Low-Frequency Fatigue in Individuals With Spinal Cord Injury

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Abstract

Background/Objective: This study examined magnitude and recovery of low-frequency fatigue (LFF) in the quadriceps after electrically stimulated contractions in spinal cord-injured (SCI) and able-bodied subjects.

Subjects: Nine SCI (ASIA A–C, levels C5–T9, injured 13.6 ± 12.2 years) and 9 sedentary able-bodied subjects completed this study.

Methods: Fatigue was evoked in 1 thigh, and the nonfatigued leg served as a control. The fatigue test for able-bodied subjects lasted 15 minutes. For SCI, stimulation was adjusted so that the relative drop in force was matched to the able-bodied group. Force was assessed at 20 (P20) and 100 Hz (P100), and the ratio of P20/P100 was used to evaluate LFF in thighs immediately after, at 10, 20, and 60 minutes, and at 2, 4, 6, and 24 hours after a fatigue test.

Results: The magnitude of LFF (up to 1 hour after fatigue) was not different between able-bodied and patients with SCI. However, recovery of LFF over 24 hours was greater in able-bodied compared with patients with SCI in both the experimental ($P < 0.001$) and control legs ($P < 0.001$). The able-bodied group showed a gradual recovery of LFF over time in the experimental leg, whereas the SCI group did not.

Conclusions: These results show that individuals with SCI are more susceptible to LFF than able-bodied subjects. In SCI, simply assessing LFF produced considerable LFF and accounted for a substantial portion of the response. We propose that muscle injury is causing the dramatic LFF in SCI, and future studies are needed to test whether “fatigue” in SCI is actually confounded by the effects of muscle injury.

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INTRODUCTION

Spinal cord injury (SCI) leads to dramatic alterations in skeletal muscle morphology and function depending on the level of injury. The use of neuromuscular electrical stimulation (NMES) is commonly used in individuals with SCI to facilitate contraction of the paralyzed musculature for both rehabilitation and fitness conditioning. It is well established that the use of NMES requires greater energy demand and consequently leads to a disproportionate amount of fatigue compared with similar voluntary efforts because of differences in recruitment of skeletal muscle (1–4). In addition, muscle fatigue has been

reported to be greater in individuals with SCI during NMES-evoked contractions compared with able-bodied controls (5–8). The affected musculature of SCI can undergo dramatic losses in force during NMES-evoked contractions, equating two- to fourfold greater relative force loss than able-bodied subjects (6,7).

Although a great deal of literature exists regarding fatigue during NMES-evoked contractions in patients with SCI and able-bodied subjects, few studies have examined recovery of muscular force after a fatiguing bout of exercise. After exercise, prolonged decrements in muscular force have been documented and occur mainly at low frequencies of electrical stimulation (9,10). This has appropriately been termed low-frequency fatigue (LFF) and is defined as a preferential loss of force at low activation frequencies (ie, 20 Hz) compared with high frequencies (ie, 100 Hz). LFF is commonly assessed by the ratio of force produced at low and high activation

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frequencies after muscular fatigue (9,11–13). Although the exact mechanism is unclear, LFF is thought to be caused by impaired excitation–contraction coupling with evidence specifically pointing to reduced calcium release from the sarcoplasmic reticulum at low stimulation frequencies after fatigue (9,14–16).

After muscular contractions, LFF is evident in both fast and slow twitch muscle fibers of animals and humans (17). However, it has been reported that fast-glycolytic fibers show greater LFF than do fast-oxidative fibers (18,19). Powers and Binder (18) reported that fatigue-resistant motor units from cat flexor digitorum muscles exhibited less pronounced LFF than fast intermediate and fast-fatigable motor units after electrically stimulated contractions. “Slow to fast” fiber conversion has been shown to occur 1 to 2 years after SCI with increased expression of myosin heavy-chain IIa and IIx (20,21) and faster contraction speeds (22,23). This shift to predominantly fast-twitch muscle with SCI may predispose them to higher levels of LFF after contractions.

