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ABSTRACT: Effects of two different training regimens on the contractile properties of the quadriceps muscle were studied in six individuals with spinal cord injury. Each subject had both limbs trained with the two regimens, consisting of stimulation with low frequencies (LF) at 10 Hz or high frequencies (HF) at 50 Hz; one limb of each subject was stimulated with the LF protocol and the other with the HF regimen. Twelve weeks of daily training increased tetanic tension by ~20%, which was not significantly different between training regimens. Interestingly, after HF but not LF training, the unusual high forces at the low frequency range of the force–frequency relationship decreased, possibly due to a reduced activation per impulse. After LF but not HF training, force oscillation amplitudes declined (by 33%) as relaxation tended to slow, which may have opposed possible effects of reduced activation as seen after HF training. Finally, fatigue resistance also increased rapidly after LF training (by 43%) but not after HF training. These results indicate that different types of training may selectively change different aspects of function in disused muscles.

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EFFECTS OF TRAINING ON CONTRACTILE PROPERTIES OF PARALYZED QUADRICEPS MUSCLE

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Skeletal muscles have the capacity to adapt to altered functional requirements. When muscle activity is reduced, for instance as a result of immobilization[3] or paralysis after spinal cord injury (SCI),[43] muscle mass decreases, leading to a loss of strength. Reduced neuromuscular activity leads not only to a reduction in the total amount of contractile protein but also to changes in the phenotypic properties of the muscle fibers. In general, decreased neuromuscular activity leads to a transition of slow to fast fiber characteristics, with concomitant adaptations of metabolic properties and the peripheral circulation.[30,39] SCI is an example of almost complete loss of neuromuscular activity, leading to muscle atrophy,[6,8,23,37,43] muscle fiber transformation from oxidative type I to glycolytic type II fibers,[5,37] reduction in oxidative metabolism,[24,35] and limited blood flow.[18,27,42]

In the past, various programs of electrical stimulation have been used to recondition the paralyzed muscles of individuals with SCI.[16,17,26,28,33,34] A variety of stimulation regimens have been used, most with relatively short contractions (~1–5 s) elicited at various frequencies (~20–60 Hz) with duty cycles ranging from ~1/1 to ~1/3. However, comparisons as to how paralyzed muscles respond to different types of training regimens are limited. Furthermore, the effects of training are usually assessed in relation to muscle strength, mass, or fatigue resistance, whereas other contractile properties, such as speed or the force–frequency relationship, have rarely been reported for SCI muscle.

The purpose of the present investigation was to compare the effects of training with two patterns of electrical stimulation (repetitive high-frequency stimulation and more continuous low-frequency

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Abbreviations: FOA, force oscillation amplitude; HF, high frequency; LF, low frequency; MFR, maximal rate of force rise; ½Rt, half-relaxation time; SCI, spinal cord injury
Key words: contractile properties; fatigue; paraplegia; relaxation; tetanus; training; twitch
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stimulation) on the strength, contractile properties, and fatigability of SCI muscle. There is reason to believe that intermittent bursts of high-frequency stimulation (eliciting high mechanical forces) may have some advantages over more continuous low-frequency stimulation in that it may preserve, or even enhance, muscle mass, whereas low-frequency stimulation may lead to a greater improvement of fatigue resistance.[20,38] We have recently reported an unusual feature of SCI muscles in that the twitch and low-frequency forces were unexpectedly high,[11] and it was of particular interest to see how this feature responded to a period of training.

METHODS

Subjects. Six subjects (five men) with SCI and complete motor lesions for more than 2 years participated in the study. Subjects' details are reported in Table 1. Exclusion criteria were: presence of a pacemaker; decubitus ulcers; severely reduced mobility in knee joints; absence of spinal reflexes; and previous bone fractures. Muscle biopsies were also obtained from all individuals for the assessment of muscle metabolism, fiber type expression, and capillarization. These data have been presented elsewhere.[14] The procedures and risks during the training and tests were explained to all volunteers before written informed consent was obtained. The medical ethics committee of the University of Nijmegen approved the study. The patient data are compared, in some instances, with those obtained from 14 normal subjects (7 men, 7 women; age 27 ± 2 years, height 176 ± 2 cm, weight 66 ± 1 kg).

