Low-frequency depression of tension in the cat gastrocnemius muscle after eccentric exercise

S. Parikh, D. L. Morgan, J. E. Gregory, and U. Proske

Low-frequency depression of tension in the cat gastrocnemius muscle after eccentric exercise. J Appl Physiol 97: 1195–1202, 2004. First published May 7, 2004; 10.1152/japplphysiol.00268.2004.—Subjecting a muscle to a series of eccentric contractions in which the contracting muscle is lengthened results in a number of changes in its mechanical properties. These include a fall in isometric tension that is particularly pronounced during low-frequency stimulation, a phenomenon known as low-frequency depression (LFD). Reports of LFD have not taken into account the shift in optimum length for active tension generation to longer muscle lengths that takes place after eccentric contractions. Given the length dependence of the stimulation frequency-tension curve, we would have expected the observation that the change in this relationship after eccentric exercise is due to the shift in optimum length. We measured LFD by recording tension in response to a linearly increasing rate of stimulation of the nerve to medial gastrocnemius of anesthetized cats, over the range 0–100 pulses per second. Tension responses were measured before and after 50 eccentric contractions consisting of 6-mm stretches starting at 3 mm below optimum length and finishing at 3 mm above it. An index of LFD was derived from the tension responses to ramp stimulation. It was found that LFD after the eccentric contractions was partly, but not entirely, due to changes in the muscle’s optimum length. An additional factor was the effect of fatigue. These observations led to the conclusion that the muscle length dependence of LFD was reduced by eccentric contractions. All of this means that after eccentric exercise the tension deficit at low rates of muscle activation is likely to be less severe than first thought.

ECCENTRIC EXERCISE IS EXERCISE during which the contracting muscle is forcibly lengthened. Examples of everyday activities that include components of eccentric exercise are skiing, horse riding, and downhill walking. All forms of exercise are followed by fatigue, but recovery from fatigue is normally completed within 1 h. After eccentric exercise, muscle force output remains depressed for several days because of damage to muscle fibers. This is accompanied by the delayed onset of muscle soreness, as a result of the inflammatory process triggered by the damage. For reviews of this subject, see Armstrong et al. (1), Morgan and Allen (17), and Proske and Morgan (22).

There remains some controversy over the primary event leading to muscle damage from a series of eccentric contractions. A prominent histological sign to which we assign particular importance is the presence of sarcomeres or half sarcomeres with disrupted contractile filaments. It forms the basis of our proposal that sarcomere length instability causes nonuniform sarcomere lengthening, leading to disruption of contractile proteins (16). This is followed by damage to the excitation-contraction (E-C) coupling system, perhaps by shearing of T tubules (28). It remains uncertain whether damage to E-C coupling is also a primary event (31), leading to nonuniform excitation and hence the observed sarcomere nonuniformities. The view that we have adopted here is that the starting point for the damage process is overstretch of some sarcomeres (22). A further contributing factor may be the operation of stretch-activated channels in the muscle membrane (15, 35). The supporting evidence is the observation in single fibers of frog muscle that after a series of eccentric contractions the tension at long lengths can be more than it was before the contractions (18), which is an observation that is inconsistent with E-C coupling dysfunction being the primary mechanism.

It has repeatedly been reported that one consequence of a series of eccentric contractions is low-frequency depression (LFD), that is, a reduction in tension in response to low-frequency stimulation that is greater than the reduction during high-frequency stimulation (2, 3, 9, 13, 36). Some authors have interpreted this as evidence in support of the idea that the primary event in the damage process is disruption of the E-C coupling system (17). However, when measuring LFD after eccentric contractions, account must be taken of any change in the muscle’s length-tension relation produced by the contractions. After a series of eccentric contractions, account must be taken of any change in the muscle’s length-tension relation produced by the contractions.

It has previously been demonstrated that the stimulation frequency-tension relationship is length dependent; that is, proportionately less force is produced at shorter lengths with low stimulation rates (24). The most obvious interpretation is that more Ca²⁺ is released at higher stimulation rates and that Ca²⁺ activates short sarcomeres less effectively than longer sarcomeres (10). In addition, there may be length-dependent changes in Ca²⁺ release (8). Here we have tested the hypothesis that the observed depression of tension with low stimulation rates after a series of eccentric contractions is a direct consequence of the changes in the muscle’s length-tension relationship.
relation. That is, it is an expression of the fiber length dependence of the stimulation frequency-tension relationship. We have identified changes in LFD attributable to the shift in optimum length and compared this to the observed amount of depression.

