Effects of activation frequency and force on low-frequency fatigue in human skeletal muscle

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Binder-Macleod, Stuart A., and David W. Russ. Effects of activation frequency and force on low-frequency fatigue in human skeletal muscle. J. Appl. Physiol. 86(4): 1337–1346, 1999.—No comparison of the amount of low-frequency fatigue (LFF) produced by different activation frequencies exists, although frequencies ranging from 10 to 100 Hz have been used to induce LFF. The quadriceps femoris of 11 healthy subjects were tested in 5 separate sessions. In each session, the muscle was stimulated before and after fatigue and at 2, ~13, and ~38 min of recovery. Brief (6-pulse), constant-frequency trains of 9.1, 14.3, 33.3, and 100 Hz and a 6-pulse, variable-frequency train with a mean frequency of 14.3 Hz were delivered at 1 train/s to induce fatigue. Immediately postfatigue, there was a significant effect of the stimulation protocol frequency. Muscles exhibited greater LFF after stimulation with the 9.1-, 14.3-, and variable-frequency trains. These three trains also produced the greatest mean force-time integrals during the fatigue test. At 2, ~13, and ~38 min of recovery, however, the LFF produced was independent of the fatiguing protocol frequency. The findings are consistent with theories suggesting two independent mechanisms behind LFF and may help identify the optimal activation pattern when functional electrical stimulation is used.

muscle fatigue; electrical stimulation; excitation-contraction coupling

MUSCLE FATIGUE IS THE DECREASE in the force-generating ability of a muscle as a result of recent activation (4, 18). Fatigue may result from failure at one or more sites in the periphery, including the neuromuscular junction; propagation of the action potential along the muscle membrane and into the transverse tubule system; Ca$^{2+}$ release from the sarcoplasmic reticulum; Ca$^{2+}$ binding to troponin C (which causes the exposure of active myosin-binding sites on the thin filament); actin-myosin interactions during cross-bridge cycling; and active reuptake of Ca$^{2+}$ by the sarcoplasmic reticulum Ca$^{2+}$-ATPase pump (for recent reviews, see Refs. 17 and 18). In addition, an inhibition, or lack of, central drive may play a role during voluntary contractions. The number of sites of potential failure highlights the complex, multifactorial nature of fatigue. Muscle fatigue is often described as task dependent to convey the concept that fatigue, and the mechanisms underlying it, varies according to the task a muscle performs and that no single mechanism can account for the decline in muscle performance associated with fatigue under all conditions (17). Fatigue may result from simultaneous failure at a number of sites, but for any particular task one site may be primarily responsible for the loss of force-generating ability.

One example of the task dependency of fatigue is the phenomenon known as low-frequency fatigue (LFF). LFF was first described by Edwards et al. (16) and is characterized by three main features: a proportionately greater loss of force with low-frequency muscle activation than with high-frequency activation, a slow rate of recovery of force-generating ability, and persistence of this low force in the absence of marked electrical or metabolic disturbances (20). Although the mechanism for the production of LFF is unknown, both metabolite build-up and elevation in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) have been suggested to play a role in the development of LFF (14).

Studies investigating LFF have induced it by using voluntary contractions (23, 27) and both high-frequency (100-Hz) (12, 14, 34) and low-frequency (10- to 40-Hz) (19, 21) electrical stimulation. Other researchers have produced LFF by repetitive muscle activation by using a preset stimulation program, consisting of 2 s each of 1-, 10-, 20-, 50-, and 100-Hz stimulation (31, 33). Although all of these methods successfully generated LFF, only one type of activation was used per study. Thus, although activation frequency has been shown to affect the rate and amount of muscle fatigue (3, 6), the effects of activation frequency on the production of LFF have not been examined.

Production of LFF may have significant implications for functional electrical stimulation (FES). FES uses artificial electrical activation of skeletal muscles to aid individuals with central nervous system dysfunction to perform functional movement patterns (24, 26). One major limitation to the use of FES is rapid muscle fatigue (20, 26). If LFF accompanies an FES application, it could interfere with the ability of the user to perform a given task, even after a period of rest.

