

The biology of long-term denervated skeletal muscle

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Abstract

This review concentrates on the biology of long-term denervated muscle, especially as it relates to newer techniques for restoring functional mass. After denervation, muscle passes through three stages: 1) immediate loss of voluntary function and rapid loss of mass, 2) increasing atrophy and loss of sarcomeric organization, and 3) muscle fiber degeneration and replacement of muscle by fibrous connective tissue and fat. Parallel to the overall program of atrophy and degeneration is the proliferation and activation of satellite cells, and the appearance of neomyogenesis within the denervated muscle. Techniques such as functional electrical stimulation take advantage of this capability to restore functional mass to a denervated muscle.

Key Words: skeletal muscle, long-term denervation

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In the absence of innervation, skeletal muscle undergoes an inexorable course of atrophy, leading finally to a state in which the functioning muscle tissue is largely replaced by fibrous connective tissue and fat, along with a scattering of extremely attenuated muscle fibers.¹⁻³ These muscle fibers are almost unrecognizable at the light microscopic level and are largely devoid of the ability to contract.

As a denervated muscle proceeds toward terminal atrophy, it passes through several recognizable stages, but the duration of these stages varies depending upon the species. In rats and most laboratory animals, the stages are measured in months, whereas human muscle requires years to pass through similar stages. Three main stages have been described for the progressive decline of denervated muscles.^{1,4} The first stage, which begins immediately after nerve section, is characterized by immediate loss of function, followed by rapid weight loss and muscle fiber atrophy. The second stage is characterized by increasingly severe muscle atrophy, including the loss of most sarcomeric organization. This is followed by a long third phase in which interstitial fibrosis and the appearance of adipocytes dominates the tissue architecture. The number of muscle fibers is greatly diminished, and those remaining bear little resemblance to normal muscle fibers. The post-denervation atrophy of the rat extensor digitorum longus muscle has been divided into three phases, based upon its restorative ability.⁵⁻⁷ During the first phase, lasting two months, the

denervated muscle retains a capacity for restoration equal to that of a normal control muscle despite the fact that by the end of the first month the muscle has lost 90% of its original mass. The second phase (from 2-7 months), corresponding to the middle stage described by Lapolombella et al.⁴ is one in which the restorative ability progressively and dramatically declines. The terminal phase (beyond 7 months) is characterized by minimal restorative ability and deepening morphological atrophy along with tissue substitution.

In addition to their value in descriptive pathology, studies on the properties of long-term denervated muscle are clinically relevant because of the possibility of restoring denervated human muscle through either reinnervation or functional electrical stimulation. After a brief summary of short-term changes in denervated muscle, this review will concentrate principally on the biology of the later stages of postdenervation atrophy in skeletal muscle.

Short-term changes in denervated muscle

Following early descriptions by Gutmann and Zelená,³ Midrio⁸ has provided an excellent contemporary account of changes and mechanisms underlying the denervation atrophy of muscle. Overall, the principal tissue dynamic seen in short-term denervated muscle is the progressive atrophy and functional deterioration of the original muscle fibers along with an activation of

the capability of the denervated muscle for producing new muscle fibers.

Within days, atrophy of muscle fibers in rodents becomes measurable, but the nature of the atrophy depends upon the type of muscle fiber and the muscle itself. Slow muscles typically undergo gross atrophy more quickly than do fast muscles. In fast muscles of the rat, type II fibers undergo rapid atrophy, whereas type I fibers retain their cross-sectional areas for at least two months.⁹⁻¹²

At the individual muscle fiber level, the initial stages of atrophy are characterized by the preservation of sarcomeric structure, but as stage one merges into the second stage of atrophy, ultrastructural changes become increasingly prominent.^{8,9,13,14} These include a loss of myofibrillar alignment, as evidenced by disruption of registration at the Z-line, and the loss of individual actin and myosin filaments. Changes in size, number and orientation of components of the sarcoplasmic reticulum system reflect a structural basis for functional changes in the excitation-coupling system.^{15,16} Both numbers and the structural complexity of individual mitochondria are also progressively reduced.^{17,18} Data on the numbers of myonuclei per fiber during denervation atrophy vary greatly, ranging from reports of no change to a significant reduction.¹⁹⁻²⁴ It appears that preservation of myonuclei is more likely to occur in atrophying muscles in the mouse and in the rat diaphragm, whereas reductions have been more generally reported in limb muscles of the rat. Such variation has added complexity to discussions about the importance of nuclear domains in muscle fibers.²⁵

Muscle spindles persist after denervation.^{9,26} The rate of intrafusal fiber atrophy is less than that of extrafusal fibers, but differences between nuclear bag and chain fibers diminish as atrophy proceeds. Interestingly, the number of intrafusal fibers typically increases after denervation.