LFF has been shown to be influenced by metabolic changes within muscle (24). Pronounced fatigue causes large increases in inorganic phosphate and hydrogen ions. It is hypothesized that high levels of inorganic phosphate may be taken up into the sarcoplasmic reticulum, where it may precipitate with calcium (14). The formation of calcium phosphate would lower free calcium concentrations in the sarcoplasmic reticulum, thus reducing subsequent calcium release. A study by McCully et al (24) determined that protocols that elicit high levels of fatigue, and therefore metabolic byproducts, can increase the magnitude of LFF observed during recovery.

LFF is more pronounced when muscle injury has occurred (19,25–27). Because the origin of LFF has been attributed to disruption in the excitation–contraction coupling process, studies have shown that muscle injury can impair calcium release/reuptake rates and affect muscular force (28), most predominately at low activation frequencies (27). Unloaded skeletal muscle, as in SCI, has been shown to be more susceptible to muscle injury upon reloading (5,29–33). A study by Bickel et al (5) showed that individuals with long-term SCI had significant muscle injury in the quadriceps muscle after NMES-evoked isometric contractions, whereas able-bodied subjects had essentially no muscle injury. Because SCI have greater metabolic impairment, increased risk of contraction-induced muscle injury, as well as a near complete transformation from slow to fast-twitch muscle fibers, it seems plausible that they would have greater LFF after contractions, compared to sedentary, ambulatory individuals. LFF has clinical implications in the SCI population who may use low-frequency NMES daily for rehabilitation to make weak or paralyzed muscles contract. In addition, many electrically stimulated exercise modalities used in the SCI population are evoked by low-frequency NMES, such as ambulation and cycling. This is important as long-lasting reductions in muscle

force at low activation frequencies could severely limit rehabilitation and/or fitness goals.

To our knowledge, no studies have examined LFF between able-bodied subjects and individuals with SCI. The aim of this study was to examine the magnitude and recovery of LFF between SCI and able-bodied subjects after a fatigue protocol designed to cause the same mean force reduction in both groups. We hypothesize that able-bodied subjects will have less magnitude of LFF and a faster recovery of LFF compared with patients with SCI.

METHODS

Subjects

Nine SCI (2 women) and 9 able-bodied (4 women) subjects participated in this study. Motor and sensory function of SCI participants was previously assessed by the American Spinal Association (ASIA) classification system. All SCI participants were nonambulatory and consisted of those with complete and incomplete spinal lesions ranging from C5 to T9. Three of these 4 individuals had limited voluntary motor function in the thigh area and were able to extend 1 or both knees with maximal isometric forces less than ~9 kg. Mean duration of spinal injury was 13.6 ± 12.2 years. Only 1 person with SCI had used NMES to evoke contractions of the thighs in the previous 6 months before taking part in this study. Able-bodied subjects were relatively sedentary and had not performed any regular exercise 1 year before testing.

The participants gave written consent before testing. This study was approved by the Institutional Review Boards of the University of Georgia and Shepherd Center. All subjects were asked to refrain from intense or unaccustomed exercise 24 hours before testing and to remain relatively inactive for the total duration of testing. In addition, subjects were asked to abstain from caffeine on testing days as it has been shown to affect muscular force at low activation frequencies (34).

Force Measurements

For isometric contractions of the thigh, subjects were seated in a custom-built force chair with the hip and knee secured at approximately 70° of flexion. Both legs were firmly secured to a rigid lever arm with an inelastic strap to ensure that the knee extensors could only perform isometric contractions. The moment arm was kept the same for all subjects tested and was established by placing a load cell (model 2000A; Rice Lake Weighing Systems, West Coleman Street, Rice Lake, WI) parallel to the line of pull and perpendicular to the lever arm. Force was recorded from the load cell using a MacLab A-D converter (model ML 400; AD Instruments, Milford, MA) sampling at 100 Hz and interfaced with a portable Macintosh computer (Apple Computer, Cupertino, CA). All force tracings were displayed and recorded on a computer.