One method to combat the rapid rate of force decline seen with FES presently being examined is modifying the activation pattern (7, 8). The stimulation pattern shown to maximize the force production in fatigued muscle is a variable-frequency train that begins with one or two brief (5- to 10-ms) interpulse intervals (i.e., a doublet or a triplet), followed by a constant-frequency burst of pulses with an interpulse interval slightly greater than or equal to the twitch-contraction time of the muscle (9, 11, 35). We found, however, that although variable-frequency train stimulation produced more force in fatigued muscles, it also produced greater fatigue than did comparable constant-frequency stimulation despite the use of the same number of pulses, same mean frequency, and same train duration as the...
constant-frequency trains (10). One factor that might account for the observed differences in fatigue was the force-time integral of the responses to the trains. Over the course of the fatigue test, the variable-frequency trains produced greater mean force-time integrals than did any of the other trains tested. The force-time integral is often used as a measure of “isometric work” (2, 35), and the decline in force is thought to be related to the metabolite build-up within the muscle (14). A previous study from our laboratory (10), however, did not test for the presence of LFF, nor did it examine recovery of force, when LFF often becomes apparent.

The present study, therefore, explored the effects of stimulation frequency and pattern on LFF in healthy human quadriceps femoris muscles in response to intermittent isometric contractions of the human quadriceps femoris muscle. The muscle was tested with a wide range of activation frequencies and patterns designed to produce a variety of force outputs and a range of muscle fatigue. The number of pulses per train, which has been identified as a factor in fatigue (25), was kept constant at six. For a description of the force-frequency relationship that results from using such short trains, the reader is referred elsewhere (9).

Because it was not possible to control both the train duration and the number of pulses when frequency was tested, the train durations varied widely. Similarly, because of the differences in train duration, either the train period or rest time between trains could have been controlled, but not both. For this study, we elected to control the train period to make the data more applicable to the common clinical practice of FES.

The force responses to the various stimulation trains were evaluated immediately after the fatiguing stimulation and at three different recovery times, when it was hypothesized that LFF would become more apparent. In addition, the mean force-time integral produced by the various fatigue stimulation trains was calculated to see whether the effect of a given stimulation pattern was related to the work it produced. Preliminary results have been presented in abstract form (30).

Fig. 1. Schematic of pulse trains used in fatiguing protocols and as testing trains. Each vertical line represents one 600-µs pulse. FP9.1, FP14.3, FP33.3, and FP100: stimulation train used during 9.1-, 14.3-, 33.3-, and 100-Hz fatiguing protocol, respectively; FPV, stimulation train used during variable-frequency train fatiguing protocol; TT8.3, TT12.5, TT20, TT50, and TT100: 8.3-, 12.5-, 20-, 50-, and 100-Hz testing train, respectively. See MATERIALS AND METHODS for details.
with an initial interpulse interval of 5 ms and four interpulse intervals of 86.25 ms (referred to as FPV). The variable-frequency train thus had a mean frequency (14.3 Hz) and train duration (350 ms) equal to that of FP14.3. This variable-frequency train has been shown to produce greater force-time integrals in fatigued muscles than any of the constant-frequency trains tested (9). The order in which each subject received the five fatiguing protocols was randomly determined to control for any ordering effects.

