

Contractile Properties and the Force-Frequency Relationship of the Paralyzed Human Quadriceps Femoris Muscle

Background and Purpose. Following spinal cord injury (SCI), paralyzed muscles undergo physiological changes that alter their force responses to electrical stimulation. The purpose of this study was to investigate the effects of SCI on the contractile properties and force-frequency relationship (FFR) of the paralyzed human quadriceps femoris muscle of adolescents and young adults. **Subjects.** Thirteen subjects (11 male, 2 female; age range=11–24 years) with motor complete SCIs and 13 matched control subjects (11 male, 2 female; age range=9–23 years) without SCI participated in the study. **Methods.** Both groups of subjects underwent the same testing protocol using similar equipment. **Results.** The paralyzed muscles of the subjects with SCI produced 62% of the peak twitch force and had a fatigue ratio that was 65% of that of the control subjects. The paralyzed muscles contracted 14% and 25% faster and relaxed 38% and 46% faster than the nonparalyzed muscles in nonfatigued and fatigued conditions, respectively. Compared with the control subjects, the subjects with SCI had twitch-to-tetanus ratios that were 84% and 127% greater in nonfatigued and fatigued conditions, respectively. Relative to the control subjects, the FFR of the subjects with SCI was shifted to the left in the fatigued condition. Relative to their respective nonfatigued conditions, the FFR of both groups of subjects shifted to the right with fatigue. **Discussion and Conclusion.** These findings may have important implications for designing stimulation strategies to reduce the rapid fatigue that limits the clinical efficacy of functional electrical stimulation. [Scott WB, Lee SCK, Johnston TE, et al. Contractile properties and the force-frequency relationship of the paralyzed human quadriceps femoris muscle. *Phys Ther.* 2006;86:788–799.]

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Electrical stimulation of paralyzed skeletal muscle can be used to assist people with spinal cord injury (SCI) in performance of functional movements.¹ This therapeutic intervention is called *functional electrical stimulation* (FES). People with SCI also can use electrical stimulation to activate their paralyzed muscle to induce muscle strengthening (increase in the force-generating capacity of a muscle) and endurance adaptations that counter many of the deleterious effects that occur following SCI, such as loss of muscle mass and osteoporosis.² Most work exploring the effects of varying the frequency of the pulses within electrical stimulation trains on the force output of human muscle has been carried out with subjects without disabilities or known pathologies.³⁻⁷ However, the paralyzed muscles of people with SCI undergo physiological changes that affect the response of the muscle to electrical stimulation.^{8,9} Due to these differences, it is important to extend the findings regarding the effects of stimulation frequency on force output to people with SCI. The paralyzed quadriceps femoris muscle is particularly important to study because it is often stimulated during FES for functionally important tasks such as standing or walking and is one of the muscles stimulated for training purposes.¹⁰

Following SCI, paralyzed muscles typically show marked atrophy¹¹⁻¹⁵ due to loss of contractile proteins and weakness.^{9,16,17} There are fiber-type changes as well, with a transition from type I, slow-twitch, fatigue-resistant

fibers toward type IIB(x), fast-twitch, fatiguable fibers.^{13,18,19} Initially following SCI in humans, fiber-type transitions occur mostly within fast-twitch fibers, with myosin heavy chain (MHC) IIa replaced by MHCIIx.^{12,20,21} As time from injury increases, transitions from type I to type II muscle also occur as MHCI is replaced by MHCIIa.^{13,18,22} Proteins associated with the Ca²⁺-ATPase of the sarcoplasmic reticulum responsible for re-sequestering Ca²⁺ into the sarcoplasmic reticulum from the myoplasm (SERCAs) also undergo transitions following SCI. As a consequence of the MHC and SERCA changes following SCI, paralyzed muscles contract and relax faster than nonparalyzed muscle.^{8,17,23} In addition to changes in the contractile and Ca²⁺ re-uptake proteins, studies have shown that the oxidative capacity of the muscle is reduced as enzymes associated with oxidative production of energy^{22,24} and blood flow to the muscles^{14,22,25} decrease. Consequently, following SCI, the fatigue resistance of paralyzed muscle is reduced.^{8,20,24,26}

Due to the faster contraction and relaxation speeds observed in paralyzed muscle,^{8,17,23} higher frequencies of electrical stimulation are expected to be required to reach the same relative force in people with SCI as compared with people without SCI because the electrical stimuli must be closer in time to allow the muscle forces to summate and fuse.²³ This is described as a rightward shift in the force-frequency relationship (FFR) and has been reported in human chronic versus acutely paralyzed soleus muscles²⁷ and rat soleus muscles following

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SCI.²⁸ The FFR is the relationship of the electrical stimulation frequency to the isometric peak force produced by the activated muscle. Unexpectedly, a leftward shift (ie, lower frequencies produce the same relative force in people with SCI as compared with people without SCI) has been observed in human paralyzed quadriceps femoris and tibialis anterior muscles.^{8,17} Gerrits and colleagues⁸ demonstrated nearly a 2-fold increase in the twitch-to-tetanus ratio and a leftward shift in the FFR of the paralyzed quadriceps femoris muscle of subjects with SCI as compared with the nonparalyzed muscle of control subjects.

In paralyzed human quadriceps femoris muscle, some responses to electrical stimulation can be explained by physiological changes, whereas other responses, such as the elevated twitch-to-tetanus ratio, remain unexplained. In addition, changes in the nonfatigued FFR of the paralyzed human quadriceps femoris muscle are counterintuitive given the observed faster contraction and relaxation speeds. The purpose of this study was to confirm, in a sample of adolescents and young adults with SCI, previous observations of the contractile characteristics and FFR of the nonfatigued paralyzed human quadriceps femoris muscle made with adult subjects and extend those observations to the fatigued condition. According to the Centers for Disease Control and Prevention Web site,²⁹ more than half of all SCI injuries occur in people 15 to 30 years of age; thus, many people with SCI who are seen in physical therapy clinics are likely to be adolescents or young adults. We hypothesized that a leftward shift in the fatigued FFR would be associated with an elevated twitch-to-tetanus ratio and the fatigued FFRs of both subjects with SCI and control subjects would be shifted to the right as compared with their respective nonfatigued FFRs. We believe that it is particularly important to understand the response of the paralyzed quadriceps femoris muscles to electrical stimulation when fatigued because most FES and training applications necessarily involve activating the muscles in a fatigued condition a large proportion of the time. These data also may lead to the design of more effective protocols for FES and training interventions for people with SCI.

