Damage to different motor units from active lengthening of the medial gastrocnemius muscle of the cat

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ALL FORMS OF EXERCISE, IF performed vigorously enough, lead to a feeling of exhaustion and, in extreme circumstances, a sensation of pain. But only one form of exercise, eccentric exercise, where the contracting muscle is forcibly lengthened, leads to sensations of stiffness and pain the day after the exercise. Such delayed soreness, called delayed-onset muscle soreness, cannot be attributed to the action of the metabolic end products of the exercise because these are long gone by the time of its onset. Current ideas for the generation of delayed-onset muscle soreness are based on a mechanism for damage to muscle fibers, which leads to an inflammatory response and sensitization of muscle nociceptors (for review, see Ref. 24).

The initial event that leads to muscle fiber damage during eccentric exercise remains the subject of debate (3, 19). Morgan (18) has put forward a theory for the initiation of damage to muscle fibers during eccentric contractions, based on sarcomere-length inhomogeneities. Briefly, stretch of the active muscle to beyond the optimum length is distributed nonuniformly among sarcomeres. A few take up most of the length change, whereas most are stretched very little. The stretched sarcomeres, distributed randomly along muscle fibers, are extended to beyond myofilament overlap, and, during repeated lengthenings, a few become disrupted. The area of disruption spreads until a point is reached at which membranes are ruptured, which may ultimately lead to the fiber’s death (3).

Previous work in our laboratory has focused attention on changes in mechanical properties of a muscle after a period of eccentric exercise, that is, a series of stretches of the contracting muscle (3, 12, 33). A measure used by us as an indicator of damage immediately after the eccentric exercise is a shift of the muscle’s optimum length for active tension in the direction of longer lengths (35, 13, 26). Such a shift does not occur with other forms of exercise. We have postulated that the shift is due to the presence of lengthened, disrupted sarcomeres lying in series with still functional sarcomeres (26). The size of the shift is proportional to the mean number of disrupted sarcomeres in fibers, which, in turn, will determine other indicators of damage.

A question that has been raised repeatedly concerns the susceptibility to damage from eccentric exercise of different types of motor units in muscles of mixed-fiber composition. It is known that the further a muscle is stretched onto the descending limb of its length-tension curve by an active lengthening, the more likely it is that muscle fibers will become damaged (18). Consequently, our working hypothesis is that, for a given stretch applied to the whole muscle, units with a shorter optimum length should become more damaged than units with longer optima. If motor unit type is correlated with optimum length, this could explain the reports that susceptibility to damage depends on unit type (14, 16).

In the medial gastrocnemius muscle (MG), length-tension curves were constructed for single motor units, and their optimum length for active tension was related to the whole muscle optimum length. Motor units were then subjected to 10 active lengthenings, and
their length-tension curves were remeasured. Shift in the optimum length for tension and the remaining tension were taken as indicators of damage and were correlated with motor unit type.

METHODS

Experiments were carried out on three cats, in the weight range of 2.5–4.2 kg. All experiments were carried out with approval of the Monash University Committee for Ethics in Animal Experimentation. Anesthesia was induced with an intraperitoneal dose of pentobarbionate sodium (40 mg/kg) and maintained, during the course of the experiment, with additional doses, given when necessary into the cephalic vein. Depth of anesthesia was routinely monitored by using eye-blink, ear-flick, and limb-withdrawal reflexes. The trachea was cannulated, and end-tidal CO2 concentration was monitored. Rectal temperature was measured, and body temperature was maintained at 38°C by the use of a feedback-regulated heating blanket.

