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Postfatigue potentiation of the paralyzed soleus muscle: evidence for adaptation with long-term electrical stimulation training

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Abstract

Understanding the torque output behavior of paralyzed muscle has important implications for the use of functional neuromuscular electrical stimulation systems. Postfatigue potentiation is an augmentation of peak muscle torque during repetitive activation after a fatigue protocol. The purposes of this study were 1) to quantify postfatigue potentiation in the acutely and chronically paralyzed soleus and 2) to determine the effect of long-term soleus electrical stimulation training on the potentiation characteristics of recently paralyzed soleus muscle. Five subjects with chronic paralysis (>2 yr) demonstrated significant postfatigue potentiation during a repetitive soleus activation protocol that induced low-frequency fatigue. Ten subjects with acute paralysis (<6 mo) demonstrated no torque potentiation in response to repetitive stimulation. Seven of these acute subjects completed 2 yr of home-based isometric soleus electrical stimulation training of one limb (compliance = 83%; 8,300 contractions/wk). With the early implementation of electrically stimulated training, potentiation characteristics of trained soleus muscles were preserved as in the acute postinjury state. In contrast, untrained limbs showed marked postfatigue potentiation at 2 yr after spinal cord injury (SCI). A single acute SCI subject who was followed longitudinally developed potentiation characteristics very similar to the untrained limbs of the training subjects. The results of the present investigation support that postfatigue potentiation is a characteristic of fast-fatigable muscle and can be prevented by timely neuromuscular electrical stimulation training. Potentiation is an important consideration in the design of functional electrical stimulation control systems for people with SCI.

Keywords

plasticity; low-frequency fatigue; excitation-contraction coupling; spinal cord injury

PRESERVATION OF THE HEALTH, function, and integrity of paralyzed extremities is a critical complement to the development of a cure for spinal cord injury (SCI). An important area of inquiry is the use of electrical stimulation to preserve the force-generating capabilities of paralyzed muscle and the integrity of the skeletal system (8, 39, 40). Although a cure for SCI may be imminent within the life spans of people injured today, the challenge will be to understand the appropriate dose of physiological load necessary to preserve the musculoskeletal system during a long-term regenerative process.

The soleus muscle, by virtue of its normally homogenous fiber type and ease of whole nerve activation, is an ideal model to advance our knowledge of the physiological factors that influence paralyzed muscle force. The acutely paralyzed soleus muscle is fatigue resistant and can maintain nearly 80% of its original force output after a bout of fatiguing contractions. On the other hand, chronically paralyzed soleus muscle (>2yr postinjury) generates as little as 25% of its original force after a fatiguing bout (34, 36, 37, 41). These changes in fatigability are believed to reflect, in part, the gradual transformation from a primarily slow muscle with ~80% type I fibers (15) to a more fatigable fast muscle with mainly type IIb fibers (10, 19). This conversion occurs gradually over the first several years after an individual's SCI.

Repetitive stimulation of paralyzed muscle induces a long-lasting form of fatigue [low-frequency fatigue (LFF)] in which peak forces decline during activation but recover with high-frequency stimulation (7, 14, 36). Force depression during LFF is believed to be caused by impaired Ca^{2+} release from the sarcoplasmic reticulum (SR) (50). A competing process is potentiation, in which repetitive stimulation of a fatigued muscle yields augmentation of peak force (26, 27). Potentiation may occur after repetitive single-pulse activation (28) ("staircase effect") or after tetanic stimulation [posttetanic potentiation (PTP)] (22). The mechanism underlying both forms of potentiation is believed to be phosphorylation of myosin regulatory light chains (RLCs) (21, 45–47), which enhances Ca^{2+} sensitivity of the actin-myosin complex during subsequent twitches. After periods of disuse, alternative mechanisms have also been implicated during the staircase effect (27).

It is unknown whether postfatigue potentiation in paralyzed human muscle varies according to time postinjury. Significant torque instabilities induced by potentiation would compromise the control needed during FES.

Many studies have shown that potentiation occurs most readily in muscles that consist of primarily fast-twitch fibers (11, 21), such as would be expected in chronically paralyzed muscle (35). Our laboratory has previously noted the existence of postfatigue potentiation in chronically paralyzed muscle (36) but has not documented its prevalence or magnitude. Similarly, the degree of potentiation present in acutely paralyzed muscle is currently unknown. It seems plausible that postfatigue potentiation may be absent in the acutely paralyzed soleus muscle, which functions as a composite of slow fibers (34). We recently reported the effects of early long-term electrical stimulation training on bone density and soleus torque, speed properties, and fatigue index in individuals with SCI (39). However, to our knowledge, no previous report has examined long-term training on postfatigue potentiation in paralyzed muscle.

