Reports of the length dependence of fatigue are greatly exaggerated

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MacNaughton, M. B., and B. R. MacIntosh. Reports of the length dependence of fatigue are greatly exaggerated. J Appl Physiol 101: 23–29, 2006.—Relative force depression associated with muscle fatigue is reported to be greater when assessed at short vs. long muscle lengths. This appears to be due to a rightward shift in the force-length relationship. This rightward shift may be caused by stretch of in-series structures, making sarcomere lengths shorter at any given muscle length. Submaximal force-length relationships (twitch, double pulse, 50 Hz) were evaluated before and after repetitive contractions (50 Hz, 300 ms, 1/s) in an in situ preparation of the rat medial gastrocnemius muscle. In some experiments, fascicle lengths were measured with sonomicrometry. Before repetitive stimulation, fascicle lengths were 11.3 ± 0.8, 12.8 ± 0.9, and 14.4 ± 1.2 mm at lengths corresponding to −3.6, 0, and 3.6 mm where 0 is a reference length that corresponds with maximal active force for double-pulse stimulation. After repetitive stimulation, there was no change in fascicle lengths; these lengths were 11.4 ± 0.8, 12.6 ± 0.9, and 14.2 ± 1.2 mm. The length dependence of fatigue was, therefore, not due to a stretch of in-series structures. Interestingly, the rightward shift that was evident when active force was calculated in the traditional way (subtraction of the passive force measured before contraction) was not seen when active force was calculated by subtracting the passive force that was associated with the fascicle length reached at the peak of the contraction. This calculation is based on the assumption that passive force decreases as the fascicles shorten during a fixed-end contraction. This alternative calculation revealed similar postfatigue absolute active force depression at all lengths. In relative terms, a length dependence of fatigue was still evident, but this was greatly diminished compared with that observed when active force was calculated with the traditional method.

The force-length relationship is a fundamental property of skeletal muscle (7, 15) that is altered by fatigue. Gauthier and colleagues (5) suggested that the changes observed in the positions of twitch optimal length and the passive force-length relationship may have been due to an increased compliance of the series elastic structure or a stretching of the connection to the tendon. They believed that these changes were too small to fully account for either the disproportionate loss in force at short lengths or the increase of the length intercept after repetitive contractions, because the shift of optimal length was less than the shift of the length intercept. Neither sarcomere nor fascicle lengths were measured in this experiment; therefore, this proposed mechanism for the observed rightward shift of these force-length relationships could not be confirmed.

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Rijkelsikhuizen et al. (16) reported a downward shift in the force-length relationship after lengthening contractions. However, they masked a rightward shift by reestablishing optimal length after the lengthening contractions. On average, the new optimal length was 1 mm longer than the initial optimum. They suggest that the lengthening contractions resulted in damage to the muscle, increasing compliance and making the sarcomeres shorter at any given muscle-tendon unit length. Butterfield and Herzog (4) showed that the shift in the force-length relationship was almost as great when fatigue was induced with auxotonic as with lengthening contractions. They argued that the damage that is typically associated with lengthening contractions would not occur with auxotonic contractions, so it would be unlikely that local damage caused the rightward shift after the lengthening contractions. They propose that a common mechanism, “fatigue,” could explain the rightward shift in the force-length relationship for both auxotonic and lengthening contractions. Considering the results of Gauthier et al. (5), a stretch of in-series structures could be the mechanism in these studies as well, but such a stretch has never been confirmed.

There is an alternative mechanism for the frequently observed rightward shift of the submaximal force-length relationship. This shift has consistently been demonstrated using the traditional method of calculating active force (peak force minus passive force measured before or after the contraction at the same muscle-tendon unit length). However, this method of calculation has recently been shown to underestimate the active force at long lengths in the rat medial gastrocnemius muscle (10). Considering that passive force measured at long initial muscle-tendon lengths is lower after repetitive contractions (5), the active force may be underestimated to a lesser extent under this condition. This would effectively cause the submaximal shift in the passive force-length relationship to be also observed. Others have reported similar shifts in the active force-length relationships after lengthening and/or fatigue exercise (4, 16, 19).
active force-length relationship to appear to be tilted or shifted to the right, similar to that which has been shown for control (no fatigue) experiments (10).

The purpose of this study was to determine how fatiguing repetitive contractions lead to a rightward shift in submaximal force-length relationships. It was hypothesized that the length dependence of fatigue is caused by contraction-induced stretch of in-series structures. An alternative hypothesis was also considered: that the rightward shift is an artifact of the inappropriate calculation of active force.

