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Electrical stimulation of denervated muscle: is it worthwhile?

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ABSTRACT

EBERSTEIN, A. and S. EBERSTEIN. Electrical stimulation of denervated muscle: is it worthwhile? *Med. Sci. Sports Exerc.*, Vol. 28, No. 12, pp. 1463–1469, 1996. Research conducted over the past 25 years has demonstrated that muscle activity, not neurotrophic substances, is the most important factor in the regulation of specific physiological and biochemical properties of muscle fibers. Application of this knowledge has led to considerable experimentation with chronic electrical stimulation as a possible clinical tool for the treatment of denervated muscles. Evidence accumulated from animal studies has indicated that direct electrical stimulation of denervated muscles can to a large extent substitute for innervation and preserve or restore the normal properties of the muscles. Appropriate stimulation parameters were critical for a successful intervention, and the best results were obtained when the stimulation pattern resembled the firing pattern of the normal motoneuron. Thus, fast muscles required intermittent, brief, high frequency stimulation and slow muscles needed continuous, low frequency stimulation. For human denervated muscles, critical questions still remain to be resolved before electrical stimulation will yield the optimum benefit. Research must be performed in human subjects to define the appropriate stimulation parameters, the stimulation current, and the type and placement of electrodes.

HUMAN MUSCLE, MUSCLE PLASTICITY, MUSCLE REINNERVATION, MUSCLE ATROPHY

Treatment of denervated muscle with electrical stimulation is controversial. Recent reviews conclude that many questions remain to be answered and further research is necessary (50,60,69). The lack of consensus after close to a century of experimentation is probably because denervated muscle is more complex than originally thought, and the variety of stimulus characteristics, training regimens, and species used in studies over the years have made comparisons very difficult. For many and obvious reasons most of the research has been performed with animals.

This review will be limited largely to studies performed during the past 25 years and will discuss findings from both animal and human experiments as they pertain to electrical stimulation of denervated muscle. In this paper denervated muscle refers to skeletal muscle that is anatomically isolated from motor nerve fibers. Two excellent reviews have been published recently regarding the effect of electrical stimulation on healthy muscles (26,41).

We will focus on two questions that are crucial in determining the usefulness of electrical stimulation of denervated muscles. The first one concerns the value of electrotherapy in preventing or reducing muscle atrophy and the second concerns the claim that stimulation will prevent reinnervation. We will begin the review by describing the time course of denervation.

CHARACTERISTICS OF DENERVATED MUSCLE

Muscle atrophy. The biochemical, morphological, and physiological changes that occur in muscle fibers following denervation have been well described (1,74). Muscle atrophy is the most striking change. The rate of atrophy varies considerably among species, among individuals of the same species, among muscles in the same individual, and even among fibers in the same muscle. For example, rat limb muscle weight may be reduced by 50% after 2 wk of denervation, whereas human muscle may show only a small loss of weight and imperceptible reduction in fiber size after 2 wk (1,56).

In human muscle, most fibers are reduced in diameter by 50% or more after 2–3 months of denervation (1,2). Occasional fiber fragmentation may be seen at this stage (2). After 4 months the atrophic process slows and remains relatively stable. The muscle striations are still intact. The number of myofibrils progressively decrease and the surviving ones are atrophic with a relative increase in sarcolemmal nuclei (76). The striations begin to fade near the end of the first year (11). The muscle fibers will fragment and disintegrate after about 2 yr and eventually will be replaced by fat cells (1,11).

The rate of atrophy can vary considerably in different muscles in the same species. In the rat the soleus muscle fibers atrophy earlier and to a greater extent than those of the extensor digitorum longus muscle (EDL) (5,82), and the diaphragm may even hypertrophy (71). During the later periods of chronic denervation, mixed rat muscles are transformed into almost pure fast muscles (14). At-

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rophy of the gastrocnemius muscle is slower than the tibialis anterior muscle in denervated dog muscles (18). This variation in the rate of atrophy has been attributed to differences in the amount of passive stretch imposed on the muscles (18,23,24). Thus, contractile activity is not the only mechanical determinant of muscle bulk and force, but muscle length is also important. In addition, other factors may contribute to the atrophic process, such as endogenous hormones (32,33,40) and blood flow (44).

Excitability. A decrease in the resting membrane potential is the earliest sign of muscle denervation (6,78). Denervation also causes a dramatic and largely uniform increase in the sensitivity of the extrajunctional membrane to acetylcholine (47). In rat soleus muscle the increase occurs between the second and third day after nerve section (43), while in human muscle the increase occurs after about 5–10 d (74).