Electrical Stimulation. The quadriceps muscles of both legs were electrically stimulated with portable stimulators. Three subjects used custom-made devices (Technical Services, Vrije University, Amsterdam, The Netherlands) and the other three used a commercially available portable stimulator (Elpha 2000, Danica, Leusden, The Netherlands). Stimulation was with monophasic square-wave electrical pulses of 0.25-ms width delivered via self-adhesive 50 × 89 mm surface electrodes (Bioflex, PE 3590, Danica) placed over the proximal and distal part of the anterior thigh. Electrode placements were marked with a waterproof felt-tip pen to ensure the same portion of the muscle was stimulated throughout the training period.

Training Procedure. Training consisted of a daily series of isometric quadriceps contractions of both legs for a period of 12 weeks. During a training session, the subjects were seated in their wheelchair with their knees at an angle of approximately 90°. The lower limbs were fixed with a tight elastic strap placed around the ankles. An elastic strap was used instead of a rigid strap, to slacken sudden force increments, for instance during occasional muscle spasms. Consequently, some movement was allowed but this was only marginal during training (i.e., <5° change in knee angle), leading to near-isometric muscle contractions.

Two different training regimens were used to train the quadriceps muscles. Although the total number of pulses was nearly the same for both training regimens, they differed significantly in the frequency of stimulation and the duty cycle. One protocol, which will be referred to as the low-frequency (LF) protocol, consisted of 35 min of repeated quadriceps activation with 10-Hz stimulation trains of 20-s duration, followed by a 50-s rest (duty cycle 28%). The second protocol, which will be referred to as the high-frequency (HF) protocol, consisted of 50 min of repeated stimulation, with 50-Hz trains of 2-s duration and followed by a 50-s rest (duty cycle 4%). Both left and right quadriceps muscles of each subject were stimulated, one limb with the LF protocol and the other with the HF protocol. The two regimens were randomly assigned to each of the limbs.

Measurements of Contractile Properties. The forces of isometric contractions of the knee extensors were recorded before and after 2, 5, and 12

Table 1. Characteristics of subjects with spinal cord injury.

Subject	Gender	Age (years)	Height (m)	Weight (kg)	Lesion level	ASIA* class	Lesion duration (years)
1	Female	52	1.62	60	C-7	A	29
2	Male	44	1.80	90	T-5	A	2.5
3	Male	29	1.85	55	C-5	A	13
4	Male	33	1.76	65	T-7	A	13
5	Male	56	1.78	89	T-9	A	12
6	Male	37	1.72	62	C-5	A	20

**ASIA (American Spinal Injury Association[25]) score is used to classify the completeness of the lesion: A, sensory and motor complete.*

weeks of training with subjects seated upright on a specially designed chair, with the pelvis and upper thigh securely fixed to the seat and the knee angle at 100° extension. Electrically stimulated quadriceps contractions were generated with a high-voltage constant-current stimulator (Model DS7A, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), delivering electrical pulses of 0.2-ms width to surface electrodes (10 × 13 cm; Electro-Medical Supplies, Greenham Ltd., Wantage, Oxfordshire, UK) placed over the anterior thigh. The muscles were activated with a stimulation intensity of 130 ± 6 mA, and the same intensity was used for each subject during all tests. During previous experiments in our laboratory it was observed that this current intensity activates a representative portion (approximately 25–30%) of the paralyzed muscle.[11] Furthermore, it appears that the precise current is not critical for the assessment of the changes in contractile properties as Binder-Macleod et al.[2] found little systematic change in contractile properties with variation in stimulation intensity during the tests.

Knee extension forces were recorded with a force transducer connected to a nonextendable strap placed around the lower leg. Stimulus trains of 1-s duration were applied to the muscle at frequencies of 1, 10, 20, 50, and 100 Hz with a rest period of 2 min between each train.

Susceptibility to fatigue was assessed by activating the quadriceps muscle repetitively for 2 min at 30-Hz stimulation of 1-s duration every 2 s. To minimize possible variations of muscle temperature, room temperature was kept constant at 25°C during the testing procedure and subjects were in the room at least 45 min before the testing was begun.

Data Analysis. Off-line analysis of force records was performed with custom-made software programs. Force–frequency relationships and several indices of contractile speed were obtained before and after training.

