Rises in whole muscle passive tension of mammalian muscle after eccentric contractions at different lengths

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Whitehead, N. P., D. L. Morgan, J. E. Gregory, and U. Proske. Rises in whole muscle passive tension of mammalian muscle after eccentric contractions at different lengths. J Appl Physiol 95: 1224–1234, 2003. First published May 9, 2003; 10.1152/japplphysiol.00163.2003.—This is a report of experiments carried out on the medial gastrocnemius muscle of the anesthetized cat, investigating the effects of eccentric contractions carried out at different muscle lengths on the passive and active length-tension relationships. In one series of experiments, the motor supply to the muscle was divided into three approximately equal parts; in the other, whole muscles were used. Fifty eccentric contractions were carried out over different regions of the active length-tension curve for each partial or whole muscle. Active and passive length-tension curves were measured before and after the eccentric contractions. When eccentric contractions were carried out at longer lengths, there was a larger shift of the optimum length for active tension in the direction of longer muscle lengths and a larger fall in peak isometric tension. Passive tension was higher immediately after the eccentric contractions, and if the muscle was left undisturbed for 40 min, it increased further to higher values, particularly after contractions at longer lengths. A series of 20 passive stretches of the same speed and amplitude and covering the same length range as the active stretches, reduced the passive tension which developed over a subsequent 40-min period. It is hypothesized that there are two factors influencing the level of passive tension in a muscle after a series of eccentric contractions. One is injury contractures in damaged muscle fibers tending to raise passive tension; the other is the presence of disrupted sarcomeres in series with still-functioning sarcomeres tending to reduce it.

When a muscle is lengthened while active, known as an eccentric contraction, damage to muscle fibers is likely. This type of muscle injury gives rise to the commonly experienced delayed-onset muscle soreness, a day or so after the exercise (15, 26, 31). Muscle damage induced by a period of eccentric exercise is commonly quantified by using the reduction in the active tension generated by the muscle (2, 16, 30). Although the mechanisms underlying the active tension deficit remain the subject of debate, a number of factors are likely to be involved, factors such as fatigue due to metabolic exhaustion, structural damage to the contractile filaments, and failure of the excitation-contraction coupling process (18, 23). An additional indicator of muscle damage revealed by our studies is a shift to longer muscle lengths of the optimum length for peak active tension. This is believed to be the result of an increased series compliance in the muscle as a result of sarcomere disruption (12, 32, 36).

The study of passive mechanical properties after eccentric contractions has received relatively little attention. It has been reported that passive stiffness of human elbow flexors increases immediately after eccentric exercise and remains elevated for a further 4–5 days (5, 11, 13). Recently, Whitehead et al. (34) have shown similar increases in passive torque for human triceps surae as well as a rise in passive tension and increased work absorption for cat medial gastrocnemius immediately after a period of eccentric contractions. We attributed these mechanical changes to the development of localized regions of injury contractures in some muscle fibers, because of structural damage to membranes from overstretch of parts of fibers, and a subsequent elevation of intracellular calcium concentration (1).

Previous work from our laboratory has demonstrated that the amount of damage from eccentric exercise, measured as the fall in isometric tension and shift in optimum length, depends on the length range over which the stretches are carried out but not on the speed of stretch or level of isometric tension (27). In the study by Whitehead et al. (34), the eccentric contractions were all carried out over a length range that lay symmetrically about the optimum length for active tension. Here, we have posed the question, does the amount of passive tension measured after eccentric contractions show a length dependence, as do the other damage indicators? The point was explored in a preliminary series of experiments in which the motor supply of the muscle was divided into three portions, equal in terms of the tension each generated. Eccentric contractions were then carried out, so arranged that the contractions produced by stimulating each nerve fragment covered a different region of the muscle's length-tension relation, in order from the shortest to
the longest length range, predicted to produce progressively increasing amounts of damage.

It was found that when the eccentric contractions were carried out at longer lengths, which included more of the descending limb of the muscle’s length-tension relation, interpretation of changes in passive tension proved not to be straightforward. The aims of the experiments described here were to confirm this result and then to simplify the experiment by reexamining in whole muscle preparations the question of the length dependence of the increase in passive tension.

METHODS

The experiments were carried out on eight cats of both sexes with a weight range of 2.5–5.9 kg. The experiments were undertaken with approval from the Monash University Committee for Ethics in Animal Experimentation.