The first purpose of this study was to quantify postfatigue potentiation in the acutely and chronically paralyzed soleus. Second, we wished to determine the effect of long-term soleus electrical stimulation training on the potentiation characteristics of recently paralyzed soleus muscle. We hypothesized that postfatigue potentiation would be present in chronically paralyzed muscle but not in recently paralyzed muscle and that training would preserve the early postinjury potentiation characteristics of paralyzed soleus muscle.

METHODS

Subjects

Fifteen men with complete SCI (American Spinal Injury Association class A) (1) participated in this study (Table 1). The University of Iowa Human Subjects Institutional Review Board approved the protocol. All subjects provided written, informed consent before participating. Inclusion criteria were complete SCI above T₁₂ as determined by neurological

examination, passive ankle dorsiflexion to neutral, passive knee flexion to at least 90° in a seated position, and intact skin over the electrical stimulation site. Exclusion criteria were lower motoneuron injury below T₁₂ (which would prevent tibial nerve electrical activation), lower extremity trauma, pressure ulcers, or peripheral and/or systemic infection. All subjects had some spasticity, consistent with an upper motoneuron lesion. None of the subjects had severe spasticity that interfered with the measurement of muscle mechanical properties during electrical stimulation.

In the first arm of the study, five men with chronic SCI [mean (SE) 8.97 (1.85) yr postinjury] underwent a single test session to determine the magnitude of soleus postfatigue potentiation. In the second arm of the study, 10 additional subjects enrolled within the first 6 mo after SCI. Five of these subjects underwent a single testing session during their initial hospitalization (before 6 wk post-SCI). The remaining five subjects were tested immediately after they returned to the local area after inpatient rehabilitation. After initial testing, 7 of the 10 acute SCI subjects elected to enroll in the electrical stimulation training protocol. This subset of subjects appears in a previous report that describes the effects of this training protocol on torque, fatigability, and bone mineral density (39). Training commenced in all of these subjects early after SCI (mean = 0.21 yr). These subjects trained for a minimum of 2 yr. One subject with acute SCI (*subject A10/C6*, Table 1) elected not to participate in the training protocol, but returned 1.5 yr later for repeat testing as a chronic SCI subject.

Test Apparatus and Protocol

The subjects remained in their wheelchairs during the test procedure. The ankle was stabilized in a system that measured isometric plantar flexion torque of one leg, as described previously (34, 36, 41). The knee was positioned at 90° of flexion, and the ankle was secured to the test apparatus in the neutral joint position. The tibial nerve was activated using a double-pronged stimulation electrode that was secured in the popliteal space behind the knee. The test position (knee flexed) minimized the contribution of the gastrocnemius to the plantar flexor torque (33). The constant-current electrical stimulator had a range of 0 – 200 mA at 400 V. It was triggered by digital pulses from a data-acquisition board (Metrabyte DAS 16F, Keithley Instruments, Cleveland, OH) housed in a microcomputer under custom software control. The stimulation intensity was supramaximal (~1.5 times the intensity required to produce a maximum compound muscle action potential). The stimulator was programmed to deliver a 10-pulse train (15 Hz; train duration 667 ms) every 2 s. A bout of exercise consisted of 125 trains. Subjects completed four bouts of exercise during each training session. Each bout was separated by a 5-min rest period. Our aim was to investigate potentiation that occurred after the muscle entered LFF (or was repetitively activated, because the acute group fatigued very little); as such, this report focuses only on data obtained in the fourth fatigue bout (15 min after the first set of repetitive stimulations).

Two silver-silver chloride electrodes (8 mm in diameter, with an interelectrode distance of 20 mm) recorded soleus compound muscle action potential activity to verify supramaximal activation. Each electrode contained an on-site preamplifier with a gain of 35. The signal was further amplified by a GCS 67 amplifier (Therapeutics Unlimited, Iowa City, IA) with adjustable gain from 500 to 10,000. The amplifier utilized a high-impedance circuit (>15 MΩ at 100 Hz), with a common mode rejection ratio of 87 dB at 60 Hz and a bandwidth of 40 – 4,000 Hz.