A laminectomy was carried out to expose ventral roots L7-S1. These were cut at their point of entry into the spinal cord and deflected onto a dissection plate. Electrical stimulation established where motor axons to MG ran in the ventral roots, typically L7-S1. The left hindlimb was dissected to expose the MG muscle. For this, it was necessary to free MG from adjacent muscles and their tendons, leaving just the tendon of MG attached to the calcaneum. All hindlimb nerves other than the MG nerve were cut, including those to hip muscles. The hindlimb was fixed to a rigid metal frame by means of steel pins in the pelvis and clamps at each end of the tibia. Exposed tissues were covered with mineral paraffin oil retained in baths fashioned from skin flaps. Temperature in the paraffin pool was maintained within 2°C of core body temperature by the use of heating lamps.

At the start of each experiment, the maximum physiological length of the muscle (Lmax) was determined. To do this, the ankle was flexed maximally, with the knee and hip in the approximate positions that they would adopt during the experiment, and the distance was noted between markers placed on the Achilles tendon and on the adjacent fibula. Measurements made at different muscle lengths were related to Lmax. The calcaneum was severed, and the piece attached to the tendon was clamped between a pair of nuts and washers on a threaded rod. Tension was measured with a semiconductor load cell (Entran). The rod and supporting strain gauge screwed into the shaft of a servo-regulated muscle stretcher. Compliance of the system was 2 μm/N.

Length-tension curves were constructed by stimulating the ventral root at 80 pulses/s (pps) for 250 ms. Tension was measured at 1-mm intervals between Lmax — 16 and Lmax — 4 mm. In one experiment, whole muscle tension reached levels (>100 N) at which the stretcher was no longer able to maintain a set length, and it began to yield. Because it was desirable to stimulate the muscle maximally, at 80 pps, to measure the length-tension relation, on this occasion the nerve supply to only 50–60% of the muscle was stimulated. Values for optimum length were assumed to be the same as for the whole muscle. This assumption was supported by data from another series of experiments in which the motor supply to the muscle had been divided into three nearly equal portions. Here the range of optima between portions and the whole muscle for five animals was 0.43 mm. In addition, it should be noted that errors in measuring whole muscle optimum length affect comparisons between animals but not between units within the same muscle. To put this to the test, experiment number was included as a factor in the statistical analysis, but it never reached significance.

Portions of ventral root were subdivided into progressively smaller portions, and each subdivision was stimulated until a portion was reached that, on graded stimulation, produced a muscle twitch behaving in an all-or-nothing manner. Slow-twitch units in MG develop very low tensions. On two occasions, where the tensions were close to noise level, pairs of slow units were stimulated together. Motor units were identified as slow if their twitch time to peak was >60 ms and fast if it was <50 ms. Further evidence of unit type was provided by their force-frequency relation using stimulation rates of 20, 30, 40, 50, and 80 pps. For each unit, a length-tension curve was constructed using 250-ms-duration tetani at 80 pps, measured over the same length range as for the whole muscle.

When up to six units had been isolated and identified, their motor nerve filaments were mounted on separate stimulating electrodes, and they were subjected to a series of 10 active stretches. Here, each filament was stimulated at 80 pps for 400 ms, and 150 ms after the start of stimulation the muscle was stretched at 50 mm/s over 6 mm (Fig. 1). Each active stretch was followed by a 40-s rest period.

Fiber length in MG is ~20 mm, and tendon length is 100 mm (31). Peak tension during an active lengthening of the collection of six motor units was ~5 N (Fig. 1). At that force level, the length change is distributed approximately equally between muscle fibers and tendon (31). A 6-mm stretch, therefore, represented an ~15% length change of muscle fibers.

Stretches were arranged so that they started at a length corresponding to the whole muscle’s optimum length. A stretch, therefore, finished well down on the descending limb of the muscle’s length-tension relation. After the active stretches, each unit’s length-tension relation was remeasured. Because the ventral root filament supplying each motor unit had been mounted on a separate stimulating electrode, it meant that length-tension curves could be constructed before and after the active stretches without disturbing the filaments and so risking a change in stimulating conditions. For each unit, the optimum length for active tension was determined by fitting a Gaussian curve to the data points. Curve fitting was performed using a computer program (Igor Pro WaveMetrics, Lake Oswego, OR).