Within days after denervation in rodents, satellite cells become activated, and in fast limb muscles of rodents, their frequency trebles during the first two months.^{19, 27,28} This is a morphological reflection of a general activation of a myogenic program in denervated muscle. Molecular investigations show an early increase of myogenic regulatory factors during the early post-denervation period.²⁹⁻³²

Functional studies mirror the morphological changes outlined above. One of the earliest indications of denervation is the presence of spontaneous fibrillation activity.^{33,34} This is temporally correlated with the early post-denervation spread of acetylcholine receptors along the muscle fiber membrane^{35,36} and the general disruption of the sarcoplasmic reticulum.³⁷⁻³⁹

A dramatic reduction in tension produced by a stimulated denervated muscle occurs within days, with a relatively greater decrease seen in tetanic than in twitch tension. Changes in speeds of contraction vary

considerably, depending upon the species, the muscle and the circumstances of stimulation.⁸ These are based on a number of factors, including the organization and types of contractile proteins.

At the tissue level, one of the most prominent changes during the first stage of denervation is seen in the capillary bed. Although paradoxically whole-muscle blood flow increases during the very early post-denervation period⁴⁰ the weeks following denervation of rodent muscle see a substantial decrease in capillarity.⁴¹⁻⁴³ This is represented in both the capillary/muscle fiber ratio and total capillarity, and necrosis of capillaries has been demonstrated at both the light and electron microscopic levels.⁴²⁻⁴⁴ Fibrosis is minimal during the early post-denervation period, but within a week after denervation in mice significant numbers of bone marrow-derived cells, mainly macrophages, appear in both the postsynaptic and general interstitial regions.⁴⁵ Growth factors secreted by these cells stimulate local fibroblasts to produce type I collagen, which progressively accumulates with increasing denervation time.

Long-term changes in denervated muscle

The biology of long-term denervated muscle is dominated by several main themes. First is the continuing atrophy and actual degeneration of original muscle fibers. Second is the replacement of muscle fiber mass by fat and fibrous connective tissue. Third is the dramatic loss of capillaries associated with the muscle fibers and degenerative changes within intramuscular nerve trunks. Fourth is the activation of satellite cells and the appearance of newly forming muscle fibers. Detailed studies on these processes have been carried out largely on laboratory animals, but in recent years an increasing clinical literature has allowed meaningful comparisons between long-term denervated muscles in humans and laboratory animals. Although differences in the rate of atrophy between fast and slow muscles and similar muscles in different species have been well documented, Wu et al.⁴⁶ have recently reported a more rapid rate of atrophy in denervated muscles of the hand than of the arm of rats. Following the rapid phase of muscle fiber atrophy seen during the first month after denervation in the rat, later post-denervation periods consist of major disorganization of the sarcomeric apparatus in most muscle fibers, although cross-sectional areas of type I fibers continue to be more resistant to atrophy than are type II fibers.^{9,10,14} A functional indication of the disorganization of the contractile apparatus of denervated muscle fibers is the report of Squecco et al.⁴⁷ who found residual contractility in only 8% of muscle fibers isolated from 16-week denervated rat soleus and tibialis anterior muscles and 5 and 3% respectively from 44-week denervated muscles. These same authors noted that excitation-contraction coupling is minimally impaired before 13 weeks, but

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that significant changes in the T tubule system occur after four months. Considerably reduced energy metabolism is strongly related to the significant decline in mitochondrial number and complexity. Although frankly necrotic muscle fibers can be seen as early as two months post-denervation in the rat,^{10,48} such changes are much more prominent after the first year. In contrast, rabbit muscle, denervated up to year, exhibits atrophy but not necrosis of muscle fibers.⁴⁹