General anesthesia was induced by an intraperitoneal injection of sodium pentobarbitone (40 mg/kg) and was maintained throughout the experiment with additional doses into the cephalic vein. At the end of the experiments, animals were killed with an overdose of anesthetic. The trachea was cannulated, and end-tidal CO2 concentration was monitored and used as both an indicator of the adequacy of ventilation and the depth of the anesthesia. Rectal temperature was measured and core body temperature was maintained at 35 °C. The nerve to MG was identified and separated from the rest of the nerve. All hindlimb nerves, including nerves to hip muscles, were then cut, except for the nerve to MG. The distal tendon was fashioned from skin in baths fashioned from skin flaps, the temperature of which was maintained between 35 and 38°C. The nerve to MG was located and carefully dissected free from the posterior tibial nerve. All hindlimb nerves, including nerves to hip muscles, were then cut, except for the nerve to MG. The distal tendon of MG was identified and separated from the rest of the Achilles tendon, leaving only the MG tendon attached to the calcaneum. The calcaneum was cut, and a 2-mm-diameter hole was drilled through it. A threaded rod was passed through the hole and secured to the bone with a pair of nuts and washers. At the other end, the rod was attached to the shaft of an electromagnetic servomotor, which regulated muscle length changes. Muscle tension was measured with an Entrant strain gauge attached to the metal rod. The system had a compliance of 5 μm/N.

Experimental Procedure

A preliminary length-tension curve for MG was carried out over a range of muscle lengths by stimulating the MG nerve, via platinum bipolar electrodes, at a rate of 80 pulses per second (pps) for 250 ms. These measurements were performed to establish the optimum length for active tension generation of the whole muscle (Lopt), which was used as a reference length (34). Typically, Lopt was ~10 mm shorter than the maximum physiological length, and, therefore, measurements were made over a 20-mm length range from Lopt – 10 mm to Lopt + 10 mm.

For the “three-part” experiments, involving five animals, the ventral root supply to MG was split into three portions that, on stimulation, produced twitch tension at Lopt that differed by <10%. The division of the muscle into three parts enabled eccentric contractions to be performed over three different length ranges, in order from short to progressively longer lengths. Here it was assumed that the response of one third of the muscle approximated that of the whole muscle. The effects of eccentric contractions of each part on the passive and active length-tension relationships were determined by comparing measurements before and immediately after each set of contractions. For passive properties, changes in passive tension of the whole muscle were measured after eccentric contractions of each part. Thus it was assumed that the changes in passive tension brought about by the eccentric contractions of each successive third were much larger than any effect the passive stretches might have had on the previously damaged portions. Because we were anticipating the largest changes to take place after contractions at the longest muscle lengths, these were carried out last.

In a second series of experiments in which measurements were made on the whole muscle, MG of both hindlimbs was dissected free. In addition, ventral roots on both sides were prepared for stimulation. In these experiments, whole muscles were subjected to eccentric contractions that were carried out over a different portion of the length range for each muscle. This was done on the basis of the assumption that intrinsic properties of muscles of the same animal were likely to be more similar than for muscles from different animals.

Active Tension Measurements

All measurements of active tension were made after subtraction of passive tension.

The length-tension relationship for each third of the muscle was established, by using the same stimulation parameters as previously described for the whole MG (see Fig. 1A). The measured optimum length for each part was determined. In general, it was 1–2 mm shorter than that for the whole muscle. This difference was attributed to the fact that activation of part of a muscle will not stretch the series elastic component quite as much as activation of the whole muscle. Thus, for a given muscle-tendon length, the muscle fibers will be at a longer length during part activation, resulting in a shorter optimum length (25). To calculate the optimum length and peak active tension, Gaussian curves were fitted to tension–length data over the range above 75% of maximum by using the software package Igor Pro (Wavemetrics, Lake Oswego, OR).

Passive Tension Measurements

Passive tension was usually measured over muscle lengths ranging from Lopt – 10 mm to Lopt + 10 mm. The muscle was initially moved to a length of Lopt – 10 mm, where the tendon lay slack and passive tension was close to zero. In three large animals the muscle had to be shortened by 1–2 mm further before the muscle fell slack. The muscle was conditioned with an isometric contraction (80 pps, 100-ms duration) and held at a constant length for 30 s. The conditioning ensured that passive properties from muscle thixotropy were controlled for (32). The passive muscle was then stretched at a velocity of 1 mm/s. To quantify changes in passive tension after the eccentric contractions, we calculated the change at each muscle length and expressed it as a percent of active tension (see Figs. 3B and 4B).

Whitehead et al. (34) recently reported for cat MG that, at a muscle length of Lopt – 10 mm, the tendon was visibly slack and the tension was near zero. Consequently, during the lengthening phase of dynamic passive tension measurements, the muscle had to be stretched by 1–2 mm to take up this slack and for tension to begin to rise. We also noted that, after 150 eccentric contractions, the muscle had to be
was chosen because it provided a means of accurately determining the length at which passive tension began to rise, independently of differences in the shapes of the tension profiles. The magnitude and direction of the change in the length of passive tension onset after the eccentric contractions of each part could then be calculated (see Fig. 6A).

**Eccentric Contractions**

Fifty eccentric contractions were carried out, one every 40 s, on each of the three parts of the muscle or on the whole muscle. For the eccentric contractions, each nerve fragment was stimulated at 80 pps for 400 ms. At 150 ms after the onset of stimulation, the muscle was stretched by 6 mm at a velocity of 50 mm/s (Fig. 1A). Given an average fiber length for MG at $L_{opt}$ of 18 mm (28), a 6-mm stretch represents approximately a 30% fiber length change.