Normalized maximal rate of force rise (MFR) was calculated from the positive differential (30-Hz filter) of the force at 100 Hz and expressed as a percentage of peak force according to the method described by Buller et al.[4] Half-relaxation time (½Rt) was defined as the time taken for force to decline from 50% to 25% of the peak 100-Hz force.[10]

The degree of fusion of the force record during stimulation at 10 Hz was calculated as force oscillation amplitude (FOA) relative to mean force according to the method described by Gerrits et al.[11] The mean amplitude of three consecutive force oscillations of the 10-Hz tetanus was taken after force reached its peak value and was divided by the mean force during that time.

During the fatigue protocol, force responses resulting from 30-Hz stimulation were continuously recorded. To compare differences in fatigue resistance, force at the end of the 2-min fatigue protocol was expressed as a percentage of the prefatigue force.

Statistical Analysis. A two-way analysis of variance with repeated measures on time during training was used to test for differences between training regimens, training duration, and their interaction on force, resistance to fatigue, and various indices of contractile speed. To test for differences between training regimens in the changes over time in force–frequency relationships, a three-factor analysis of variance with repeated measures on time during training and stimulation frequency was used. This analysis included tests of main effects of training regimen, training duration, stimulation frequency, and their interaction on relative tension. In case of significant effects of training regimen or interaction effects between training regimen and duration, repeated-measures analysis of variance was performed on separate training regimens. If significant main effects of time during training were observed, post hoc tests for repeated measures were used to determine the time at which a significant change occurred. All values are expressed as mean ± SE, unless otherwise stated, and levels of significance were set at $P < 0.05$.

RESULTS

All subjects finished the 12-week training period. They had no problems with the training or testing procedures and generally reported that their muscles felt larger. In one subject there was an improved orthostatic tolerance during the training procedures. The majority of the subjects found the training program rather boring, however, and some mentioned that an alternative training program, such as electrically induced cycle training, would be preferable as an activity. Measurements of contractile properties were obtained before, after 2 and 5 weeks of training, and after the end of the training period. In one subject it was not possible to perform measurement after 2 weeks of training.

Force–Frequency Relationship. After 2 weeks of training, maximal tetanic (100-Hz) force values of the trained muscles were slightly lower than pretraining values (Table 2). However, after longer training periods, maximal tetanic force of this muscle was

Table 2. Changes in maximal (100-Hz) force, force oscillation amplitude (FOA), and fatigue resistance (% of prefatigue force) after 2, 5, and 12 weeks of low- and high-frequency training.

	Force at 100 Hz (N)			FOA (%)			% of prefatigue force		
	Week			Week			Week		
Subject	2	5	12	2	5	12	2	5	12
Low-frequency training									
1	87	89	116	113	57	43	104	100	105
2	85	115	141	76	64	70	153	160	178
3	77	98	151	113	110	93	212	173	208
4	100	112	105	140	118	100	117	139	123
5	102	117	113	109	54	43	128	179	172
6		91	105		66	55		148	165
Mean ± SE	90 ± 5	104 ± 5	122 ± 8	110 ± 10	78 ± 12	67 ± 10	143 ± 19	150 ± 12	159 ± 15
High-frequency training									
1	82	108	121	135	81	88	98	111	106
2	84	86	102	93	111	135	235	167	233
3	80	105	134	103	85	97	143	124	180
4	98	82	87	108	105	176	88	101	92
5	104	108	106	205	219	219	95	92	90
6		98	156		64	69		67	108
Mean ± SE	90 ± 5	98 ± 5	118 ± 10	129 ± 20	111 ± 23	131 ± 24	132 ± 28	110 ± 14	135 ± 24

Values are expressed relative to pretraining values (%).

increased: post hoc analysis showed a significant increase after 12 weeks of training ($P < 0.05$). There were no main effects of regimen and no interaction effects between regimen and training duration, indicating that there were no significant differences between training protocols.