Training Protocol

Subjects in the training group attended laboratory-based stimulation sessions one to four times monthly, depending on factors such as work obligations and driving distance. In addition, these subjects performed the plantar flexion stimulation protocol to one limb at

home using a custom-designed portable stimulator and home training system. The home training system duplicated the limb position and stabilization design used in the laboratory. Subjects affixed reusable adhesive carbon electrodes over the plantar flexor muscles of one leg. Stimulation parameters were identical to parameters used in the laboratory sessions. Subjects performed four stimulation bouts per day on 5 days each week, with 5 min of rest between each bout.

Justification for Training Protocol

We designed the muscle stimulation training protocol to “overload” the muscle to induce hypertrophy and repetitively stress the muscle to increase endurance (39). In a previous study of the torque-frequency relationship of acutely and chronically paralyzed muscle, our laboratory determined that muscular overload (~60% of maximal torque) can be generated via 15-Hz supramaximal stimulation (36). We also previously determined (36) that eliciting muscle contractions with a 1 on: 2 off work-rest cycle with a 15-Hz frequency induced significant low-frequency fatigue without compromising neuromuscular transmission. Thus the muscle itself (excitation-contraction coupling) would be stressed repetitively, meeting our endurance training criterion (5, 34, 35, 37). The protocol also needed to be feasible to assist with subject compliance (that is, it had to avoid being burdensome to subjects). We designed a home-based stimulation system that required minimal daily time commitment from subjects, was easy to use, and obviated the need for daily laboratory visits. The entire protocol took 35 min/day to complete.

Training Compliance

The electrical stimulation system was programmable by laboratory personnel, but it only had a start or stop button at the subject interface. The stimulators were designed to operate when the subject's skin impedance was recognized (based on a range previously established in the laboratory). The stimulators did not engage unless the electrodes were in place and the skin impedance matched that of the subject. Once activated, the preprogrammed stimulus frequency, intensity, repetitions, and duration were delivered unless the subject aborted the bout. A microprocessor and memory chip within the stimulator recorded the date, time, and number of stimulus pulses delivered to the subject. These data were downloaded each month during a laboratory session. In this way, we were able to precisely quantify each subject's compliance with the training protocol. The training protocol specified that 10,000 electrically stimulated contractions be completed each month (4 bouts of 125 contractions per day $\times$ 5 days $\times$ 4wk = 10,000 contractions). Compliance was calculated as the percentage of the recommended number of contractions a subject completed in each month.

Quantification of Fatigue, Potentiation, and Rate of Torque Development

For each 10-pulse stimulus train for *bout 4*, we obtained a single torque value. We then obtained the peak torque for the 125-train bout. We divided the final torque in the bout (*contraction 125*) by this peak torque to obtain a fatigue index. Higher values indicate higher resistance to fatigue.

We next divided the peak torque for the bout by the torque of the first train in the bout, thus obtaining a potentiation index. We also noted the train number at which the peak torque occurred to investigate the timing of the potentiation process in response to repetitive stimulation. Four subjects from the training cohort also underwent testing of the untrained limb near the end of the study. Data from the untrained limbs were processed in the same manner as data from the trained limbs.

Potentiation of torque may occur in association with twitch contractile speed changes. We therefore examined the rate of torque development from 0 to 50 ms during the first twitch of

the summated torque response (Fig. 1, *inset*). For *contraction 1* and *contraction 30*, we obtained the absolute and normalized rate of torque development after the stimulus pulse using the following two equations: 1) (change in *twitch 1* torque/change in time); and 2) (change in *twitch 1* torque/change in time)/(peak *twitch 1* torque). The rate calculation (2), adjusted to twitch torque, delineated muscle speed changes that were independent of peak torque amplitude (potentiation) (12). We then created a ratio of the rate of torque development using *contraction 30* and *contraction 1*. A ratio >1 indicated that the contractile speed increased over the 30 contractions. A ratio <1 indicated that the contractile speed decreased over the 30 contractions. We compared the mean ratio of the acute limbs with the chronic limbs and the untrained limbs with the trained limbs to determine whether relative changes in contractile speed were associated with potentiation.

