Effects of muscle activation on fatigue and metabolism in human skeletal muscle

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Russ, David W., Krista Vandenbome, Glenn A. Walter, Mark Elliott, and Stuart A. Binder-Macleod. Effects of muscle activation on fatigue and metabolism in human skeletal muscle. J Appl Physiol 92: 1978–1986, 2002.—Increasing stimulation frequency has been shown to increase fatigue but not when the changes in force associated with changes in frequency have been controlled. An effect of frequency, independent of force, may be associated with the metabolic cost resulting from the additional activations. Here, two separate experiments were performed on human medial gastrocnemius muscles. The first experiment (n = 8) was designed to test the effect of the number of pulses on fatigue. The declines in force during two repetitive, 150-train stimulation protocols that produced equal initial forces, one using 80-Hz trains and the other using 100-Hz trains, were compared. Despite a difference of 600 pulses (23.5%), the protocols produced similar rates and amounts of fatigue. In the second experiment, designed to test the effect of the number of pulses on the metabolic cost of contraction, 31P-NMR spectra were collected (n = 6) during two ischemic, eight-train stimulation protocols (80- and 100-Hz) that produced comparable forces despite a difference of 320 pulses (24.8%). No differences were found in the changes in P concentration, phosphocreatine concentration, and intracellular pH or in the ATP turnover produced by the two trains. These results suggest that the effect of stimulation frequency on fatigue is related to the force produced, rather than to the number of activations. In addition, within the range of frequencies tested, increasing total activations did not increase metabolic cost.

31P-NMR; electrical stimulation; frequency

Muscle fatigue is defined as an impairment in the force-generating ability of muscle associated with recent contraction (44). A complex phenomenon, fatigue has been associated with impairment of function at a number of sites, from central activation to myofilament interaction (for a recent review, see Ref. 39). Although it is unlikely that any single mechanism accounts for fatigue under all conditions, for a given task one site or mechanism may primarily be responsible for the decline in muscle performance (15). Because muscle contraction increases muscle metabolism by an order of magnitude (41) and fatigue is a consequence of muscle contraction, it has long been held that the metabolic cost of muscle activation is a primary factor in fatigue (30). Proposed mechanisms include alterations of the cellular environment by the build-up of metabolic by-products (13, 33, 39) and depletion of substrate (36). The results of metabolic studies do not demonstrate consistently that any one metabolite is the cause of fatigue, but they do show that several substances can alter force generation under specific conditions (9, 11, 13, 16, 18, 33, 44). In further support of a metabolic basis for fatigue, several studies have demonstrated that during short-duration, high-intensity exercise (both voluntary and electrically elicited), protocols that produce the greatest metabolic changes also produce the greatest fatigue (2, 10, 22, 34, 35, 38), although other factors, such as activation failure, are likely to be involved in the decline in force (2, 15).

The metabolic demand of muscle contraction is associated with the ATP hydrolysis occurring at three ATPases: 1) the Na⁺-K⁺-ATPase associated with maintaining the resting membrane potential of the sarcolemma, 2) the actin myosin (AM) ATPase associated with cross-bridge cycling and force production, and 3) the sarcoplasmic reticulum (SR) Ca²⁺-ATPase associated with Ca²⁺ reuptake at the SR. The demand of the AM-ATPase is related to the force produced by a muscle as demonstrated by Boska (8), who found that ATP consumption increased proportionately with force during voluntary contractions of the plantarflexors. Stienen et al. (40) also found that ATP turnover was proportional to force in amphibian muscle fibers. ATPase activity, however, was lower in fibers that had been chemically skinned to remove the SR, thus eliminating the metabolic demand associated with the SR.
Ca\textsuperscript{2+} -ATPase. This finding is in accord with the assertion that between 20 and 40% of the ATP hydrolysis that occurs with muscle contraction is thought to result from noncontractile (i.e., non-AM-ATPase) ATPase activity (1, 22, 23).