Myonuclear loss in the denervated rat EDL muscle proceeds at a rate of about one nucleus per day over the first seven months of denervation.¹⁹ Both apoptotic⁵⁰ and non-apoptotic mechanisms^{27,48} have been proposed. As the time of denervation and the severity of atrophy progresses, a significant distribution in the arrangement of the nuclei takes place. In rat muscle denervated for more than seven months, it is common to find severely atrophic fibers, the outlines of which resemble a chain of elongated beads. The bulging part of an individual bead-like compartment is anucleate, and at the attenuated neck between cytoplasmic beads is an aggregation of 5-15 nuclei.^{19,51} Neither the mechanism underlying the nuclear redistribution nor the functional significance of this arrangement is understood, but its appearance in both rat and human muscle suggests that it is not an uncommon phenomenon. In contrast to rat limb muscles, myonuclei do not appear to decrease in limb muscles of the mouse up to 4 months after denervation.²³

Another poorly understood phenomenon is the presence of large muscle fibers in very long-term denervated muscle. Sometimes seen in rats, it is particularly prominent in human muscle denervated for more than three years.⁵² Although these large muscle fibers have often been considered to be products of selective reinnervation, they do not typically possess ultrastructural features of reinnervated muscle fibers. Reinnervation is unlikely to account for many instances of the presence of such large myofibers.

One of the most characteristic features of very long-term denervated muscles, whether in laboratory animals or in humans, are massive fibrosis and the presence of fat cells within the interstitium.^{9,10,53,54}

Individual atrophic muscle fibers are typically embedded in dense mats of collagen fibers and are frequently not associated with capillaries. Adipocytes tend to occur in clumps with no obvious geometrical relationship to the atrophic muscle fibers. In the rat, the adipocyte presence is greater in red than in white muscles.⁵⁵

Capillary loss is one of the most prominent features of denervated muscle, with a 90% reduction in capillary/muscle fiber ratio by 18 months in rat fast muscle.⁴² The rate of loss is most rapid between two and four months of denervation. The rate and extent of capillary loss is greater around type II than type I muscle fibers, suggesting that this could be related to the more rapid atrophy of type II than type I fibers.

Mitochondrial numbers, especially in type I fibers, are dramatically reduced. The corresponding compromise in oxidative metabolism, along with the production of reactive oxygen species in denervated muscle,⁵⁶ may play a significant role in the overall atrophic process. One of the prominent features associated with capillary loss is the number of muscle fibers that are not associated with capillaries, but are rather surrounded by dense masses of collagen fibers. The source of these collagen fibers is not clear, especially in view of the low number of fibroblasts seen in atrophying muscle. The morphological evidence strongly suggests that many of the atrophying muscle fibers are situated in a highly hypoxic environment because of the linear distance between the muscle fibers and the nearest capillaries. A prominent feature of the diminution of the capillary network in a denervated muscle is the patchy distribution of vascular changes, with some areas of a muscle much more severely affected than others at a given time. The basis for this is not known.

A factor of considerable importance in denervated muscle is the condition of distal nerve channels within the muscle, because of their role in guiding regenerating nerve fibers to muscle fibers. The large literature on changes in distal trunks of transected nerves will not be reviewed here.⁵⁷⁻⁶¹ After an early period of activation, Schwann cells undergo progressive atrophy. Many of them disappear as the endoneurial spaces become filled with increasing deposits of collagen fibers. Nevertheless, some Schwann cells within small nerve branches can survive and produce some neurotrophic molecules for more than two years after denervation.^{57,62} A critical issue for reinnervation is whether a small population of competent Schwann cells can compensate for the mechanical obstruction of the nerve channels by collagen fibers and the attendant hypoxic environment resulting from capillary depletion.