Method

Subjects

Data were collected on 13 subjects (11 male, 2 female) with motor complete SCIs (American Spinal Injury Association [ASIA] classification A or B).³⁰ The subjects in this group were a sample of convenience and were recruited from Shriners Hospitals for Children in Philadelphia, Pa. The subjects in the SCI group were adolescents or young adults aged ≤ 24 years. A control group of 13 subjects without disabilities or known pathologies was

recruited from the University of Delaware and the surrounding community. The control subjects were matched to the subjects with SCI based on age (± 2 years), body mass index (BMI), and sex. The characteristics of the subjects with SCI and the control subjects are shown in Tables 1 and 2. The BMI for each subject was calculated using the formula:

$$(1) \text{ BMI} = [\text{weight (in kilograms)} / \text{height}^2 \text{ (in meters)}]$$

Subjects then were matched based on the National Institutes of Health–defined categories of BMI: < 18.5 =underweight, 18.5 – 24.9 =normal weight, 25 – 29.9 =overweight, and ≥ 30 =obese.³¹ Inclusion criteria for the SCI group consisted of a motor complete SCI, at least 1 year postinjury or, if < 1 year postinjury, neurologically stable as determined by an evaluation by a Shriners Hospitals for Children rehabilitation staff member at time of admission, no lower motoneuron involvement of the quadriceps femoris muscle or history of lower-extremity fractures, and passive knee joint flexion of at least 100 degrees in a sitting position. Inclusion criteria for the control group consisted of matching one of the subjects with SCI by age, sex, and BMI category. Subjects were excluded from both groups if they had a history of orthopedic knee injuries, heart disease, peripheral vascular disease, current neoplasms, or neurological disorder (other than SCI) affecting the lower extremities. Participation was voluntary, and subjects were free to withdraw from the study at any time. All subjects and the legal representatives of minors signed an informed consent form that was approved by the University of Delaware Human Subjects Review Committee and the Institutional Review Board of Temple University, which serves as the oversight committee for Shriners Hospitals for Children. In addition, minors signed an assent form that was approved by both oversight committees.

Experimental Protocol

Both groups of subjects underwent the same testing protocol using similar equipment. The subjects with SCI were tested at Shriners Hospitals for Children in Philadelphia, and the control subjects were tested at the University of Delaware. Each subject was tested during a single session that lasted approximately 1 hour. The control subjects' quadriceps femoris muscles were tested isometrically using a computer-controlled dynamometer (Kin-Com III)* with their knees flexed to 90 degrees and with approximately 75 degrees of hip flexion. A Kin-Com II dynamometer with the Kin-Com III seating system was used to test the subjects with SCI. The force transducer was positioned against the anterior aspect of the lower leg proximal to the lateral malleolus. The control sub-

* Chattecx Corp, 101 Memorial Dr, PO Box 4287, Chattanooga, TN 37405.

Table 1.Individual and Muscle Contractile Characteristics of the Subjects With Spinal Cord Injury (SCI)^a

Subject No.	Sex	Age (y)	BMI Category	Peak Twitch Force (N)	Fatigue Ratio	CT (ms)	½RT (ms)	Twitch-to-Tetanus Ratio	Months From Injury	SCI Level
1	M	11	Underweight	48 ^b	0.38	38	40	0.22	45	T6
2	F	24	Normal	44 ^b	0.44	42	22	0.24	108	C6/C7
3	M	16	Normal	54	0.51	93	66	0.32	10	C6/C7
4	M	15	Normal	218 ^{b,c}	0.43	66	66	0.16	22	T7/T8
5	M	22	Normal	128 ^b	0.33	85	33	0.31	63	T6/T7
6	F	12	Underweight	48 ^b	0.71	50	38	0.22	60	T8
7	M	12	Normal	16	0.39	55	54	0.48	80	T6
8	M	17	Normal	70	0.19	70	51	0.13	8	C5/C6
9	M	21	Overweight	85	0.24	75	40	0.34	144	T5
10	M	20	Normal	85	0.34	45	18	0.18	17	T4
11	M	15	Normal	54	0.50	68	73	0.34	8	C7
12	M	19	Overweight	133 ^b	0.22	51	50	0.13	17	T5/T6
13	M	15	Normal	101	0.19	79	69	0.20	14	T5/T6

^aThe sex, age, and body mass index (BMI) categories were used to match subjects with SCI with control subjects. The contraction time (CT), half-relaxation time (½RT), and twitch-to-tetanus ratios are from the nonfatigued condition.

^bSubject reported "conditioning" the quadriceps femoris muscles by activating them on a regular basis with electrical stimulation. Typically, this consisted of lying prone in bed and generating isometric contractions with the legs fully extended.

^cSubject reported doing knee extension exercises versus weighted resistance.

Table 2.Individual and Muscle Contractile Characteristics of the Control Subjects^a

Subject No.	Sex	Age (y)	BMI Category	Peak Twitch Force (N)	Fatigue Ratio	CT (ms)	½RT (ms)	Twitch-to-Tetanus Ratio
1	M	9	Underweight	108	0.54	41	30	0.10
2	F	22	Normal	125	0.80	74	73	0.24
3	M	16	Normal	180	0.54	93	64	0.12
4	M	17	Normal	169	0.51	69	67	0.20
5	M	23	Normal	173	0.59	77	67	0.17
6	F	13	Underweight	65	0.54	69	73	0.20
7	M	12	Normal	106	0.58	85	70	0.13
8	M	17	Normal	126	0.61	76	66	0.08
9	M	23	Overweight	113	0.74	73	71	0.10
10	M	22	Normal	142	0.42	71	71	0.11
11	M	15	Normal	143	0.52	69	67	0.10
12	M	21	Overweight	116	0.50	62	62	0.09
13	M	14	Normal	192	0.66	70	67	0.21

^aThe contraction time (CT), half-relaxation time (½RT), and twitch-to-tetanus ratios are from the nonfatigued condition.

jects' quadriceps femoris muscle was stimulated using a Grass S8800 stimulator with a Keaton RB SIU8T stimulus isolation unit.[†] For the subjects with SCI, a Grass S88 stimulator with a SIU8T stimulus isolation unit[†] was used. The maximum output of the 2 stimulators was identical (150 V). Two 7.5- × 12.5-cm self-adhesive electrodes were used for transcutaneous electrical stimulation of the muscle. One electrode was placed distally over the muscle belly of the vastus medialis muscle, and the other electrode was placed proximally over the rectus femoris muscle. Smaller (5- × 9-cm) electrodes

were used for subjects with thin legs. Following placement of the electrodes, 1-second 20-Hz stimulation trains were delivered to test the electrode location. A smooth rate of rise and a plateau in force indicated that a consistent population of motor units was being recruited throughout the stimulation train. If a consistent population of motor units was not being recruited, the electrode placement was shifted until a smooth rate of rise and plateau in force were achieved.