For each unit, the shift in optimum length was determined, as well as the amount of active tension remaining after the active lengthenings. The ratio of tension remaining to tension before the contractions was calculated using values measured at the optimum length determined both before and after the active stretches.

Statistical analysis. For all parameters measured, means and SEs of the mean were calculated. Differences between slow and fast units were tested for significance using a two-sided t-test. Coefficient of determination (r2) analysis was used to examine the relationships between preexercise optimum length, shift in optimum, and tension remaining postexercise. Preexercise optimum length, unit type, and experiment number were tested as factors determining the shift in optimum length postexercise, using an ANOVA with no interactions. Residuals of the regression were calculated, and another ANOVA was carried out with residuals as the dependent variable and motor unit type and experiment number as factors. Equivalent analysis was performed on the other damage indicator, postexercise tension. Tests were carried out using the statistical package Data Desk (Data Description, Ithaca, NY).
RESULTS

In three experiments, 35 units were studied. Of these, eight were of the slow-twitch variety. Here it was not our intention to study properties of the three types of motor units known to be found in MG exhaustively. Others had already done that (see, for example, Refs. 6, 22). We simply wanted to be able to distinguish between fast and slow units. This was done on the basis of the twitch time to peak, tetanic tension, and the force-frequency relation, using stimulation rates of 20–80 pps (Fig. 2). Tension in fast units remained unfused at all stimulation frequencies >60 ms were all slow. Their tetanic tensions all lay <0.6 N (mean 0.21 ± 0.06 N). Fast units covered a much wider range of tensions, 0.1–2.2 N (mean 0.91 ± 0.10 N), and their time to peak was always <50 ms.

For each motor unit, a length-tension curve was constructed (Fig. 3). The slow units had, on average, the longer optimum lengths (Fig. 4). The muscle lengths over which the slow unit optima were distributed lay between −0.2 and +4.0 mm relative to whole muscle optimum (mean, 0.8 ± 0.5 mm). For the fast units, the range was −3.2 to +1.7 mm (mean, −1.3 ± 0.3 mm).

After a motor unit’s optimum length had been determined, it was subjected to 10 active lengthenings. Examples from one experiment of length-tension curves from before and after the active lengthenings are shown in Fig. 3. Two of the units, one slow and one fast, had initial optimum lengths for a contraction that were longer by ~1 mm than the whole muscle optimum.

![Fig. 1. Active lengthenings of motor units. Top: plot of whole muscle tetanic tension against muscle length for medial gastrocnemius (MG), expressed in millimeters below maximum physiological length (L_max). Shaded bar below the curve indicates the length range over which the active lengthenings were carried out. Line drawn through the data points is a Gaussian curve fitted by computer to locate the optimum length for peak tension (arrow). Bottom: tension (top traces) and length (bottom trace), when a collection of 6 motor units was subjected to a series of active lengthenings. Units were stimulated at 80 pulses/s (pps), and 150 ms after the start of stimulation they were stretched 6 mm at 50 mm/s. The stretch began at a muscle length corresponding to the whole muscle optimum. Tensions after the 1st, 5th, and 10th contractions have been superimposed. Solid bar below the tension trace indicates the period of stimulation.](image1)

![Fig. 2. Motor unit tensions. Top: example of tension responses of a fast-twitch (left traces) and a slow-twitch (right traces) motor unit of the MG muscle of the anesthetized cat to stimulation of their motor axons at 20, 40, and 80 pps. Bottom: plot of twitch time to peak for all of the 35 motor units studied against their tetanic tension. ● Slow-twitch units; ○, fast-twitch units.](image2)
length. After the active lengthenings, the optimum in the curves of both units had shifted to the right, in the direction of longer muscle length: the slow unit by 1.9 mm, and the fast unit by 2.4 mm. However, although the fast unit developed approximately twice the tension of the slow unit before the active lengthenings, afterward the percent tension remaining for the two units was about the same. The third unit shown, also fast, had its initial optimum to the left of the whole muscle optimum by 2.0 mm. After the active lengthenings, its optimum lay 3.5 mm to the right; that is, it had shifted in the direction of shorter muscle lengths by 5.5 mm, representing ~30% of its working range. It also showed a larger fall in tension than the other two. Notice for all three units that values at long lengths measured after the active lengthenings lie above those measured beforehand; that is, the length-tension curves have crossed at the long lengths. The mean shift in optimum length for the 27 fast-twitch units was 4.3 ± 0.3 mm, and for the eight slow-twitch units it was 2.1 ± 0.4 mm. A t-test showed these means to be significantly different (P < 0.01).