Regeneration and myogenesis in long-term denervated muscle and during aging

Traditionally, denervated muscle has been thought to undergo an inexorable atrophic decline, leading to an essentially functionless remnant of severely atrophic muscle fibers embedded in a dense matrix of fibrous connective tissue and fat. Research over the past two decades, however, has shown a surprising amount of developmental potential and activity. The basis for this is activation of the satellite cell population. Within days after denervation the satellite cell population begins to increase until by two months in the rat the number of satellite cells per muscle fiber is approximately three times control values.¹⁹ From this peak, the satellite cell population undergoes a steady decline even into old age. The increase in satellite cell numbers is accompanied by morphological signs of satellite cell activation and patterns of gene activity characteristic of a myogenic response.^{4,28,31} However,

by the time denervation is well advanced (e.g. >2 years), satellite cell numbers fall well below control levels, even when advanced age is taken into consideration.^{19,63} Nevertheless, despite the reduced population of satellite cells, both MyoD and myogenin mRNA and proteins are expressed at elevated levels.⁶³ In denervated human laryngeal muscle, Donghui et al.⁶⁴ found upregulation of MyoD and myogenin up to two years post-denervation, but after that time levels were no higher than those in control muscle tissue.

A tangible result of the satellite cell activity in long-term denervated muscle is the formation of new muscle fibers among the atrophying original muscle fibers.^{51,53,64} In non-stimulated muscle, these muscle fibers remain very small and undergo poor differentiation.⁶⁵ In addition, these regenerated muscle fibers are not associated with satellite cells in the rat.⁶³ Available evidence suggests that in long-term denervated muscle there are repeated cycles of neomyogenesis and subsequent atrophy of these newly formed muscle fibers.

Several laboratories have demonstrated a substantial regenerative capacity in denervated muscle after some form of injury, whether induced by myotoxic agents or ischemia.⁶⁶⁻⁶⁸ Using a grafting model, Billington and Carlson⁶⁹ found improved regeneration if the grafted muscles were treated with bupivacaine as compared with untreated grafted muscles.

The formation of new muscle fibers in long-term denervated muscles is paralleled by the formation of new muscle fibers in control muscles of extremely old rats.^{70,71} This could be a reflection of the greatly diminished innervation in the muscles of very old rats. Nevertheless, the reactions of satellite cells in 24-month-old rats to denervation is very similar to that seen in young animals,⁷² suggesting that aging per se does not repress the capacity of satellite cells to become activated after denervation.

The restoration of long-term denervated muscle in humans

Two fundamental strategies have been employed to restore function to long-term denervated muscle. The classical surgical technique has been to facilitate reinnervation through nerve repair.^{73,74} Although successful for the repair of short-term denervated muscle if the distance required for axonal regeneration is not too great, the recovery of contractile function is typically greatly diminished if regenerating motor axons do not contact denervated muscle fibers within 12-18 months. Because of this limitation, considerable effort has been expended in devising means of restoring long-term denervated human muscle by functional electrical stimulation (FES).

Techniques of FES in humans have now developed to the point where substantial maintenance and quantifiable improvement of muscle fiber structure and function can be produced if a treatment regimen is

instituted within two years.^{51,53,75,76} The reduction of effectiveness of this technique after two years of denervation corresponds to the substantially poorer recovery of rat muscle after 4-7 months of denervation. These post-denervation times are roughly parallel in terms of the cellular and tissue architecture of the two species. Human muscle denervated for shorter periods still maintains a metabolic profile that is more compatible with restoration.⁷⁷ Yet, the exact nature of the factor(s) inhibiting full restoration remains incompletely understood.

Summary

Over the previous two decades our understanding of the biology of long-term denervated muscle has undergone a dramatic change. Before that time, denervated muscle was generally considered to progress inexorably down a long course of atrophy and degeneration, with replacement of functioning muscle mass by dense sheets of connective tissue and fat. It is now apparent that denervated muscle exhibits another dimension, namely a system that has both the capability and actuality of participating in active myogenesis. Left alone, this developmental capability is of little practical use, but when regeneration is stimulated, either by myotoxic agents or through FES, a remarkable degree of functional restoration can be attained. Nevertheless, there are presently limits on the capacity of denervated muscle for functional return. In human muscle, at roughly two years - the boundary between a principally atrophic response to denervation and the phase of degeneration - the success of restorative programs becomes sharply diminished. This corresponds to the period of from 4-7 months post-denervation in the rat. The underlying basis for this reduction in restorative capacity remains to be elucidated.

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