This additional, noncontractile metabolic cost may lend some support to the theory, first put forth by Marsden et al. (27), that fatigue during electrical stimulation of muscle is a function of the number of stimulation pulses. Because of the changes in Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} that occur in response to each action potential, it seems reasonable that the metabolic demand of these pumps would be related to the number of pulses. This cost might result in an increase in fatigue with increasing frequency, independent of the force produced during stimulation. Frank et al. (17) demonstrated that the SR Ca\textsuperscript{2+} -ATPase activity increased with increased frequency of electrical stimulation in cardiac muscle. Garland et al. (20) directly examined the effect of the number of pulses by altering the pulse frequency (15 vs. 30 Hz) for trains of fixed duration and found that the total number of pulses did not affect the rate of fatigue. Using such low frequencies, however, introduced two confounding variables. First, a 15-Hz train is more susceptible to low-frequency fatigue than a 30-Hz train. Low-frequency fatigue is characterized by a proportionately greater decline in the force response to low-frequency stimulation vs. high-frequency stimulation (24). Second, the two frequencies produced different forces. Other studies (5, 38) have shown that stimulation protocols with equal numbers of pulses do not produce equal fatigue when the pulse frequency, the number of tetani, and/or the train duration is varied. Here again, however, the lack of an effect of the number of pulses is complicated by differences in force production. No studies to date have determined the specific contribution that the number of pulses makes to fatigue during stimulation with trains that use different frequencies but produce equal forces.

The first goal of the present study was to evaluate the effect of increasing the number of stimulation pulses delivered to a muscle, while controlling for initial force, on the fatigue produced during repetitive electrical stimulation. It is commonly accepted that increasing the frequency of stimulation will increase fatigue (25, 31), although an effect of frequency, independent of force, has not been established. The second goal was to compare metabolic cost associated with stimulation trains that vary in the number of stimulation pulses but produce comparable forces. We were able to address each of these goals by taking advantage of the sigmoidal nature of the force-frequency relationship. We compared the responses to two high-frequency trains (80 and 100 Hz) that did indeed produce nearly identical forces. Because the durations of the two trains were equal, the 80-Hz train contained ~20% fewer pulses. In the first set of experiments, we evaluated the changes in isometric force that occurred during repetitive, intermittent stimulation with 80- and 100-Hz trains to determine the additional fatigue associated with the extra pulses, independent of force.

Because exercise and stimulation protocols that produce greater fatigue tend to produce greater metabolic changes, we also examined the metabolic cost of increasing the number of pulses within a stimulation train. In the second set of experiments, we therefore compared the metabolic cost of 80- and 100-Hz trains through the use of in vivo \textsuperscript{31}P-NMR.

METHODS

Subjects

Thirteen healthy subjects (6 men) ranging in age from 20 to 33 years of age (mean of 26.9 ± 4.9) with no history of muscle or joint problems participated in this study. All subjects were informed of the purpose and procedures of the study and gave written, informed consent to their participation. The experimental protocols were approved by the Human Subjects Review Boards of both the University of Delaware and University of Pennsylvania.

Experimental Procedures

Fatigue experiments. These experiments were conducted at the Muscle Performance Laboratory at the University of Delaware and assessed the force responses to 150 brief, intermittent stimulations with trains of 80 and 100 Hz. Eight subjects (4 men) participated in this experiment.

Training session. Each subject participated in one training session. During this session, subjects were trained to perform maximum, volitional, isometric contractions (MVICs) of the planter flexors and to relax their muscles during electrical stimulation. In addition, one of the investigators (D. W. Russ) located the motor point of the medial gastrocnemius (MG) muscle on each subject and recorded its relationship to the following bony landmarks: the posterior fibular head, the medial malleolus, and the tibial tubercle. These measurements were used to assist the investigators in finding the motor point during subsequent testing sessions. During the training session, the subject’s plantarflexion MVIC was assessed. We confirmed that the subject was performing a true MVIC with a burst-superimposition technique, previously described in the quadriceps muscle (37). Self-adhesive, 5 × 5-cm electrodes were used. One electrode was placed directly over the MG motor point, and the other was placed over the distal muscle belly. The subject was positioned supine, with the foot at a 90° angle to the shank, in a KinCom III isokinetic dynamometer (Chattecx, Chattanooga, TN) that was set up according to the manufacturer’s recommended plantar flexion-testing configuration. The subject was stabilized by use of inelastic, nylon straps with Velcro closures and a custom-made shoulder harness.