The testing protocol used five 6-pulse, constant-frequency testing trains (8.3, 12.5, 20, 50, and 100 Hz) to assess the force-generating ability of the muscle (Fig. 2). These testing trains are referred to as TT8.3, TT12.5, TT20, TT50, and TT100, respectively. The sequence of the testing trains was randomly determined for each subject, to control for any effects of order or activation history. The five testing trains were delivered in the randomly determined order and then repeated in reverse order for a total of 10 trains. The testing protocol was delivered before the fatiguing protocol (prefatigue responses) and at four fatigue states: when the muscle was fatigued (R0), and at 2, 13.36, and 37.36 min after completion of R0 (R1, R2, and R3, respectively) (Fig. 2). During the prefatigue portion of the testing protocol, the testing trains were delivered at a rate of one train every 10 s. Pilot testing indicated that these short trains produced no fatigue when delivered once every 10 s. Five minutes after the prefatigue testing, the fatigue protocol was begun. Immediately (i.e., 1 s) after the fatigue protocol, the R0 portion of the testing protocol began (see Fig. 2). During the R0 portion, the testing trains were delivered in the same order that was used in the prefatigue portion. Each testing train, however, was separated by three of the fatiguing trains (Fig. 2), and trains were delivered at a rate of 1 train/s to maintain the muscle in a consistent state of fatigue and to control for the previous activation history of the muscle. Recovery testing (R1, R2, and R3) consisted of the same sequence of testing trains, delivered at a rate of 1 train/10 s.

To set the stimulation intensity, at the start of each experimental session, a 100-Hz, 6-pulse, constant-frequency train was delivered to the muscle once every 5 s. Stimulation intensity was initially adjusted to elicit ~20% of each subject's MVIC and was held constant until the muscle was potentiated (i.e., force did not increase over 3 successive trains). This typically required activation of the muscle with 5–10 stimulation trains. After potentiation, the intensity was readjusted to produce 20% of the subject's MVIC. The intensity was then kept constant throughout the remainder of the session in an attempt to recruit a consistent population of motor units from each subject's muscle. Pilot testing revealed that the intensity that produced 20% of the MVIC peak force with the 6-pulse, 100-Hz train produced >75% of MVIC when employed with a 1.5-s train of equal frequency and intensity. Thus, although the peak force was low, a sizable portion of the muscle was activated during the contraction. Before the prefatigue portion of the testing protocol, the fatigue protocol, and the R1, R2, and R3 portions of the testing protocol, the muscle was repotentiated with 100-Hz, 6-pulse trains, to ensure that any differences seen were not simply due to a loss of potentiation. Although repotentiating the muscle may have attenuated the LFF observed, we felt that this procedure was necessary to allow comparisons across subjects and fatigue states.

DATA MANAGEMENT

Two force measurements, the force-time integral and peak force, were computed from the force responses to each train of the fatiguing protocol, using custom software (LabView 4.0,
National Instruments, Austin, TX). All force data were digitized on-line at a sampling frequency of 200 samples/s and stored for subsequent analyses. The response to the first train in each fatiguing protocol was used to calculate the nonfatigue values, and the average of the final six responses (i.e., trains 175–180) was used to calculate the fatigue responses. These values were used to determine the percent decline in force-time integral and peak force over the course of the 180 contractions of the fatiguing protocol. The average force-time integral and average peak force produced during each fatiguing protocol were calculated by dividing the sum of the responses to each of the 180 contractions during each fatiguing protocol by 180.

For the testing train data, only the peak forces were computed, because peak force measurements are most commonly used to assess the presence of LFF (14, 20). The averaged responses to the two occurrences of each testing train within each testing protocol (see Fig. 2) were calculated and used for all analyses. The fatigue (R0) and recovery (R1, R2, and R3) responses to each testing train frequency were divided by the prefatigue responses at each comparable frequency to normalize the forces and determine the amount of fatigue at each time and for each testing train frequency. A ratio of the normalized TT12.5 peak force response to the normalized TT50 peak force response (hereafter referred to as 12.5/50 force) was used as a measure of the amount of LFF at each time. Although other studies have used the 20/50-Hz (31) and 20/100-Hz (34) force ratios, it was felt that the comparison of the 50-Hz train with the lower frequency 12.5-Hz train more accurately illustrated the effects of LFF for the present data, while not differing widely from the 20/50-Hz ratio. In addition, the 12.5-Hz frequency was within the range of discharge rates reported for voluntary contractions of human muscles (1).