METHODS

Muscle Preparation

For all trials, female adult Sprague-Dawley rats (average weight of 229 g) were anesthetized with a ketamine-xylazine mixture [100 mg/ml ketamine 100 mg/ml xylazine (85:15), at a dose of 0.11 ml/100 g body mass iml]. The appropriate level of anesthesia was maintained throughout the experiment with additional injections, as required. Before experimentation, the rats were housed in plastic cages with a 12:12-h light-dark cycle. Standard rat chow and water were freely available. At the end of the experiment, rats were euthanized by anesthetic overdose. All procedures were approved by the Life and Environmental Sciences Animal Care Committee at the University of Calgary. Care and treatment of these animals were according to the Canadian Council on Animal Care.

Experiments involved an in situ preparation of the medial gastrocnemius muscle. The left hindlimb was shaved, and a posterior longitudinal incision was made in the skin from the calcaneus to the spine. The subcutaneous connective tissue was cleared away, thereby facilitating the use of the skin as a muscle bath. Care was taken throughout the procedure to ensure the blood supply remained intact.

The superficial hindlimb muscles were cut to expose the sciatic nerve. The connective tissue was then delicately dissected, and the distal branches of the sciatic nerve were cut, with the aid of a dissecting microscope, leaving only the innervation to the medial gastrocnemius muscle. This ensured that the force generation observed in this study was solely due to the contractions elicited by the medial gastrocnemius. A string was then tied around the proximal end of the nerve, and the nerve was transected proximal to the tie.

After removal of the connective tissue surrounding the triceps surae, the tendinous end of the plantaris, soleus, and lateral gastrocnemius were cut. This step was taken to further ensure that the recorded forces were due only to the medial gastrocnemius. A silk string (no. 1) was tied around the Achilles tendon, as close to the calcaneous as possible. The Achilles tendon was then detached from the heel along with a small piece of the calcaneous. A string was tied midway up the tibia, and the distal portion of the leg and foot was amputated.

Apparatus and Experimental Setup

A metal probe was placed into the open end of the tibia, and a drill bit (1.56 mm) was inserted into the femur. The silk string attached to the remainder of the Achilles tendon was secured to a stainless steel wire, the other end of which was connected to the force transducer. The bit in the femur and the probe in the tibia were then affixed to an aluminum frame to immobilize the bones. The skin was pulled up and secured to the frame to create a pocket that was filled with warmed paraffin oil.

The nerve was draped across a pair of hooked stainless steel stimulating electrodes (3-mm separation). The stimulating electrodes were connected to a Grass model S88 stimulator (Grass Instruments, Quincy, MA). The nerve was stimulated with 50-μs square pulses. The rectal and muscle (oil bath) temperatures were both maintained at 37°C with an adjustable infrared heat lamp and pieces of tinfoil for shielding.

The force transducer (model FT10, Grass Instruments, with black springs, resonant frequency 700 Hz) was mounted on a translation table that could be precisely positioned by a computer-controlled stepper motor (model MD2, Arrick Robotics Systems, Hurst, TX). The output of the force transducer was amplified (model PM-1000, CWE) and then analog-to-digital converted at 4,000 Hz (analog-to-digital board, PCI-MIO-16E-4 National Instruments, Austin, TX). The data were recorded and stored using custom programs in the LabVIEW v.5.1 environment (National Instruments) for subsequent analysis.

Preexperimental Preparation

The stimulating voltage was set at 1.5 times the maximum voltage (the lowest voltage that activated all motor units) to ensure maximal activation of all motor units with each stimulus. A series of double-pulse contractions (5- to 10-ms delay) at differing muscle lengths with a 20-s rest between contractions was then used to determine the muscle length that elicited the greatest traditional active force. This length is referred to as the reference length (RL). The traditional active force elicited by double-pulse stimulations was used to set the RL. These contractions have been shown to yield an optimal length, which is similar to that obtained with longer duration tetanic contractions, while preventing fatigue (14). The muscle was then made to contract auxotonically at RL by a conditioning stimulus (200 Hz, 500 ms) to set the connections between the muscle and the force transducer. After a 5-min rest to permit dissipation of the activity-dependent potentiation, the submaximal double-pulse-force-length relationship was reexamined and RL was reset.