When the shift in optimum length after the active lengthenings was plotted for each unit against its initial optimum, each relative to whole muscle optimum, a significant inverse relationship emerged (Fig. 5). Regression analysis gave an r² of 0.63 (P < 0.01). An ANOVA, carried out with shift as the dependent variable and initial optimum, unit type, and experiment number as factors, gave P values of 0.0002, 0.25, and 0.987, respectively. To confirm that unit type was not a significant factor after accounting for optimum length, the residuals of the regression of shift against initial optimum were calculated. Another ANOVA, with the residuals as the dependent variable and unit type and experiment number as factors, showed no significant difference between slow and fast units (P = 0.58); that is, shifts for all units fell on the same line. Experiment number was also not significant (P = 0.78). The data indicated that the further a unit’s optimum lay to the left of the whole muscle’s optimum; that is, in the direction of shorter muscle lengths, the larger was the shift produced by the active lengthenings. Furthermore, if differences in initial optimum length were accounted for, fiber type no longer became a significant factor.

Motor units showed a drop in tension at optimum length after the active lengthenings. Given that fast twitch units, on average, showed a larger shift in their length-tension relation after the active lengthenings than the slow-twitch units, they were predicted to show a larger tension drop due to fiber damage. This was, indeed, found to be so (Fig. 5). The mean tension remaining for fast units was 51 ± 18%, and for the slow units it was 70 ± 15%. By t-test, these means were significantly different (P = 0.01). Motor units with long
The aim of these experiments was to test the hypothesis that, in a muscle of mixed fiber composition, motor units differ in their susceptibility to the damage from eccentric exercise because of differences in optimum length. In response to a series of active lengthenings, it was hypothesized that the length change experienced by a motor unit, rather than motor unit type, determined the extent of damage. Consistent with that view, after the active lengthenings, the fast-twitch units showed a larger shift in optimum length for active tension and had less tension remaining than the slow-twitch units. This result, therefore, suggests that fast-twitch units are more prone to damage from eccentric contractions than slow-twitch units, and analysis showed that this was because of their shorter optimum length. Therefore, optimum length is a better predictor of damage than unit type.

Observations have been made on exercising animals that have led to claims that slow motor units show a predisposition to damage (2, 17, 29). The reasons put forward included the low recruitment threshold and important postural roles of these motor units. However, in an isolated muscle preparation, when muscles of mixed-fiber composition were subjected to maximal active lengthenings, it was found that the large, fast-fatiguable motor units were the more vulnerable. It was suggested that the lack of oxidative capacity (10) or the higher tensions generated by them might be responsible (1). In another recent study demonstrating preferential fast-oxidative glycolytic fiber damage, it was suggested that fiber phenotype or lower contractile workload might be responsible (28). Others have suggested a combination of factors involving both active and passive properties of muscle fibers (16). Summarizing their position in a recent review, Lieber and Friden (14) proposed that the larger amount of fiber injury in fast-glycolytic fibers after eccentric exercise was a result of the “increased strain and injury due to their short fiber length.” Our own results suggest that the apparent correlation between damage and motor unit type derives from the correlation between unit type and optimum length (Fig. 4).