For the three-part experiment, the eccentric contractions were carried out over a different range of lengths for each part. Pooling the data from the five experiments in which the muscle had been divided into three parts, the lengths at which the eccentric contractions ended were $L_{opt} -3$ mm ($n = 4$), $L_{opt}$ ($n = 3$), $L_{opt} + 3$ mm ($n = 3$), $L_{opt} + 6$ mm ($n = 1$), and $L_{opt} + 9$ mm ($n = 4$).

For the whole muscle experiments it was found that peak tensions during the eccentric contractions of the whole muscle exceeded the force-sustaining capacity of the muscle stretcher and strain gauge. To overcome this, the eccentric contractions were carried out in three steps. The motor supply at the ventral root was subdivided into three equal portions, and each portion was stimulated in turn, with the eccentric contractions for each part carried out over the same length range. The eccentric contractions of each portion were carried out at 1-s intervals and repeated every 40 s until 50 eccentric contractions had been completed.

**Statistics**

For all parameters measured, the mean and SE were calculated. Statistical significance was set at $P < 0.05$. A two- or three-factor analysis of variance (ANOVA) with interactions was used to determine the significant factors determining the effects of the eccentric contractions. The factors used were animal, final length for the eccentric contractions, and condition, that is, whether the measurement was made immediately after the eccentric contractions, after a delay or after passive stretches. Where significance was found, a least significant difference post hoc test was used. For some variables, a linear regression analysis was also performed. The statistical program used was Data Desk (Ithaca, NY).

**RESULTS**

**Three-Part Experiments**

**Active length-tension relation.** Sample records of the 1st and 50th eccentric contractions for the three portions of the muscle stretched to $L_{opt} - 3$ mm, $L_{opt}$, and $L_{opt} + 9$ mm, respectively, highlight the length-dependent reduction in isometric tension as a result of the eccentric contractions (Fig. 1A). Mean values for the prestretch isometric tension of the 50th eccentric contraction, expressed as a percentage of the 1st, ranged from $45.3 \pm 8.6\%$ when the eccentric contraction ended at $L_{opt} - 3$ mm to $19.5 \pm 2.1\%$ at $L_{opt} + 9$ mm. These values, however, do not take into account the shift of the active length-tension relation to longer muscle

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**Fig. 1.** A: eccentric contractions of 3 parts of the muscle. Sample tension traces of the 1st (continuous trace) and 50th (dashed trace) eccentric contraction of each of 3 portions of the medial gastrocnemius muscle of the anesthetized cat. Top 3 pairs of traces, tension: bottom trace, length. The muscle was stretched by 6 mm at 50 mm/s. Solid bar below length trace shows period of stimulation at 80 pulses/s. Length range over which the eccentric contractions were carried out for each part is shown by the hatched bars in B. B: active length-tension curves of 3 parts of the muscle. A plot of the isometric tension (80 pulses/s stimulation for 250 ms) against muscle length with respect to the optimum length for active tension ($L_{opt}$), for each of the 3 parts of the muscle before (open symbols) and after (solid symbols) 50 eccentric contractions. Each third of the muscle was subjected to eccentric contractions carried out over a different range of muscle lengths: part 1 (circles) from $L_{opt} - 9$ mm to $L_{opt} - 3$ mm; part 2 (squares) from $L_{opt} - 6$ mm to $L_{opt}$; and part 3 (triangles) from $L_{opt} + 3$ mm to $L_{opt} + 9$ mm. Length range is indicated by hatched bars at bottom.
lengths (see below). Figure 1B shows length-tension curves before and after the eccentric contractions for the three parts of the muscle from one experiment. Note that when the eccentric contractions ended at longer lengths, there was a progressively larger drop in peak active tension and shift in optimum length, in the direction of longer lengths. Mean values for the shift in optimum length for eccentric contractions ending at $L_{opt} - 3$ mm and $L_{opt} + 9$ mm ranged from 2.1 ± 0.5 to 7.1 ± 0.3 mm, respectively (Fig. 2A). The mean peak active tension measured at the new optimum length and expressed as a percentage of the preeccentric value was 81 ± 3.5% when the contractions ended at $L_{opt} - 3$ mm and 31.4 ± 2.8% when they ended at $L_{opt} + 9$ mm (Fig. 2A). Analysis showed a significant effect of the

length at which the eccentric contractions were carried out on both the shift and tension drop ($P < 0.05$). There was also a significant linear relationship between these parameters ($r^2 = 0.87; P < 0.05$; Fig. 2B).

During the course of these experiments, the observation was made that submitting one third of the muscle to a series of eccentric contractions did not change active tension of the other two thirds. That is, because the stretches were applied to the whole muscle, the passive stretches of the second and third portions during the active stretches of the first third did not change their active length-tension relations.