Electrical Stimulation (NMES)

A high-voltage electrical stimulation unit (Rich-Mar Theratouch 4.7) was used to assess LFF and to evoke contractions during fatigue protocols. Stimulation for assessment of LFF and performance of fatigue tests was delivered through 2 electrodes (8×10 cm) to evoke isometric contractions of the quadriceps femoris muscle group. One electrode was placed 2 to 3 cm above the superior aspect of the patella over the vastus medialis muscle. The second electrode was placed lateral to and 30 cm above the patella over the vastus lateralis muscle. Permanent ink marker was used to trace the electrodes to ensure the same placement for the 24-hour assessment of LFF or at any other time the electrodes were taken off during testing.

LFF was assessed by measuring force elicited at 20 (P20) and 100 Hz (P100). The ratio of P20/P100 was calculated and the percentage reduction in this ratio (from prefatigue values) was used to evaluate LFF (9,35). A greater percent reduction in P20/P100 from prefatigue values indicated greater LFF. P20 and P100 were assessed in the quadriceps femoris muscle group before, immediately after, and at 10, 20, and 60 minutes, as well as 2, 4, 6, and 24 hours after an NMES-evoked fatigue protocol. At each assessment of LFF, the quadriceps femoris muscle group was given two 1-second contractions at 20 Hz followed immediately by two 1-second contractions at 100 Hz. These contractions were elicited to potentiate the muscle. Approximately 10 seconds after these 4 contractions, a 1-second contraction at 20 Hz was given followed immediately by a 1-second contraction at 100 Hz for the assessment of LFF, and these contractions will be referred to as "evaluation stimulations." These 1-second contractions at 20 and 100 Hz were elicited 3 times for each assessment of LFF. In essence, over the 24-hour assessment of LFF, 45 contractions were elicited at both 20 and 100 Hz for a total of 90 contractions for both the experimental and control leg.

Fatigue tests for both SCI and able-bodied subjects were elicited through NMES. All contractions during the fatigue tests were evoked with 30-Hz trains of 450- μ s biphasic pulses with a 33% duty cycle (3 seconds on/6 seconds off). This protocol was chosen as longer contractions have been shown to elicit greater LFF compared with shorter ones (10). The fatigue test was evoked in 1 thigh (experimental leg). The contralateral, unfatigued leg served as control. The experimental leg was assigned by counterbalancing dominant/nondominant leg for each subject. The measurements of P20 and P100 were assessed in both thighs.

Experimental Protocol

Familiarization. Subjects were seated in the custom-designed isometric force chair, and their legs and torso were firmly strapped into the chair. Subject's legs were cleaned with alcohol, and 2 electrodes were placed on each thigh as previously described. Electrical stimulation

at a frequency of 100 Hz was given to evoke 1-to 3-second isometric contractions of the knee extensors at increasing current levels until 22.7 kg of isometric force was produced in able-bodied subjects. If this level of NMES current was tolerated well and subjects were still interested in participation, they were asked to return to the laboratory 1 to 2 days later for testing. For participants with SCI, the current was increased until they could produce a minimal of 4.5 kg of isometric force with maximum not exceeding 18 kg. Force in the SCI group was kept less than 18 kg to substantially reduce the risk of fracture when performing isometric contractions (36). Force values in able-bodied subjects were not based on a percentage of maximal voluntary contraction. This study used low absolute force values in each group as individuals with long-term SCI have substantial muscle atrophy and typically produce low force values and this allowed for a better comparison of the LFF response between these 2 groups.

Test Session. Subjects were seated in the custom-designed isometric force chair, and their legs and torso were firmly strapped into the chair as previously described. Electrical stimulation amplitude at 100 Hz was slowly increased over several 1-second contractions to achieve ~18 to 22.7 kg of isometric force in each thigh for SCI and able-bodied subjects, respectively. After the NMES amplitude was determined, it remained the same in each leg throughout the duration of the test session for the measurements of P20 and P100, as well as for the fatigue test. LFF (ie, P20/P100) was measured before, immediately after, at 10, 20, and 60 minutes, and 2, 4, 6, and 24 hours after the NMES-evoked fatigue test.