In a related experiment, Macpherson et al. (16) used permeabilized fiber segments from a fast and a slow rat muscle, applying single stretches starting from the optimum sarcomere length. Force deficits were greater in fast fibers. We would have predicted similar force deficits in the two fiber types. In seeking an explanation for their result, the authors hypothesized that sarcomere lengths in fast fibers were more heterogeneous than those in slow fibers. This is against a background of a greater tendency for fast fibers to develop sarcomere length heterogeneities than slow fibers during contractions after the permeabilization process (R. L. Moss, personal communication). Therefore, it may be that the difference in susceptibility to

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**Fig. 5.** Shift in optimum length and fraction of tension remaining after active lengthenings. Top: plot of shift in optimum length against initial optimum, after 10 active lengthenings for the 35 motor units. Initial optimum length is expressed relative to whole muscle optimum, which is given as 0 mm. A unit optimum <0 means that it is at a length shorter than the whole muscle optimum. Bottom: fraction of motor unit tension remaining after 10 active lengthenings, plotted against the motor unit’s initial optimum length, expressed relative to the whole muscle optimum length. In both panels, the lines drawn through the data represent fitted regression lines. ●, Slow-twitch units; ○, fast-twitch units.

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optima, relative to whole muscle optimum, tended, on average, to show more tension remaining after the active lengthenings. There was a significant correlation ($r^2 = 0.42, P < 0.01$) between the initial optimum and the fraction of tension remaining after the active lengthenings. The ANOVA returned $P = 0.0032$ for initial optimum, 0.56 for fiber type, and 0.63 for experiment number. Calculating the residuals of the regression analysis for tension remaining against initial optimum and performing the ANOVA with fiber types and experiment as factors gave $P = 0.59$ and $P = 0.63$, respectively. Thus the trend of the data for tension was similar to that for the shift in optimum, although there was more scatter of values.

Despite these differences between slow units and fast units, as a group, it should be noted that some of the fast units had an optimum length and a shift in optimum length comparable to that of the slow units (Fig. 5).
damage of different fiber types reported by these authors was the result of the permeabilization process.

Before the present experiments were begun, a pilot study was carried out in which 150 active stretches were used rather than 10. Although there was a trend in the data, differences in damage indicators between slow and fast units did not reach significance. It was concluded that 150 contractions were too many. We hypothesized, much as had Vijayan et al. (28), that, during the contractions, the fatiguable motor units would rapidly lose tension, which meant that fatigue-resistant units would effectively be subjected to a larger number of active stretches. We, therefore, decided to restrict the number of active stretches to 10, with a 40-s interval between each contraction, in an attempt to keep fatigue to a minimum (34).

The active lengthenings were carried out over a length range from whole muscle optimum to 6 mm down the descending limb. For all of the motor units studied, this meant that they too were stretched onto their descending limb. More importantly, units with shorter optima would have more of the stretch applied on their descending limb so that they were more likely to become damaged. It has been proposed that the descending limb of the length-tension curve is a region of sarcomere length instability (18). Sarcomeres that lengthen over this range become progressively weaker and stretch to beyond filament overlap. Some become disrupted. The larger shift in optimum for the fast motor units is, therefore, consistent with the hypothesis that fast units experienced a larger amount of sarcomere disruption than slow units.

A shift of the optimum length in the direction of longer muscle lengths after a series of active lengthenings has been interpreted as an indicator of sarcomere disruption. For a discussion, see Morgan and Allen (19). To explain the shift, a change in length-dependent sensitivity of the myofilaments to Ca$^{2+}$ (8) is unlikely to be involved because increasing stimulation rate after the active lengthenings to >80 pps did not increase tension further. Furthermore, the length-tension curves determined after the active lengthenings crossed the precontraction curves at long lengths (Fig. 3). If active tension after the active lengthenings is higher at long lengths, the shift cannot be explained by a reduction in Ca$^{2+}$ release or reduced Ca$^{2+}$ sensitivity of the myofilaments, because tension at these lengths is more, not less. Consistent with our interpretation, in a recent study of passive tension changes in MG after a series of active lengthenings, onset of the tension rise in response to a slow stretch was delayed after a series of active lengthenings, consistent with an increase in whole muscle compliance (33).