Statistical Analysis

Separate one-way analyses of variance were used to determine whether the dependent variables (torque, fatigue index, potentiation index, and rate of torque development ratio) were different between the acute and chronic groups and between the trained (1 and 2 yr) and untrained limbs. One-way analyses of variance were used to determine whether differences existed among the acute group, the trained limbs, the untrained limbs, and the chronic group. A significant main effect for the trained and untrained limb analysis was analyzed with a Tukey post hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

All results pertain to *bout 4* of the testing protocol. Thus all groups received the same previous three bouts of 125 contractions with 5 min of rest between each bout before *bout 4*. Representative examples of torque and M waves for the chronic and acute conditions appear in Fig. 1, A and B. Notice the considerable potentiation of the chronically paralyzed soleus, despite stable M waves (Fig. 1, A and B).

Chronic vs. Acute SCI

Mean (SE) fatigue index was 56 (6.4) % and 81 (1.6) % for the chronic and acute soleus groups, respectively ($P < 0.05$). Although the acutely paralyzed limbs generated higher absolute torque than chronically paralyzed limbs (Fig. 2A), mean normalized torque over the first 30 contractions (expressed as a percentage of the *train 1* torque) was higher for the chronic SCI group than the acute SCI group (Fig. 2B). On average, the peak torque potentiated by nearly 100% by *contraction 18* and remained elevated to *contraction 30* for the chronic group. Conversely, the peak torque always occurred at *train 1* for the acute group. One subject who was tested within 3 wk of SCI (acute group) and then later retested at 1.5 yr (chronic group) shows the extent of *bout 4* potentiation as a function of time postinjury (Fig. 2C). This subject's potentiation index at 1.5 yr postinjury was 1.443. Overall mean (SE) potentiation index was 1.900 (0.233) and 1.000 (0.000) for subjects with chronic and acute SCI, respectively ($P < 0.05$; Fig. 3).

Both the acute and chronic subjects had a more fused torque profile during the first contraction than during later contractions (Fig. 4A). For the acute subjects, the absolute rate of torque development increased by 55% ($P < 0.001$) from *train 1* to *train 30* (Fig. 4B; rate ratio = 1.55). As would be expected, torque declined when the rate of torque development increased in the acute group (and thus the degree of between-twitch fusion decreased). Interestingly, the chronic group showed a 116% increase in the absolute rate of torque development from *train 1* to *train 30*, which was significantly higher than the acute group (Fig. 4B; rate ratio = 2.16; $P < 0.01$). Such an increase in contractile speed should contribute to a decline in twitch fusion, less torque summation, and therefore a decrement in peak

torque (as was evident in the acute limbs). However, torque potentiated (rather than declined) in the chronic group (recall Fig. 3).

The rate of torque development from *train 1* to *train 30* increased by 10 and 0% for the acute and chronic groups, respectively, after we adjusted for changes in peak torque (potentiation) (Fig. 4B; rate ratio = 1.1 and 0.98, respectively; $P < 0.05$). Thus the change in rate of torque development covaried with postfatigue potentiation in the chronic group.

Effects of Long-Term Training

The group that trained was, on average, 83% compliant with the training protocol (Table 1). After 2 yr of training, the *bout 4* fatigue indexes for the trained and untrained limbs were 82 (1.08) and 62 (4.3) %, respectively ($P < 0.05$). Torque responses to electrical stimulation for the trained and untrained limbs of one subject appear in Fig. 5, A and B, respectively. Notice that the trained limb shows no potentiation (similar to the acute condition) (Fig. 1C), but the untrained limb shows extensive potentiation. There was no difference between the torque produced by the trained limbs after 1 and 2 yr of training ($P = 0.32$); however, trained limb torque was always significantly higher than untrained limb torque at all contractions ($P < 0.01$; Fig. 6A). The peak torque in the trained limb was also significantly greater than that in the acutely paralyzed group ($P < 0.05$). Peak torque potentiated $< 1\%$ in the trained limbs and did not significantly differ from the acutely paralyzed limbs ($P = 0.21$) (Figs. 6B and 2B). Conversely, the untrained limbs potentiated by $> 40\%$ (Fig. 6B). On average, the peak torque occurred by *contraction 14*. Mean (SE) potentiation index for the trained limbs after 1 and 2 yr of training was 1.000 (0.000) and 1.065 (0.006), respectively (Fig. 7). Mean (SE) potentiation index for the untrained limbs was 1.545 (0.229), which was significantly higher than the potentiation index for the trained limbs ($P < 0.01$; Fig. 7).