Seven testing positions were then designated at the following relative lengths: RL − 3.6 mm, RL − 2.4 mm, RL − 1.2 mm, RL + 1.2 mm, RL + 2.4 mm, RL + 3.6 mm. Considering that fascicle length in these muscles was ~13 mm at RL and that fascicle length changed about half as much as whole muscle length over this range, these length adjustments represent a range that is RL ± 14%. This length range was chosen because it provided a good sense of the shape of submaximal force-length curves without inflicting obvious damage to the muscle. Muscle damage can occur at extremely short lengths in mammalian muscle (12).

Rest periods were included between all contractions when obtaining data for the submaximal force-length relationships to ensure the dissipation of activity-dependent potentiation (20-s rest between twitch and double-pulse contractions and 1-min rest between the 50-Hz contractions). Lingering potentiation (11) would have resulted in an inaccurate estimation of RL (13).

Experimental Protocol

Submaximal active force-length relationships were determined for twitch, double-pulse (delay = 5 ms), and 50-Hz (duration = 300 ms) contractions by sampling contractions at the seven test lengths. The muscle then underwent a repetitive stimulation fatigue protocol at 50 Hz for 300 ms, repeated at 1-s intervals for 5 min at RL. During the fatiguing protocol, the progressive changes in the muscle contractions were monitored with contractions recorded at 0 s, 30 s, 1 min, 2 min, 3 min, 4 min, and 4 min 55 s. Once the fatigue protocol was completed, the muscles were left at RL for a 45-min recovery period. The recovery was monitored with 50-Hz contractions at intervals until 45 min. After this time, submaximal active force-length relationships were reevaluated for the twitch, double-pulse, and 50-Hz contractions.

Sonomicrometry Experiments

A second set of experiments was done using sonomicrometry to measure fascicle length. The sonometric technique for measurement of fascicle length is valid and has often been used for this purpose (3,
Before positioning of the rat and the muscle in the apparatus, piezoelectric crystals (1 mm) were placed in the muscle (one at each end of a fascicle). An individual muscle fascicle was identified by microstimulation applied directly to the muscle surface. Crystals were inserted into small pockets, created with an 18-gauge needle, and were secured with a small drop of tissue glue (Vetbond, 3M, St. Paul, MN). The crystals were connected with a thin wire to a sonomicrometer (Sonometrics, London, ON, Canada). The distance between the crystals was calculated by the Sonometrics software, based on transmission time and assumed speed of sound in muscle (1,540 m/s). Submaximal active force-length relationships were obtained for double-pulse contractions by sampling at the seven test lengths. The muscle was then subjected to the same fatiguing protocol outlined above (50 Hz for 300 ms, repeated at 1-s intervals for 5 min at RL). At the end of the fatiguing protocol, the muscle was held at RL for a 45-min recovery period. At the end of the recovery period, the force-length relationship was reevaluated with double-pulse stimulation.

Calculation of active force. Active force is the force generated by the contractile proteins, and the calculation of active force requires measurement of the peak force during a contraction and subtraction of the appropriate passive force. Traditionally, the passive force has been measured before or after a contraction, at the muscle-tendon unit length at which a contraction was elicited. This approach was demonstrated to be appropriate for muscle with very little series elastic property (9). However, MacIntosh and MacNaughton (10) have recently shown that passive force is likely to decrease during contraction in a muscle with substantial series elasticity, such as the rat medial gastrocnemius muscle. To estimate the passive force contribution at the time of peak force, it is necessary to know the compliance of the muscle-tendon complex and the passive force-length relationship. The compliance for the rat medial gastrocnemius muscle preparation as used in this study is known to be 0.33 mm/N. When this information is taken into consideration with the measured passive force-length relationship, as obtained in these experiments, active force can be calculated. The apparent fascicle length change during the contraction can be determined from this compliance, and the expected passive force contributed at this length can be calculated, as described by MacIntosh and MacNaughton (10). We used their method, as well as the traditional method, to calculate the active force. In this paper, we refer to these as the corrected and traditional active force, respectively.

Curve fitting. After comparing second-, third-, and fourth-order polynomials, it was apparent that third-order polynomials most closely fit the traditional and corrected active force data and fourth-order polynomials suited the passive force data (Microsoft Excel, Microsoft, Redmond, WA). For each submaximal force-length relationship, this equation for the passive forces was used to estimate the passive force that was expected to be present at the peak of the contraction. The polynomial equations associated with each traditional and corrected active force curve were differentiated and solved to find the length at the true peak active force (change in force/change in length = 0). This was then designated as the true position of optimal length, and it is expressed relative to RL.