In a second control experiment, the fatigability of the preparation was measured as well as its length-tension relation and passive tension levels after a series of 50 isometric contractions. The nerve supply to the muscle was divided into two portions, and the first portion was stimulated to give 50 isometric contractions at 40-s intervals at a length of $L_{opt} - 3$ mm. After each contraction, the muscle was stretched passively from $L_{opt} - 3$ mm to $L_{opt} + 3$ mm. Passive tension at the start of the experiment was 2 N at $L_{opt} - 3$ mm. It remained within 0.1 N of that level throughout the series, whereas isometric tension had fallen by 7% after the 50 contractions. This fall is an expression of the muscle’s fatigue property (35). The only other detectable change was a 0.6-mm shift in the optimum length for active tension, in the direction of longer muscle lengths. It compares with a shift of 4.2 mm when eccentric contractions were carried out over a similar length range (Fig. 2). A small shift after isometric contractions has been reported previously (12) and is presumably the result of sarcomeres in some muscle fibers undergoing lengthenings as a result of internal motion within the muscle.

The series of isometric contractions and passive stretches applied to the first half of the muscle did not change active or passive tension levels or the length-tension relation in the second half. A similar series of 50 isometric contractions was then carried out on the other half of the muscle. This time the muscle was held at $L_{opt} + 3$ mm. The passive stretches were carried out over the range $L_{opt} + 3$ mm to $L_{opt} + 9$ mm. Passive tension at the start of the series was correspondingly higher, at 20 N, and did not change, within measurement accuracy, over the 50 contractions. Peak isometric tension fell by 13%. There was also a 0.6-mm shift in optimum length in the stimulated portion.

Passive length-tension relation. It was found that passive tension increased after the eccentric contractions and the increase was dependent on the length range over which the contractions were carried out. However, for eccentric contractions that ended at longer muscle lengths, the percent increase in passive tension was less, and it reached its peak value at longer lengths.

Such a result was contrary to our predictions. We had anticipated that for eccentric contractions carried out at longer muscle lengths, there would be more damage. This prediction was borne out for the shifts in the active length-tension relation and the falls in active

![Figure 2](https://example.com/figure2.png)

Fig. 2. A: dependence of active tension and optimum length on length range of eccentric contractions. Changes in the optimum length for active tension (○, solid line) and fall in active tension, measured at the new optimum (○, dashed line) against the final length of the stretch for the eccentric contractions. Final length is expressed relative to $L_{opt}$. Fall in isometric tension is expressed as a percent relative to the value before the eccentric contractions. B: fall in active tension plotted against shift in optimum length. Fall in active tension measured at the new optimum length after 50 eccentric contractions, expressed as a percentage of the preeccentric value, plotted against the size of the shift, in the optimum of the active length-tension relation, after the eccentric contractions. Data are from 5 animals, with each muscle subdivided into 3 near-equal portions. A regression analysis gave an $r^2 = 0.87$ ($P < 0.05$).
force (Figs. 1 and 2). However, it was not true for the passive tension changes and therefore threw doubt on the three-part approach for measurements of changes in passive tension. We speculated that whenever eccentric contractions were carried out on the second or third part of the muscle, the first part was also being subjected to passive stretches, because the stretches were applied to the whole muscle. Any rise in passive tension after eccentric contractions of the first part might therefore be reduced by such stretches. In view of this we decided to resort to whole muscle eccentric contractions for additional measurements of changes in passive tension.

Whole Muscle Experiments

Active length-tension relation. The aims of these experiments were fourfold. First it was necessary to confirm with whole muscles that when eccentric contractions were carried out at longer lengths more damage would result, as demonstrated by a larger shift in active length-tension relation. Second, changes in passive tension were measured after eccentric contractions carried out at different lengths. If our interpretation of the changes in passive tension seen in the partial muscle experiments was correct, a third objective was to confirm this on the whole muscle and determine the precise length dependence of passive tension changes. Finally, in the three-part experiment it was noticed that the level of passive tension measured depended on how soon after the eccentric contractions the measurements were carried out. During the period immediately after the eccentric contractions, passive tension continued to rise for some minutes. It was decided therefore to make additional measurements 40 min later, on the assumption that this time passive tension had reached a steady level.

Experiments were carried out on MG of both legs in three animals, that is, on six muscles. For one muscle in each animal the eccentric contractions were arranged to be symmetrical about the optimum length, that is, stretches were from \( L_{\text{opt}} - 3 \) mm to \( L_{\text{opt}} + 3 \) mm. For the other muscle they were from \( L_{\text{opt}} + 3 \) mm to \( L_{\text{opt}} + 9 \) mm. The mean shift in optimum length for the eccentric contractions carried out on the plateau of the length-tension curve was \( 4.8 \pm 0.6 \) mm, and this compared with \( 8.1 \pm 0.6 \) mm for stretches on the descending limb. This difference was not quite significant (\( P = 0.07 \); unpaired t-test). The accompanying falls in active tension were \( 37.0 \pm 9 \) and \( 50.5 \pm 0.8 \)%, respectively. This was significant (\( P < 0.05 \), 2-factor ANOVA).