There were noteworthy adaptations in the low-frequency components of the force–frequency relationship in the SCI muscles as a result of training. As we recently reported,[11] the paralyzed muscles before training produced relatively high forces at low frequencies of stimulation compared with normal subjects, and this changed after training. Figure 1 shows original data of a 10-Hz force signal from one subject that illustrates the general trend. With LF training there was a tendency for the mean force to increase slightly but, at the same time, the amplitude of the oscillations decreased. With HF training there was a decrease in mean force; although the absolute amplitude of the oscillations was less, when expressed as a proportion of the mean force (FOA) the oscillations were unaffected by training. Data for all subjects are summarized in Figure 2. This figure presents the changes in the force–frequency relationships over time (values are normalized for 100-Hz force) for the two training protocols. As a result of training, there was a significantly different effect of HF and LF training on the force–frequency relationship, indicated by a significant interaction effect between training regimen, stimulation frequency, and training duration ($P < 0.05$). The force–frequency curve of the HF-trained muscles clearly shifted downward during the 12-week training period ($P < 0.01$) so that higher stimulation frequencies were required to produce similar amounts of force compared with pretraining values. Interestingly, the changes in the force–frequency relationship following HF training occurred very rapidly (i.e., after 2 weeks), as indicated by post hoc analysis. No changes were present in the LF-trained muscles.

The most striking difference between SCI and able-bodied subjects is indicated by the FOA. As can clearly be seen in Figure 3, FOA of the SCI subjects was around twice the value of that in normal subjects. In addition, the effect of training was also clearly demonstrated by this variable (Table 2). Statistical analysis showed a significant interaction effect between training duration and regimen, indicating a different change in FOA over time between the HF and LF protocols ($P < 0.05$). LF training resulted in a gradual reduction in FOA to 67 ± 10% of pretraining values, after 12 weeks of training ($P < 0.05$). Post hoc analysis revealed that changes from pretraining values were significant, starting after 5 weeks of training. In contrast, FOA showed no significant time-course changes as a result of HF training.

Speed of Contraction and Relaxation. Results from training-induced changes for indices of contractile speed are provided in Figure 4. When the average data of ½Rt before training are compared with those obtained from able-bodied individuals, it seems that

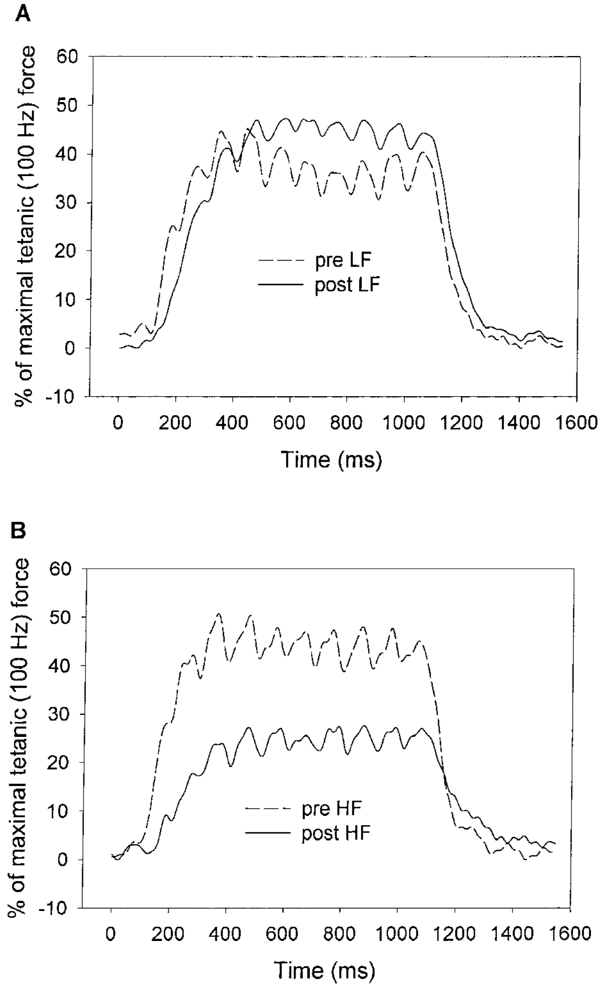

FIGURE 1. Typical example of a 10-Hz force response before (pre) and after (post) training by low-frequency (LF) stimulation **(A)** or high-frequency (HF) stimulation **(B)** in one subject with spinal cord injury.

values for SCI muscles are similar to normal values. With LF training, there was a trend, although not significant, to slower relaxation over the 12 weeks of training but this was not evident after HF training. The difference between these two regimens approached statistical significance ($P = 0.064$).

In agreement with our previous results,[11] MFR seemed greater in the SCI group than in the able-bodied group, before training. Furthermore, no effects of training duration, regimen, or an interaction between these factors were observed on MFR, indicating that MFR was not significantly changed at any time (2, 5, or 12 weeks) after LF or HF training.

