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doi:10.1152/japplphysiol.00403.2009

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Combined effects of fatigue and eccentric damage on muscle power

Seung Jun Choi1 and Jeffrey J. Widrick2

1Department of Nutrition and Exercise Sciences, Oregon State University, Corvallis, Oregon; and 2Department of Physical Medicine and Rehabilitation, Harvard Medical School, and Spaulding Rehabilitation Hospital, Boston, Massachusetts

Submitted 16 April 2009; accepted in final form 1 August 2009


Many physical activities can induce both transient and long-lasting muscle dysfunction. The separate and interactive effects of short-term fatigue and long-lasting contraction-induced damage were evaluated in an in vitro mouse soleus preparation (35°C) using the work loop technique. Repetitive fatiguing work loops reduced positive work (work produced by the muscle), increased negative work (work required to reextend the muscle), and reduced cyclical power (net work/time) immediately after treatment. These changes were readily reversible. The fatigue treatment had no long-term effects on optimal muscle length (L0) and isometric force (P0). High strain lengthening work loops, where the muscle contracted eccentrically, resulted in both immediate and long-lasting positive work, power, and P0 deficits as well as a shift in L0 to longer lengths. When the treatments were combined, i.e., fatigued muscles subjected to eccentric activity, the immediate power deficit exceeded the sum of the power deficits noted for the other two treatments. Much of this effect was due to an exaggerated rise in negative work. However, in the long term, power and P0 deficits and the shift in L0 were reduced compared with the damage-only treatment. These results show that 1) the immediate effects of combined fatigue and damage on cyclical power are synergistic, in large part because of a reduced ability of the muscle to relax; and 2) fatigued muscles are less susceptible to long-term contraction-induced dysfunction. Fatigue may protect against long-term damage by reducing the probability that sarcomeres are lengthened beyond myofilament overlap.

pliometric; lengthening contractions; popping sarcomere hypothesis; work loops

OBER A CENTURY AGO, Hough (18) identified two distinct types of dysfunction that can occur after repetitive muscular activity. Muscle fatigue was relatively short lived and was attributed to the accumulation and subsequent dispersion of the byproducts of contractile activity. In contrast, some types of muscular activities induced prolonged dysfunction that required days for full recovery. Hough proposed that this long-lasting dysfunction was due to physical damage to the muscle. It is now known that long-lasting contractile dysfunction is most likely to occur when active muscles are lengthened by external forces (1, 36, 49).

Many physical activities, with locomotion being a prime example, require muscles to undergo repetitive cycles of shortening and lengthening. These activities therefore have the potential to induce both short-term fatigue and long-term muscle damage. Studies addressing the interaction of fatiguing and damaging contractile activity on muscle function are limited in number and their findings equivocal. Frödin and Lieder (16) proposed that fatigued muscles would be more susceptible to damage by subsequent lengthening contractions based on their reasoning that the rapidly fatiguing fast fibers would enter a rigor state and be disrupted as they were stretched. However, McCully and Faulkner (30), using in situ and in vitro rat extensor digitorum longus preparations, and Nosaka and Clarkson (34), who studied the voluntary muscular performance of human subjects, reported that fatigue had the opposite effect, i.e., it reduced the severity of the long-term dysfunction that occurs after lengthening contractions. More recently, Morgan et al. (32) found that fatigued and nonfatigued motor units of the cat gastrocnemius muscle displayed similar susceptibility to lengthening contractions, a finding that was interpreted as being consistent with the popping sarcomere theory of muscle damage (31, 37).

A better understanding of the interaction between muscle fatigue and damage would be expected to have important practical, clinical, and mechanistic implications. In this study, we used the work loop procedure to subject isolated mouse soleus muscles to repetitive cycles of shortening and lengthening. This approach mimics the repetitive, cyclical activity that muscles may undergo during in vivo locomotion (20, 23, 28). We varied the number, strain, and frequency of these cycles as well as the portion of the cycle when the muscle was stimulated to induce 1) short-term dysfunction, or fatigue; 2) longer-term dysfunction, referred to as damage; or 3) a combination of fatigue and damage. We evaluated the effects of these treatments on the recovery of cyclical power, a key determinant of skeletal muscle performance (23, 28). We also evaluated postrecovery isometric force (P0) and optimal muscle length (L0), two other indexes of muscle damage (1, 36, 37).