A comparison of the shift in optimum length between the whole muscle preparations and the three-part experiments (Fig. 2) showed that for eccentric contractions carried out over a given length range the shifts in optima were not significantly different.

Passive tension. An example of passive tension changes after eccentric contractions carried out across the optimum length of the muscle's length-tension curve is shown in Fig. 3. In the period immediately after the eccentric contractions, there was a significant rise in passive tension (Fig. 3A). It was found that the amount by which passive tension had increased depended on the muscle length at which the comparison was made. That is, the passive tension did not increase by equal amounts at each muscle length, when expressed as a percentage of the pre eccentric values. The increase was largest at lengths near the optimum length for active tension (34).

![Graph](http://jap.physiology.org/)

**Fig. 3.** A: passive tension after eccentric contractions. Passive tension, measured in response to a slow stretch of the muscle at 1 mm/s, covered the full physiological range, \( L_{\text{opt}} - 12 \) mm to \( L_{\text{opt}} + 8 \) mm. Solid line, passive tension measured before 50 eccentric contractions carried out over the length range indicated by the hatched bar, \( L_{\text{opt}} - 3 \) mm to \( L_{\text{opt}} + 3 \) mm; dotted line, passive tension measured immediately after these 50 contractions. Dashed line, passive tension measured 40 min after the eccentric contractions; dashed-dotted line, passive tension measured immediately after 20 passive stretches of 6-mm amplitude at 50 mm/s and covering the same length range as the active stretches. B: changes in passive tension. Continuous measurement, by computer, of the length-dependent increase in passive tension after 50 eccentric contractions expressed as a percentage of values measured before the eccentric contractions. Hatched bar indicates the length range of the eccentric contractions. Symbols for the curves as in A.
In this experiment, passive tension increased to a peak of 142% at $L_{\text{opt}} + 3$ mm (Fig. 3B). If the muscle was then left undisturbed for 40 min, the passive tension rose further to peak at 191% at $L_{\text{opt}} + 1$ mm. At this point, applying a series of 20 passive stretches to the muscle, using the same speed, amplitude, and length range as for the active stretches, led passive tension to fall to 118%. Not shown on the figure, waiting for a further 40 min saw passive tension return to 202%. So the results of this experiment were that passive tension continued to rise over at least 40 min after the eccentric contractions, and it could be collapsed by a series of passive stretches but would then redevelop, with time, back to values similar to those it had maintained before the passive stretches.

The experiment was repeated on MG of the other leg but with the eccentric contractions carried out over the length range $L_{\text{opt}} + 3$ mm to $L_{\text{opt}} + 9$ mm, that is, with the stretches entirely on the descending limb of the muscle's length-tension relation (Fig. 4). The changes seen at these longer lengths were similar although more dramatic than for the eccentric contractions carried out at the shorter lengths. Immediately after the eccentric contractions, the passive tension peaked at $L_{\text{opt}}$ with an increase of 114%. That is, the increase was a little less than after the eccentric contractions at the shorter length. However, during the subsequent 40-min delay period, passive tension rose further to reach a peak increase of 239%. The passive stretches reduced this to 130%, and a second delay period saw a recovery back to 234% (Fig. 4).

A summary of the data is shown in Fig. 5A. For three animals, when the eccentric contractions were carried out symmetrically across the whole muscle optimum, passive tension reached a mean peak of 170.3 ± 21.6% immediately after the eccentric contractions and 103.2 ± 7.6% when carried out entirely on the descending limb. Mean peak values after waiting 40 min were 210.8 ± 15.5 and 232.3 ± 8.9%, respectively. The passive stretches reduced passive tension to 151.8 ± 23.2 and 119.8 ± 5.8%, with recovery during the second delay period being to 216.2 ± 18.9 and 237.3 ± 8.9%, respectively (Fig. 5A). Statistical analysis showed that there was no significant overall passive tension increase with length but there was a significant interaction between the condition and length ($P < 0.05$, 3-factor ANOVA). The condition was whether the measurement had been made immediately after the eccentric contractions, after a delay, or after passive stretches.

In two experiments, an attempt was made to measure passive tension changes continuously over time after completing a series of 50 eccentric contractions, carried out symmetrically about the optimum length. Data from one experiment are shown in Fig. 5B. Passive tension was measured every 2 min at $L_{\text{opt}} + 0.5$ mm during a slow stretch over the range $L_{\text{opt}} - 11$ mm to $L_{\text{opt}} + 1$ mm. Smaller stretches were used here to minimize any effect they might have on passive tension. Before the eccentric contractions, passive tension was 2 N. This rose to 8.5 N immediately after the contractions and then continued to rise over the next 54 min to 12 N, with an approximate time constant of 10 min. A series of large rapid stretches from $L_{\text{opt}} - 11$ mm to $L_{\text{opt}} + 10$ mm caused passive tension to fall by 3.4 N. Passive tension then began to rise again with a similar time course. Two further series of large stretches again caused passive tension to fall followed by a slow recovery (Fig. 5B).