Resistance to Fatigue. Figure 5 presents the force responses during 2 min of repeated 30-Hz stimulation for the HF- and LF-trained quadriceps muscle before training and after 2, 5, and 12 weeks of stimulation. Before training, force declined progressively during the stimulation protocol and this decline was more pronounced in SCI subjects than in able-bodied subjects. During the 12-week training period, a difference was observed between training regimens for the changes in fatigue resistance, which approached statistical significance ($P = 0.056$). The resistance to fatigue improved in the LF-trained quadriceps, as indicated by a significant increase in relative force at the end of stimulation from 35 ± 7%, before training, to 50 ± 5%, after training ($P < 0.05$).

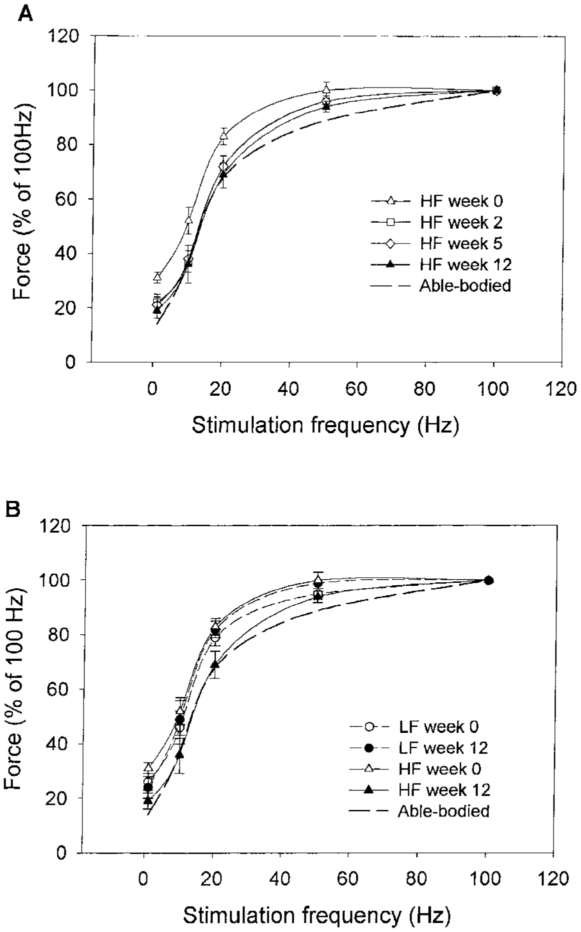

FIGURE 2. Effect of low-frequency (LF) and high-frequency (HF) training on the force–frequency relationship of the quadriceps muscle in subjects with spinal cord injury. Values of able-bodied humans are also included. **(A)** The mean force–frequency relationships before and after 2, 5, and 12 weeks of the HF training. **(B)** Data before and after training, including both HF- and LF-trained quadriceps muscles. Error bars represent SE. HF training resulted in a significant change (downward shift) in the force–frequency relationship after 12 weeks of training ($P < 0.05$). Post hoc analysis revealed that this occurred already after 2 weeks of training. LF training did not result in a significant change.

Post hoc analysis revealed that these training-induced changes occurred rapidly, as an improved fatigue resistance became apparent ($P < 0.05$) as

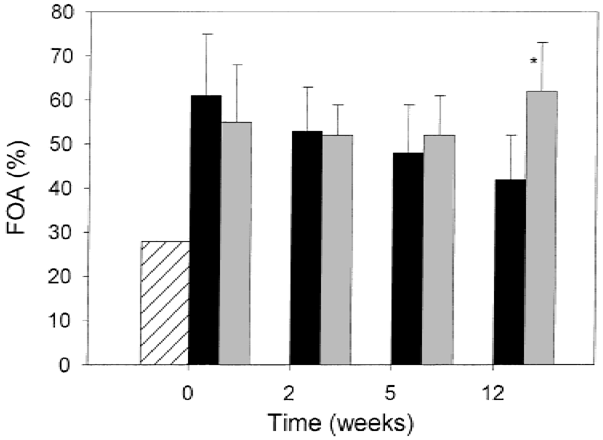

FIGURE 3. Effect of low-frequency (LF) training (black bars) and high-frequency (HF) training (gray bars) on the force oscillation amplitude (FOA) of the quadriceps muscle in subjects with spinal cord injury. Values of able-bodied subjects (hatched bars) are also presented. Data represent mean values (±SE) before and after 2, 5, and 12 weeks of training. Asterisk indicates a significantly different change in FOA between HF and LF training ($P < 0.05$). Post hoc tests showed a decline in FOA after LF training starting at 5 weeks of training. No changes in FOA were observed after HF training.

early as after 2 weeks of training. In contrast, fatigue resistance of the HF-trained quadriceps muscles showed no significant change during the 12-week training period.