MATERIALS AND METHODS

Animals. Male ICR mice (Harlan, Indianapolis, IN, body weight: 40–50 g) were used in this study. All mice were housed under the same environmental conditions (12:12 h light-dark cycle at 22°C) with ad libitum access to a standard rodent diet and tap water. The use of these animals was approved by the Institutional Animal Care and Use Committee of Oregon State University.

Muscle preparation. On the day of an experiment, animals were anesthetized with pentobarbital sodium (40 mg/g body wt). Intact soleus muscles were dissected, and the animals were humanely euthanized. Silk sutures were used to attach the proximal tendon to the lever arm of a dual-mode muscle lever system (model 300B-LR or model 305C, Aurora Scientific, Aurora, ON, Canada) and the distal tendon to a stable hook. We (40) have previously reported that the myosin heavy chain isoform distribution of the ICR soleus muscle is ∼50% slow and ∼50% fast.

The muscle was suspended vertically in bicarbonate buffer [containing (in mM) 137 NaCl, 5 KCl, 1.25 CaCl2, 1.0 MgSO4, 1.0 NaH2PO4, 24 NaHCO3, 11 glucose, and 0.025 tubocurarine chloride] equilibrated with 95% O2 and 5% CO2. Buffer temperature was maintained at 35°C. Functional data were collected using a personal computer and a data acquisition board (model AT-MIO16E-10, Na-
Assessment of Po. Tetani were induced with supramaximal 500-ms trains consisting of 200-μs square-wave pulses delivered at a frequency of 200 Hz. For protocol step 1, the muscle length was systematically altered every 3 min and force was reevaluated. The muscle length that produced maximal isometric force was defined as $L_o$. This length was measured using a digital micrometer. At protocol step 7, muscles were shortened by 0.5 mm, and $L_o$ was reassessed systematically. In all $L_o$ assessments, the two muscles under study received the same number of contractions.

Assessment of cyclical power. The ability of muscles to produce cyclical power was assessed using work loops. Muscles were subject to sinusoidal changes in length centered about their $L_o$. Cycle amplitude and frequency were ±5% of fiber length and 5 Hz, respectively. These parameters are optimal for cyclical power output of the mouse soleus muscle at 35–37°C (4, 20). Power was optimized by initiating stimulation 15 ms before the muscle attained its maximum length (phase = 17.5%, where phase is defined as a percentage of the entire work loop cycle) and terminating stimulation 50 ms before the muscle reached its minimum length (phase = 50%). When power was assessed, muscles received three consecutive sinusoidal cycles. Force and length data were recorded and plotted to form a loop (see Fig. 1). Loop force was defined as the maximum force attained during the loop (measured from the $L_o$ resting tension baseline). The work