After 10–21 d of human muscle denervation, spontaneous contractions appear in individual fibers, each accompanied by a fibrillation potential. Fibrillation potentials will persist as long as any muscle fiber remains, but they are difficult to detect after 1 yr (7). Thesleff (77) noted that fibrillations and an increase of acetylcholine receptors are adaptive changes of the denervated muscle that maintain the muscle fiber until reinnervation.

Following nerve section and after the last fragments of the motor axon and the intramuscular nerve terminals disappear, the excitability of the muscle fibers decreases. As muscle excitability falls, the stimulus required to elicit a muscle fiber contraction must be increased in amplitude and duration (74). For completely denervated human muscle, the minimum duration of the stimulation current required to produce a contraction is approximately 1 ms (74). The decline in excitability progresses very slowly so that a strong stimulus can still produce a response as long as some contractile tissue is present up to 1–3 yr after denervation (1).

ELECTRICAL STIMULATION OF DENERVATED MUSCLE

Plasticity of denervated muscles. Many studies (3,15,16,25,42,44,46,51,55,62) have demonstrated that the metabolic and contractile properties of denervated muscles can be influenced by their pattern of activity. Salmons and Vrbová (63) prompted these studies by showing that the key factor in determining the contractile properties of striated muscles was the activity pattern transmitted to the muscles. The studies were a major shift from previous research because stimulation patterns which resembled the normal activity of the motor neurons were now applied to denervated muscles.

The paradigm for these experiments involved chronic direct stimulation at low frequency. Almost all research was performed in animals, with the majority carried out on the soleus muscle of the rat. Early experiments demonstrated that properties of denervated soleus muscle could be altered by the stimulation pattern imposed on the muscle. For example, denervated rat soleus muscles stimulated at 10 Hz for 10 s every 50 s maintained their slow contraction times, whereas soleus muscle stimulated at 100 Hz for 0.5 s every 25 s became fast contracting (46,48). The alterations in properties were attributed to the increase in stimulation frequency.

Other investigators (3,40) argue against this conclusion. Al-Amood and Lewis (4) stimulated denervated rat soleus muscles with short bursts of impulses at frequencies ranging from 10 to 100 Hz and obtained the same contraction times regardless of frequency used. However, 10-Hz stimulation given continuously did not change the slow properties of the soleus. They concluded that the time allowed between muscle contractions rather than the pulse frequency during contractions was crucial in determining the effect on contractile properties. Later Westgaard and Lømo (81) attributed the increase in twitch speed by the denervated soleus muscle to high frequency stimulation but agreed that this conclusion is not unequivocal because changes in pulse frequency will give rise to changes in train duration and intervals between trains.

Similarly, direct stimulation can transform a fast muscle to a slow muscle (15,16,20,62), but the conversion may be incomplete. Low direct stimulation (10 Hz, 24 $h \cdot d^{-1}$) of denervated fast muscle for several weeks in rat and sheep increased the amount of slow myosin (15,16); low-frequency stimulation (10 Hz, 12 $h \cdot d^{-1}$ for 21–56 d) of denervated rabbit tibialis anterior led to an increase in aerobic-oxidative enzyme metabolism, a decrease in glycolytic enzyme activities, and a shift in LDH isozyme pattern toward that of a slow muscle (62). In the rat conversion of muscles may not be as complete in fast muscles as in slow muscles, whereas in the rabbit there may be a more complete fast-to-slow transformation. Therefore, differences apparently exist not only between different muscles in the same animal but also between corresponding muscles in different species (20,81).

These findings suggest that denervated muscles have properties of plasticity and can be influenced by electrical stimulation. The effect produced by stimulation, however, can be modulated by other factors such as muscle load (24), hormones (33,40), or blood flow (44). A consequence of these other factors is that denervation atrophy may not be prevented completely by electrical stimulation (25,81).

Prevention of muscle weight loss and atrophy. The evidence that electrical stimulation can influence denervated muscle properties generated renewed interest in the use of electrical stimulation to prevent or reverse the changes produced by denervation, such as muscle atrophy (46,51,54), weight loss (1,56) and loss of force (20,29). In recent years investigators (4,10,15,36,50,54,55,79,81) have been experimenting with various stimulation protocols to determine the efficacy of electrical stimulation and its ultimate relevance in clinical practice.