For this session, the NMES-evoked fatigue test for able-bodied individuals lasted 15 minutes and consisted of 3-second isometric contractions of the thigh with 6-second rest between contractions (33% duty cycle). This allowed for a total of 100 3-seconds contractions, which were evoked at the same NMES amplitude used for measurement of P20 and P100. All able-bodied subjects were tested first to determine the mean group force loss during this fatigue protocol. The SCI group performed a fatigue test that was terminated when mean force loss was equivalent to that of the able-bodied group (from 15-minute fatigue protocol). This was done in an effort to match relative fatigue between groups and to assess LFF. The duty cycle for the SCI fatigue test was the same as in able-bodied (33%; 3 seconds on/6 seconds off) and ranged from 2 to 8 minutes, which was dependent on individual force reductions in the patients with SCI.

Analysis of Force Tracings. Force tracings for the first and last 3 contractions of the fatigue protocol were analyzed by measuring force-time integrals (FTIs). Fatigue was calculated as follows: Fatigue = [FTI (first 3 contractions) – FTI (last 3 contractions)]/FTI (first 3 contractions). Single contractions for the assessment of P20 and P100 were always 1 second in duration. For each force tracing at 20 and 100 Hz, the average value for the

Table 1. Individual and Mean Data for Participants With SCI

SCI Classification	Subject	Sex	Injury Level	Duration of Injury (years)	Age (years)	Body mass (kg)
ASIA A	1	Male	T9	8.0	30	63.6
	2	Male	T7	3.9	32	79.5
	3	Male	T8	24.2	40	70.5
	4	Male	T6	25.0	43	84.1
ASIA B	5	Male	C6	3.5	41	86.4
	6	Female	T9	21.4	40	43.2
ASIA C	7	Female	C5	2.9	19	47.7
	8	Male	C6	32.9	48	90.9
	0	Male	C6	0.5	18	61.4
	Mean	NA	NA	13.6	34.6	69.7
	SD	NA	NA	12.2	10.6	17.0

ASIA (American Spinal Injury Association Classification 1992) score is used to classify completeness of the spinal lesion: A, motor and sensory complete; B, sensory incomplete but motor complete; C, sensory and motor incomplete but no functional motor activity. NA, not applicable.

first 0.5 seconds after the initial rise was analyzed. The highest mean force at 20 and 100 Hz was used in the calculation of the P20/P100 ratio at each time-point.

Statistical Analyses

The SCI subject population in this study consisted of individuals with both complete and incomplete spinal injuries. Because we found no prior statistically significant differences in LFF results between complete and incomplete subjects, the entire SCI population was combined for comparison with able-bodied subjects.

Data analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL). Independent *t* tests were used to assess potential differences between the groups in subject age, body mass, and percent force loss during NMES-evoked fatigue protocols. In the *t* test analyses, the homogeneity of variance was tested using Levene test for equality of variance. Statistics were adjusted accordingly if the assumption of equality of variance was violated.

For all analyses of LFF, the ratio of the P20/P100 was calculated for each time-point. These time-points were reported, as the percentage reduction from baseline and from here on will represent LFF. The magnitude of LFF was assessed by the percentage reduction from baseline P20/P100 values up to 1 hour after the fatigue protocol. This time period was selected because it tests our first hypothesis about the magnitude of LFF after the fatigue protocol. The recovery of LFF was examined over the entire 24-hour postfatigue period because prior empirical research suggests that SCI would have muscle injury after NMES-evoked isometric contractions (5), which can dramatically affect LFF (25).

The magnitude of LFF in the experimental leg was analyzed using a 2 (group: SCI, able-bodied) $\times$ 4 (time: 0, 10, 20, and 60 minutes after fatigue) mixed-model ANOVA with a repeated measure on the time variable.

Magnitude of LFF was not examined in the control leg because it did not perform a fatigue protocol.

A separate analysis for recovery of LFF was performed for both the experimental leg and the control leg in each group. Recovery of LFF was analyzed using a 2 (group) $\times$ 8 (time: 0, 10, 20, 60 minutes; 2, 4, 6, and 24 hours) trend analysis to examine differences in the rate of recovery between groups after the fatigue protocol. Because the recovery of LFF was significantly different in the “control leg” between groups, a secondary analysis was performed. This assessment included a 2 (group) $\times$ 8 (time) trend analysis for the experimental leg with control leg LFF values used as covariates. This allowed us to determine what effect the evaluation stimulations had on the LFF response above and beyond that caused by the fatigue protocol.