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Fig. 1. Optimal and lengthening work loop examples. A: optimal work loops. Sinusoidal changes in length were imposed upon the muscle (indicated by L). The cycle frequency was 5 Hz, and the cycle amplitude was ±5% of the fiber length [centered at optimal muscle length ($L_o$)]. Muscle stimulation (indicated by S) was timed to optimize power (see METHODS for details). Force was recorded (indicated by F) and plotted against muscle length to form a work loop. In this example, the work loop obtained from the third cyclical contraction is shown. The arrow indicates the direction of loop. The length of 0.0 mm indicates $L_o$. As shown by the shaded areas, negative work and positive work were calculated as the force $\times$ length integral during muscle lengthening and shortening, respectively. Net work was defined as positive work minus negative work, i.e., the area enclosed by the work loop. Net work per unit time defined cyclical power. The time scale is identical for L, S, and F records. The force scale pertains to both the F record and work loops. B: lengthening work loops. Sinusoidal changes in length were imposed upon the muscle (indicated by L) at a frequency of 2 Hz and an amplitude of ±25% of the fiber length (centered at $L_o$). Muscles stimulation (indicated by S) was timed to maximize force during lengthening (see METHODS for details). Force was recorded (indicated by F) and plotted against muscle length to form a work loop. In this example, only the initial 3 of 10 cyclical contractions are shown, and the work loop obtained from first cyclical contraction is shown. The arrow indicates the direction of loop. The length of 0.0 mm indicates $L_o$. Negative work, positive work, net work, and power were calculated as described in A. The time scale is identical for L, S, and F records. The force scale pertains to both the F record and work loops. Note that the time scale and amplitude of the L, F, and work loop records differ from A. For a direct comparison of optimal and lengthening work loops, see Fig. 2.
produced by the muscle (shortening or positive work) and the work absorbed by the muscle (lengthening or negative work) were defined as the force by length integrals during muscle shortening and muscle lengthening, respectively. Net work was calculated as work produced minus work absorbed. Cyclical power was defined as net work per unit time. We expressed work and power relative to muscle mass to be consistent with the animal locomotion literature (4, 20). Because muscle density is only slightly greater than 1.00 (1.06 mg/mm³), values expressed in this manner are within a few percent of work or power per unit muscle volume.

Experimental treatments. Both soleus muscles were studied from each mouse. Each soleus muscle was assigned to one of four treatments: control (C), fatigue (F), damage (D), or fatigue and damage (FD). Assignment was arranged so that two consecutively studied mice completed one replicate of all four experimental treatments. All of the experimental treatments consisted of a 5.0-s stage 1 and a 5.0-s stage 2, separated by 200 ms, for a total treatment time of 10.2 s. For the C treatment, muscles were not administrated any length change or stimulation during this time period. The F, D, and FD treatments are shown in Fig. 2. For the F treatment, muscles were administrated 25 consecutive cyclical contractions using the same parameters and stimulation timing as used for the assessment of optimal power. The first cyclical contraction coincided with the initiation of the treatment period. Thus, work loops occurred during the initial 5.0 s of the treatment period with the muscle quiescent during the remaining 5.2 s.

For the D treatment, muscles were administrated 10 consecutive cyclical contractions at an amplitude of ±25% of fiber length and a cycle frequency of 2 Hz. To activate the muscle during the lengthening portion of the cycle, stimulation was initiated 15 ms before the muscle attained its shortest length (phase = 22%) and continued until the muscle had attained its maximal length (phase = 75%). This treatment commenced at the treatment time point of 5.2 s and concluded at the time point of 10.2 s.

For the FD treatment, the muscle was administrated 25 fatiguing cyclical contractions (identical to those used for the F treatment) followed 200 ms later by 10 damaging cyclical contractions (identical to those used for the D treatment). Thus, the fatiguing cycles occurred between the treatment time points of 0.0 and 5.0 s and the damaging cycles between 5.2 and 10.2 s, identical to the timing of the F-only and D-only treatments, respectively.

Statistical analysis. As has been reported by others (4, 20), we found that power was maximized on either the second or third work loop, with a <1% difference between these loops. Therefore, data from the third loop were used for statistical analysis.

All data are presented as means ± SE. Variables were analyzed with one-way ANOVA (main effect of treatment) or two-way repeated-measures ANOVA (main effects of treatment and time, treatment × time interaction). In the event of a significant F ratio, the Holm-Sidak post hoc procedure was used to identify differences between specific means. The type I error rate was <0.05. All analyses were performed using SigmaPlot 11.0 (Systat Software, San Jose, CA).

RESULTS

Characteristics of the muscles before treatment. Observed mean values for P₀ (254 ± 9 kN/m², n = 28) and cyclical power (35.5 ± 1.4 W/kg, n = 28) were in good agreement with literature values for the mouse soleus muscle at 35–37°C [212–287 kN/m² (Refs. 3, 4, 20, and 48) and 33–34 W/kg (Refs. 4 and 20)]. There were no between-group differences in soleus mass, L₀, P₀, or any work loop parameters before treatment (Table 1). The maximal force attained during the pretreatment cyclical contractions, or loop force, averaged 46 ± 1% (n = 28) of peak P₀ (no differences between treatments).