Following testing for electrode placement, the peak twitch force (PTF) of the subjects' quadriceps femoris muscle was recorded when stimulated with a series of

[†] Grass-Telefactor, 600 E Greenwich Ave, West Warwick, RI 02893.

single 600-microsecond pulses delivered at a rate of 1 every 10 seconds. The stimulator voltage was incrementally increased until the maximum of 150 V was applied. Next, the stimulation intensity for the session was set by using a 1-second 20-Hz stimulation train to produce a peak force equivalent to the greatest PTF recorded for each subject. We believe that the PTF was either the subjects' maximum twitch force or very close to it, because the twitch force responses of the subjects either reached their greatest value prior to the maximum stimulator output of 150 V or were on the asymptote of an intensity-force curve. Once the intensity was set, it remained unchanged for the remainder of the testing session. The elevated twitch response of the subjects with SCI led to a twitch-to-tetanus ratio for the quadriceps femoris muscle of approximately 0.26 versus 0.14 in the control subjects. We believe, therefore, that the 1-second 20-Hz train was producing approximately 26% and 14% of the force-generating ability of the paralyzed and nonparalyzed muscles, respectively. Based on the FFRs of the quadriceps femoris muscles, the 1-second 20-Hz train produces approximately 60% to 65% of the force produced in response to the 100-Hz train. Thus, we estimated that, in response to the higher frequencies tested, we were recruiting approximately 35% and 20% of the force-generating ability of the paralyzed and nonparalyzed muscles, respectively.

Nonfatigued Muscle Testing

Following a 5-minute rest, the nonfatigued portion of the testing protocol was begun. There were 2 non-fatigued protocols that subjects received in a random order separated by a 5-minute rest. During both protocols, stimulation trains were delivered at a rate of 1 every 20 seconds to avoid producing fatigue. One protocol consisted of 6-pulse testing trains of various frequencies and patterns. These data will be presented in a separate report. The other protocol consisted of a single pulse and eight 1-second constant-frequency trains (CFTs) of 10, 20, 25, 33, 40, 50, 80, and 100 Hz, delivered in a random order and then repeated in reverse order. All subjects received the same random order of testing trains. We did not potentiate the muscles prior to testing because pilot work showed that it was difficult to produce potentiation without simultaneously producing fatigue in the subjects with SCI.

Fatiguing Stimulation and Fatigued Muscle Testing

Following another 5-minute rest, the fatiguing stimulation consisting of 110, 13-pulse, 40-Hz CFTs delivered at a rate of 1 train every second (300 milliseconds on, 700 milliseconds off, 30% duty cycle) was delivered. Immediately following the last train of the fatiguing stimulation, the fatigued muscle testing started. The same 6-pulse testing trains followed by the single pulse and eight 1-second CFT testing trains that were delivered in

the nonfatigued condition were now delivered to the fatigued muscles. However, in the fatigued condition, the testing trains were separated by 2 of the 13-pulse 40-Hz CFTs, a 700-millisecond off time separated each train, there was no rest period between the sequences of 6-pulse and 1-second testing trains, and there was only one occurrence of each of the testing trains. The 13-pulse, 40-Hz CFTs were included to control for prior activation history of the muscle to ensure a consistent level of fatigue throughout the fatigued testing.

Data Management and Analysis

For each subject, the entire force response to each train in the nonfatigued testing protocol, the fatiguing stimulation, and the fatigued testing protocol was digitized online at a sampling frequency of 200 Hz and stored for subsequent analysis. Data were analyzed using custom-written software (Labview 5.0[†]). The PTF of each subject was used to compare the force-generating ability of the quadriceps femoris muscles between groups. A fatigue ratio was calculated for each subject by dividing the average of the final 3 peak force responses in the 110-train fatigue-producing protocol to the peak force response to the first train of the fatiguing protocol to compare the fatigue resistance of the 2 groups' muscles. The PTF and fatigue ratios of the 2 groups were compared using paired *t* tests.

The contraction time (CT) and the $\frac{1}{2}$ relaxation time ($\frac{1}{2}$ RT) of each subject's nonfatigued and fatigued twitch responses were calculated. A third-order polynomial was fitted to each subject's twitch data to smooth the data. From the fitted curve, the CT was calculated as the amount of time that it took the muscle to reach the peak force once the muscle began to develop force, and the $\frac{1}{2}$ RT was calculated as the amount of time that it took for the force to decline to 50% of the maximum peak force value. The CT and $\frac{1}{2}$ RT were analyzed separately with 2-way (group \times condition), repeated-measures analyses of variance (ANOVAs). If there was a significant interaction, paired *t* tests were used for *post hoc* testing.

A normalized FFR from both the nonfatigued and fatigued conditions was calculated for each subject. Each subject's peak force responses to the nonfatigued and fatigued single pulses and 1-second CFTs were normalized to the 100-Hz response from the respective conditions. For the nonfatigued data, the 2 occurrences of each testing train were averaged. To compare FFRs, a nonlinear curve fitting routine (SigmaPlot[§]) was used to fit the normalized peak forces of each subject to the nonfatigued and fatigued data with the following 4-parameter Hill equation:

[†] National Instruments, 11500 N Mopac Expwy, Austin, TX 78759-3504.