The potentiation observed in the untrained limb ($\sim 40\%$) was significantly less than that seen in the long-term chronic limbs ($\sim 90\%$; $P < 0.05$; Figs. 2B and 6B). Both the trained and untrained limbs showed a more fused torque profile during *train 1* than in *train 30* (Fig. 8A). The absolute rate of torque development was 61 (13.9) and 60 (4.2) % faster from *train 1* to *train 30* for the trained limbs and untrained limbs, respectively. These ratios did not differ between trained and untrained limbs ($P = 0.97$) (Fig. 8). Both the trained and untrained limbs therefore experienced similar relative increases in contractile speed during the first 30 contractions. The rate of torque development from *train 1* to *train 30* decreased by $\sim 8\%$ for both the trained and untrained limbs after we adjusted for changes in peak torque (potentiation) (Fig. 8B; rate ratio = 0.91 and 0.93, respectively; $P = 0.32$). Thus the change in peak torque (potentiation) explained the increase in the rate of torque development from *contraction 1* to *contraction 30*.

DISCUSSION

The objectives of this study were to determine the extent to which chronic paralysis influences postfatigue potentiation and to determine whether long-term electrical stimulation training would alter the development of potentiation characteristics. Longitudinal training studies are complicated because they are difficult to control and they require substantial commitment from subjects. We addressed these issues by utilizing within-subject controls and by developing a method to quantify subject compliance with the long-term intervention. We were therefore able to quantify the dose of stimulation (on average, 8,300 contractions/mo) that yielded the observed effects presented in this paper.

Acute vs. Chronic SCI

Paralyzed Muscle Fatigue—In neurologically intact muscle, potentiation occurs most readily in muscles that contain primarily fast-twitch fibers (11, 21). The low fatigue index in the chronically paralyzed soleus confirms that these muscles functioned as a composite of fast-fatigable fibers (22, 34, 36). As would be expected for the functionally slow soleus muscle (48) (acute fatigue index was ~81%), no potentiation was observed in the acute SCI condition at the pretraining measurement point.

At the cellular and molecular level, there is evidence that paralysis from spinal cord transection leads to hybrid fibers that express a “mixture” of fast and slow properties (44). For many years, the prevailing belief was that the soleus muscle retains its fatigue-resistant properties after spinal transection, regardless of other cellular transformations, because 12 mo after spinal transection, the cat soleus maintained a fatigue index of 0.93 (2). However, more recently, the spinally isolated cat soleus muscle showed fatigue indexes of 0.80 (4 mo posttransection) (52), 0.64 (8 mo posttransection) (31), and 0.87 (6–8 mo posttransection) (32). In other species, the rat soleus fatigue indexes were 0.66 and 0.50 at 3 and 6 mo after spinal transection, respectively (43), consistent with findings in humans (34–37, 39, 41). However, the time post-SCI, method of assessing fatigue, spinal transection procedures, and species may be important factors contributing to the variations observed in paralyzed muscle fatigue. Importantly, the postparalysis variation in management between humans (wheelchair) and animal models (quadrupeds in cage) may cause differences in overall muscle length, stiffness, and spasticity, all of which may influence neuromuscular plasticity (12, 13, 53).

Muscle fatigability and speed properties (34, 41), neuromuscular transmission (5, 37), torque-frequency relationship (36), low-frequency fatigue (5, 35, 36), paralyzed muscle training effects (39, 40), and now postfatigue potentiation (this report) support the view that the human chronically paralyzed soleus muscle physiologically performs like a fast-fatigable muscle. In this paper, we now report the extent to which our intervention prevented postfatigue potentiation, a property specific to fast-fatigable muscle, from developing in one limb while the opposite limb served as a control. We also present, for the first time, the dose of training (~1–1.5 times body weight muscle force, ~35 min/day) that successfully prevented the development of potentiation (see method for intervention rationale).

Potentiation—The magnitude of potentiation was extensive in both the chronically paralyzed soleus muscle (~90%) as well as the untrained limb (~40%) of the training group. The difference in potentiation between the chronic group and the untrained limbs was significant and may be attributable to several factors. First, it is conceivable that the untrained limb benefited, via segmental cross-education, from the training that occurred on the contralateral side. Second, the periodic assessments of the untrained limb over the course of the study may have been enough to partially adapt the excitation-contraction coupling system (T tubule, SR, and associated receptors), leading to improved force generation with the initial stimulation trains (thus limiting potentiation). Third, the process of neuromuscular adaptation toward fast-fatigable muscle, with respect to potentiation, may not be complete within 2 yr after SCI (chronic group ~8 yr; untrained group ~2 yr). Evidence to support that time postinjury influenced potentiation was provided by the single subject assessed acutely and later at 1.5 yr. This individual’s soleus potentiated in a manner quite similar to that of the untrained limbs (~40%), despite no contralateral training. We believe that the extensive transformation of the excitation-contraction coupling system after paralysis may therefore be time dependent. Previous studies support that fast sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) enzymes and myosin heavy chain enzymes do not transform concurrently in the vastus lateralis after SCI in humans (42).