Previous measurements of motor unit properties in MG have suggested that, on average, slow and fast units had essentially similar length-tension relations (25). However, for other cat hindlimb muscles, there is good evidence that slow units have longer optima than fast units. Thus Bagust et al. (4) found for flexor digitorum longus a difference of 2.7 mm in mean optimum length between slow and fast units. Filippi and Troiani (9) reported a longer twitch optimum for slow units of cat peroneus longus but a shorter tetanic optimum. For rat gastrocnemius, Huijing and Baan (12) concluded that one of the explanations of their data was that smaller motor units had longer optimum lengths. Similarly, Van Eijden and Raadsheer (27) commented on the heterogeneity of fiber and sarcomere lengths in human masseter.

Two factors determine the optimum length for contractions in muscle fibers. These are sarcomere number and tendon length. The simplest structural arrangement to achieve a longer optimum length is to have muscle fibers with a larger number of sarcomeres but with a similar tendon length. That is, for a given muscle length, more sarcomeres would have packed into the same fiber length. Such an arrangement would lead to a steep fall in tension at short muscle lengths. No such fall was apparent in the length-tension curves (Fig. 3). To achieve a longer optimum length and maintain tension-generating capacity over the same length range as for fibers with shorter optima, fibers with more sarcomeres would have to have a shorter tendon. It would mean that, for a given length of the whole muscle, motor units with longer optimum lengths would have longer muscle fibers. This latter possibility seems to be the most likely.

The only measurements of fiber length, made on type-identified fibers, were reported for the cat tibialis anterior muscle by Ounjian et al. (21). The mean lengths of muscle fibers from four fast-fatigue units, one fast-fatigue resistant unit, and two slow units were 29, 35, and 41 mm, respectively. Thus, whereas the sample is obviously very small, the trend of the data is in a direction consistent with our own findings. Interestingly, the authors mentioned that, for fast fibers, cross-sectional area changed progressively along the lengths of the fibers. This would be expected to increase nonuniformities further in these fibers during active stretches.

It remains to explain the functional significance of differences in optimum length between motor units. A clue comes from the comment of Bagust et al. (4) that the slow unit optimum for flexor digitorum longus lay closer to the length of the muscle adopted during standing. Similarly, it is known that, in the cat during standing and slow walking, force levels in MG are <10% of maximum (30). Only when the cat is running or jumping do large parts of the muscle become active. The authors concluded in relation to MG that “quadrupedal standing may involve, primarily if not exclusively, activation of the type S motor unit complement.” Like many other mammals, the cat walks on its toes. This means that, during the weight-bearing (E2) phase of locomotion, MG is stretched (11). Any contracting motor units will, therefore, routinely undergo active lengthening.

Muscle injury from eccentric exercise leads to a rapid training effect so that, whereas unaccustomed exercise leads to soreness, the same exercise 1 wk later produces much less soreness (7, 20, 23). Recently, a shift in the length-tension curve was shown to accompany the
adaptation, suggesting that the training effect is achieved by increasing the number of sarcomeres in series in muscle fibers (5). Such an adaptation process was also reported in muscle fibers of rats that were subjected to regular eccentric exercise (15).

We, therefore, suggest that the slow motor units in MG are continuously active during standing and slow walking in the cat. They are, therefore, regularly subjected to active lengthenings during the load-bearing phase of locomotion, and we suggest that they have adapted to this role by increasing their number of sarcomeres in series. As a result, the slow units have longer optimum lengths than the normally inactive sarcomeres in series. As a result, the slow units have adapted to this role by increasing their number of sarcomeres.  

Eccentric Exercise-Induced Injury to Single Motor Units

Modeling Sarcomere Lesions


REFERENCES