Responses to the fatigue protocol (F and FD treatments). Because they had received no prior treatment, muscles in the F and FD treatments responded similarly to the fatigue protocol. On the 25th (final) cycle of the protocol, loop force was reduced by 16% (Fig. 3A) and power was reduced by 48% (Fig. 3B) compared with the peak values measured on the 3rd cycle. As shown in Fig. 3B, the reduction in power was substantially greater than the reduction in loop force.

Fig. 2. The experimental treatments. A: force and work loop records obtained during the experimental treatments. Each row represents records from a single muscle, with a pretreatment isometric contraction included for reference. The fatigue treatment (F treatment) was designed to induce short-term dysfunction. This treatment, administrated during stage 1 (initial 5 s) of the experimental protocol, consisted of 25 consecutive work loops using the parameters described in Fig. 1. The damage treatment (D treatment) was designed to induce long-term dysfunction. This treatment, administrated during stage 2 of the protocol (from 5.2 to 10.2 s), consisted of 10 cyclical contractions at an amplitude of ±25% of fiber length (centered at L₀) and a frequency of 2 Hz. Stimulation was timed to maximize force during the lengthening portion of each cycle (see METHODS for details). The combined fatigue and damage treatment (FD treatment) consisted of the F treatment followed 200 ms later by the D treatment. The work loop direction is indicated by an arrow. All records in A are scaled identically. P₀, isometric force. B: expanded view of the stage 1 work loops obtained during the F treatment (i) and FD treatment (ii) of A. Both work loops are scaled identically.

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1.87-fold greater than pretreatment Po (Table 1 and Fig. 4 on average, 3.95-fold greater than the pretreatment loop force and force attained on the initial lengthening cycle of the D treatment was, treatment. For clarity, only B cycle. force expressed as a percentage of the peak loop force attained on the third

Table 1. Pretreatment morphological and functional characteristics of soleus muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mass, mg</th>
<th>Optimal Muscle Length, mm</th>
<th>Isometric Force, kN/m²</th>
<th>Loop force, kN/m²</th>
<th>Work produced, J/kg</th>
<th>Work absorbed, J/kg</th>
<th>Power, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.2±0.7</td>
<td>13.2±0.4</td>
<td>253±20</td>
<td>111±9</td>
<td>9.6±0.7</td>
<td>2.9±0.4</td>
<td>33.5±3.7</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9.9±0.6</td>
<td>13.1±0.3</td>
<td>246±17</td>
<td>112±8</td>
<td>9.9±0.7</td>
<td>3.2±0.3</td>
<td>33.4±2.6</td>
</tr>
<tr>
<td>Damage</td>
<td>9.5±0.5</td>
<td>13.3±0.3</td>
<td>271±13</td>
<td>127±5</td>
<td>10.9±0.4</td>
<td>2.9±0.3</td>
<td>39.9±2.2</td>
</tr>
<tr>
<td>Combined fatigue and damage</td>
<td>9.3±0.3</td>
<td>12.8±0.2</td>
<td>248±19</td>
<td>117±9</td>
<td>10.1±0.8</td>
<td>3.1±0.4</td>
<td>35.1±2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 muscles/treatment. All work loop data are from the third cycle of the pretreatment assessment. There were no between-treatment differences for any variable (P > 0.05).

because the change in power reflected a reduction in positive work (17% reduction from peak) coupled with an increase in negative work (50% increase above minimum).