Data Analysis

Fatiguing portion. One-way ANOVAs were used to test for differences among the initial and fatigue responses to each fatiguing protocol. One-way, repeated-measures ANOVAs were also used to test for the effect of the fatiguing protocol on the percent decline and average forces produced during the 180-train fatiguing protocol. If significant effects were present, post hoc testing using paired t-tests with a Bonferroni correction for multiple comparisons was performed.

Testing portion. One-way, repeated-measures ANOVAs of the peak force data found no significant differences among the prefatigue testing train responses collected before the different fatiguing protocols (e.g., the TT50 before FP14.3 = the TT50 before FP9.1 = the TT50 before FP100, and so on). Thus all subsequent analyses used values that were normalized to the prefatigue responses. A three-way, repeated-measures ANOVA was performed on the effects of fatigue state, testing train frequency, and fatiguing protocol. As anticipated, significant interactions among the variables were present. Therefore, main effects of each fatiguing protocol on each frequency were investigated. To do this, one-way, repeated-measures ANOVAs were performed on the normalized R0, R1, R2, and R3 peak force responses at each testing train frequency to test for the effects of the fatiguing protocol. One-way, repeated-measures ANOVAs were also performed on the normalized 12.5/50 force ratios to compare the LFF resulting from each fatiguing protocol at each fatigue state (i.e., R0, R1, R2, and R3). In addition, one-way ANOVAs were performed on the responses within each fatiguing protocol to test for the effect of fatigue state on the 12.5/50 force ratio. If any of these one-way ANOVAs showed significant effects, paired t-tests with a Bonferroni correction were used for post hoc comparisons. For all tests, \( P \leq 0.05 \) was considered significant.

RESULTS

Complete data sets were collected in 11 subjects. The remaining subject missed two experimental sessions because of an injury to the right lower extremity. This injury was unrelated to the present testing. These data were thus excluded from analysis.

Fatiguing Protocol

Examination of the initial force-time integral responses to each fatiguing protocol showed that FP14.3 produced the greatest responses, followed closely by FPV (see Fig. 3). Both trains produced initial force-time integrals that were \( \sim 12\% \) greater than those produced by FP9.1, a difference that was not statistically significant. FP9.1, FP14.3, and FPV all produced initial force-time integral responses that were substantially greater than those produced by the initial trains of FP33.3 (41–65\% greater, all \( P \leq 0.005 \)) and FP100 (93–126\% greater, all \( P \leq 0.005 \)). At the conclusion of the fatiguing protocols, FP9.1, FP14.3, and FPV produced forces only 2–28\% and 35–68\% greater than for FP33.3 (FP9.1 was not significant; \( P \leq 0.005 \) for FP14.3 and FPV) and FP100 (\( P \leq 0.005 \) for FP9.1; \( P \leq 0.005 \) for FP14.3 and FPV), respectively. The only change in order was that the FPV response was 8.5\% greater than that of FP14.3 at the end of the protocol.

The peak force values also showed a pattern that was maintained over the course of the fatigue test. FP33.3 consistently produced the greatest peak forces, followed by FP100, FP14.3, FPV, and FP9.1, respectively (see Fig. 3). At the commencement of the test, the peak force responses of FP33.3 were 69, 6, and 16\% greater than FP9.1, FP14.3, and FPV responses, respectively (\( P \leq 0.005 \) for FP9.1 only), and the FP100 responses were 62, 1, and 11\% greater than FP9.1, FP14.3, and FPV responses, respectively (\( P \leq 0.005 \) for FP9.1 only). The fatigue responses of FP33.3 were 42–127\% greater than FP9.1, FPV, and FP14.3 responses (all \( P \leq 0.005 \)), and FP100 responses were 33–112\% greater than FP9.1, FPV, and FP14.3 responses (all \( P \leq 0.005 \)).