All mixed model analyses were based on the *F*-statistic. Effects sizes for *F*-statistics were expressed as partial eta-squared (η^2). The Greenhouse-Geisser epsilon (ϵ) was reported, and degrees of freedom were adjusted when the sphericity assumption was violated (ie, if Mauchly test of sphericity was statistically significant at $P < 0.05$). The family-wise error rate was controlled using the Bonferroni adjustment when tests of simple effects were conducted.

RESULTS

Participant Characteristics

All subjects completed this study without incidence. Individual subject characteristics for the SCI group are shown in Table 1. Subjects with SCI were significantly older (34.6 ± 10.6 years) than able-bodied (24.8 ± 5.3 years) subjects ($F_{16} = 5.61$, $P = 0.025$). Body mass for SCI and able-bodied subjects was 69.7 ± 17.0 and 71.5 ± 19.5 kg, respectively, which was not different between groups ($F_{16} = 0.303$, $P = 0.838$).

Figure 1. Representative force tracings (raw data) at 20 and 100 Hz, before and 1 hour after fatigue for 1 participant with SCI.

Force Reduction During NMES-Fatigue Protocols

The fatigue tests in this study were designed match force loss between groups. Mean percentage force loss during the fatigue tests was $-50.3 \pm 9.0\%$ for SCI and $-49.6 \pm 9.5\%$ for able-bodied subjects, which were not significantly different ($F_{16} = 0.010$, $P = 0.870$). The range of percentage force loss was 41% to 62% in the SCI group and 33% to 61% for the able-bodied group. The able-bodied fatigue test lasted 15 minutes and consisted of 100 contractions (3 seconds on/ 6 seconds off). The SCI fatigue test was performed at the same duty cycle (3 seconds on/ 6 seconds off) and lasted an average of 4.3 ± 1.8 minutes, which was dependent on individual force loss in this group. This protocol was used with the SCI group to produce an equivalent force loss as the able-bodied group during the 15-minute fatigue protocol.

Force Tracings. Representative force tracings at 20 and 100 Hz are shown in Figure 1 for 1 participant with SCI before and 1 hour after fatigue. Using the ratio of these forces allowed for quantification of LFF. One hour after the fatigue protocol, 100 Hz force has returned to near baseline values, whereas 20-Hz force is still depressed, indicating substantial LFF.

Magnitude of LFF. The magnitude of LFF, expressed as percent change from baseline P20/P100 ratio values up to 1 hour after fatigue, was examined between groups. In the experimental leg, the analysis showed that a significant group $\times$ time interaction existed ($F_{3,16} = 8.599$, $P < 0.001$, $\eta^2 = 0.350$; Figure 2a). However, when tests for simple effects were performed and adjusted for multiple comparisons, there were no significant differences noted at any time-point. Therefore, we concluded that no differences existed for the magnitude of LFF between groups.

Recovery of LFF. Trend analysis was used to examine differences between groups in the recovery of LFF across the 24-hour postfatigue period in both the experimental and control legs. In the experimental leg, the recovery of LFF was significantly faster in the able-bodied group compared with SCI (Figure 2b). This was based on a significant group by trend interaction for the linear recovery of LFF in the experimental leg over the 24-

Figure 2. (a) Magnitude of LFF was not different in the experimental leg of SCI and able-bodied subjects more than 1 hour after fatigue. Values are mean $\pm$ SD. (b) Recovery of LFF in the experimental leg over the 24-hour postfatigue period between SCI and able-bodied subjects. $^{\dagger}P < 0.001$, group $\times$ trend interaction. Recovery was 18% faster in able-bodied subjects based on differences in slopes between groups.

hour period between groups ($F_{7,128} = 4.91$, $P < 0.001$, $\eta^2 = 0.212$). Decomposition of the interaction showed that able-bodied recovered from LFF in the experimental leg at an 18% greater rate than the SCI group ($t = 5.68$, $P < 0.001$), which was based on differences in slopes for the linear trend between groups. In essence, the SCI group had little or no recovery of LFF over 24 hours.