Statistics
A two-factor repeated-measures ANOVA (Statistica, Statsoft, Tulsa, OK) was used to compare the pre- and postfatigue submaximal active force-length relationships for each series: twitch, double-pulse, and 50-Hz contractions. When interaction was significant (α < 0.05), a post hoc paired t-test was done (Excel, Microsoft) to evaluate the differences at specific lengths; a Bonferroni correction was applied. When interaction was not apparent, indicating a parallel shift, the main effects were considered to compare the pre- and postfatigue data regardless of length. A paired t-test was used to detect significant differences between passive forces measured at long lengths at the beginning and end of the experiment. Values are means ± SE.

RESULTS
Repetitive Contractions
During repetitive intermittent stimulation, corrected active force increased briefly, reaching a peak of staircase within 7 s, and then it decreased dramatically to 34 ± 3% of the initial active force by the end of the 5 min (see Fig. 1). Early in recovery there was initially a sharp increase in corrected active force, and this was followed by a slight decrease. Subsequently, there was a slow increase such that active force after 45 min was 77 ± 2% of the initial (prefatigue) corrected active force.

Sonomicrometry Measurements
Passive (before contraction) and peak (at the peak of a double-pulse contraction) fascicle lengths were measured at the seven test lengths before and after a 1-Hz fatiguing protocol and recovery period. Average passive and peak fascicle lengths at any given muscle-tendon unit length did not change after the 1-Hz fatiguing protocol (Table 1).

Fig. 1. Active force during repetitive stimulation and recovery. Relative corrected active force is shown during 5 min of repetitive stimulation (A) and 45 min of recovery (B). Inset, a sample plot of the relative corrected active force for sequential contractions during the first 30 s of repetitive stimulation. There was a significant reduction in relative active force at the end of the repetitive contraction protocol when compared with initial force (P < 0.002). The inset graph shows that the active force actually increases over the first 7 s of the repetitive contractions protocol. Tetanic (50 Hz) active force (means ± SE for 6 rats) for the 45-min recovery period after the 5-min fatiguing protocol showed a rapid initial increase followed by a decrease over the first 5 min. The active force steadily increased for the remainder of the recovery period. A significant amount of active force depression persisted at the end of the recovery period (significantly different from control, P < 0.012).
Changes in Passive Force

Passive force measured before any contraction at lengths corresponding to RL and longer decreased progressively throughout the experiment. An example of this is shown in Fig. 2. It appeared as though passive force decreased each time a force-length relationship was obtained, and this occurred as a result of the fatiguing contractions. The initial passive force at $RL = 3.6$ mm was $1.9 \pm 0.3$ N, and this had decreased significantly ($P < 0.02$) to $1.3 \pm 0.1$ N during the time when the postfatigue 50-Hz force-length relationship was being evaluated.

Submaximal Force-Length Relationships

The impact of the repetitive contractions and recovery period on the submaximal active force-length relationships for twitch, double-pulse, and 50-Hz contractions varied according to calculation method and contraction type. The prefatigue-corrected active force-length curves (see Fig. 4) were broader with a longer optimal length than was evident with the traditional calculation of active force (see Fig. 3), similar to that reported earlier by MacIntosh and MacNaughton (10). The corrected active forces were always greater than traditional active forces of a given contraction type at lengths longer than the reference length (cf. Figs. 3 and 4).

In general, the postfatigue submaximal traditional active force-length relationships were shifted down and either tilted, as was the case for the twitch and double-pulse contractions, or flattened, as was the case for the 50-Hz contractions compared with the prefatigue curves (Fig. 4). The differences between pre- and postfatigue were significant at short lengths but not at long lengths.

The submaximal corrected active force-length relationships were also shifted down after fatigue; however, this shift was nearly parallel in nature (see Fig. 4). The difference between

Table 1. Passive and peak fascicle lengths

<table>
<thead>
<tr>
<th>Prefatigue</th>
<th>Postfatigue</th>
</tr>
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<tbody>
<tr>
<td>RL = 3.6 mm</td>
<td>11.3 ± 0.8</td>
</tr>
<tr>
<td>RL = 2.4 mm</td>
<td>11.7 ± 0.9</td>
</tr>
<tr>
<td>RL = 1.2 mm</td>
<td>12.8 ± 0.9</td>
</tr>
<tr>
<td>RL = 1.2 mm</td>
<td>13.4 ± 1.1</td>
</tr>
<tr>
<td>RL = 2.4 mm</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>RL = 3.6 mm</td>
<td>14.4 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE given in mm for 3 rats. Passive, force before contraction; Peak, fascicle length at the time of peak force during a contraction; RL, reference length, the muscle-tendon unit length at which traditional active force (peak minus passive) was the greatest.