Testing sessions. Each subject participated in two sessions, separated by at least 48 h. Patient positioning and setup were the same as described for the training session. The subject’s plantar flexion MVIC was determined as described for the training session. If the subject could not produce an MVIC within 5% of that achieved in the training session after three attempts, the session was cancelled and another session was scheduled.

If the session did proceed, the next step was to set the stimulation intensity to produce 20% of the plantar flexion MVIC, by using a 200-ms, 100-Hz stimulation train with the same electrode placement used for the burst-superimposition test. The MG has been estimated to comprise ~30% of the cross-sectional area of the human plantar flexor muscle mass (14, 19). Thus stimulation at this intensity corresponded to
~60% of the MG MVIC force. Pilot work determined that this force level was well tolerated by subjects and produced consistent force responses (35). After the stimulation intensity was set, the muscle was potentiated with ten 200-ms, 100-Hz trains, delivered at a rate of 1 train per 10 s. Within 5 s of completion of the potentiating stimulation, the fatiguing protocol commenced. The fatiguing protocol consisted of 150 200-ms trains, with an intertrain interval of 1.2 s. All stimulation pulses were 300 μs in duration. The stimulation frequency was set to 80 Hz in one session and 100 Hz in the other. We studied this range of frequencies, as we were concerned that force would be reduced with stimulation at less than 80 Hz and that electrical fatigue might occur with stimulation higher than 100 Hz. The large number (150) of trains delivered offset the small difference (4 pulses) in number of pulses per train. The order of the two sessions was randomly determined for each subject.

**DATA ACQUISITION.** Force responses to each train in the fatiguing protocols were collected and digitized on-line at a sampling rate of 200 Hz by using customized software (LabView 4.0, National Instruments, Austin, TX) and used to calculate peak force and force-time integral (FTI). The FTI is the area under the force-time curve during the muscle contraction and is often used as a measure of isometric work (20). The percent decline in each variable during the fatigue test was calculated by subtracting the mean of the final six responses from the response to the first train of the fatiguing protocol and dividing by the first train’s response. To compare the rates of fatigue, a nonlinear curve fitting routine was used to fit the peak force responses of each subject to each protocol with Eq. 1, a four-parameter Hill equation (SigmaPlot, Jandel Scientific)

\[ y = y_0 + \frac{a(x^b)}{c^b + x^b} \]  

where \( y \) = force, \( x \) = contraction number, \( y_0 \) = minimum peak force produced by a given train, \( a \) is a scaling factor, \( c \) is the contraction at which 50% of the force decline is achieved, and \( b \) is a measure of the slope.

**STATISTICAL ANALYSIS.** Paired-sample \( t \)-tests were performed on the initial peak force and FTI values, the fatigued peak force and FTI values, and the percent decline in peak force and FTI to compare the two frequencies that were tested. Paired-sample \( t \)-tests were also used to test for significant differences in \( b \) and \( c \), as calculated from Eq. 1, which would indicate differences in the rates of force decline.

**Metabolic cost experiments.** The second set of experiments, conducted at the University of Pennsylvania, also tested the MG isometrically, but the testing was performed in a 1-m bore, 2.0-T superconducting magnet, interfaced with a custom-built spectrometer (43). These experiments were performed to compare the ATP turnover associated with an 80-Hz vs. a 100-Hz stimulation train. Six subjects (3 men) participated in these experiments.

An adaptation of the QUEST protocol, previously described by Blei and colleagues (7), was used to measure the metabolic cost associated with each train. Briefly, this protocol separates ATP utilization from resynthesis. Changes in the energy-rich phosphate content are measured with \(^{31}\)P-NMR spectroscopy during electrically induced contractions in the absence of oxygen. For this purpose, a blood pressure cuff was placed around the thigh and inflated to 230 mmHg for 5 min before stimulation. It has been demonstrated that 5 min of ischemia is sufficient to eliminate the oxygen supplying the muscles (7, 26).