Rate of torque development—The acute group's peak torque always occurred at the first contraction, followed by a small but consistent decrease in torque. We discovered that the absolute and normalized rates of torque development increased by ~55 and 10%, respectively, in the acute group after the first contraction, which made the train torque “less fused” for all subsequent contractions. Dubose and colleagues (6) reported that slow motor units displayed a 16.2-ms decrease in twitch contraction time after 30 s of repetitive activation. Klass and colleagues (16) noted that rate of torque development increased by 22% after a bout of volitional fatiguing contractions (16). Changes in Ca^{2+} kinetics likely influenced the rate of torque development with repetitive activation and contributed to the decreased torque after the first contraction in the acute group. It should be noted that the rate of torque development ratio, as used in this study, only provides an index of the change in contractile speed from *contraction 1* to *contraction 30* within a given group (acute vs. chronic), and it does not provide a direct comparison of contractile speeds between the acute and chronically paralyzed limbs. For example, the normalized rate of torque development of the acute group was significantly slower than that of the chronic group (0.0015 and 0.0021 ms^{-1} ; $P < 0.01$) for *contraction 1*, consistent with the transformation from slow to fast (34).

The chronic group also showed an ~116% increase in rate of torque development by *contraction 30*. However, unlike the acute group, the chronic group's torque increased (potentiated), despite becoming less fused (Fig. 4). Thus, in the presence of an impaired excitation-contraction coupling system (36), the mechanism leading to potentiation clearly covaried with the mechanism that contributed to faster contractile speeds. When we normalized the rate of torque development to each respective peak *twitch 1* torque, there was less change in rate from *contraction 1* to *contraction 30* in the chronic group. Although the mechanisms for rate change and potentiation appear to covary, previous reports suggest that two independent processes (rather than a single process) are involved (25).

Training Effects

Potentiation—The trained limbs at 1 and 2 yr postinjury did not potentiate, and therefore they appeared similar to the pretraining acute subjects. The training protocol also yielded enhancements in *bout 4* torque by 1 yr of training. Thus the trained limb generated ~200% more torque than the untrained limb at the first contraction (Fig. 6A). After the untrained limb potentiated, the trained limb generated ~110% more torque than the untrained limb. This finding underscores the importance of the training program in increasing the ability of chronically paralyzed muscle to perform work.

The dose of activity delivered (~35 min/day; targeting 1–1.5 times body weight in muscle force) (40) was adequate to maintain the excitation-contraction coupling system in a state that prevented postfatigue potentiation. These same doses of musculoskeletal stress successfully maintained fatigue resistance, torque production, and some of the bone mineral density for over 2 yr after SCI (39, 40). Thus a minimal amount of daily muscle activity was capable of preventing the significant potentiation that develops as a function of chronic paralysis. Unfortunately, routine laboratory assessments of the untrained limb included only one bout (not 4 bouts), which precludes us from determining the exact temporal onset of potentiation in the untrained limbs.

Rate of torque development—The rate of torque development ratios did not differ between the trained and untrained limbs during the first 30 contractions. This does not indicate that the absolute speed properties between the trained and untrained limbs were similar. For example, the normalized rate of torque development of the trained limb was significantly slower than that of the untrained limb (0.0018 and 0.00194 ms^{-1}). Even in the latter stages of the fatigue protocol (beyond *train 30*), the untrained limbs demonstrated

contractile slowing that is typical for functionally fast muscle (39). However, early after resumption of stimulation (*trains 1–30*) in *bout 4*, the relative change in contractile speed (as indicated by the rate ratio) was similar for trained and untrained limbs. This finding supports the idea that, in the presence of similar relative changes in rate, the magnitude of potentiation may be quite varied. This finding gives further support to the suggestion that independent processes (that may or may not covary) underlie potentiation and contractile speed.