Fig. 2. Sample successive evaluations of passive force-length relationships. Submaximal force-length relationships were evaluated, and passive forces were obtained before contraction at each length. Solid symbols, prefatigue; open symbols, postfatigue; squares, passive forces taken before twitch contraction; diamonds, passive forces taken before double-pulse contractions; triangles, passive forces taken before 50-Hz contractions. RL, reference length, which corresponds to the length at which the amplitude of the traditional active force for double-pulse contractions was greatest.

Fig. 3. Traditional active force-length relationships. Traditional active forces (means ± SE) for twitch (A), double-pulse (B), and 50-Hz (C) contractions before (top) and after (bottom) the 5-min repetitive-contraction protocol (1 Hz) and a 45-min recovery period are shown. Values are means ± SE for 6 rats. Posttreatment absolute and relative active force depression was greater at short vs. long lengths. *Significant absolute difference after the fatigue/recovery period at that length, $P < 0.05$. 

Fig. 4. Corrected active force-length relationships. Corrected active forces (means ± SE) for twitch (A), double-pulse (B), and 50-Hz (C) contractions before (top) and after (bottom) the 5-min repetitive-contraction protocol (1 Hz) and a 45-min recovery period are shown. Values are means ± SE for 6 rats. Posttreatment absolute and relative active force depression was greater at short vs. long lengths. *Significant absolute difference after the fatigue/recovery period at that length, $P < 0.05$. 

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pre- and postfatigue was significant at all lengths, except the longest one tested for twitch and double-pulse contractions, and at all lengths for 50-Hz contractions. It is important to point out that there is still a length dependence of fatigue in these measurements. The relative reduction in both the traditional and corrected active force was greater at short lengths than it was at long lengths for all contraction types.

To evaluate the parallel nature of the decrease in corrected double-pulse active force, the prefatigue and postfatigue differences were compared across lengths. There were no significant differences in the magnitude of corrected active force change from -2.4 to +3.6 mm. This is a substantially different result than was obtained when active force was calculated in the traditional way (no significant fatigue was evident at the long lengths for twitch and double-pulse contractions).

Comparing the length dependence of fatigue for traditional and corrected active force revealed a marked difference between these methods. No differences in calculated active force were evident at lengths below RL. The relative traditional active force was 83 ± 4% of the prefatigue value at RL and 94 ± 5% at +3.6 mm. In contrast, relative corrected active force was 83 ± 4% at RL and 87 ± 4% at +3.6 mm. Therefore, it can be stated that traditional active force overstates the length dependence by a factor of 2.4.

The length at which the highest active force occurs appears to have shifted to the right for traditional active force. This significant shift for traditional active force was 0.77 ± 0.1 mm for the 50-Hz (P < 0.001) contractions, 0.49 ± 0.1 mm for the twitch, and 0.37 ± 0.1 mm for double-pulse contractions (P < 0.001). Unfortunately, assessing changes in the position of optimal length with the corrected active force before and after fatigue proved difficult because the majority of lengths evaluated in this study appeared to be on the ascending limb of the submaximal force-length relationship.

DISCUSSION

The purpose of this paper was to determine how fatiguing repetitive contractions lead to a rightward shift in submaximal force-length relationships. Two potential explanations for this observation were considered: stretch of a series elastic structure may lead to shorter fibers at any muscle-tendon unit length, or miscalculation of active force may result in the apparent shift in this relationship. Our results indicated that there is no change in the fascicle length after repeated contractions associated with fatigue and that calculation of corrected active force yielded results with an essentially parallel shift downward in the submaximal active force-length relationship. These results suggest that the reported rightward shift in the force-length relationship of skeletal muscle in fatigue is an artifact of the traditional method of calculating active force.