DISCUSSION

The present study has confirmed previous observations made on SCI muscle concerning the loss of force, changes in contractile properties, and susceptibility to fatigue. In addition, we have shown that two different training regimens have very different effects on the paralyzed muscle and, contrary to expectations, high-frequency stimulation did not provide any benefits in terms of muscle strength.

Effects of SCI on Quadriceps Contractile Properties. This study confirms the results of previous research, demonstrating that the paralyzed muscles of SCI individuals adapt to reduced neuromuscular activity. SCI muscles showed a faster speed of contraction and greater susceptibility to fatigue than normal muscles, which coincides with previous work on paralyzed quadriceps[11,12] and tibialis anterior muscles.[36,41]

Differences between SCI and normal muscle are most clearly seen in the large relative oscillation amplitudes in low-frequency force responses, which are twice as large as in normal muscles. The large force oscillation is characteristic of fast muscle and corresponds to the changes in myosin phenotype expression and the predominance of glycolytic type II fibers found in the muscles of our subjects[14] and in those reported by others.[5,37] This is consistent with the extensive research on animal models that has shown a slow-to-fast transition in myofibrillar protein isoforms and fiber types.[30,39]

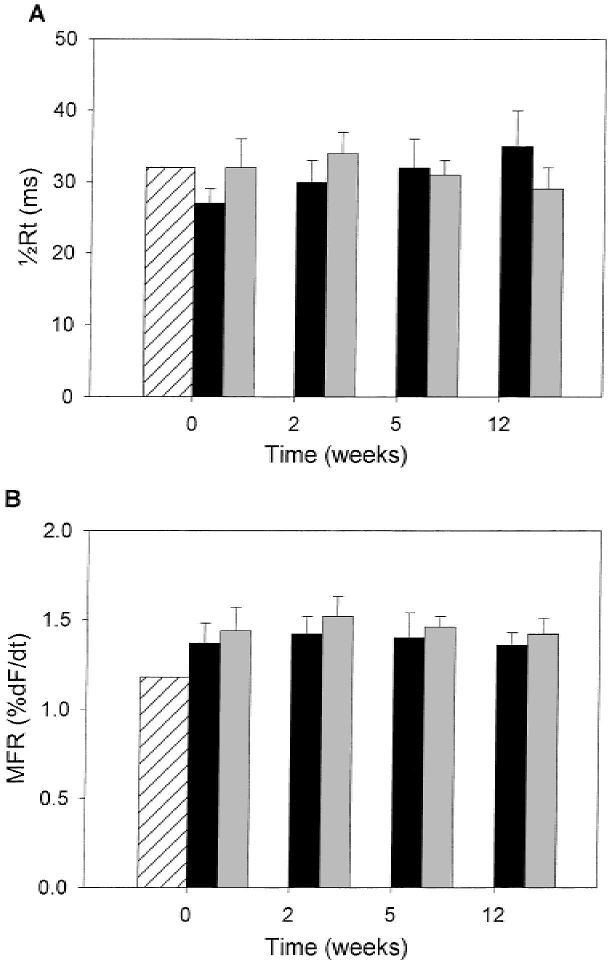

FIGURE 4. Effect of low-frequency (LF) training (black bars) and high-frequency (HF) training (gray bars) on **(A)** half-relaxation time (½Rt) and **(B)** maximal rate of force rise (MFR) of the quadriceps muscle in subjects with spinal cord injury. Values of able-bodied subjects (hatched bars) are also presented. Data represent mean values (±SE) before and after 2, 5, and 12 weeks of training. No significant changes were observed in ½Rt or MFR after training.