We did not collect force data during the acquisition of the phosphorous spectra. Simultaneous operation of the spectrometer, the stimulator, and the force transducer introduced an unacceptable amount of noise. To confirm that similar forces were produced throughout the 80- and 100-Hz protocols, additional experiments at the University of Delaware were performed on three of the six subjects tested at the University of Pennsylvania. During these experiments, force data were collected by using the same eight-train, 80- and 100-Hz protocols described below for the \(^{31}\)P-NMR experiments. The order in which the two protocols were delivered was randomly determined. No formal statistical tests were performed on these data because of the low number of subjects. There were, however, no apparent differences in the force responses to the two protocols (see Fig. 1).

The largest difference in peak force (100-Hz force − 80-Hz force) was 5.59 ± 6.56% for the first train, and the overall mean difference (all eight trains) was 2.51 ± 6.29%. The largest difference in FTI was 3.67 ± 9.67% for the first train, and the overall mean difference was 1.41 ± 7.54%. Thus the 100-Hz train did show a tendency to produce slightly (<5%)
more force than the 80-Hz train, but the two protocols produced very similar patterns of force decline.

TRAINING SESSION. The training session for this experiment was essentially the same as that described for the first experiment.

TESTING SESSION. The motor point of the MG was located as described above, and the subject was positioned in a similar fashion to that used for the first experiment, except that a custom-made, nonmagnetic, variable-resistance, plantar flexion/dorsal flexion ergometer locked in neutral position was used instead of the KinCom dynamometer. Straps around the shoulders, waist, knee, and ankle were used to stabilize the subject during exercise, and a blood pressure cuff was placed around the subject’s proximal thigh to induce circulatory occlusion during the experiments. Carbonized rubber electrodes (4.5 × 4 cm), coated with a conductive gel and held in place with paper tape, were used instead of the self-adhesive electrodes used in the first set of experiments. It was found that the signal-to-noise ratios of the spectra were improved with these electrodes vs. the self-adhesive variety. The electrode placement was the same as that described above. The subject then performed three plantar flexion MVICs, and the greatest of the three values was used to set the stimulation intensity. Again, the session continued only if the subject was able to produce a MVC that was ≥95% of the MVC produced during the training session. Stimulation intensity was set to produce 20% MVC.

31P-NMR spectra were acquired during two stimulation protocols. One consisted of eight 80-Hz, 2-s trains with an intertrain interval of 30 s (total contraction/relaxation time of 32 s), and the other consisted of eight 100-Hz, 2-s trains with a 30-s intertrain interval. We chose long stimulation trains (2 s) to maximize the difference in number of pulses between the two trains while minimizing the number of contractions during ischemia in an effort to limit subject discomfort. Because only eight total trains were delivered, we were less concerned about electrical fatigue than we were during the fatigue tests. As recommended by Matheson and colleagues (28), we tested both protocols on each subject within a single session, to control for the effects of any sampling of inactive tissue with the surface coil. The order in which these protocols were delivered was randomly determined for each subject. 31P-NMR data were acquired by using a 6-cm × 8-cm oblong surface coil, double-tuned to 1H and 31P (43). The coil was placed over the upper one-third of the MG. Spectra were collected at rest, during ischemic stimulation, and during recovery.

The session began with collection of NMR spectra for 5 min with the subject at rest, after which circulation was occluded by inflating the blood pressure cuff. An additional 5 min of resting spectra were collected during circulatory occlusion. At the end of these 5 min, the first stimulation protocol commenced. The blood pressure cuff was released 30 s after the last train was delivered, and spectra were collected throughout 5 min of recovery. The subject was allowed to rest for 15 min after the end of the protocol, and then the process was repeated by using the second ischemic protocol.

DATA ACQUISITION. Phosphorous spectra were collected using an adiabatic 90° pulse with a sweep width of 3 kHz and 1,024 complex data points. Pulse repetition time (TR) was set at 4 s. Homogeneity of the magnetic field was adjusted using the proton signal (full width at half-maximal height ≤ 30 Hz), and the spectral data were filtered with an exponential filter corresponding to a line broadening of 5.1 Hz. These spectra were collected into eight-sum bins, providing a temporal resolution of 32 s. This allowed us to collect spectra that included one stimulation train per bin during exercise while improving our signal-to-noise ratio through signal averaging. Fully relaxed spectra (TR = 30 s) were collected to correct for saturation during the 4 s TR.