[§] Systat Software Inc, 501 Canal Blvd, Suite E, Point Richmond, CA 94804-2028.

$$(2) y = y_0 + [(ax^b)/(c^b + x^b)]$$

where y =peak force, x =frequency, y_0 =minimum peak force produced by a tested frequency, a is a scaling factor, c is the frequency at which 50% of maximum peak force is produced, and b is a measure of slope. From the fitted Hill equations for the nonfatigued and fatigued conditions for each subject, c was used as the frequency that produced 50% of the maximum tetanic force (F50) to compare the FFRs between groups and conditions. The nonfatigued and fatigued twitch-to-tetanus values (ie, the normalized twitch responses) also were analyzed. The F50 and twitch-to-tetanus values were analyzed separately with 2-way (group \times condition), repeated-measures ANOVAs. If there was a significant interaction effect, paired t tests were used for *post hoc* testing.

Results

All subjects completed the data collection session. However, because of spasms interfering with the force response to the electrical stimulation, it was not possible to use all of the data from the subjects with SCI. Six of the 13 subjects with SCI reported using electrical stimulation on a regular basis to activate their quadriceps femoris muscles for training and FES (1 subject). However, 1-tailed, paired t -test comparisons of PTF ($P=.23$), CT ($P=.08$), $\frac{1}{2}$ RT ($P=.13$), fatigue ratios ($P=.18$), F50 ($P=.18$), and twitch-to-tetanus ratios ($P=.12$) of the 7 subjects with “unconditioned” quadriceps femoris muscles and the 6 subjects with “conditioned” quadriceps femoris muscles identified no significant differences. The trends were for the conditioned subjects as compared with the unconditioned subjects to have faster CTs and $\frac{1}{2}$ RTs as well as lower F50s. This is the opposite of what would be predicted, because the use of electrical stimulation should attenuate or reverse the typical shift toward fast-twitch fibers following SCI injury^{32–34} and lower F50s.⁸ Consequently, we collapsed all of the subjects with SCI into a single SCI group because it did not appear that the subjects with “conditioned” muscles and those with “unconditioned” muscles were significantly different from one another in any of the variables of interest. All results are reported as group means with standard errors.

Contractile Properties

The quadriceps femoris muscles of the subjects with SCI displayed lower PTFs and fatigue ratios than those of the control subjects (Fig. 1). The PTFs of the subjects with SCI (83.4 ± 14.6 N) were 62% of those produced by the control subjects (135.2 ± 10.0 N) ($P < .005$). The fatigue ratio of the subjects with SCI (0.38 ± 0.04) was significantly lower than that of the control subjects (0.58 ± 0.03) ($P < .005$).

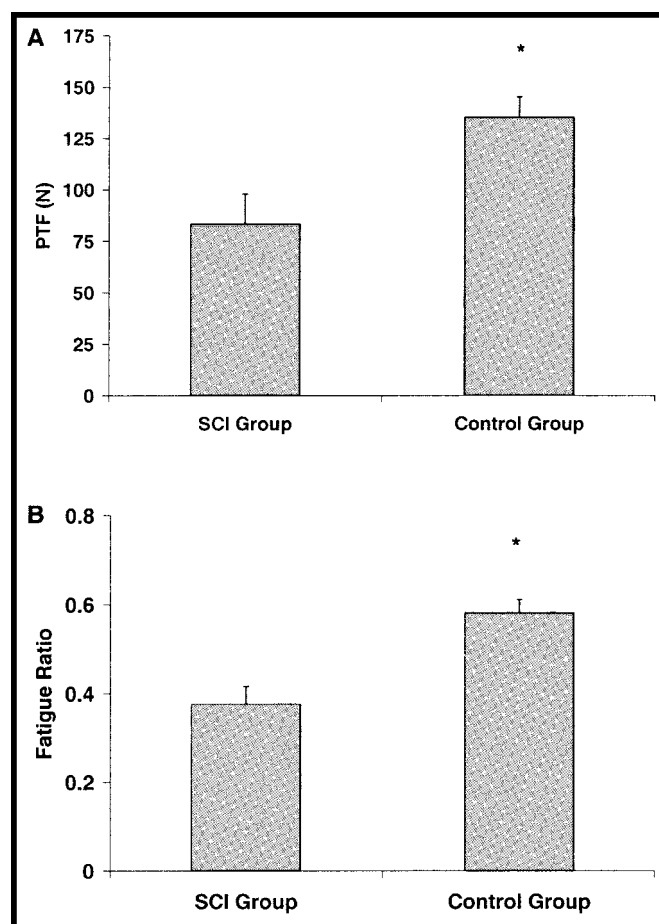


Figure 1.

Mean (\pm SE, $n=13$) peak twitch forces (PTFs) in response to the maximum stimulator output (150 V) (A) and fatigue ratios (B) for the subjects with spinal cord injury (SCI) and control subjects. The fatigue ratios were calculated from the 110-train fatiguing stimulation protocol as the average of the final 3 peak force responses divided by the peak force response to the first train of the fatiguing protocol. Asterisk (*) indicates control group significantly different from SCI group ($P < .005$).

The paralyzed quadriceps femoris muscles of the subjects with SCI contracted and relaxed faster than those of the control subjects based on the twitch responses collected during the nonfatigued and fatigued testing protocols (Fig. 2). The CT of the quadriceps femoris muscles of each group did not slow significantly with fatigue, whereas the relaxation time of each group slowed with fatigue (Fig. 2). One of the subjects with SCI (#8 in Tab. 1) had spasms in the fatigued condition during the twitch response that prevented an accurate calculation of his CT and $\frac{1}{2}$ RT in the fatigued condition. Consequently, data for 12 subjects with SCI and their matched control subjects were used for the CT and $\frac{1}{2}$ RT analyses. There was a main effect of group ($F=10.85$, $P < .01$) but not condition (ie, nonfatigued versus fatigued) on the CT. There was no interaction effect. The SCI group’s quadriceps femoris muscles contracted 14% and 25% faster than those of the control subjects in the nonfatigued and fatigued conditions, respectively.