METHODS

The experiments were carried out on 19 cats of both sexes with a weight range of 1.9–3.3 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 pentobarbital sodium (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 measured, and the depth of the anesthesia. Rectal temperature was measured, and feedback regulated heating blanket.

The animal was secured to a steel frame with pins in the pelvis and tibia of one or both hindlimbs. In 12 animals, only the left hindlimb was used; in the remaining 7, muscles were prepared on both sides. The leg was dissected to expose the calf muscles. Markers were placed on the tibia and the distal tendon of medial gastrocnemius (MG). With the leg in the position it would occupy during the experiment, the distance between these markers was measured with the ankle fully flexed. This determined the maximum physiological length of MG (Lm). MG was separated from lateral gastrocnemius and soleus. The exposed muscle was covered by a pool of paraffin in a bath 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. It was severed above the knee and mounted on electrodes for stimulation.

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 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 a muscle stretcher, which controlled muscle length changes. The muscle stretcher consisted of a moving-coil electromagnet whose position was controlled by feedback from a linear variable differential transformer. Muscle tension was measured with a load cell (Entron Devices, Fairfield, NJ) attached to the metal rod. The system had a compliance of 5 μN/N.

Length-tension curves. Isometric tension for MG was measured over a range of muscle lengths by stimulating the nerve, using platinum bipolar electrodes, at a rate of 80 pulses per second (pps) for 250 ms. To determine the optimum length for each muscle, isometric tension was measured over the length range at 2-mm intervals. Muscle length was expressed relative to Lm. The length range normally covered was Lm – 20 mm to Lm. Optimum length for active tension lay at about Lm – 10 mm. Optimum length was determined by fitting a smooth curve to the data points. The curve used was a combination of two parabolas, one to fit values to the left of optimum, one to fit values to the right of the optimum. This was necessary because the relation between tension and length typically had different curvatures on either side of optimum. The two parabolas used therefore had different curvatures, but both had a zero slope at the optimum and values at the optimum were equal. Fitting of the curves as well as all other data acquisition and analysis was done by using the program Igor (Wavemetrics, Lake Oswego, OR). All curve fits were checked by eye, and, if necessary, adjustments were made to the range of lengths fitted until an acceptable fit was achieved around the optimum length, with a minimum of six data points on the curve.

Eccentric contractions. The servomotor was used to apply controlled stretches to the contracting muscle. Each eccentric contraction consisted of an isometric tetanic contraction using 80 pps stimulation for 400 ms. At 150 ms after the onset of stimulation, the muscle was stretched by 6 mm at 50 mm/s. The stretches were arranged to lie symmetrically about the optimum length for active tension, i.e., from 3 mm short of optimum to 3 mm beyond it. Individual eccentric contractions were separated by 40-s rest intervals. Each muscle was subjected to between 2 and 50 eccentric contractions. Experience had shown that active stretches of this size and over the chosen length range were effective in generating the signs typical of muscle damage from such exercise (32, 33). Damage from eccentric exercise can also be induced by only one or a small number of contractions in which each stretch extends further beyond optimum length and that is therefore associated with less fatigue. With large stretches, out to long lengths, there is, however, the risk of “tearing” muscle fibers, rather than inducing the microscopic damage more typical of this kind of exercise.

Low-frequency depression. LFD has been quantified in many different ways. We chose to stimulate the muscle with pulses at a linearly increasing frequency (frequency ramp) and to compare tensions for low and high frequencies of stimulation. This technique has the advantage over measurements using steady rates of stimulation at different frequencies in that all contractions had the same history of preceding stimulation. The stimulation rate rose from zero to 100 pps over 1.5 s, followed by a more rapid decrease back to zero. Only the rising tension in response to the increasing stimulus rate was analyzed.

It was found that, for the low rates of stimulation, tension in response to the first of two or more frequency ramps was higher than for subsequent contractions. This was some kind of potentiation also seen with fixed-rate contractions (6). Therefore two frequency ramps were always given, 40 s apart, and tension in response to only the second ramp was analyzed. Differences between the second and subsequent responses were very much smaller (Fig. 1).