Responses to the damage protocol (D and FD treatments). Loop force attained on the initial lengthening cycle of the D treatment was, on average, 3.95-fold greater than the pretreatment loop force and 1.87-fold greater than pretreatment Po (Table 1 and Fig. 4A). For the D treatment, loop force declined 25% across 10 successive lengthening cycles (Fig. 4A). The total work absorbed by the muscle averaged 1,347 ± 49 J/kg (Fig. 4B). During the FD treatment, muscles showed similar rates of absolute force decline and work absorption as the D treatment (Fig. 4, A and B; no significant interactions). However, because of the prior fatigue protocol, force on the initial lengthening cycle averaged 19% less for the FD treatment. Thus, the FD muscle experienced less relative decline in force during the damage protocol (Fig. 4B), and the total work absorbed by the muscle was 11% less (Fig. 4B).

Immediate posttreatment power. Cyclical power measured immediately after the C treatment averaged 100 ± 0% (range: 98.5–101.2%) of the pretreatment value (Figs. 5 and 6A). The F, D, and FD treatments all showed significant reductions in cyclical power compared with the C treatment. These changes are evident in the representative experiments shown in Fig. 5 as reductions in the area enclosed by the work loop, although the extent that power differed greatly across treatments. The average smallest reduction was observed after the F treatment, where power was 27% lower than the pretreatment value (Fig. 6A). This decline was attributed to a reduction in positive work (Fig. 6B) coupled with an increase in negative work (Fig. 6C). The reduction in positive work was proportional to a loss in the ability of the muscle to produce force (Fig. 6D).

Power was reduced 34% by the D treatment, which was a greater decline than noted after the F treatment (Fig. 6A). However, in contrast to the F treatment, the power deficit after the D treatment was due entirely to a reduction in positive work (Fig. 6, B and C). In fact,
negative work fell after the D treatment, a change that would tend to increase power output.

The greatest power deficit was observed after the FD treatment, where power was 84% lower than the pretreatment value (Fig. 6A). The representative FD experiment shown in Fig. 5 demonstrates a greatly impaired ability of the muscle to produce force and to fully relax at the conclusion of the posttreatment stimulation, resulting in a reduction in work during shortening and an increase in work during the lengthening portion of the cycle. On average, the FD treatment induced the greatest reduction in positive work coupled with the greatest increase in negative work compared with all other treatments (Fig. 6, B and C).

Recovery power and $P_o$. Cylindrical power remained constant for the C treatment during recovery (Figs. 5 and 6A). As shown in the example in Fig. 5 and compiled in Fig. 6, the F treatment showed a rapid recovery of loop force, positive work, negative work, and cylindrical power all within the initial 6 min of the recovery period.

For the D treatment, small increases in loop force, positive work, and power occurred over the initial 6–12 min of recovery (Fig. 6). Additional recovery did not occur beyond those time points so that at the end of the recovery period, loop force (−22%), positive work (−26%), negative work (−41%), and power (−20%) were all depressed relative to pretreatment values. Figure 5 shows that the FD treatment showed an almost complete restoration of power by the end of the recovery period. This was confirmed by the mean data shown in Fig. 6A, which demonstrates that cylindrical power after 30 min of recovery was significantly greater for the FD treatment versus the D treatment and statistically similar to the C and F treatments. This complete recovery of power was attributed to a partial restoration of positive work coupled with a decline in negative work.

Recovery $P_o$. As shown by the solid bars in Fig. 7A, there were no long-term changes in $P_o$ for the C and F treatments. However, the D treatment resulted in a long-term $P_o$ deficit of 26%. A $P_o$ deficit also existed after the FD treatment, but its magnitude was half that observed for the D-only treatment.

Reassessment of $L_o$ and $P_o$. As shown by the open bars in Fig. 7A, the C and F treatments had no effect on $L_o$ and, consequently, no effect on the final $P_o$ evaluation (shaded bars in Fig. 7A). In contrast, $L_o$ was 9.6 ± 0.7% longer after the D treatment. When force was evaluated at this new length, the $P_o$ deficit was reduced from 26% to 18%. The FD treatment also showed a significant increase in $L_o$ after recovery, but the magnitude of this increase was roughly half (4.3 ± 0.5%) of the increase noted for the D treatment. When $P_o$ was reevaluated at this new $L_o$, the $P_o$ deficit was reduced from 13% to 9%.

