expert microsurgical technique (note that there was 30% specific reinnervation when no surgery was performed), but rather in identifying the appropriate biological signals (Lundborg, 1988). In fact, one of the pioneers in this area, the Swedish hand surgeon Dr. Göran Lundborg has performed a long-term follow-up to his original study that compared the effects of direct microsurgical nerve repair to nerve repair in which the axons were allowed to "grow across" a small tube placed between the severed ends. Lundborg and colleagues had already shown that axons would grow across the gap, and there was evidence that the specificity was better in the presence of the tube (Lundborg, 1988). Much to their credit, they published an excellent report stating that the long-term functions of these patient groups were identical (Lundborg, 2000).

CHAPTER SUMMARY

Skeletal muscle response to decreased used is characterized by muscle fiber atrophy, decreased muscle force-generating capacity, and a slow-to-fast muscle fiber type conversion if the disuse is extreme enough. Generally, muscle oxidative capacity and endurance do not change. This essentially represents a reversal of the increased use response. Glycolytic response seems to match the fiber type: Increased percentage of fast fibers is accompanied by increased glycolytic capacity. The magnitude of the atrophic and adaptive response is related to the change in use experienced by the muscle. Thus, the often-used antigravity muscles atrophy to a greater extent than their less often–used antagonists. Regulation of muscle mass represents the balance between protein synthesis and degradation. In the hindlimb unloading model, muscle loss was initiated at the translational level. Pretranslational and posttranslational regulation have been observed in unloading and other models of decreased use. When the nerve to a muscle is cut, the muscle fiber becomes a qualitatively different entity. A reversal of some of the developmental processes, which serve as signals to nerves for reinnervation to occur, is observed. The reinnervation process itself

does not appear to be fiber type specific, although some signals are conveyed between the reinnervating stump and its target. At this point, muscle adaptation to the relatively "normal" perturbations has been discussed. However, in some cases the level of use is so intense or extreme that the muscle itself becomes injured or even killed. In still other cases, muscle malformation during development imparts specific properties to the muscle. In the next chapter, these muscle responses to exercise-induced injury, surgical trauma, and muscle disease will be considered.

CLINICAL PROBLEM

Continuation of the Patient Example in Chapter 2

In Chapter 2, we discussed a patient who had a combination of a tendon tear and, perhaps, a denervation injury to her supraspinatus muscles. On the basis of the information provided in this chapter, would you expect normal supraspinatus muscle function?

The answer is probably not. If the denervated muscle fibers become reinnervated, it is likely that the number of muscle fibers per motor neuron will increase, which impairs normal neurophysiological control of muscle force generation. The good news is that it may be possible for the muscle to adapt to its new environment. With appropriate exercise and recovery time, global shoulder function can improve to the point where local changes to the supraspinatus do not impair function. ■

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