Similarly in the control leg, the recovery of LFF was significantly faster in the able-bodied group compared with SCI (Figure 3). This was based on a significant group by trend interaction for the linear recovery of LFF in the control leg over the 24-hour period between groups ($F_{7,128} = 2.30$, $P = 0.031$, $\eta^2 = 0.112$). Decomposition of the interaction showed that able-bodied recovered from LFF in the control leg at a 12% greater rate than the SCI group ($t = 3.95$, $P < 0.001$), which was based on differences in slopes for the linear trend between groups.

Because significant group differences were found for the recovery of LFF in the control leg, a secondary analysis was performed. When the experimental leg was

Figure 3. Recovery of LFF in the control leg over the 24-hour postfatigue period in SCI and able-bodied subjects. Values are mean $\pm$ SD. $^{\dagger}P = 0.031$, group $\times$ trend interaction. Recovery was 12% faster in able-bodied subjects based on differences in slopes between groups.

statistically adjusted for control leg LFF values, recovery was not different between able-bodied and SCI individuals (Figure 4). This was based on a lack of significant group by trend interaction for the recovery of LFF over the 24-hour period between groups ($F_{7,127} = 1.98$, $P = 0.063$, $\eta^2 = 0.098$). There was a significant main effect for time ($F_{7,127} = 6.34$, $P < 0.001$, $\eta^2 = 0.259$) but not for group ($F_{1,127} = 0.76$, $P = 0.386$, $\eta^2 = 0.006$) for the recovery of LFF.

Indicator of Muscle Injury. Significant reductions in 100-Hz muscle force at 24 hours (from prefatigue values) were used as an indicator of muscle injury. Force at 100 Hz in the experimental leg was significantly lower ($-30.8 \pm 19.6\%$) in the SCI group at the 24-hour time-point compared with baseline values ($P < 0.001$). However, 100-Hz force in the able-bodied group was not different at 24 hours ($P = 0.354$). This suggests that muscle injury was likely more severe in the SCI compared with the able-bodied group.

DISCUSSION

For this study, participants were administered an NMES fatigue test that matched relative force loss between SCI and able-bodied subjects and LFF was assessed up to 24 hours after fatigue. The fatigue test lasted 15 minutes in the able-bodied group, and this group had a mean reduction in force of $\sim 50\%$. As expected, fatigue in SCI was rapid, and as a group, they achieved $\sim 50\%$ drop in force in only 4.3 minutes. Although not related to LFF, one can appreciate the effects of unloading and disuse and how it can impact muscle force during repeated contractions.

We were unable to accept our first hypothesis, as our results indicate that no differences were found between SCI and able-bodied subjects for the magnitude of LFF (up to 1 hours after fatigue). This was an unexpected finding as we predicted that even with the same force loss, the SCI group would have a greater metabolic impairment, which might cause the magnitude of LFF to be greater in

Figure 4. Recovery of LFF in the experimental leg when statistically adjusted for LFF in the control leg of SCI and able-bodied subjects. Values are mean $\pm$ SD. When LFF values in the control leg were used as covariates, no difference in recovery rate was observed between groups ($P = 0.064$; $\eta = 0.098$).

this group. We predicted that the extreme disuse and unloading of paralyzed muscle would cause the magnitude of LFF to be more severe than normally loaded muscle, but our results did not support this hypothesis.

To our knowledge, no one has examined the differences in LFF between able-bodied and SCI subjects. However, Shields et al (37) examined reductions in LFF (ie, 20 Hz) in the soleus muscle of chronic (>3 years after injury) and acute SCI (<5 weeks after injury) after a 3-minute bout of intermittent contractions. Immediately after exercise, 20-Hz force was reduced by 75% in the chronic SCI but only 16% in acute SCI group. After 5 minute after fatigue, 20-Hz force had completely recovered in the acute SCI group but was still suppressed nearly 60% in the chronically injured group. Direct comparisons to the study of Shields et al (37) is difficult because of differences in the subject groups tested and because they caused extremely different amounts of fatigue in these 2 groups on examining LFF. A strength of this study is that we examined LFF after causing the same amount of force loss (from fatigue test) in both groups. It is possible that if the SCI group performed the same 15-minute fatigue protocol as the able-bodied group that the magnitude of LFF may have been different between groups.