Reassessment of cylindrical power. Figure 7B shows mean cylindrical power measured in a subset of muscles after the reassessment of $L_o$. There were no changes in cylindrical power from the original $L_o$ to the final $L_o$, for the C or F treatments. However, cylindrical power decreased for the D and FD treatments at the final, longer $L_o$. This reduction in power at the reassessed $L_o$, despite an increase in force at this muscle length, was due mainly to an elevation in negative work.
DISCUSSION

Definitions. Both muscle fatigue and muscle damage can be characterized by a loss in muscle function (13, 37). This does not imply that the mechanisms responsible for these functional changes are similar. Indeed, the mechanisms differ because fatigue and damage can be distinguished by their time course of recovery: fatigue is a relatively transient depression in function, whereas damage requires days to weeks for full recovery (13, 37). We used repetitive work loops, conducted using parameters that optimized pretreatment power output, to induce a transient and reversible loss in cyclical power (F treatment), which we define as fatigue. Within the 30 min postrecovery limitation imposed by this study, a long-term functional depression, which we define as damage, were induced by a protocol in which the muscle performed lengthening or eccentric contractile activity during larger magnitude work loops (D treatment). While these descriptive definitions may have limitations [for instance, they are inconsistent with a long-lasting dysfunction termed low-frequency fatigue (7, 22)], they are generally accepted and have been used in this report.

The work loop model. The work loop procedure was developed to model the repetitive, cyclical muscular activity that is characteristic of aquatic, airborne, and terrestrial locomotion (23, 28). Because movement requires a net positive work output by skeletal muscles, cyclical power is a key determinant of animal locomotion. The magnitude of a muscle’s cyclical power is dependent on its length-tension relationship, its force-velocity relationship, and its rate of activation and relaxation (23, 28). Damaging lengthening contractions have long-term effects on all of these properties (32, 48, 53), whereas fatigue has transient effects on the force-velocity relationship and muscle activation/relaxation (12, 15, 44, 51). We therefore reasoned that work loops would provide a comprehensive approach for assessing the separate and interactive effects of fatigue and damage on muscle performance.

We attempted to study work loops under physiologically relevant conditions. The cycle amplitude and cycle frequency used for optimal power determination fell well within the range of soleus strain amplitudes and stride frequencies calculated during normal mouse gait (20). The strain magnitude during the lengthening contractions also fell within the physiological range (8). However, the sinusoidal changes in length used here are probably a simplification of the more complex patterns of shortening and lengthening that muscles may undergo in vivo (28). Thus, the preparation studied here should be considered a generalized model of muscle function, but not necessarily representative of any specific muscle.

One limitation of our approach is the possibility that the D treatment induced some fatigue as the muscle was being damaged. Lengthening contractions result in less perturbation of intracellular high-energy phosphate homeostasis compared with shortening contractions (38) and are therefore less likely to induce fatigue. While we tried to take advantage of this in the design of our experiment, there is a small increase in power over the initial minutes of recovery from the D treatment. This suggests that some fatigue may have occurred during the D portion of the D treatment. Lengthening contractions result in less perturbation of intracellular high-energy phosphate homeostasis compared with shortening contractions (38) and are therefore less likely to induce fatigue. While we tried to take advantage of this in the design of our experiment, there is a small increase in power over the initial minutes of recovery from the D treatment. This suggests that some fatigue may have occurred during the D portion of the D treatment. However, the recovery after the D treatment was clearly limited and substantially different from the complete recovery of power observed 6 min after the completion of the F treatment. Thus, for the D treatment, any fatigue effect was considerably less than the amount of damage that occurred. For the FD treatment, any fatigue produced during the D portion would mean that slightly more fatigue occurred in this treatment than during the F-only
treatment. Neither of these would appear to be quantitatively important when one considers the very clear differentiation between the responses of the F, D, and FD treatments shown in Fig. 5 and compiled in Fig. 6. Thus, any fatigue contaminating the D or FD treatments is unlikely to alter the general conclusions drawn from this work.