In a typical experiment, a frequency ramp was applied at the optimum length for peak active tension. This provided the reference tension trace. Then a series of eccentric contractions was carried out. After the eccentric contractions had been completed, the length-tension relation of the muscle was remeasured and, typically, optimum length had moved in the direction of longer muscle lengths by 3–4 mm. Frequency ramp responses were then remeasured, both at the original optimum length and at the new optimum length (Fig. 2).

To quantify LFD, the tension response to the frequency ramp given after the eccentric contractions was divided by the preeccentric ramp tension. This gave a ratio of the test to the reference tension as a function of time and hence stimulation rate (Fig. 3). To reduce peak-to-peak oscillations in the ratio of tensions at the low frequencies, tension traces were box-car filtered, whereby each point of the trace was replaced by the average of n neighboring points, n being the number of data points in the interval between the first two stimulus pulses.

A Hill plot (11) was then fitted to the ratio curve, and the high- and low-frequency asymptotes (A_H and A_L) of the fitted curve were found (Fig. 3). LFD was defined as the difference in asymptotes as a fraction of the high-frequency value.

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\text{LFD} = \frac{A_{H} - A_{L}}{A_{H}}
\]

An LFD value of zero implied that tensions at the high and low frequencies of stimulation were affected equally by the treatment, or the tension ratio was independent of frequency. An LFD of 1 implied that, at the low frequency, tension had fallen to zero. A negative LFD meant that tension at low frequencies had increased relative to that at high frequencies, or that tension at low frequencies had decreased less.

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Statistical analysis. Statistical tests, as described under RESULTS, were carried out by using Data Desk (Data Description, Ithaca, NY). All means are quoted ± SE.

RESULTS

The first series of experiments, carried out on 15 muscles, examined the effect of 50 eccentric contractions on LFD.

Evidence of muscle damage from the eccentric contractions was apparent from the shift of the length-tension curve in the direction of longer muscle lengths and from an accompanying fall in active tension. It was found that 50 eccentric contractions, given symmetrically about the optimum length for active tension, led to a mean shift in optimum of 3.2 mm, accompanied by a mean tension drop of 45%, measured from the length-tension relation at the original optimum length.

Because optimum length had shifted after the eccentric contractions, values of tension measured at the old optimum were now on the ascending limb of the length-tension relation and so were less than at the new optimum. Example records are shown in Fig. 2. At the high stimulation rate, tension in this muscle reached a peak value of 77 N before the eccentric contractions. After the eccentric contractions, it reached only 35 N when measured at the original optimum length. When muscle length was adjusted to the new optimum length, the posteccentric value rose to 47 N (Fig. 2).

Because both peak isometric tension and the stimulation frequency-tension relation varied between preparations, it was necessary to standardize the measurements. This was done by assigning a numerical value to LFD (see METHODS). If tensions at high and low frequencies had been affected equally by the eccentric contractions, the value of LFD would be zero. A value of 1 meant that tensions at low frequencies had fallen to zero. In practice, values lay in the range of 0.3–0.7. Example traces of tension ratios calculated from the traces shown in Fig. 2 are illustrated in Fig. 3.

After the eccentric contractions, tension in response to frequency ramp stimulation was measured at both the old and new optimum lengths, yielding two values of LFD. It is apparent from Fig. 3 that LFD calculated by using values measured at the new optimum was less than when measured at

Fig. 1. Frequency ramp stimulation. Two successive stimulus ramps comprising linearly increasing rates of stimulation between 0 and 100 pulses per second (pps) over 1.5 s followed by a more rapid fall in rate were applied to the nerve supply of the medial gastrocnemius muscle of the anesthetized cat. Top trace, isometric tension; bottom trace, stimulus rate. Tension was always measured in response to the second of the 2 frequency ramps, given 40 s apart, because there were small differences in tensions at low stimulation rates. Analysis was restricted to the tension response to the rising ramp.