DATA ANALYSIS. Spectra were manually phased and the areas of ATP, phosphocreatine (PCr), phosphomonoester (PME), and P, peaks were manually integrated using customized software (42). Intracellular pH was calculated from the chemical shift of P, based on the equation

\[ p\mathrm{H} = 6.75 + \log \left[ (6 - 3.27)/(5.69 - \delta) \right] \]  

where \( \delta \) is the chemical shift of the P, peak in parts per million relative to P Cr. Absolute concentrations of phosphorous metabolites were calculated based on a resting ATP concentration ([ATP]) of 8.2 mM (21). Saturation correction factors were determined from fully relaxed spectra (30 s) and were 1.6, 2.1, and 1.6 for P, PCr, and ATP, respectively. The ATPase rate during ischemic exercise was determined by using Eqs. 3 and 4 as has been done previously (26, 42)

\[ \text{ATPase} = 1.5L + d\text{PCr/dt} + d\text{ATP/dt} \]  

where L represents anaerobic glycolysis and can be calculated as follows

\[ L = (-\beta_{\text{tot}} \text{dpH/dt}) - (0 \text{dPCr/dt}) \]  

The \( \beta_{\text{tot}} \) is the apparent buffer capacity of the muscle in millimoles of acid added per unit change (\( \Delta \)) in pH (slykes) and is determined initially from \( \Delta \text{PCr} \) concentration ([PCr])/\( \Delta \text{pH} \) during ischemic exercise. \( \theta \) represents the millimolar concentration of protons released by PCr when coupled to P i and is determined initially from Eqs. 3 and 4.

RESULTS

**Fatigue Experiments**

A total of 150 trains were delivered during each protocol. Because each 80-Hz train contained 17 pulses and each 100-Hz train contained 21 pulses, the 100-Hz protocol delivered 600 more pulses (23.5% more) to the muscle than the 80-Hz protocol. However, no differences between the 80- and 100-Hz trains in peak force or FTI were noted at either the initiation or the completion of the fatiguing protocols (Fig. 2). Peak force began to decline within the first few trains for both fatiguing protocols, but the FTI remained at or above the initial level for 40–50 trains (Fig. 2). This atten-
ation in the decline of FTI can be attributed to the marked slowing of relaxation that occurred during the protocol (see Fig. 2D). Although peak force was clearly decreasing, the increase in relaxation time resulted in the maintenance of a high FTI.

The percent declines in peak force for the 80- and 100-Hz protocols were 65.1 and 63.9%, respectively. The declines in FTI were 49.2 and 48.4%, respectively. Fitting the peak force responses of each subject to Eq. 1 produced $R^2$ values of 0.987 ± 0.003 and 0.985 ± 0.004 for the 80- and 100-Hz protocols, respectively. The corresponding values of $c$, indicating the number of the contraction at which 50% of the decline is achieved, were 46 ± 2.95 and 47 ± 2.25. The values for $b$, representing the steepness of the linear portions of the slopes, were −3.31 ± 0.39 and −3.32 ± 0.40. There were no significant differences between protocols for any of these variables, indicating that the rate, as well as the amount, of fatigue was similar for the two protocols (Fig. 2C), despite the 23.5% greater number of pulses in the 100-Hz protocol.

**Metabolic Cost Experiments**

A total of eight 2-s trains were delivered in each protocol. Because the 100-Hz protocol consisted of 201-pulse trains and the 80-Hz protocol consisted of 161-pulse trains, there was a total difference of 320 pulses between the two protocols. This corresponded to a 24.8% increase in the number of pulses when going from the 80- to the 100-Hz protocol.

Throughout the experiment, ATP levels did not change significantly and they were not significantly different across protocols (see Figs. 3 and 4). Significant changes in the other metabolites occurred during both protocols, but, despite the difference in the number of pulses, there were no significant differences between protocols in the percent changes in [PCr], [Pi], and PME concentration or the change in pH (Fig. 4).