Summary of the findings and relationship to previous work. The absence of any functional change during the C treatment verifies that the observed changes during the F, D, and FD treatments can be attributed to the treatments per se and not to any time-dependent change in the preparation. Fatiguing contractions (F treatment) induced an immediate, but transient, reduction in cyclical power due to a reversible reduction in positive work coupled with a reversible rise in negative work. Similar changes in positive work, negative work, and cyclical power have been reported by others studying fatiguing contractions (29) and allow $Ca^{2+}$ influx into the cell (39) present only when lengthening is immediately power loss but 2) reduced the long-term power, force, and $L_o$ changes associated with lengthening contractions.

Immediate effects. The mechanism responsible for the loss of cyclical power immediately after the FD treatment appears to be related to an exacerbated increase in negative work as muscles failed to completely relax as they were extended in preparation for another cycle of shortenings. While the mechanism responsible for this effect in the FD treatment cannot be determined from the present study, it would have to have the following characteristics: 1) present only when lengthening is combined with fatigue and 2) readily reversible in a time course roughly similar to the restoration of positive work. Stretch-activated channels that are activated during lengthening contractions (29) and allow $Ca^{2+}$ influx into the cell (39) may be a mechanism underlying these observations. A nonfatigued fiber subjected to lengthening contractions may be able to adequately buffer $Ca^{2+}$ entering through these channels. A fatigued fiber, where sarcoplasmic reticulum $Ca^{2+}$ uptake may already be compromised (52), may be unable to buffer any additional $Ca^{2+}$ influx that occurs as a result of lengthening contractions. This inability to buffer $Ca^{2+}$ might then impair relaxation. Additional work will be required to test this hypothesis.

Long-term effects. The long-term effect of fatigue was to reduce dysfunction due to lengthening contractions. This result, obtained on a mixed-fiber composition, oxidative muscle, is in agreement with the results of McNally and Faulkner (30), who studied the glycolytic, fast extensor digitorum longus muscle. Taken together, these two studies suggest that the interactive effect of fatigue and damage is not confined to muscles of a particular fiber type or metabolic profile. Nosaka and Clarkson (34) reported that prior fatigue reduced indexes of muscle damage and soreness in the elbow flexors of human volunteers. Our results suggest that the observations of Nosaka and Clarkson can be explained by processes within the muscle per se.

In contrast to the present work, Morgan et al. (32) concluded that fatigue had no effect on long-term damage from eccentric contractions in that it did not prevent a fall in $P_o$ or a shift in $L_o$. In the Morgan et al. study (32), different portions of the cat
gastrocnemius muscle were subjected to 10 eccentric contrac-
tions to induce damage, 200 concentric contractions to induce
fatigue, or 200 contractions followed by 10 eccentric contrac-
tions. Because the eccentric contractions were separated by
30 s of rest, it required almost 5 min to subject a previously
fatigued muscle to all 10 eccentric contractions. It is possible
that the muscle could have recovered from some or all of the
effects of the fatiguing contractions while awaiting the entire
eccentric treatment. This might have blunted any effects of
fatigue and explain why their conclusions differ from ours.

An important question is the mechanisms by which prior
fatigue reduced subsequent damage from lengthening contrac-
tions. One possibility is that by reducing force, fatigue lowers
the stress experienced by the muscle as it is actively stretched.
Maximum force during lengthening is clearly associated with
the extent of eccentric-induced muscle damage (30, 47). How-
ever, this strong association may simply be due to covariation
of force with another factor, or factors, that are truly causative.

Talbot and Morgan (45) studied toad muscles with very low
passive force characteristics to vary sarcomere length without
inducing large changes in total force. They reported that the
change in sarcomere length, but not force, was significantly
correlated with the posteccentric isometric force deficit and
increase in optimal length. Likewise, Lieber and Friden (25)
found that mammalian muscles lengthened at the onset of
stimulation showed force deficits similar to muscles allowed to
attain peak force before lengthening, even though the maxi-
imum force attained in the latter case was 33% greater. Because
maximum attained force does not appear to be a causative
factor in lengthening-induced muscle damage, it seems un-
likely that the fatigue protocol studied here conferred its
protective effects via a reduction in force per se.