A fast muscle fiber type is generally associated with fast rates of contraction and relaxation associated with fast cross-bridge cycling[1] and fast calcium handling processes.[29] This leads to reduced fusion of single twitches and a shift of the force–frequency relationship to the right so that higher frequencies of stimulation are required to generate the same relative force in fast muscles as in slow muscles. Para-

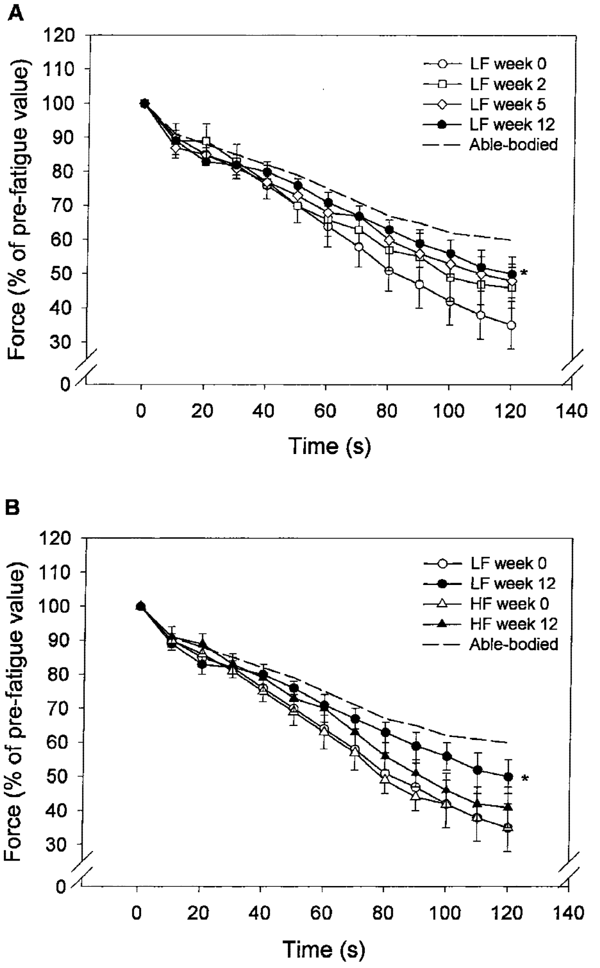

FIGURE 5. Effect of low-frequency (LF) and high-frequency (HF) training on the resistance to fatigue of the quadriceps muscle in subjects with spinal cord injury. Values of able-bodied subjects are also included. **(A)** Mean data before and after 2, 5, and 12 weeks of LF training. **(B)** Data before and after training for both LF- and HF-trained quadriceps muscles. Error bars represent SE. There was a tendency for a different change in fatigue resistance between training regimens. Asterisk indicates a significant change in fatigue during 12 weeks of LF training ($P < 0.05$). Post hoc analysis showed that values were significantly different from pretraining starting after 2 weeks of LF training. HF training did not result in a significant change.

doxically, despite a clear reduction in the degree of fusion in the SCI muscles, the opposite effect is observed on the force–frequency relationship, with greater relative forces being generated at low frequencies of stimulation. We have previously suggested[11] that this phenomenon indicates two separate changes in SCI muscle, which may not be strictly linked.

First, the change in myosin expression toward a faster phenotype[14] would account for the greater force oscillation, similar to that seen in hyperthyroid patients. Unlike the SCI patients, however, this leads in hyperthyroid patients to a reduction in force at low frequencies of stimulation.[44] Second, the leftward shift of the force–frequency relationship may indicate some alterations in the calcium-handling processes as a result of long-term disuse. Altered calcium handling may, for instance, be related to changes in calcium uptake. Although muscles composed of fast type II fibers usually have higher rates of calcium uptake than those composed of slow type I fibers,[29] there may be some variation in paralyzed muscles. In fact, relaxation speed seems similar (present study) or only slightly increased in chronic SCI,[11] and even decreased in acute SCI,[7] which would at least partly result in greater summation of successive twitches. It may be expected that this would reduce the FOA, but other processes involved in calcium handling may be more important. The level of activation achieved for each electrical impulse may, for example, be increased in SCI muscle. This greater activation could be a result of increased calcium release (similar to that seen with low doses of caffeine in isolated muscle preparations[21]), a greater affinity of troponin for free calcium, or changes in light-chain phosphorylation similar to those seen with posttetanic potentiation.[19] High activation levels would lead to high twitch forces, further enhancing FOA. Furthermore, a combination of the aforementioned adaptations in calcium handling (i.e., slow reuptake, greater activation) may more than compensate for the increased cross-bridge cycling and give rise to the greater force seen at low frequencies of stimulation.