Fig. 2. Tension in response to frequency ramp stimulation before and after eccentric contractions. Superimposed isometric tension traces (top) in response to stimulation (bottom) recorded before (pre) and after 50 eccentric contractions of the muscle. Tension after the eccentric contractions was measured at both the original optimum length (old) and at the new, longer optimum (new).
the old optimum. Values of LFD measured at the new optimum length plotted against values measured at the old optimum length are shown for the 15 experiments in Fig. 4. It can be seen that all values lie below the line of proportionality. That is, LFD at the new optimum length was always less than at the old optimum length. Our original hypothesis had been that any changes in LFD after eccentric contractions could be entirely attributed to the change in whole muscle optimum length. That is, LFD would be reduced to zero once muscle length had been adjusted to the new optimum. Statistical analysis showed that values of LFD measured at the new length were significantly smaller than when measured at the old length (paired t-test; \( P < 0.0001 \)), but they had not fallen to zero, lying in the range 0.1–0.6.

It can be seen from Fig. 4 that LFD was often >0.5; that is, tension during low-frequency stimulation was reduced by more than a half after the eccentric contractions, even after allowance for the depression during high-frequency stimulation. Notice in Fig. 4 that there was a tendency for lower values of LFD to lie further from the line of equality. When LFD values were low, 0.3–0.5, adjusting optimum had a larger effect, leading values to lie further from the line of equality. This trend suggested that the lower values of LFD were more length sensitive.

It appeared that when LFD was large, factors other than a shift in the length-tension relation, resulting from the eccentric contractions, were playing a role. It was therefore decided to measure LFD after varying numbers of eccentric contractions to see whether these factors were less evident after fewer contractions. The results from six muscles from different animals are shown in Fig. 5. For each animal, a total of 50

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**Fig. 3.** Tension ratios at new and old optimum lengths. Isometric tension in response to frequency ramp stimulation after a series of eccentric contractions expressed as a fraction of the tension measured before the eccentric contractions, using measurements made at both the original optimum length (bottom trace) and at the new, posteccentric optimum (top trace). Hill plots were fitted to the smoothed data to determine high- (\( A_H \)) and low-frequency (\( A_L \)) asymptotes and hence to calculate low-frequency depression (LFD).

**Fig. 4.** LFD after eccentric contractions measured at new and old optimum lengths. LFD was calculated as described in METHODS. Two values of LFD were calculated, one from tension ratios determined at the original optimum length and the other from tension ratios using the new optimum. The 2 values were plotted against one another. Dotted line indicates identical values for LFD at the 2 lengths. Each point represents the data from 1 muscle.

**Fig. 5.** LFD measured after different numbers of eccentric contractions. LFD calculated at the new, posteccentric optimum length was plotted against its value determined at the original, preeccentric optimum length. Data from 6 different experiments are shown, each represented by a different symbol. For each experiment, measurements were made after varying numbers of eccentric contractions, typically 2, 5, 10, 20, and 50. For the sake of clarity, all of the values belonging to 1 experiment have been joined by straight lines. In each case, the lowest value for LFD was measured after the smallest number of eccentric contractions. The dashed line indicates identical values for LFD at the 2 lengths.
eccentric contractions were given consecutively. At various points in the series, typically after 2, 5, 10, and 20 contractions, optimum length for tension was redetermined and LFD measured, at both the old and new optimum lengths.

Again, all values of LFD lay below the line of proportionality, and for all muscles increasing the number of eccentric contractions increased LFD (Fig. 5). However, the difference in values between the old to the new optimum lengths was relatively independent of the number of eccentric contractions. So, as damage increased, the change in LFD became proportionately smaller. Otherwise, the larger values should have deviated further from the line of equality. Second, the amount of LFD seen with only a small number of eccentric contractions differed from one muscle to another, suggesting that factors such as fatigue properties and susceptibility to damage from eccentric contractions varied considerably between muscles.

In a final series of five muscles, it was decided to try to apportion changes in LFD to the various possible contributing factors: fatigue, the shift in muscle length from the eccentric contractions, and any additional effects. Length dependence was studied by simply measuring LFD over a range of muscle lengths without giving any eccentric contractions. The effect of fatigue was measured by examining changes in LFD resulting from a series of concentric contractions. It is known that concentric contractions, while producing fatigue, do not induce any muscle damage (20, 21). The concentric contractions used shortening ramps of 6 mm at 50 mm/s, applied across the same length range as had been used for the eccentric contractions. Here the combined protocol used was measurement of LFD before and after different numbers of concentric contractions, then over a range of different muscle lengths, and finally after a series of eccentric contractions. To calculate LFD, the reference trace was taken as the tension recorded at the optimum length during the length dependence series. Because this was measured after the concentric contractions, the muscle was already fatigued and the values of LFD calculated for the concentric contractions were therefore negative.