Changes in the relative forces produced by each stimulation pattern over the course of the 180 fatiguing trains were due to the lower frequency protocols (FP9.1, FP14.3) and FPV, exhibiting much greater percent declines in force-time integral and peak force than with the higher frequency protocols (FP33.3, FP100) (see Fig. 4). Declines in the force responses of the FP9.1, FP14.3, and FPV ranged from 40 to 48\% for force-time integral and 45–48\% for peak force. In contrast FP33.3 and FP100 declined by only \( \sim 24\% \) for force-time integral and \( \sim 29\% \) for peak force. The percent declines for FP9.1, FP14.3, and FPV were not significantly different from each other, nor were those for FP33.3 and FP100. The declines during FP9.1, FP14.3, and FPV were all significantly greater than those produced during FP33.3 and FP100 (see Fig. 4 for details). Generally, protocols that produced the highest average force-time integrals demonstrated the greatest percent declines in force...
responses during the fatigue test (see Figs. 4 and 5). This was indicated by the significant main effect for fatiguing protocol on average force-time integral ($F = 33.59, P < 0.005$). The relationship was not exactly proportional, however. FP9.1 exhibited a greater, and FPV a smaller, percent decline than would be expected from an exactly proportional relationship. There was also a significant effect of fatiguing protocol on peak

![Fig. 3. Force responses to 180 fatiguing trains for each of the 5 fatiguing protocols. A and B: force-time integral (FTI) and peak force group data, respectively (means ± SE; n = 11) for every 20th contraction. Generally, differences between lower (FP9.1, FP14.3, and FPV) and higher frequency (FP33.3 and FP100) FTI responses decreased over course of test, whereas differences in peak force between higher and lower frequency protocols increased (see text for a report of significance). C and D: FTI and peak force data, respectively, from a typical subject. Responses are shown for every 5th contraction. Note that decline in force follows a fairly regular pattern, indicating that a consistent population of motor units was recruited throughout tests.](image)

![Fig. 4. Group data from fatiguing protocols (means ± SE; n = 11). A and B: average FTI per train and average peak force per train, respectively, during fatiguing protocol. C and D: percent decline in FTI and percent decline in peak force, respectively, over course of fatiguing protocols. Generally, protocols that produced highest average FTIs also exhibited the greatest percent declines in force responses during fatigue test. FP9.1 exhibited a greater, and FPV a smaller, percent decline, however, than would be expected from an exactly proportional relationship between FTI and percent decline in force. Significantly greater than value for FP100: † $P < 0.05$; ‡ $P < 0.005$. Significantly greater than value for FP33.3: * $P < 0.05$; ** $P < 0.005$. Significantly greater than value for FP9.1: § $P < 0.05$; §§ $P < 0.005$. Significantly greater than values for both FPV and FP14.3: ¶ $P < 0.05$.](image)
for each low-frequency testing train but not for either of the high-frequency testing trains. The lower frequency fatiguing protocols and FPV protocol produced greater attenuation of the TT8.3 and TT12.5 responses than did the higher frequency fatiguing protocols (see Fig. 6). The only differences that were found to be statistically significant, however, were between the TT8.3 responses after the higher and lower frequency fatiguing protocols and the TT12.5 responses after the FPV and the higher frequency protocols.

During recovery (i.e., R1, R2, R3), the low-frequency testing trains continued to exhibit greater attenuation of peak force responses than the high-frequency testing trains. By R3, all of the TT50 and TT100 responses had recovered to ≥90% of the nonfatigue peak force values. In contrast, the TT8.3 and TT12.5 had recovered only to ≈75% of the nonfatigue values by R3 (see Fig. 6). The differences in the effects of the various fatiguing protocols on the testing trains during recovery were, however, much less pronounced than at R0. The low-frequency fatiguing protocols no longer produced greater attenuation of the lower frequency testing trains. In fact, FP100 demonstrated the greatest fatigue in all testing trains at all three recovery states. This effect, however, was only statistically significant for TT12.5 at R1 (see Fig. 6). Thus, other than FP100, all of the fatiguing protocols appeared to affect each of the testing trains similarly during recovery.