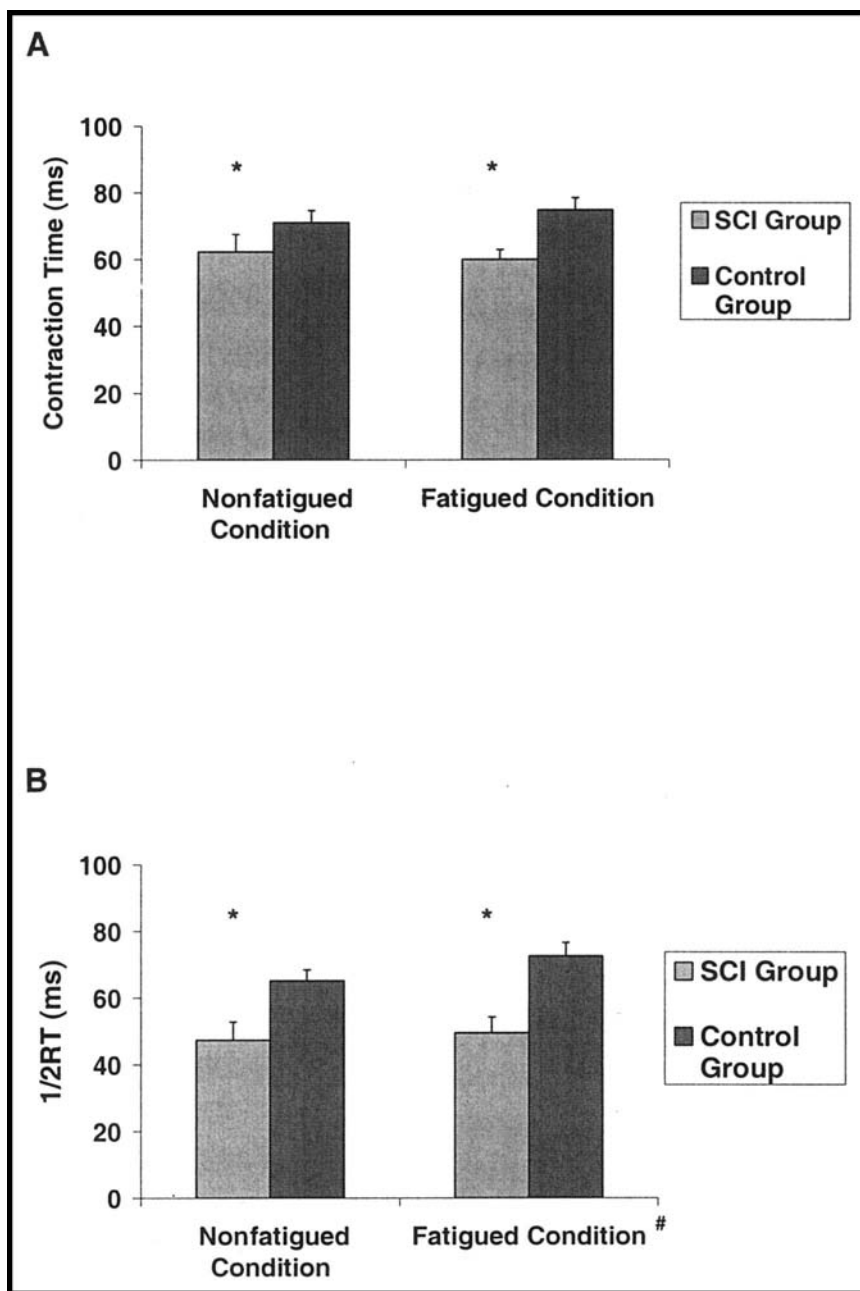


Figure 2. Mean (\pm SE, $n=12$) contraction times (time to peak twitch force) (A) and $1/2$ relaxation times ($1/2$ RTs) (B) for the subjects with spinal cord injury (SCI) and control subjects in the nonfatigued and fatigued conditions. There was a main effect of group on both the contraction and relaxation times and a main effect of condition on the relaxation times. No interaction effects were observed. Asterisk (*) indicates SCI group significantly different from control group ($P<.01$ for both). Number sign (#) indicates fatigued condition significantly different from nonfatigued condition ($P<.05$).

There was a main effect of both group and condition on the $1/2$ RT ($F=9.88$, $P<.01$ and $F=6.09$, $P<.05$, respectively). There was no interaction effect. The $1/2$ RT of the quadriceps femoris muscles of the subjects with SCI were 38% and 46% faster than those of the quadriceps femoris muscles of the control subjects in the non-

fatigued and fatigued conditions, respectively. There was 5% and 11% slowing of the $1/2$ RT with fatigue for the subjects with SCI and control subjects, respectively.

Twitch-to-Tetanus Ratios and Force-Frequency Relationship

Compared with the control subjects' quadriceps femoris muscles, the paralyzed quadriceps femoris muscles of the subjects with SCI had higher twitch-to-tetanus ratios in the nonfatigued and fatigued conditions and lower F50s in the fatigued condition. Furthermore, both groups had higher F50s in the fatigued condition as compared with their F50s in the nonfatigued condition (Fig. 3). One of the subjects with SCI (#8 in Tab. 1) had spasms in the fatigued condition that prevented an accurate calculation of his twitch-to-tetanus ratios and F50. Consequently, data for 12 subjects with SCI and the matched control subjects were used for the twitch-to-tetanus and F50 analyses. There was a main effect of group ($F=24.9$, $P<.001$) on the twitch-to-tetanus ratios (Fig. 4A). The twitch-to-tetanus ratios of the paralyzed quadriceps femoris muscles were 84% (0.26 ± 0.03 versus 0.14 ± 0.02) and 127% (0.28 ± 0.03 versus 0.12 ± 0.01) greater than those of the nonparalyzed muscles in the nonfatigued and fatigued conditions, respectively. There was no main effect of condition or an interaction effect on the twitch-to-tetanus values.