Several experimental approaches have suggested that dam-
age may be causally linked to strain magnitude and sarcomere
length heterogeneity (25, 26, 35, 45). We propose that the
fatigue-induced long-term reduction in damage observed here
can be interpreted in terms of sarcomere heterogeneity, as put
forward by Morgan and colleagues (31, 37) in their popping
sarcomere hypothesis of muscle damage. The popping sarco-
mere hypothesis states that much of the length change during
stretch of an active muscle is taken up by a relatively small
number of sarcomeres. When one of these sarcomeres is
extended onto the descending limb of its length-active tension
relationship, it will continue to lengthen until its total tension is
equal to the tension produced by in series sarcomeres (which
remain on the plateau of their length-tension relationship). If
this sarcomere is overextended beyond thin and thick myofil-
ament overlap, myofilaments may fail to reinterdigitate when
the muscle is returned to its original length, rendering the
sarcomere nonfunctional. The mechanical strain resulting from
the continued overextension of this now-noncompliant sarcomere
may then be propagated longitudinally and radially to neigh-
boring sarcomeres, causing the disruption of cross bridges,
t-tubules, the sarcoplasmic reticulum, and sarcolemma (1).

In a short communication, Morgan and Prosko (33) proposed
that sarcomere popping may be reduced or eliminated under
conditions of partial activation because a sarcomere does not
need to be extended as far along its total tension curve before
its force is equal to the (reduced) active force of in-series
sarcomeres. Under these conditions, there is a reduced likeli-
hood that the thin and thick myofilaments will be extended
beyond overlap and a reduced likelihood of sarcomere pop-
ning. We propose that fatigue, by reducing the force of the
sarcomeres that remain on the plateau of the length-tension
relationship, causes similar behavior.

In this model, fatigue would reduce long-term dysfunction
by decreasing the likelihood of sarcomere overextension be-
yond thin and thick filament overlap. In the nonfatigued state,
the length at which the total tension of an overextended sarcomere equilibrates (with the tension of the sarcomeres
remaining on the plateau of the length-tension relationship) is
only slightly longer than the length at which thin and thick
filaments no longer overlap (see Fig. 1 of Ref. 32 and Fig. 7 of
Ref. 41). Thus, even a small decrease in the active tension of
in-series sarcomeres (a decrease in the plateau of the length-
tension relationship) has the potential to substantially reduce
sarcomere popping. This is consistent with the present results
in which a relatively mild fatigue protocol prevented much of
the long-term loss in power and increase in optimal length
attributed to lengthening contractions. This model also ex-
plains why force deficits were reduced in skinned fibers that
were only partially Ca2+ activated during lengthening (27) and
why postlengthening force deficits were greater in fibers in
which force had been potentiated by phosphorylation of the
regulatory light chain (10).

There may be other factors contributing to our results. The
optimal length for twitches occurs at a longer sarcomere length
than for tetanic contractions (11). If fatigue causes a similar
shift to a longer L0 due to a reduction in activation, then the
relative number of sarcomeres extended onto the descending
limb would be reduced. Repetitive contractions may also alter
the passive tension-sarcomere length relationship. The stiffness
of the titin filament, which confines passive tension to the
sarcomere (17), has recently been shown to be increased by
elevations in intracellular Ca2+ and by repetitive contrac-
tions (9, 24). A shift in the length-passive tension relationship to
the left (i.e., toward shorter sarcomere lengths) would be expected
to reduce the overextension of sarcomeres and lower the
probability of sarcomere popping. There is a rise in intracel-
lular Ca2+ in mildly fatigued muscle (50), and it may be
important to test whether this is sufficient to stiffen titin and
stabilize sarcomeres extended onto the descending limb of their
length-tension relationship.

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