The decline in [PCr] was 14.27 mM for the 100-Hz protocol and 13.01 mM for the 80-Hz protocol. The increases in [Pi] were 11.03 and 11.89 mM for the 100- and 80-Hz protocols, respectively. By the sixth contraction, PME peaks became observable above the baseline.
noise during both protocols and reached its maximum area at the eighth contraction. The peak PME concentrations observed for the 80- and 100-Hz protocols were $1.90 \pm 0.36$ and $1.53 \pm 0.19$ mM, respectively. These PME values were used to calculate the buffer capacity due to G6P ($\beta_{G6P}$) values for each protocol. Consistent with previous observations (7), pH exhibited an initial increase before showing a decline that persisted for $\sim 90$ s after the end of the ischemic stimulation. The pH values declined from 7.01 and 7.00 to 6.80 and 6.77 for the 100- and 80-Hz protocols, respectively.

The calculated ATP costs per contraction were 4.02 and 4.08 mM/contraction for the 80- and 100-Hz protocols, respectively, and they were not significantly different. These values correspond to mean ATPase rates of 120.61 and 122.17 mM/min, respectively. Because the total contraction time for each protocol consisted of 16 s (eight 2-s contractions), the total ATP hydrolyzed during the protocols was 32.16 and 32.65 mM, respectively.

**DISCUSSION**

This study was designed to examine changes in fatigue and metabolic cost associated with an increase in the number of activations while keeping force constant. We used two different experimental setups to address the different phenomena (fatigue and metabolic cost), each of which tested the outcome of altering the frequency and number of pulses while controlling for force. We were able to accomplish this by examining two high-frequency trains (80 and 100 Hz) that produced comparable forces but differed by $>20\%$ with regard to the number of stimulation pulses.

The principal finding of this study was that increasing the frequency and number of pulses had no effect on the fatigue produced during repetitive activation, when the force produced by the stimulation was controlled. This finding contradicts the frequently made assertion that increasing frequency will increase fatigue (25, 31). These observations build on earlier work that found other factors to be more important than the number of pulses and/or frequency of stimulation as determinants of fatigue (5, 20, 38). In addition, we observed no significant increase in the metabolic demand placed on the muscle when the number of stimulation pulses was increased by $>20\%$. Because of the variability of the data and the small sample size ($n = 6$), the lack of a statistically significant effect of fre-
quency on metabolic cost may have been due to insufficient power with regard to these data. However, the extremely small difference in metabolic cost (~1.3%) observed is consistent with what one would anticipate given our finding that frequency did not affect fatigue. Given this context, we contend that the observed lack of difference is real and not simply due to insufficient statistical power. We did not, however, collect any metabolic data during the fatiguing protocols, and thus we cannot comment on any metabolic changes that might have occurred during those protocols.

The mean rates of ATP consumption calculated in the present study (120.61 and 122.17 mM ATP/min for the 80- and 100-Hz protocols, respectively) were less than the peak rate of 287 mM ATP/min reported by Walter and colleagues (42) for high-intensity voluntary exercise in the human plantar flexors. Walter and colleagues, however, examined repeated, maximal, dynamic contractions at a rate of 2–3 s⁻¹. Thus there were a greater total number of contractions within a given time period and the muscle was allowed to shorten during contraction. Both of these factors have been shown to increase ATP demand (10, 34). If resting ATP is assumed to be 5.5 mmol/kg wet wt (3), the ATPase rates in the present study become 1.35 and 1.37 mmol ATP·kg wet wt⁻¹·s⁻¹ for the 80- and 100-Hz trains, respectively. These values are ~10× the rate reported for single, isometric twitches (7, 12). The rates can be converted to units of millimoles ATP per kilogram dry weight per second by multiplying the millimoles ATP per kilogram wet weight per second values by ~4.5 (4, 10), giving rates of ~6.1 and 6.2 mmol ATP·kg dry wt⁻¹·s⁻¹, respectively. These values are comparable to those obtained during intermittent, 20-Hz stimulation in the human quadriceps muscle (4, 10).