Passive tension increases were greater after eccentric contractions at a longer length, giving a mean peak increase of 232 vs. 211% (see above). In an attempt to demonstrate a muscle length dependence of the passive tension increase (Fig. 5C), we included consideration of some of the passive tension measurements made in the three-part experiments. We reasoned that a passive tension increase observed after eccentric con-
tensions of the first third of the muscle was likely to be reliable because the rest of the muscle had not yet been subjected to any eccentric contractions. For three animals, there were data for eccentric contractions of the first third of the muscle over the range \( L_{\text{opt}} - 6 \) mm to \( L_{\text{opt}} - 3 \) mm and for one animal over the range \( L_{\text{opt}} - 6 \) mm to \( L_{\text{opt}} - 3 \) mm. For passive tension increases in these animals, because they involved only one-third of the muscle, values had to be multiplied by the appropriate fraction to obtain an estimate of the passive tension increase of the whole muscle. They were likely to be slight underestimates because the measurements had been made immediately after the eccentric contractions. However, in previous experiments we had found that passive tension increases after eccentric contractions at short lengths changed rather little with time. For eccentric contractions ending at \( L_{\text{opt}} - 3 \) mm the mean peak increase in passive tension was \( 127.2 \pm 30.9\% \). For the one animal in which the eccentric contractions ended at \( L_{\text{opt}} \), the value was 192%. So, within the limits of error for these approximations there did appear to be a weak, positive length dependence for the increase in maximum passive tension (Fig. 5C). That is, the negative effect observed with the three-part experiments was not confirmed.

**Onset of passive tension.** We have reported previously that when a passive muscle is stretched from a slack length, as tension begins to rise, it does so with an initial steep increase, followed by a more gradual change (Ref. 34, Fig. 6). It was observed that after a series of eccentric contractions, tension onset was delayed. We argued that the delay was due to the presence of disrupted sarcomeres in muscle fibers, leading to an increase in whole muscle slack length. Here we sought evidence for similar changes in tension onset. The length at which tension began to rise was determined using a curve-fitting method (6). Examples are shown in Fig. 6A. For eccentric contractions carried out about the muscle optimum length (plateau), tension onset measured immediately after the eccentric contractions was little different. For two muscles it required an average of 0.4 mm further stretch than before the contractions. After 40-min delay, the onset of passive tension shifted back in the direction of shorter lengths for two muscles by an average of 0.6 mm. A series of 20 passive stretches led to a fall in passive tension, and tension onset was shifted by an average of 0.13 mm from its original starting length. After the second delay and recovery of passive tension, the onset length reverted to 0.9 mm shorter than before the eccentric contractions.

The changes in tension onset after eccentric contractions carried out at longer lengths were similar but larger (descending limb, Fig. 6A). Immediately after the eccentric contractions, the muscle had to be stretched further, by 1.3 mm before passive tension began to rise. During the next 40 min, starting length shortened by 1.0 mm. It then lengthened again by 0.6 mm after a series of passive stretches to shorten a second time by 0.5 mm after a further 40-min delay (not shown).

All of these observations suggested a negative correlation between the increase in passive tension after the eccentric contractions and the onset length for the tension rise. When passive tension increases were

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**Fig. 5.** A: passive tension measured under different conditions. Maximum increase in passive tension is expressed as a percentage of control values after a series of eccentric contractions were carried out either across the plateau (●) or on the descending limb (□) of the whole muscle active length-tension curve. Pre, passive tension measured before the eccentric contractions; Post Ecc, passive tension measured immediately after the eccentric contractions; Delay 1, value measured 40 min later; Pass Stretch, passive tension measured after 20 passive stretches of the muscle; Delay 2, value measured after a second 40-min delay. Each point is the mean ± SE of values from 3 muscles. B: passive tension measured at regular intervals after a series of 50 eccentric contractions. Test stretches from \( L_{\text{opt}} - 11 \) mm to \( L_{\text{opt}} + 1 \) mm were given at 2-min intervals, and passive tension was measured during each stretch at \( L_{\text{opt}} + 0.5 \) mm. At 54 min after the eccentric contractions had ended, a series of large, rapid stretches was given that covered the full physiological range, from \( L_{\text{opt}} - 11 \) mm to \( L_{\text{opt}} + 10 \) mm. Similar large stretches were given at 82 and 116 min after the eccentric contractions. ●, Passive tension; trace at bottom, length. Dashed line indicates rise in passive tension during the eccentric contractions. C: passive tension increase after eccentric contractions at different lengths. A plot is shown of the maximum percent increase in passive tension, above the preeccentric control value, against the final length to which the muscle was stretched during the eccentric contractions. ●, Values measured after Delay 1 from whole muscle measurements; □, measurements obtained from the 3-part experiments. Squares, means ± SE. Values from 3-part experiments include only measurements made after eccentric contractions of the first part. Data for \( L_{\text{opt}} - 3 \) are from 3 muscles. Data from only 1 muscle were available for stretches that finished at \( L_{\text{opt}} \).
large they were accompanied by a shorter length at which passive tension began to rise during a slow stretch (Fig. 6B). A regression line fitted to the data gave an $r^2$ of 0.7 ($P < 0.05$).