Fitting the peak force responses of each subject to the 4-parameter Hill equation resulted in R^2 values for the nonfatigued and fatigued conditions of 0.988 ± 0.009 and 0.965 ± 0.034 for the subjects with SCI, respectively, and 0.983 ± 0.009 and 0.97 ± 0.018 for the control subjects, respectively. The results of the 2-way, repeated-measures ANOVA of c , the frequency that produced 50% of the maximum force (F50), revealed a main effect of condition ($F=66.62$, $P<.001$) and a strong trend for a main effect of group ($F=4.01$, $P=.071$). However, there was also an interaction effect ($F=5.30$, $P<.05$). Consequently, because of the interaction effect, paired t tests were used to compare the 2 groups within each condition and the 2

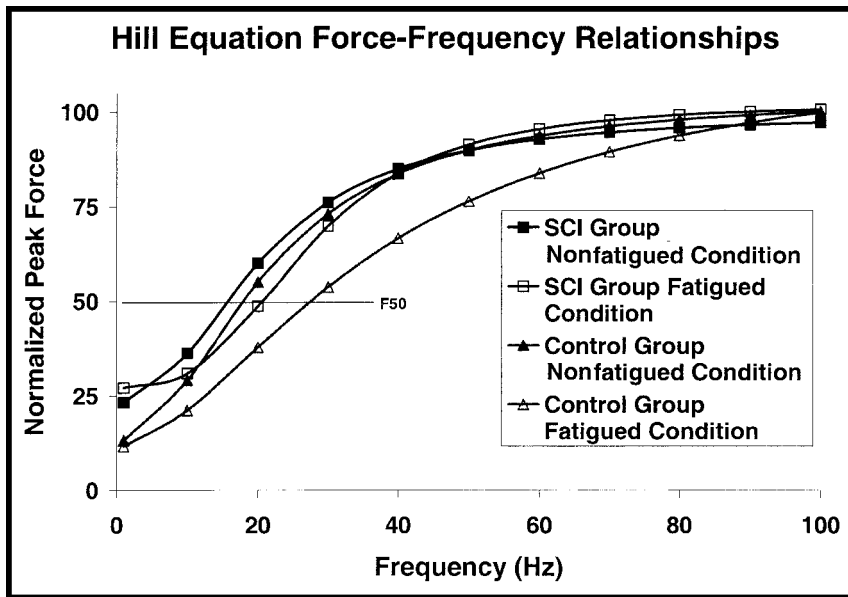


Figure 3. Force-frequency relationships of the subjects with spinal cord injury (SCI) and control subjects in the nonfatigued and fatigued conditions based on the 4-parameter Hill equation. The 4 parameters (α , b , c , and γ_0) were calculated as group means ($n=12$) for each condition and then used to calculate the normalized peak force at each of the frequencies tested. F50=frequency that produced 50% of the maximum tetanic force.

conditions within each group. The F50 was greater in the fatigued condition as compared with the nonfatigued condition, indicating a rightward shift in the FFR for both the SCI group (20.7 ± 1.6 versus 28.7 ± 2.3 Hz) and the control group (21.6 ± 2.0 versus 37.7 ± 2.2 Hz) ($P < .005$ and $P < .001$, respectively). The SCI group had a significantly lower F50 (28.7 ± 2.3 versus 37.7 ± 2.2 Hz), which indicates that their FFR was to the left of the FFR of the control group in the fatigued condition $P < .01$. There was no difference in the nonfatigued F50s of the 2 groups (Fig. 4B).

Discussion and Conclusion

In this study, we demonstrated that, consistent with previous reports on the effects of paralysis on the adult human quadriceps femoris muscle,^{8,20,26} the paralyzed quadriceps femoris muscle of adolescents and young adults with SCI contracts and relaxes faster and is weaker and less resistant to fatigue than its nonparalyzed counterpart. Furthermore, we confirmed the findings of Gerrits and colleagues⁸ that the nonfatigued paralyzed human quadriceps femoris muscle has a greatly elevated twitch-to-tetanus ratio, but we did not observe a significant leftward shift of the nonfatigued FFR of the paralyzed muscle as they did. For the first time, we showed that, for subjects with SCI as compared with control subjects, the twitch-to-tetanus ratio remains elevated when the quadriceps femoris muscles are fatigued. Finally, we showed that higher frequencies are required to produce the same normalized force output from

fatigued quadriceps femoris muscles as compared with the nonfatigued condition, with a rightward shift of the FFR following fatigue being larger in the nonparalyzed muscles than in the paralyzed muscles.

Contractile Properties

The lower PTF of the subjects with SCI suggests that their paralyzed muscles were weaker than the nonparalyzed muscles of the control subjects, as has been reported previously.^{8,16,17,20,24} The use of the peak twitch to quantify the force-producing capability of the quadriceps femoris muscles probably caused an underestimation of the actual weakness resulting from paralysis of the human quadriceps femoris muscle because of the greatly elevated twitch response of the paralyzed muscle we observed. We observed the paralyzed muscles producing 62% of the PTF of the nonparalyzed muscles. The PTFs and the twitch-to-tetanus ratios of the 2 groups can be used to estimate that

the subjects with SCI were capable of producing only approximately 35% of the maximum tetanic force of the control subjects.

Our observation of decreased fatigue resistance following paralysis of the human quadriceps femoris muscle is consistent with previous reports from the literature; however, direct comparisons are problematic due to methodological differences in the stimulation parameters used to produce fatigue.^{8,20,35} The elevated twitch response of the paralyzed human quadriceps femoris that we observed and the method that we used to set the stimulation intensity for this study may affect the interpretation of the decreased fatigue resistance that we observed in the subjects with SCI. We may have been activating a greater percentage of the quadriceps femoris muscles of the subjects with SCI than the control subjects with the 1-second, 20-Hz train we used to set the stimulation intensity. Although it is generally recognized that higher forces result in greater fatigue during intermittent electrical stimulation,³⁴ this conclusion is based on activating a consistent percentage of the muscle or motor units at different force levels (ie, recruitment is static, frequency or train duration is varied). Adams and colleagues³⁶ have shown that when different percentages of the muscle are activated with the same electrical stimulation parameters (ie, recruitment varies, frequency and train duration are static), similar levels of fatigue are produced. This finding suggests that even if the 1-second 20-Hz stimulation train that we used to set