Given that the present study used higher stimulation rates than 20 Hz, our ATPase rates appear somewhat low, particularly because we calculated ATPase rate based on the contraction time only, whereas many of the previous studies determined ATPase rates from the entire contraction and relaxation cycle (4, 7, 10, 42). Furthermore, the rates in the present study do not achieve the maximum rate of 2.0 mmol ATP·kg wet wt⁻¹·s⁻¹ suggested by Meyer and Foley (30) for human skeletal muscle. The present study may, however, have underestimated the rate of ATP consumption during the ischemic exercise. The ³¹P-NMR spectra collected in this study reflect an average based on the volume of muscle “seen” by the coil. Thus inactive muscle sampled by the coil would have attenuated metabolic changes reflected in the spectra (28). It is likely that a...
variable amount of such inactive tissue was sampled for each subject. This may explain the fact that the ATPase rates in the present study were lower than those reported in studies that examined comparable ischemic contraction protocols (32). In addition, the entire gastrocnemius may not have been activated at the stimulation intensity employed. However, because each subject received both protocols within a single session and the position of neither the electrodes nor the coil was changed, the error associated with this sampling issue was similar for both protocols. This study was concerned with relative differences between protocols rather than absolute values, and thus this potential sampling error does not affect the validity of the results.

Our finding that an increase in the number of stimulation pulses because of an increase in frequency had no effect on either fatigue or metabolic cost of contraction suggests that the noncontractile cost of activation is a minor factor in contractile metabolism compared with the AM-ATPase, which contradicts the findings of Frank et al. (17). One caveat, however, is that only high (nonphysiological) frequencies of stimulation were employed in this study, whereas Frank et al. studied frequencies from 0.5 to 3 Hz. We chose high frequencies to produce similar force responses with different frequencies, to test our hypothesis that changes in fatigue due to changes frequency were the result of the accompanying changes in force. It is possible that the number of pulses might have played some role in fatigue at lower, more physiological discharge rates.

The bulk of the noncontractile ATP-costs associated with contraction are due to the activity of the Na\(^+\)-K\(^+\)-ATPase and the SR Ca\(^{2+}\)-ATPase. Although the Na\(^+\)-K\(^+\)-ATPase should respond to each stimulation pulse at the frequencies used in this study (29), it may be that the SR Ca\(^{2+}\)-ATPase is running at its maximum rate in response to the 80-Hz stimulation used here. In this scenario, the increase to 100 Hz would not increase the metabolic demand and thus the fatigue. Indeed, Stienen et al. (40) demonstrated that the SR ATPase activity was at maximum at pCa levels that corresponded to relative forces that were on the ascending, linear portion of the force-pCa curve. Given the similarity in the shape of the force-pCa and force-frequency curves, it seems reasonable that both the 80- and 100-Hz stimulation used in this study resulted in maximum SR ATPase activity. At lower frequencies of stimulation, the addition of pulses by increasing frequency may increase the activity of the SR ATPase and substantially increase the metabolic demand. Previous research on the metabolic cost of the Na\(^+\)-K\(^+\)-ATPase in isolated amphibian muscle suggests that it accounts for \(\approx10\%\) of the total metabolic cost during a contraction (23, 30). Although we did not directly assess the cost of the Na\(^+\)-K\(^+\)-ATPase in the present study, our results are consistent with these findings.

In summary, increasing stimulation frequency did not increase fatigue during repetitive, electrical stimulation of the MG muscle, when the force produced by the stimulation was controlled. This finding contradicts the commonly accepted view that fatigue increases in response to increasing frequency (25, 31) and suggests that the force produced by stimulation is a more important determinant of fatigue than the frequency of stimulation. Similarly, the additional pulses associated with the increase in stimulation frequency did not increase the metabolic cost of contraction. However, this study only examined high frequencies of stimulation, on the flat portion of the force-frequency relationship. Thus there is potentially still an effect of frequency on fatigue, independent of force, at lower stimulation frequencies, on the steep portion of the force-frequency curve.

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