**DISCUSSION**

**Shift in Active Length-Tension Relation**

One important objective of this study was to demonstrate for the first time for mammalian muscle that the size of the shift, in the direction of longer muscle lengths, of the muscle’s active length-tension relation, after a series of eccentric contractions depended on the lengths over which the eccentric contractions were carried out. Our laboratory has previously demonstrated such a length dependence for toad sartorius muscle, a uniform, parallel-fiber muscle with rather short tendinous attachments (27). Here we have shown a similar length dependence for three different portions of the same muscle (Fig. 1) and demonstrated a close correlation between the shift in optimum length for active tension and the size of the fall in active tension after the eccentric contractions (Fig. 2).

An assumption central to the three-part experiment was that dividing the muscle’s nerve supply, at the level of the ventral root, apportioned motor units of different types (4, 24) approximately equally between the three parts. In a recent study of the fatigue properties of cat MG (35), our laboratory carried out the same kind of experiment and concluded that axons of different motor units were distributed at random throughout the ventral root and that similar proportions of fatigue-resistant and fatigable motor units were included in each stimulated portion.

The point is important because we have recently shown that length-tension relations for different motor units in MG were not the same. Some units had optimum lengths shorter than the whole muscle optimum, whereas others had longer optima. Motor units with short optima showed more evidence of damage after a series of eccentric contractions. Slow-twitch units, as a group, tended to show less damage than fast-twitch units, although not all fast-twitch units were damage prone (3).

Interpretation of the shift in optimum length for active tension is based on our view of the initial event in the damage process (17). In regions of myofibrils that resist the active lengthening less than others, sarcomeres are stretched to beyond myofilament overlap and some become disrupted. These regions of disrupted sarcomeres increase the total series compliance of the muscle. It is this increase in compliance that leads to the shift in the active length-tension relation. Experiments on isolated frog fibers with minimal tendinous attachments have revealed similar shifts (19), making it unlikely that tendon properties play a significant role.

The observations reported here reinforce the view that a shift in active length-tension relation is a useful indicator of damage. In fact, it could be considered more reliable than the fall in active tension because immediately after the eccentric contractions this is likely to be confounded by metabolic fatigue, independent of any damage effects. Furthermore, any fall in active tension measured at the original optimum is
likely to be an overestimate, given that a change in optimum length has taken place. A disadvantage revealed by these experiments is that the passive tension increase becomes a saturating function at longer muscle lengths.

Increase in Passive Tension

We have introduced consideration of a second damage indicator, which is present immediately after a series of eccentric contractions and which is also independent of fatigue: the rise in whole muscle passive tension. It has been reported previously that after a series of eccentric contractions there is a rise in muscle stiffness (5, 11, 13). This has been shown to be the result of a rise in whole muscle passive tension (34).

We have hypothesized that passive tension rises as a result of some damaged fibers developing an injury contracture. We interpret this as a consequence of damage to the sarcofibrillar membranes and T tubules, leading to uncontrolled release of Ca$^{2+}$ into the sarcoplasm (18, 23, 34). One important objective of this study was to demonstrate that the amount by which passive tension rose depended on the length range over which the eccentric contractions had been carried out. Only if it exhibited such a dependence could a passive tension rise be considered a reliable damage indicator.

We carried out an initial series of observations using a muscle with its motor supply separated into three portions. Although such a preparation was valid for measurements of changes in active length-tension relations, it turned out to be inappropriate for measurements of passive tension. We had presumed that the damage in a third of the muscle undergoing eccentric contractions would remain relatively unaffected by the passive stretches when the next third was subjected to eccentric contractions at a longer length, where more damage was expected. Such stretches were unavoidable because they were applied to the whole muscle. When it was observed that passive tension levels did not increase with length, it was decided to resort to a whole muscle preparation. The additional data showed that passive tension increases were, indeed, positively length dependent; they could be collapsed by a series of passive stretches, which is also independent of fatigue: the rise in whole muscle passive tension (34).