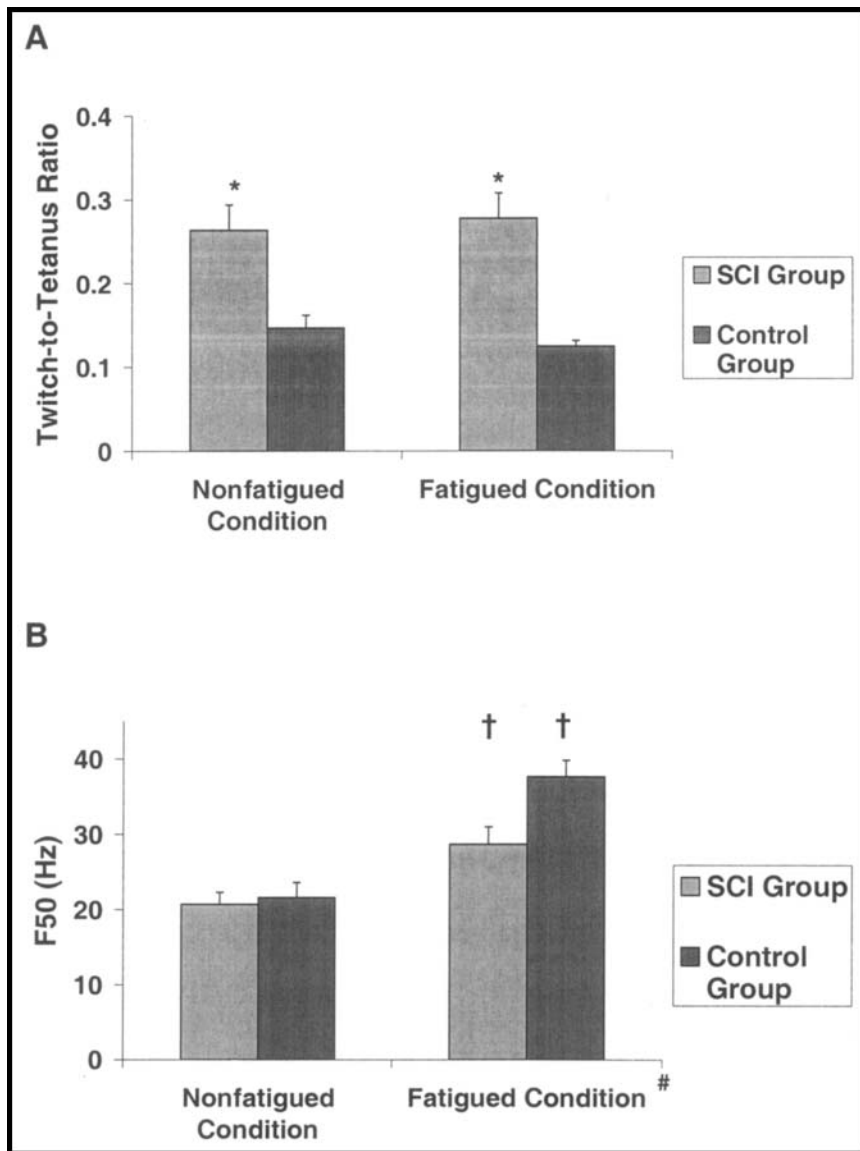


Figure 4. Mean (\pm SE, $n=12$) twitch-to-tetanus ratios (A) and frequency that produced 50% of the maximum tetanic force (F50) (B) for the subjects with spinal cord injury (SCI) and control subjects in the nonfatigued and fatigued conditions. For the twitch-to-tetanus ratios, there was a main effect of group but no effect of condition or interaction effect. Asterisk (*) indicates twitch-to-tetanus ratio for SCI group significantly different from twitch-to-tetanus ratio for control group ($P<.001$). For the F50, paired t tests were used to compare groups within conditions and conditions within groups. The F50 was determined by c from the fitting of the 4-parameter Hill equation to the normalized peak forces. Dagger (†) indicates F50 was significantly different in the nonfatigued and fatigued conditions for the SCI and control groups ($P<.01$ and $.001$, respectively). Number sign (#) indicates F50 was significantly different in the fatigued condition for the SCI and control groups ($P<.01$).

the stimulation intensity was recruiting a greater percentage of the paralyzed muscle because the PTF was relatively greater in the subjects with SCI, both groups should have demonstrated similar levels of decline in force if their resistance to fatigue were similar. At the end of the fatigue protocol, the control subjects were producing 58% of their initial force, whereas the sub-

jects with SCI were producing only 38% of their initial force. Although we did not biopsy the paralyzed muscles to examine their protein content, our observations of faster contraction and relaxation times from the paralyzed muscles are consistent with a shift toward faster MHC and SERCA isoforms.

Twitch-to-Tetanus Ratios and Force-Frequency Relationship

Gerrits and colleagues⁸ reported an 80% increase in the normalized (to the 100-Hz tetanic force) twitch force of the nonfatigued paralyzed human quadriceps femoris muscle. We report a similar difference (84%), although the actual twitch-to-tetanus ratios are different. The twitch-to-tetanus ratios of the subjects with SCI and control subjects that Gerrits and colleagues⁸ reported (0.18 versus 0.10, respectively) were lower than those that we observed (0.26 versus 0.14, respectively). This disparity could be accounted for by methodological differences or different characteristics (eg, age, time since injury) of the samples studied. Interestingly, in a subsequent study that examined the effects of training on the contractile properties of the paralyzed quadriceps femoris muscle, Gerrits and colleagues³² reported a pretraining twitch-to-tetanus ratio of 0.27, which is similar to our observation. We believe that our study is the first to report that the twitch-to-tetanus ratio of the paralyzed quadriceps femoris muscle is greater than that of the nonparalyzed muscle in a fatigued condition.

An elevated twitch-to-tetanus ratio may be a general phenomenon that occurs following decreased contractile activity of human muscle. It has been observed by Seki and colleagues³⁷ in the human first dorsal interosseous muscle following 6 weeks of immobilization, as well as in the human paralyzed thenar muscles¹⁵ and tibialis anterior muscle.¹⁷ Although it may be attractive to attribute an elevated twitch-to-tetanus ratio to a shift in fiber type toward the fast-twitch phenotype, the studies of Gerrits and colleagues^{32,33} that showed a decrease in the twitch-to-tetanus ratios of the paralyzed quadriceps femoris muscle following training did not show an

associated decrease in the maximum rate of force rise as would be expected with a shift toward slow-twitch fibers.