Some investigators (50,55) have designed experiments with clinical application in mind and evaluated only short-term stimulation procedures. Nix and Dahm (55) measured the effect of short-term stimulation with two different stimulation patterns in denervated fast muscle of the rabbit. One group of rabbits was stimulated isometrically with 7-ms duration pulses at 1 Hz for 20 min. A second group was stimulated for 20 min at 40 Hz for 100 $ms \cdot s^{-1}$ with 1-ms pulses. Stimulation current was adjusted in both groups to induce maximum response. Implanted electrodes were used in all animals. The muscles stimulated at 1 Hz showed statistically significant less atrophy (10%) and about 25% more force than nonstimulated controls, whereas the 40-Hz stimulation induced severe fibrosis. The short-term low frequency stimulation thus had an atrophy retarding effect. The authors attributed the positive results to stimulating with implanted electrodes and were able to impact all the muscle fibers. They surmised that stimulating denervated human muscles with surface electrodes would be of little value.

In a more recent study Nix (54) stimulated a denervated fast muscle of the rabbit for 4 wk with square-wave pulses at a rate (100 Hz) mimicking its motoneuron firing pattern and found that the stimulation had no effect on denervation atrophy or force output. However, Mokrusch et al. (50) obtained more positive results in rabbit fast muscle by using a special stimulation current. They used biphasic rectangular impulses of 20-ms duration (phase changing every 10 ms) at a frequency of 25 Hz. With stimulation by surface electrodes for up to 205 d at 6 min twice daily, the fiber diameters were preserved at 72–86% of normal while the nonstimulated fibers were 29–49% of normal diameter.

Mokrusch et al. (50) showed that strong tetanic contractions could be obtained with surface electrodes if long duration (20-ms) biphasic rectangular impulses are used. In their opinion, the long duration impulses compensated for the short daily stimulation sessions and were the determining factor in reducing atrophy. Results of other studies are consistent with this view (15,31,36). Carraro et al. (15) found in sheep a lack of atrophy of the slow fibers in denervated cricoarytenoid posterior muscle stimulated with 30-ms duration exponential pulses. Herbison et al. (31) reported that after 1 month of stimulation of denervated rat muscle with 0.2 ms of direct current square wave pulses at 20 Hz the treated gastrocnemius muscles were not significantly different than the untreated, whereas 100-ms pulses at 2 Hz produced a significant increase of treated muscle weight of 10% compared to untreated denervated muscle. But stimulation with 25-ms pulses at 20 Hz caused the most significant change: the weight of treated muscle exceeded untreated muscle weight by 10–33%.

Recent experiments involving human denervated muscle have been sparse and with mixed results. Nine patients with complete denervation of the tibialis anterior muscles had their muscles stimulated 5 $d \cdot wk^{-1}$ with 20-ms square-wave pulses at 25 Hz for 20 min, two times a day (79). After 3 wk, the level of foot dorsiflexion obtained with stimulation of the tibialis anterior muscle increased. The authors suggest that the stimulation increased muscle strength and reversed the course of atrophy.

Boonstra et al. (10) measured the effect of low frequency (1 Hz) square-wave electrical stimulation on muscle atrophy and recovery of function in patients with peripheral nerve lesions. The study included patients with totally denervated muscles that were not reinnervated. Muscles were stimulated supermaximally, causing a maximum twitch contraction. The cross-sectional areas of denervated stimulated and denervated nonstimulated muscles were obtained from computer tomographic and ultrasonographic images and compared. No difference was found between the patients treated with stimulation and those not treated. The authors speculated that this was a result of the use of twitch instead of tetanic contractions and the possibility that all the fibers of the denervated muscle were not stimulated.

In a study involving six patients, atrophy was assessed by means of monthly volumetric measurements (57). A cylindrical tank filled with water was used for volume measurements of the forearm by water displacement. Denervated muscles were electrically stimulated five times a week, once per day, with 60-Hz sine current. Stimulus intensity was set to secure a vigorous muscular contraction, and the treatment period varied from 5 months to a maximum of about 18 months. Atrophy was retarded during the period of stimulation, but any atrophy existing before the start of treatment was not reversed.

Stimulation parameters and treatment protocol. Not all stimulation patterns are equally effective in restoring normal properties to the denervated muscle. For example, Herbison et al. (31) demonstrated in the rat that stimulating with 25-ms duration direct square wave current at 20 Hz was better than 100-ms pulses at 2 Hz or 0.2-ms pulses at 20 Hz. Similarly, Eken and Gundersen (20) experimented with four different stimulation patterns in denervated rat fast and slow muscles and found that nearly normal contractile properties could be maintained in the denervated muscles only if the applied activity resembled normal motor unit activity. Other stimulation patterns induced only limited transformations toward normal muscle action.