Our interpretations of these observations are based on the assumption that during an eccentric contraction some muscle fibers develop an injury contracture. Such an interpretation is based on structural observations (7, 20) and on observations of the behavior of damaged single fibers (19). We envisage contracture formation as a dynamic process, in which sarcomeres in a damaged region shorten as a result of active cross-bridge cycling, triggered by uncontrolled release of Ca$^{2+}$ into the sarcoplasm. Sarcomeres in the region of contracture shorten, stretching sarcomeres and membranes in immediately adjacent areas and spreading the contracture. As a result, the level of passive tension generated by the injured fibers increases over time.
to the presence of larger numbers of overstretched, disrupted sarcomeres. There is some similarity in the length-dependent changes in passive tension after the eccentric contractions and changes in the active length-tension relation. However, especially after eccentric contractions at long lengths, the shapes of the curves and the locations of their optima are not the same (compare Figs. 1B and 4B).

Why do passive stretches reduce the passive tension? We have considered two mechanisms. It is conceivable that the stretches break up the contraction clots in damaged fibers, so that what had been a single large region of contracture becomes several smaller areas, distributed along the length of the fiber, separated by an empty sarcolemmal tube. The presence of segments containing no contractile material reduces the level of passive tension at the tendon. Presumably, with time, more sarcomeres will enter a state of contracture to recover the preexisting level of passive tension, and some of the distributed contractures may coalesce to reform larger clots.

A second possibility is that the passive stretches increase the numbers of overstretched sarcomeres, to increase the series compliance. This may be particularly so for fibers in which injury contractures are already present. They will behave as though they are shorter than they actually are because the contracted region will be stiffer and therefore resist the stretch more than the rest of the fiber. Such an interpretation is consistent with observations on the length at which passive tension begins to rise during a slow stretch (Fig. 6). Passive stretches both reduced the measured level of passive tension and delayed the onset of tension during the stretch. These effects were particularly marked with eccentric contractions carried out at longer lengths, again, because the proportion of overstretched sarcomeres was likely to be greater at longer lengths. Within that context, it is interesting that there is a close correlation between the maximum increase in passive tension and the onset length for passive tension rise (Fig. 6C).

Although the underlying structural changes accompanying sarcomere disruption after eccentric contractions remain unclear, recent evidence points to damage of the elastic filament titin as a potential candidate. Titin connects the Z line to the myosin molecule in each half sarcomere and is responsible for a large portion of the passive tension in skeletal muscle over the physiological range of muscle lengths (10, 14). During an eccentric contraction, the weakest sarcomeres are proposed to be overstretched beyond myofilament overlap so that tension is borne by passive structures (17). Under these conditions, it is possible that titin is stretched to a sarcomere length at which it reaches its yield tension (~3.8 μm in rabbit psoas muscle fibers) and becomes dislodged from its attachment to the thick filament (29). With repeated eccentric contractions, more of the titin may detach, until a point is reached at which the myofilaments become misaligned and the sarcomere is no longer able to generate active tension. For passive muscle, detachment of titin from its anchorage on the thick filaments increases the extensible segment length of titin, which results in a shift of the passive length-tension curve to longer lengths (29). Within this context, the compliance whose increase is postulated to be responsible for the shift in active length-tension relation should be seen as a nonlinear compliance, high at short lengths because it increases fiber rest length and lower at longer lengths when the remaining normal sarcomeres begin to be stretched.

In summary, there are two mechanisms available to account for the changes in passive muscle properties after a series of eccentric contractions. One is sarcomere disruption, increasing the optimum length for active tension, reducing passive tension, and increasing the onset length for passive tension. Detachment of titin, if it occurs, will be part of this process. Second, the presence of contractures would have an opposite effect, raising passive tension and shortening the onset length for passive tension. It seems likely that both mechanisms are operating at any one time.

To conclude, we have shown for the first time that there is a shift in the active length-tension relation for mammalian muscle after eccentric contractions that is dependent on the length range over which the eccentric contractions are carried out. Second, there is a rise in passive tension, the size of the rise depending on the length range covered by the eccentric contractions. It means that we now have two simple, reliable indicators of muscle damage present immediately after eccentric exercise. In human subjects, measurement of active angle-torque curves is not always easy or reliable (12) and it may be that measurements of passive tension are simpler and more convenient to make. However, it should be kept in mind that if damage is severe, passive tension rises are not necessarily larger and they are likely to exhibit large time-dependent changes.

Future experiments should explore further the time course of the passive tension rise after eccentric contractions and its muscle length dependence. Such measurements may provide new clues about the underlying mechanism. We know from other experiments that eccentric contractions of single motor units are sufficient to produce measurable increases in passive tension, increases that can be detected in the stretch responses of tendon organs (8). Furthermore, only two to three eccentric contractions are required to produce these changes. It suggests that in some muscle fibers the transition from overextended, disrupted sarcomeres to injury contractures is almost immediate. It raises the question of the factors determining the balance between injury contractures and disrupted sarcomeres. It is perhaps the difference in length-tension relation for individual motor units (3).

DISCLOSURES

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