Effects of Training: Strength. We found an increased maximal tetanic force after training, which was not different between LF and HF stimulation. Force initially decreased during the training period, which may have been due to some initial damage following exercise of severely disused muscles. However, after 12 weeks there was a significant increase in force of ~20%. These findings agree with numerous previous studies, which used a variety of electrical stimulation programs to recondition paralyzed muscles.[16,17,26,28,33,34] Contrary to our expectations, we found no benefit of HF training in terms of strength gain. It may be speculated that the differences in mechanical load between the regimens was insufficient to lead to differences in strength gains.

Effects of Training: Susceptibility to Fatigue. The improved fatigue resistance of the LF-trained muscles, which was evident after only 2 weeks of stimulation (Fig. 5), supports the general consensus that increased neuromuscular activity increases the oxidative capacity of exercised muscles.[22,31,40] The results also agree with previous publications demonstrating that electrical stimulation of SCI muscles can reverse the high susceptibility to fatigue of these muscles[26,34,36,41] and is associated with an increased oxidative capacity.[14,24]

The changes in fatigue resistance seen with LF stimulation were very rapid, being apparent within the first 2 weeks of training. This may suggest adaptations of mitochondrial function and possibly blood supply, which are known to respond rapidly to changes in muscle activity.[39]

Effects of Training: Contractile Properties. There were interesting and complex changes in the force–frequency relationships and FOA with training, which may indicate adaptations in molecular processes within the muscle. With the interpretation of these contractile properties it should be noted that possible mechanical adaptations in the noncontractile tissue of the muscle or tendon as a result of training might also affect the functional properties of the muscle. It has been suggested, for instance, that quantitative and qualitative changes in connective tissues may underlie the increased muscle stiffness seen after immobilization.[15,46] Furthermore, contractile activity seems important for the maintenance of normal muscle compliance[45] and training can decrease muscle stiffness.[32] In contrast, Douglas et al.,[9] who studied muscle tone in humans, reported decreased muscle stiffness in paraplegic subjects compared with noninjured controls. Furthermore, the muscle stiffness of these paralyzed muscles was increased by electrically stimulated shortening contractions and decreased by a period of passive movements. It remains unclear how the training regimens as used in the present study would affect muscle stiffness, and it is therefore difficult to speculate to what extent these possible mechanical adaptations in muscle and tendon may have affected the results of the present study. With HF training the force–frequency relationship of the SCI muscle appeared to normalize in that it approached a relationship observed in the quadriceps muscles of able-bodied subjects. This downward shift in the force–frequency relationship, which was apparent after 2 weeks of stimulation (Fig. 2), was not accompanied by a significant change in FOA or ½Rt. We have seen similar changes in the force–frequency relationship when SCI muscles were trained for 6 weeks, three times per week, with electrically stimulated (30-Hz) cycle ergometric exercise.[13] Unlike the present study, however, this was accompanied by a reduction in ½Rt and an increase in FOA, suggesting an increased calcium uptake by the sarcoplasmic reticulum. A decrease in force of the twitch is consistent with a decrease in activation per impulse (as discussed earlier) and it is of much interest to note that this occurred so rapidly within the first 2 weeks of training. The fact that FOA and ½Rt were unaffected by HF training suggests that this training primarily affected activation rather than calcium uptake or myosin expression.

In contrast, LF stimulation apparently had no effect on the force–frequency relationship of SCI muscle, but this may disguise more subtle changes in the muscle. After 12 weeks of stimulation there was a small reduction in the twitch size but small increases in 10- and 20-Hz forces. At the same time, the FOA was reduced as the rate of relaxation slowed. These changes are consistent with a small decline in the activation per pulse (smaller twitch) coupled with a slower calcium uptake and possibly a small increase in the expression of slower myosin (decreased FOA, increased half-relaxation time). The first change will tend to decrease force at low frequencies of stimulation, and the second will increase fusion and hence the mean force. Combined, these two opposing tendencies give rise to little or no change in the force–frequency relationship of the LF-stimulated muscle.

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