We were able to accept our second hypothesis that able-bodied subjects would recover faster from LFF. Our results showed that when fatigue was matched between groups that the recovery of LFF in the experimental leg of the able-bodied group was significantly faster ($\sim 18\%$) than the SCI group, who essentially had no recovery over the 24-hour postfatigue period. In the control leg, the able-bodied group showed small amounts of LFF that remained constant over the time of the experiment, whereas SCI showed a progressive appearance of LFF that nearly matched the LFF in the experimental leg by 24 hours. However, when the experimental leg LFF was statistically adjusted for LFF in the control leg, recovery of

LFF was not different between groups (Figure 4). This suggests that a large portion of the recovery from LFF in the SCI group can be attributed to the evaluation stimulations used to assess LFF. In the control leg of SCI (Figure 3), each additional set of evaluation stimulations caused a further reduction in the P20/P100 ratio (greater LFF). In the SCI experimental leg (Figure 2b), however, it seemed there was a “basement effect,” and once the P20/P100 ratio was suppressed ~50%, each additional set of evaluation stimulations did not cause a further reduction in this ratio. Therefore, it seems that both the fatigue protocol and evaluation stimulations affect LFF in the SCI group but their relative impact cannot be determined in this study. Regardless, our results show definitively that if NMES is used to contract paralyzed muscle repeatedly (ie, fatigue test in experimental leg) or used periodically over time (ie, evaluation stimulations) that LFF will be severe and the recovery will be minimal over 24 hours.

The proposed physiologic mechanism for the almost nonexistent recovery of LFF in the SCI group is probably related to contraction-induced muscle injury. It has been shown in several studies that LFF is more severe when prior muscle injury has occurred (19,25–27). For example, Child et al (25) examined the recovery of LFF after a bout of eccentric contractions of the thigh, which were evoked by NMES and caused significant muscle pain and injury. They showed that subjects had significant LFF up to 3 days after this type of exercise. Because the origin of LFF has been attributed to disruption in the excitation–contraction coupling process, disruption of myofibers with injury can impair calcium release/reuptake rates and affect muscular force (28), most predominately at low activation frequencies (27).

Unloaded skeletal muscle, as in SCI, has been shown to be more susceptible to muscle injury upon reloading (5,29–33). Models of extreme unloading have consisted of immobilization, hind limb suspension, and space flight. A more severe model of unloading occurs in those with paralysis, which results in complete inactivation of skeletal muscle below the point of spinal lesion. A previous study from our laboratory showed that individuals with SCI are more susceptible to muscle injury than able-bodied subjects (5). In a study by Bickel et al (5), T2-weighted magnetic resonance images were taken of the quadriceps femoris muscle group before and 3 days after 80 NMES-evoked isometric contractions of the thigh in both able-bodied and SCI subjects. Three days after exercise, there was a greater relative area of muscle injured for the SCI group (25%) compared with able-bodied (2%). In addition, they showed that NMES-evoked force was reduced by 22% 3 days after exercise in SCI, whereas able-bodied force was not different from baseline values.

Although this study did not quantify muscle injury, it likely played a role in the almost nonexistent recovery of LFF in the SCI group, which was most evident from the data collected in the control leg. In the control leg, approximately ninety 1-second contractions were per-

formed from pre- to 24 hours after fatigue, which was more than were evoked in the study by Bickel et al (5). In addition to these ninety 1-second contractions, the experimental leg performed a fatigue protocol that consisted of at least nineteen 3-second contractions, which was dependent on individual fatigue values. Based on the findings by Bickel et al (5), it is probable that our SCI subjects incurred muscle injury in both the experimental and control leg. In addition, the SCI group had a significant reduction (~31%) in 100-Hz force in the experimental leg at 24 hours after fatigue, which can be best explained by the long-lasting effects of muscle injury. Even more dramatic was the 20-Hz force loss in the SCI group (experimental leg) at 24 hours, which equated to ~66% reduction from baseline values (data not shown). These findings support our hypothesis that muscle injury is likely unavoidable when using NMES in previously untrained paralyzed muscle and probably leads to the dramatic LFF observed in this study.