Potential underlying mechanisms—When an action potential travels down the T tubule, a voltage-driven change in the dihydropyridine (DHP) receptor regulates the ryanodine (Rya) channel, causing Ca^{2+} to be released from the SR (30). A number of soluble molecules (ATP, Ca^{2+} , reactive oxygen species) and proteins (calmodulin) regulate the sarcoplasmic reticulum Ca^{2+} -release Rya channel as well as posttranslational phosphorylation reactions (30). Sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA) pumps Ca^{2+} back into the SR. Many of these sites are known to adapt during the slow to fast transformation of skeletal muscle (3, 4, 35, 42, 52) or training (23).

Slow-to-fast skeletal muscle transformation leads to long duration muscle fatigue. LFF, such as the chronically paralyzed fast soleus muscle demonstrated in this and previous reports (36), is caused by excitation-contraction coupling compromise. Decreased release of Ca^{2+} by the SR and altered receptor sensitivity have been implicated in LFF (17, 18, 20, 49, 50). Phosphorylation of myosin RLCs also increases when fibers transform from slow to fast as a result of reduced use (3, 4). Fast skeletal muscle, designed for phasic activity, becomes “uncoupled” (reduced Ca^{2+} and/or reduced receptor sensitivity) when activated repetitively for a long duration. As Ca^{2+} concentration falls during this repetitive activation, less free Ca^{2+} is available, yielding impaired myosin RLC phosphorylation.

Postactivation potentiation is under the influence of myosin RLC phosphorylation (45–47, 51). Because myosin RLC phosphorylation adapts with fiber type, it suggests that there may be “short-term” regulation (*contractions 1–30* in this study) as well as “long-term” regulation of the myosin RLC (weeks to yr: acute to chronic transformation) (3). The extent of potentiation, therefore, depends on the fiber type and the state of muscle fatigue. During potentiation, increased Ca^{2+} release and/or receptor sensitivity (Rya and DHP) with repetitive activation likely increases the myosin RLC phosphorylation during LFF in the chronically paralyzed soleus. Overcoming LFF during repetitive activation likely depends on posttranslational (myosin RLC) and pretranslational mechanisms. Interestingly, Rassier and colleagues (29) induced the staircase phenomenon in paralyzed rat muscle (gastrocnemius) only after impairing the Ca^{2+} release system with dantrolene sodium. Thus the impaired Ca^{2+} release associated with LFF appears to be a fundamental component of the significant potentiation observed in chronically paralyzed muscle. Perhaps the DHP and Rya receptors actively modulate their sensitivity as a function of repetitive activation once the muscle is in LFF. Potentiation, therefore, appears to be a mechanism to overcome excitation-contraction compromise in fast muscle in LFF.

The training effect observed in the present study (minimization of LFF and of potentiation in trained muscles) most likely caused adaptations at several sites involved with the excitation-contraction coupling mechanism. Consistent with a transformation from fast to slow muscle, the electrical stimulation training may have caused a decrease in myosin RLC phosphorylation as previously reported in the fast extensor digitorum longus of the rat (3). In this study, the slower rates of torque development for the acute and trained limbs, compared with the untrained limbs, support the idea that training kept the muscle speed properties slow. The slower rate for the trained and acute groups also enabled these muscles to be repetitively activated without compromising the excitation-contraction coupling system (less

LFF). Potentiation was most prevalent in the subjects with the lowest *bout 4* fatigue index, the chronic SCI group. Clearly, these findings demonstrate that the training protocol adapted segments of the excitation-contraction process, at either the SR or the contractile apparatus. The extent that SERCA (23, 42, 52), Rya (23), myosin RLCs (3), and other SR proteins (24) adapt specifically to training in humans is an important area for future investigations.

Functional Implications

The present study illustrates that trained paralyzed muscle does not potentiate after previous bouts of repetitive electrical stimulation. Untrained muscle, starting in a state of LFF, potentiates by over 40%. This varied torque output may be problematic for the optimal use of FES after SCI. An important goal of FES research is to develop neuroprostheses that can be used for grasping, standing, and walking (9). These functional activities will most certainly include periods of activity followed by quiescent intervals (such as using eating utensils or stopping at crosswalks). After resumption of the activity, postfatigue potentiation would create significant torque instabilities, complicating the control strategies needed to generate precise muscle forces. This study verified that fatigue and postfatigue potentiation can both be minimized during long-duration (~35 min) electrical stimulation that includes both high-intensity activity and quiescent intervals. Moreover, trained paralyzed muscle will generate higher torque (39), and thus greater skeletal loads (8), which may spare bone mineral density in the paralyzed extremities (39, 40). This maintenance of muscular and skeletal integrity may be important to the overall health of individuals with SCI (35, 38).