Examination of the normalized 12.5/50 forces (Fig. 7) shows that all of the protocols produced values that were <1. This indicates that all of the protocols attenuated low-frequency force responses more than high-frequency responses and that this attenuation was maintained during recovery. There was a significant main effect for fatiguing protocol on the normalized 12.5/50 forces at R0 (F = 11.121, P < 0.001), but not during later testing. At R0, FP33.3 produced significantly less LFF than did FP9.1, FP14.3, and FPV, and FP100 produced significantly less LFF than did any of the other protocols. This finding highlights the greater loss of force in response to low-frequency testing trains immediately after the lower frequency and variable-frequency fatiguing protocols vs. the higher frequency fatiguing protocols.

There was a significant effect of fatigue state on the normalized 12.5/50 forces for each of the fatiguing protocols. Post hoc testing found that the R1 ratios for each fatiguing protocol, except FP100, were significantly greater than R0 values. For each fatiguing protocol, R2 and R3 ratios were significantly smaller than the R1 ratio. There were no differences between R2 and R3 ratios. The decrease in the ratio between R1 and R2 was due, at least in part, to a decline in the force responses to low-frequency stimulation from 13 min vs. 2 min (Fig. 8).

**DISCUSSION**

This study examined the effects of different activation frequencies and patterns of electrical stimulation on the production of LFF in the human quadriceps femoris muscle. The results indicate that stimulation...
trains of different frequencies and patterns affected the production of LFF immediately after the fatiguing stimulation. The lower frequency fatiguing protocols (FP9.1 and FP14.3), which used activation frequencies within the physiological range of the human muscle (1), produced significantly greater LFF than did the higher frequency fatiguing protocols (FP33.3 and FP100). Although variable-frequency train stimulation (FPV) produced greater attenuation of peak force responses to low-frequency test trains (TT20, TT12.5, and TT8.3) than of those to high-frequency test trains (TT100 and TT50), it did not produce significantly greater LFF than did the low-frequency fatiguing protocols, because it also produced greater fatigue of the high-frequency testing trains (See Fig. 6). These results support the findings of Binder-Macleod et al. (10), who demonstrated greater fatigue of low-frequency testing trains after a variable-frequency-train fatiguing protocol than after comparable constant-frequency fatiguing protocols.

It should be noted that this experiment examined differences in effects of different fatiguing protocols, not simply different frequencies. That is, the stimulation frequencies, rest times, and mean force-time integrals varied among the different protocols. As shown in Fig. 5, the low-frequency protocols and FPV all produced greater average force-time integrals and had shorter rest times than did the high-frequency protocols. It appears, in fact, that the differences in the effects of these protocols more closely followed the differences in force-time integral than frequency.

At >30 min of recovery, there were no significant differences among the fatiguing protocols in the LFF produced. These results suggest that the force produced by low-frequency activation during recovery is unaffected by the frequency or pattern of the stimulation that induced the fatigue, if the number of pulses and trains within the fatiguing protocol are kept cons-