When active force was calculated in the traditional manner, we did see a rightward shift in the submaximal force-length relationships, similar to the shift reported by Gauthier et al. (6) and Butterfield and Herzog (5). In that respect, our results are not different from previously reported results dealing with the length dependence of fatigue. Not only was the curve shifted but also the estimate of optimal length was at a longer length after the fatiguing contractions, which is consistent with the results of Rijkelijkhuizen et al. (16). However, when we calculated active force with the corrected method, the rightward shift was not evident. In fact, with the corrected method of calculating active force, there was a nearly parallel decrease in force across the tested lengths. This means that the force deficit was similar in absolute terms across most of the tested lengths. It should be noted that this circumstance is still consistent with a length dependence of fatigue. The relative decrease in force is still greater at short lengths than at a long length; however, the magnitude of this length dependence of
fatigue is much less than that reported by others using the traditional method of calculating active force (4, 5, 17).

Notably, Gauthier and colleagues (5) state that they calculated active force by subtracting the passive force before contraction (traditional). Although this may be of minimal importance for the length range evaluated with the 100-Hz contractions (the passive forces appeared relatively small), using the traditional method of active force calculation appeared to be of significance in their twitch active force-length relationship. Passive forces at the long lengths evaluated with the twitch contractions were substantial and, in accordance with the findings of the present study, had decreased in the postfatigue evaluation. Gauthier and colleagues’ twitch active force-length relationships before and 30 min after fatigue appeared very similar to the force-length relationships obtained for traditional active force of twitches in the present study, showing a postfatigue tilt in the submaximal force-length relationship.

The recent work demonstrating similar apparent rightward shifts in the force-length relationship after repeated lengthening contractions is of particular interest. Talbot and Morgan (19) and Rijkelijkhuizen et al. (16) reported a shift in optimal length after such contractions. In both of these studies, it is suggested that local myofibrillar damage results in stretch of the sarcomeres in the damaged region, with corresponding shortening of adjacent sarcomeres. If this were the case, our measurements of fascicle length would not correspond to sarcomere length, and this shift in the force-length relationship could be due to changes in sarcomere length. However, Butterfield and Herzog (4) reported such a shift after lengthening contractions, as well as after auxotonic contractions, during which such local damage should not occur, casting doubt on the localized damage mechanism. Furthermore, histological sections showing this kind of damage do not support the notion that sarcomeres outside the damaged region shorten to accommodate the apparently stretched region (2). The lateral connections that keep adjacent fibers in register apparently preserve the sarcomere lengths of the intact fibers. Therefore, the mechanism proposed by Talbot and Morgan (19) and Rijkelijkhuizen et al. (16) to explain the apparent shift in the force-length relationship seems unlikely.

Our results indicate that a decrease in passive force combined with calculation of active force in the traditional method could account for an observed shift in the traditional active force-length relationship in experiments where substantial passive forces were present. It is important to point out that the method of calculating corrected active force used in this study is necessary only for muscles with a substantial series elastic component (10) and is not required for muscle with a stiff series elastic compliance (9). Although passive forces were not reported in the articles by Rijkelijkhuizen et al. (16) and Butterfield and Herzog (4), both studies involved in situ assessments of mammalian muscle, and substantial passive force would have been evident over the length ranges reported. The muscles used in these studies are likely to have a substantial series elastic compliance. In reports by Rijkelijkhuizen et al. (16) and Butterfield and Herzog (4), the calculation of active force using the traditional method may account for the apparent rightward shift in the position of the force-length relationship. In this case, it would not be necessary to propose a mechanism associated with damage. In contrast, the study of Talbot and Morgan (19) was with toad muscle in vitro, and they measured passive force. Not only were changes in passive force absent in their study but also the length associated with the optimum corresponded with an absence of passive force. The use of the traditional method of calculating active force is probably appropriate for this parallel-fibered muscle, which is known to have a stiff series elastic structure.

It is remarkable that, despite substantial change in passive force over the course of these experiments, there was no change in fascicle length. This observation can be interpreted to indicate that compliance of the series elastic structures must have changed in parallel with the changes in the passive force. Otherwise, it would be expected that the lower passive force would be associated with less stretch of the series elastic structures and with longer fascicle lengths at any muscle tendon unit length.

In conclusion, a relative length dependence of fatigue exists; however, it is not as dramatic as previously reported in studies using the traditional method to calculate active forces. In absolute terms, this relative length dependence of fatigue translates into a similar postfatigue-corrected active force reduction at all test lengths. These observations coupled with the fact that fascicle lengths did not appear to change as a result of repetitive contractions confirm that fatigue is not associated with stretch or increased compliance of structures in series with the fascicles and that previous reports of a shift in the force-length relationship are probably the result of inappropriate calculation of the active force. Such inappropriate calculation of active force results in an exaggeration of the length dependence of fatigue.

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