In conclusion, without intervention, chronically paralyzed soleus muscle demonstrates significant postactivation potentiation. With the early implementation of electrically stimulated muscle training, potentiation characteristics of acutely paralyzed soleus muscle can be preserved for as long as 2 yr post-SCI. Untrained limbs showed marked postactivation potentiation at 2 yr post-SCI. A single acute SCI subject who was followed longitudinally into the chronic state developed potentiation characteristics very similar to the untrained limbs of the training subjects. The results of the present investigation contribute another dimension to our understanding of the plasticity of skeletal muscle after SCI. The ability to predict the torque output behavior of muscle (potentiation, fatigability, peak torque, etc.) has important implications for the use of functional electrical stimulation in rehabilitation. In the future, electrical stimulation training may be a useful tool to maintain predictable contractile characteristics in paralyzed muscle.

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Fig. 1.

Representative examples for torque and electromyograph over several trains. Subject numbers refer to Table 1. *A*: chronic spinal cord injury torque (*subject C1*). *Inset*, point during the summated train at which we obtained the rate of torque development. *B*: M waves elicited by the first stimulus pulse of the trains depicted in *A*. The stability of the M waves suggests that potentiation of torque between *trains 1* and *25* was not due to changes in neuromuscular propagation. *C*: *subject A7*, acute spinal cord injury pretraining torque. *D*: M waves elicited by the first stimulus pulse of the trains depicted in *C*.

Fig. 2.

A: mean (SE) torque for acute and chronic spinal cord-injured subjects; *n*, no. of subjects. **B:** mean (SE) torque expressed as a percentage of *train 1*; *n*, no. of subjects. **C:** single subject who was tested during the acute stage of spinal cord injury and who returned 1.5 yr later for testing as a chronic spinal cord injury subject. The subject did no electrical stimulation training in the interim.

Fig. 3. Potentiation index for acute and chronic spinal cord-injured subjects. *Significant difference, $P < 0.05$.

Fig. 4.

A: representative examples of the final 3 summated twitches of *trains 1* and *25* for the acute spinal cord-injured and chronic spinal cord-injured cohorts. Solid lines, *train 1*; dashed lines, *train 25*. *B*: mean (SE) ratio of the absolute and normalized rate of torque development at *train 30* to the rate of torque development at *train 1*. *Significant difference from acute condition, $P < 0.05$. **Significant difference from chronic normalized condition, $P < 0.05$.

Fig. 5. Representative examples for torque and electromyograph over several trains. *A*: trained limb after 2 yr of training. *B*: untrained control limb after 2 yr of contralateral limb training.

Fig. 6.

A: mean (SE) torque for the trained and untrained limbs at 2 yr of training; *n*, no. of subjects.

B: mean (SE) torque expressed as a percentage of *train 1*; *n*, no. of subjects.

Fig. 7. Potentiation index for trained and untrained limbs of the training group. *Significant difference, $P < 0.05$.

Fig. 8.

A: representative examples of the final 3 summated twitches for *train 1* and *train 25* for the trained and untrained limbs at 2 yr of training. Solid lines, *train 1*; dashed lines, *train 25*. *B:* mean (SE) ratio of the absolute and normalized rate of torque development at *train 30* to the rate of torque development at *train 1*.

Table 1

Subject characteristics

Subject No.	SCI Level	Age at Enrollment, yr	Trained Limb	Training Duration, yr	Compliance, % of Goal
C ₁	T ₆	56	NA	NA	NA
C ₂	T ₁	37	NA	NA	NA
C ₃	T ₄	33	NA	NA	NA
C ₄	T ₅	67	NA	NA	NA
C ₅	T ₇	49	NA	NA	NA
A ₁	C ₅	30	R	2.31	84.96
A ₂	C ₆	43	L	2.35	93.40
A ₃	T ₉	29	R	3.02	72.40
A ₄	T ₁₀	22	R	2.13	101.04
A ₅	T ₄	37	L	1.87	52.01
A ₆	T ₄	22	R	3.05	83.31
A ₇	T ₄	21	R	2.27	80.16
A ₈	T ₇	39	NA	NA	NA
A ₉	C ₆	49	NA	NA	NA
A ₁₀ /C ₆	T ₈	36	NA	NA	NA

SCI, spinal cord injury; C, chronic SCI subjects; A, acute SCI subjects; R, right; L, left; NA, not applicable.