Two additional possible explanations for the increased twitch-to-tetanus ratio of paralyzed muscle are changes in muscle stiffness and changes in muscle length. If the paralyzed muscles become less compliant following paralysis because of connective tissue changes,³⁸ less cross-bridge activity may be required to take up the series-elastic component of the muscle during the twitch and, therefore, would contribute to the force generated by the paralyzed muscle to a greater extent than in nonparalyzed muscle. Another explanation could be that the chronic inactivity following paralysis leads to shortening of the muscle. Consequently, although the isometric force responses of both the subjects with SCI and the control subjects were recorded at the same joint angle in this study, the 90-degree angle may have placed the sarcomeres of the paralyzed quadriceps femoris muscle in a relatively lengthened position in the subjects with SCI. When muscles are activated submaximally, increased sarcomere length leads to increased Ca^{2+} sensitivity, probably due to increased cross-bridge kinetics as a result of decreased intermyofibrillar spacing.³⁹ The increased Ca^{2+} sensitivity associated with greater sarcomere length decreases as the force produced goes from submaximal to the maximum tetanic response. Consequently, a leftward shift in the force- pCa^{2+} curve due to the paralyzed muscle being activated in a relatively lengthened position could explain the increased twitch-to-tetanus ratio we observed.

Based on the work of Gerrits and colleagues⁸ from the paralyzed human quadriceps femoris muscle, we expected to observe a lower F50 for the subjects with SCI as compared with the control subjects in the non-fatigued condition, but we did not. Our failure to observe a lower F50 value in the nonfatigued condition was not the result of methodological differences in determining the value of the subjects' F50. Although we used c from the 4-parameter Hill equation to obtain the F50 for each subject, interpolating the F50 values from each subject's normalized FFR curve as Gerrits and colleagues⁸ did also failed to produce F50s that were significantly different (data not presented). The most likely explanation for this discrepancy is probably the small sample sizes used in both our study and the study by Gerrits and colleagues ($n=7$), coupled with the fact that paralyzed muscles display a large amount of variability in their contractile responses.³⁵ This inherent variability was probably exacerbated in our study because our subjects displayed large variability in time from injury, training of their paralyzed muscle, and degree of physical maturation both at the time of injury and during testing.

We are unaware of other studies that have examined the FFR of the paralyzed quadriceps femoris muscle in a fatigued condition. Our findings show that the subjects with SCI as compared with the control subjects have a lower F50 (28.7 ± 2.3 Hz versus 37.7 ± 2.2 Hz) when the muscles are fatigued. It is unlikely that contractile speeds of the fatigued muscles play a role in this difference. The $\frac{1}{2}\text{RT}$ of both groups slowed similarly with fatigue, and, as was the case in the nonfatigued condition, both the contraction and $\frac{1}{2}\text{RT}$ times of the subjects with SCI were faster in the fatigued condition. Faster contractile speeds should result in higher, not lower, stimulation frequencies being required in subjects with SCI as compared with control subjects to produce similar normalized forces. Another explanation, in addition to the elevated twitch-to-tetanus ratio that could account for the differences in the F50s of the 2 groups in the fatigued condition, could be different causes of fatigue. It may be the case that the paralyzed muscles of subjects with SCI do not experience failure of excitation-contraction coupling to the extent of the nonparalyzed muscles. Failure of excitation-contraction coupling would require higher stimulation frequencies to elevate the myoplasmic Ca^{2+} concentration in response to a stimulation train.⁴⁰ This would suggest that failure at the level of the cross-bridges may play a relatively greater role than excitation-contraction coupling failure in the fatigue produced in the paralyzed muscles as compared with the fatigue produced in the nonparalyzed muscles.

This study also extends previous findings of a rightward shift in the FFR with fatigue of the quadriceps femoris muscles of control subjects^{3,4,41} to the paralyzed quadriceps femoris muscles of subjects with SCI. That is, with fatigue, higher frequencies are required to generate the same relative force from both the nonparalyzed and paralyzed muscles as compared with their respective nonfatigued FFRs, as indicated by the increase in the F50s from 21.6 ± 2.0 to 37.7 ± 2.2 Hz for the control subjects and 20.7 ± 1.6 to 28.7 ± 2.3 Hz for the subjects with SCI. It has been theorized that the slowing of contractile speeds with fatigue would allow the force in response to each pulse of a stimulation train to fuse to a greater extent, resulting in a leftward shift in the FFR.⁴¹ Clearly, slowing of contractile speeds, which we observed for the $\frac{1}{2}\text{RT}$ of both groups, is not the primary determinant of the relative relationship of the nonfatigued and fatigued FFRs of the human quadriceps femoris muscle. More likely, impairments in excitation-contraction coupling led to decreased Ca^{2+} release from the sarcoplasmic reticulum with fatigue, resulting in higher frequencies being required to produce similar normalized forces.⁴⁰

Our results showed that the subjects with SCI were in a greater state of fatigue than the control subjects (fatigue ratio of 0.38 versus 0.58, respectively). As we observed, the FFR shifts toward the right with fatigue for both groups. Consequently, it is possible that if the muscles of the control subjects had been fatigued to a similar level as the muscles of the subjects with SCI, we might have seen an even greater shift to the right of the control subjects' fatigued FFR relative to their nonfatigued FFR. This would have had the effect of increasing the relative leftward shift of the FFR of the subjects with SCI as compared with the FFR of the control subjects in the fatigued condition. Despite the greater fatigue generated in the subjects with SCI, the F50 of the subjects with SCI only increased by 39% with fatigue, as compared with 75% in the control subjects. The fact that there was a smaller rightward shift in the FFR of the subjects with SCI as compared with the control subjects with fatigue suggests that excitation-contraction coupling failure may be a less important component of fatigue in paralyzed quadriceps femoris muscles as compared with nonparalyzed quadriceps femoris muscles.

The most important findings of this study were that the nonfatigued elevated twitch-to-tetanus ratio of paralyzed skeletal muscle versus muscles of control subjects remains when the muscles are fatigued and that a significant smaller rightward shift of the FFR of the paralyzed versus nonparalyzed quadriceps femoris muscles exists when the muscles are fatigued. The clinical implications of this work relate to the stimulation frequencies that should be used to activate muscle during FES or training of paralyzed muscles. The paralyzed human quadriceps femoris muscle appears to require lower frequencies to produce equivalent relative forces as compared with nonparalyzed quadriceps femoris muscle when fatigued. Furthermore, with fatigue, stimulation frequency must be increased to produce the same relative force from nonparalyzed and paralyzed muscle as compared with the nonfatigued condition. The latter observation is particularly important in FES and training protocols, which almost invariably require muscle fatigue to be countered to continue adequate performance.

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