The efficacy of stimulation depends on the stimulus parameters and the pattern of stimulation. The parameters include the stimulus amplitude, stimulus duration, pulse frequency (which is the reciprocal of pulse duration), the duration of the train of pulses, and the interval between trains. Stimulus duration is the time from the start of the stimulus to the end of that stimulus; pulse duration is the time between the start of the stimulus to the start of the next stimulus. Stimuli delivered at a given frequency and train duration over a fixed period of time is referred to as the pattern of stimulation. A treatment protocol of electrical stimulation must specify the pattern and each stimulus parameter.

Unfortunately, researchers disagree as to the most effective stimulation parameters required to reverse the deterioration process. Some investigators (41,77) state that the frequency of stimulation is the most important factor. Others (38,39,70) argue that the total amount of activity is more important than the frequency, while others (20,45) conclude that all the parameters are equally important. The pulse duration (50) and the time allowed between contractions (3,4) have also been proposed as dominant factors. The time allowed between contractions was considered important because the extent of the transformation of denervated slow soleus muscle to fast muscle was independent of the frequency of stimulation. Only 10-Hz stimulation given continuously induced the muscle to remain slowly contracting (4).

Since the mechanism by which stimulation affects the course of denervation atrophy is unknown, the choice of stimulation parameters and patterns has been by trial and error. Fortunately, successful interventions (20,25,38,39,43,45,47,57,70,81) have demonstrated that nearly normal properties of denervated muscles can be preserved with stimulation. Conflicting recommendations as to the most effective parameters and patterns of stimulation illustrate the need for more research.

A controversy has also arisen regarding the type and placement of stimulating electrodes. To be effective electrical stimulation must stimulate all the muscle fibers in a denervated muscle (29,55,81). This can only be accomplished, as Nix and Dahm (55) stated, by using implanted electrodes, not surface electrodes. This problem apparently was overcome by using surface electrodes with stimuli of long stimulus duration (20 ms) (50). In these animal experiments substantial, but not complete, reversal of denervation atrophy was observed. It thus appears that surface electrodes may be used but only with appropriate stimulation parameters. Further research is required to determine these parameters, especially in human subjects where surface electrodes are predominately used.

Impact on reinnervation. Following severance of a nerve, both the proximal and distal sections undergo a series of characteristic changes. The essential features of the changes for the distal portion are loss of excitability of the axon, degeneration of the axon terminals with Schwann cells interposed between the terminals and postsynaptic regions, degeneration and removal of the axon and its myelin sheath (Wallerian degeneration), and proliferation of Schwann cells along the edge of the endoneurial tube. At the proximal end, the injured axon is first sealed off at the lesion site, and the axon tip swells due to accumulation of axonal transport material. Growth cones, which form at the axonal tips and send out fine extensions or sprouts, play a role in pathfinding and target recognition (66). Elongation of the axon proceeds by forward movement of the sprouts. If contact is made with a denervated muscle fiber, synaptogenesis and recovery of muscle function ensues.

The rate of regeneration of peripheral nerves decreases with distance from the motor cell bodies (72,74,75) and also with increasing age (9,58). In man a nerve repaired by suture of the cut ends will regenerate at a rate of 2.5 $mm \cdot d^{-1}$ in the upper forearm, 2 $mm \cdot d^{-1}$ in the lower forearm, and 1 $mm \cdot d^{-1}$ in the wrist and hand. Similarly, the rate is 2 $mm \cdot d^{-1}$ in the upper leg below the knee, 1.5 $mm \cdot d^{-1}$ in the lower leg, and 1 $mm \cdot d^{-1}$ in the ankle (72,74,75).

Several lines of evidence (27,37,68,83) have shown that the denervated muscle fibers produce a nerve growth stimulus or trophic factor that can elicit outgrowths from nearby nerve terminals. The effective diffusion range of the growth stimulus is very limited and the axons differentiate into nerve terminals only when they are 0.01 μm or less from the muscle (8,61,67). It has been suggested that the stimulus originates from the altered surface properties of the denervated muscle fibers (13,59). If this is so, then the question arises as to whether restoration of normal properties to denervated muscle by stimulation will suppress the release of the growth stimulus and prevent reinnervation.