It seems plausible that LFF in individuals with SCI would be less pronounced with consistent NMES training, possibly by reducing muscle injury. This phenomenon of less muscle injury with subsequent bouts of similar exercise has been reported widely in the able-bodied literature and is commonly known as a “repeat bout” effect. Sabatier et al (38) used NMES resistance training of the thigh muscles in individuals with SCI and showed increases in fatigue resistance after 12 weeks of training. The subjects performed only 80 dynamic knee extensions per week, which significantly improved muscle size but was not expected to cause reduced fatigability. These researchers proposed that as training progressed muscle injury occurring during contractions was reduced and therefore caused less force loss during a bout of contractions. This potential reduction in muscle injury with training in the SCI population would likely reduce the amount of LFF. Experimental evidence to support this hypothesis comes from a study showing that 10 weeks of eccentric training in rats significantly reduced LFF after a fatiguing bout of exercise (35). Future studies need to examine how NMES-evoked training in paralyzed muscle might affect LFF.

In addition to muscle injury, fast-twitch muscle fibers have been reported to be more susceptible to LFF (18,19). Numerous studies have shown “slow to fast” fiber conversion after 1 to 2 years of SCI with increased expression of myosin heavy-chain IIa and IIx (20,21) and faster contraction speeds (22,23). Muscle biopsy data from SCI subjects 2 to 11 years after injury indicate a significantly lower percentage of slow-twitch fibers compared with able-bodied controls (39). Although not examined in this study, greater percentages of fast-twitch muscle fibers in the SCI group may have partially contributed to the LFF observed in this group.

Several potential limitations exist for this study. First, surface electrical stimulation was used, and changes in the properties of the electrodes and/or the skin over the

course of the study may have altered conductance of electrical current and generation of muscular force. Subjects were asked to remain relatively inactive over the course of the day, and the electrodes were traced to ensure the same placement for the 24-hour assessment of LFF or at any other time the electrodes were taken off during testing. Second, this study used submaximal electrical current to evoke muscular contractions of the quadriceps muscle, and therefore, only small portions of this muscle group were likely recruited. For the SCI group, this was done to keep forces relatively low in to reduce the risk of fracture in this population (36). For the able-bodied group, submaximal current was used mainly to limit the pain associated with its use in individuals with normal sensory function. Future studies may want to stimulate smaller muscles to maximize the chance of activating the entire muscle. Despite increased variability that may have occurred because of testing methods, we feel that the differences observed between these groups for recovery of LFF are indeed “real” and the robust response of the SCI group is truly remarkable.

CONCLUSION

This study showed that the magnitude of LFF after a matched fatigue was not different between SCI and able-bodied subjects. Over the 24-hour postfatigue period, LFF in able-bodied subjects recovered toward baseline values, whereas no recovery was observed in SCI. More importantly, simply assessing LFF with NMES in paralyzed muscle caused a progressive increase in LFF and accounted for a substantial portion of the response over time. We propose that muscle injury is the main factor responsible for the incomplete recovery of LFF in the SCI group and future studies need to test whether “fatigue” in SCI is actually confounded by the effects of muscle injury.

LFF has clinical implications for those with SCI and various other neuromuscular disorders. These patient populations may use low-frequency NMES daily for rehabilitation to make weak or paralyzed muscles contract. In addition, many electrically stimulated exercise modalities used in the SCI population are evoked by low-frequency NMES. From this study, it is apparent that once the paralyzed muscle is stimulated sufficiently, forces at low frequencies will be substantially suppressed for at least 24 hours or longer, which could potentially limit rehabilitation and/or fitness goals.

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