![Fig. 6. Group data (±SE; n = 11) for peak force responses to each testing train after each fatiguing protocol, at each fatigue state. Data were normalized by dividing peak force value by prefatigue force response to corresponding testing train. R0, immediately after fatiguing protocol; R1, fatigue state 2 min after completion of fatiguing protocol; R2, 10 min after completion of R1 testing protocol; R3, 20 min after completion of R1 testing protocol. A-E: responses to TT8.3, TT12.5, TT20, TT50, and TT100, respectively. At R0, each fatiguing protocol generally produced greater attenuation of peak force responses than those to high-frequency test trains (TT100 and TT50). One-way ANOVAs of normalized peak force responses at R0 demonstrated a simple main effect of fatiguing protocol for each low-frequency testing train but not for either of the high-frequency testing trains (TT8.3, P < 0.05; TT12.5, P < 0.05; TT20, P < 0.05). At R1 a simple main effect for fatiguing protocol was found for TT20 (P < 0.01), but, after correction for multiple comparisons, none of individual t-tests was significant. A simple main effect for fatiguing protocol was also present for TT12.5 at R1 (P < 0.05). TT12.5 showed significantly greater fatigue after FP100 than after FP33.3 (P = 0.022), FP14.3 (P = 0.01), and FP9.1 (P = 0.001). No other significant differences were present. Significantly less than value after FP100: **P < 0.005. Significantly less than value after FP33.3: §P < 0.05; §§P < 0.005. Significantly greater than value after FP100, P < 0.05.}
2stant. Furthermore, regardless of the fatiguing protocol, there was greater LFF at 13 min of recovery than at 2 min of recovery (see Fig. 7). This change in the amount of LFF is due to a decline in force responses to low-frequency fatiguing protocols than after higher frequency fatiguing protocols. No such effects were present at, or after, R1. There was also a significant effect of fatigue state in each of the fatiguing protocols. R1 ratios for each fatiguing protocol, except FP100, were significantly greater than R0 values. For each fatigue protocol, R2 and R3 ratios were significantly smaller than were R1 ratios. There were no differences between R2 and R3. Thus low-frequency force fatigue decreased between R1 and R2 and then stabilized. Significantly less than value for FP100: *P < 0.05; **P < 0.005. Significantly less than value for FP33.3: §§P < 0.005. Significantly less than value for corresponding fatiguing protocol at R1: †P < 0.05; ‡P < 0.005.

The responses at R0 show that the attenuation of force was generally related to the force-time integrals produced by the different protocols and was thus likely related to metabolite build-up and reequilibration. The low-frequency testing trains exhibited greater attenuation than did the high-frequency testing trains, and they also showed less recovery of force than did the high-frequency trains. These observations suggest the LFF seen here may consist of two phases: an early, rapidly recovering phase, likely related to metabolite build-up, and a long-lasting response, which is not dependent on metabolite levels. These results are consistent with recent work by Chin et al. (14), which also suggests that there may be two components to LFF. They theorize that there is a metabolic component, which recovers rapidly in the presence of glucose, and a long-lasting component that is dependent on an elevated [Ca$^{2+}$]-time integral. Chin and Allen (13) demonstrated a relationship between the elevated [Ca$^{2+}$]-time integral and an impairment of sarcoplasmic Ca$^{2+}$ release, with no changes in Ca$^{2+}$ sensitivity. This impairment is uniform across high and low activation frequencies, but because of the sigmoid nature of the force-[Ca$^{2+}$] curve, the concurrent loss of force-generating ability was selectively greater at low frequencies of activation (12). It has been suggested that increased Ca$^{2+}$ release from the sarcoplasmic reticulum is the cause of the force augmentation seen with variable-frequency train stimulation of fatigued muscles (15). In their study, Chin and Allen (13) demonstrated that greater quantities of Ca$^{2+}$ were released as frequency increased. Thus high Ca$^{2+}$ levels resulting from FP100 and the initial, high-frequency doublet of FPV may have added to the impairment of excitation-contraction coupling, causing greater LFF, and contributed to the increased force attenuation seen in this study and previous work (10). It should be noted,
however, that we did not examine Ca\textsuperscript{2+} transients or concentrations and cannot say what effects any of the protocols had on these variables.

It may be that this Ca\textsuperscript{2+}-dependent, long-lasting component of LFF has a longer onset time than the metabolic component. This would explain the rapid initial decrease in LFF at 2 min of recovery and subsequent increase in LFF at ~13 min of recovery seen in this study. Previous researchers have typically examined LFF at 10, 30, and/or 60 min of recovery (12, 14, 31, 34) and so would not have detected the changes in low-frequency force responses between 2 and ~13 min seen here, although a similar decrease and subsequent increase in LFF have previously been reported (29). By ~10 min, the metabolic component may have fully recovered, but the [Ca\textsuperscript{2+}]-time-integral-dependent component may be active enough to counteract recovery of the metabolite-dependent component. In any case, the 12.5/50-Hz ratio at 30 min shows no changes from the 10-min values, indicating that the second component is still in effect.