Studies (12,28,34,65) show that terminal sprouting can be inhibited and reinnervation prevented by stimulation of denervated muscle. It is noteworthy that in these experiments reinnervation was not completely abolished by stimulation, but only reduced. For example, in a recent well designed study in rats (28), long-term muscle stimulation significantly suppressed reinnervation, i.e., stimulation prevented reinnervation in 5–20% of the muscle fibers. The cause for nonreinnervation of some fibers was not known. The author (28) conjectures that stimulation suppressed the release of the muscle-derived trophic factor or suppressed the receptiveness of the denervated endplate to reinnervation.

Reports (17,19,22,30,35,52,53,79) of successful reinnervation of stimulated denervated muscles argue against the possibility that stimulation restrains reinnervation. That reinnervation occurs in spite of stimulation may indicate the shortcomings of the treatment. Although direct stimulation restores resting membrane potential, acetylcholine sensitivity, contractile speed, and specific membrane properties to normal values (29,43,47,80), denervation atrophy is not restored completely (29,81). This may be a result of the lack of a neurotrophic factor, damage of muscle fibers by the stimulus, or the fact that all fibers are not stimulated. Whatever the reason, the effect may be that the muscle-derived growth stimulus is still produced and axon regeneration proceeds normally.

The proper starting time. Those who advocate electrical stimulation generally agree that the procedure should be used only if there is a likelihood of muscle reinnervation (76). Likewise, a denervated muscle that will be reinnervated within a short period of time need not be treated since the influence of the nerve when reconnected to the muscle will reverse the degenerative changes (55).

A problem arises with denervated muscles that require many months of nerve regeneration before innervation is reestablished. With the rate of axon regeneration varying from 2.5 to 1 mm·d^{-1} in humans (72,74,75), muscles that are approximately 50 cm or more from the lesion site may be denervated for 1 yr or more. An example of this would be an injury of the ulnar nerve at the brachial plexus which could leave the intrinsic muscles of the hand denervated for 1 yr or longer. Although nerve transection will cause changes to both nerve and muscle, the nerve does not limit functional recovery, but the muscle rapidly loses the ability to become reinnervated (21).

If reinnervation occurs before 1 yr, good muscle function can be restored (73,76); after 1 yr function can at best be poor (49). If reinnervation is delayed for 18–24 months, irreversible changes in the muscle cells develop with no hope for return of motor function (49). Thus, any remedial activity designed to preserve the muscles must be instituted well before 1 yr has elapsed.

The optimum time to start muscle stimulation is soon after the onset of denervation (11,29,81). Direct stimulation started about 1 d after denervation prevented atrophy almost entirely in rat slow and fast muscles (29,45,81). But stimulation started some time after nerve section can also be beneficial. Mokrusch et al. (50) began stimulation after 28 d of denervation and fiber diameter was preserved after 110–117 d of stimulation at a level of 72–86% of the initial values. Stimulation started after 64 d of denervation increased the tetanic tension 37 times for the rat soleus and 8 times for the EDL above the nonstimulated muscles for up to 5 months of denervation (29). Similar results were obtained for soleus and EDL muscles denervated for a much longer time (between 4 and 10 months) and stimulated during the last 3–8 wk of denervation (5,64). Chronic stimulation increased force by 20–55 times in the soleus and 7 times in the EDL with the final tension reaching 4–5% of normal in both muscles. Unfortunately, recovery of denervated muscle declines as the interval between onset of denervation and start of stimulation increases.

SUMMARY

Research conducted over the past 25 years has clearly demonstrated that muscle activity and not neurotrophic factors is essential for the regulation of muscle fiber properties. Thus, electrical stimulation can be considered a useful procedure for preserving or restoring the normal properties of denervated muscles. However, stimulation can only be beneficial if an appropriate stimulation pattern is used. Numerous studies have shown that the best results are obtained if the evoked muscle activity resembles the stimulation pattern of the normal motoneuron.

Stimulation should be initiated well before 1 yr of denervation has elapsed. The optimum time to start stimulation is as soon as possible after nerve injury. Treatment started months after nerve injury may still reverse the degenerative process to some extent, but the longer the interval between onset of denervation and start of stimulation the less the recovery. Stimulation of the denervated muscle will not effectively hinder axon regeneration or reinnervation of the muscle.

All of the findings and recommendations with respect to stimulation of denervated muscles were derived from animal experimentation. Many of the findings are also valid for human denervated muscles. However, critical questions still remain to be resolved before chronic electrical stimulation will yield the optimum benefit in clinical operations. Research must still be performed in human subjects to define the appropriate stimulation parameters, as well as the stimulation current and the type and placement of electrodes. Then the most efficient stimulation protocols can be designed and applied to human denervated muscles.

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