Further support for the theory that the initial, rapidly recovering mechanism is related to metabolites can be found by comparing the average force-time integral during the fatigue protocol (Fig. 5) with the 12.5/50 force ratio (Fig. 7). In general, protocols that produced the greatest force-time integrals also produced the greatest LFF at R0. Force-time integral has been used as a measure of isometric “work” (2, 35), and thus greater force-time integrals should be associated with greater buildup of metabolites. The lone exception to this pattern is FP9.1, which produced greater LFF than would be expected from its average force-time integral. One possible explanation may lie in the pattern of force generation and relaxation at this frequency (see Fig. 5) compared with the other fatiguing protocols. FP9.1 produced a marked rise in force followed by relaxation in response to each pulse, whereas the other protocols generally exhibited a maintenance of a given force level. It has been shown that intermittent contractions with a rise and fall in force are more fatiguing than continuous “holding” contractions of equal intensity (12). It has been suggested that the torque “ripple” associated with low-frequency-stimulated isometric contractions may result in an increase in internal work (28). Thus FP9.1 would produce more metabolites than would be associated with the average force-time integral it produced. Alternatively, the greater-than-expected LFF produced by FP9.1 may have been due to the shorter rest time, which allows less recovery between contractions. In contrast, LFF at R1, R2, and R3 shows no effect of the fatiguing protocol, suggesting that metabolic demand is not associated with LFF during recovery.

Another new observation was that the greatest attenuation of the low-frequency testing trains during recovery occurred after FP100 (Fig. 6). Although not significant, this finding was surprising, as this protocol did not produce the highest average peak forces or force-time integrals (Fig. 5). This protocol also exhibited the smallest percent declines in peak force and force-time integral. These results are consistent with those of Chin et al. (14), which showed that reduction in force had no effect on the induction of LFF. The mechanisms behind this increased fatigue remain unclear but, as previously noted, may relate to greater [Ca\textsuperscript{2+}] levels resulting from the 100-Hz trains. Chin et al. were able to induce different levels of LFF by varying the number of tetani. If the number of tetani is a key factor in producing LFF, this has important implications for the use of variable-frequency train stimulation to offset fatigue during FES applications. Generally, a given number of contractions is necessary to perform a functional task. For example, to take 20 steps would require 20 tetani of a patient’s quadriceps femoris muscle. Theoretically, any task will induce LFF if it exceeds this hypothetical threshold number of contractions. The present results showed that FP resulted in no less recovery of the low-frequency, force-generating ability than did any other stimulation protocol after ~13 min of recovery, despite the fact that it produced greater low-frequency force attenuation immediately after a fatiguing protocol. Thus, during an FES application, the amount of LFF present during recovery should be the same whether variable-frequency or constant-frequency stimulation is employed. Furthermore, consistent with earlier work by this laboratory (10), this study showed that, although variable-frequency trains produced greater attenuation of the responses to low-frequency trains than did comparable constant-frequency trains, they still produced greater force-time integrals in fatigued muscles than did the constant-frequency trains (Fig. 4). Thus variable-frequency stimulation might be used during FES to increase force production during fatigue, without delaying the rate of force recovery, compared with constant-frequency stimulation.

Conclusion

This study showed that there are effects of both stimulation frequency and pattern on the production of LFF by brief, intermittent stimulation trains. Generally, lower frequency trains produced greater LFF immediately after the fatiguing activation. This greater LFF appeared to be related to the average force-time integral produced by the repetitive contractions. However, after ~2 min of recovery time, LFF appeared to be independent of the forces produced during the fatiguing contractions; this was a new and somewhat surprising finding. Also, LFF decreased after 2 min of recovery, increased again over the next ~10 min, and then plateaued over the next 30 min. These results support the theory that there are two components to LFF: a rapidly recovering, metabolite-dependent component and a slow-developing, slow-recovering component that is not a result of metabolite build-up. These findings may help clinicians determine the optimal activation pattern of skeletal muscle when using FES.

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