A typical experiment is shown in Fig. 6. Muscle optimum length was determined, and then a series of concentric contractions was carried out. LFD was measured after 2, 5, 10, and 50 concentric contractions. The concentric contractions did not lead to any significant change in the muscle optimum length, which for this muscle was \( L_m = 9 \) mm. There was, however, an increase in LFD with the number of concentric contractions. At this point, LFD was measured over a range of muscle lengths, beginning at \( L_m - 16 \) mm and increasing in 2-mm steps up to \( L_m - 2 \) mm. Then a series of eccentric contractions was carried out at the optimum length and LFD calculated after 2, 5, 10, 20, and 50 contractions.

The line drawn through the relation between LFD and muscle length before the eccentric contractions had a slope of \(-0.090 \text{ mm}^{-1}\) (Fig. 6A). The mean slope from five experiments was \(-0.084 \pm 0.008 \text{ mm}^{-1}\). This slope is a quantitative measure of the length dependence of LFD. It has a negative sign because, as muscle length is increased, LFD becomes less, except possibly at very long lengths.

In Fig. 6B, LFD has also been calculated for different numbers of concentric and eccentric contractions. As the number of contractions was increased, LFD predictably increased. The value of LFD measured at the original optimum length was plotted against the new optimum length, taking into account that this had been shifted by the eccentric contractions. It was argued that if the changes in LFD after the eccentric contractions were purely the result of the shift in optimum length, this relationship should have the same slope, but of opposite sign to the length dependence of LFD before the eccentric contractions. If other factors were contributing, the slope would be expected to be steeper. For this experiment the slope was 0.087 \text{ mm}^{-1}. For five experiments the slope had a mean value of 0.086 \pm 0.008 \text{ mm}^{-1}.

It therefore appeared that length dependence of LFD was large enough to be able to fully account for the changes seen after the eccentric contractions. Yet the relationship had been expected to be steeper because it now included the effects of fatigue. For the example in Fig. 6, LFD measured at the old
optimum length changed by 0.35 from before to after the 50 eccentric contractions. However, 50 concentric contractions changed it by 0.14, so the change in LFD due to the specific effects of the eccentric contractions was 0.21, assuming that the fatigue effect of the eccentric contractions was the same as for the concentric contractions. If it was less, the change due to the specific effects of the eccentric exercise would be between 0.21 and 0.35. In three experiments, estimates were made of changes in LFD after eccentric contractions, but taking into account the previously measured fatigue effects for each number of contractions. The net result was a relationship with a slope of 0.043 mm$^{-1}$ for the experiment shown in Fig. 6 and a mean value from three experiments of 0.036 mm$^{-1}$, ranging from 0.017 to 0.088.

The above results led to the conclusion that, after fatigue had been taken into account, the length dependence of LFD after the eccentric contractions was only half as steep as before. This conclusion provided an explanation for the results shown in Figs. 4 and 5. If, after the eccentric contractions, the length dependence of LFD had changed, moving the muscle to the new optimum length would not fully reverse the increase in LFD.

The same conclusion can be arrived at from inspection of Figs. 4 and 5. The data in Fig. 6 come from the same muscle as shown by the diamonds in Fig. 5, where after 50 eccentric contractions the value of LFD measured at the old and new optimum lengths was 0.34 and 0.18, respectively. Because here the shift in optimum length was 3.7 mm, the slope of the length dependence can be calculated as the difference in LFD divided by the length changes, in this case given a slope of $-0.043$ mm$^{-1}$. The mean magnitude of the slope for four experiments was $0.048 \pm 0.005$ mm$^{-1}$, that is, about half the value of the slope of the length dependence measured before the eccentric contractions. We concluded that one additional consequence of eccentric contractions was to reduce the length dependence of LFD.

**DISCUSSION**

It is now well established that eccentric exercise leads to muscle damage and the presence of damage is indicated by changes in the muscle’s mechanical properties. (For reviews, see Refs. 1 and 22.) The changes include a shift in optimum length for peak active tension in the direction of longer muscle lengths (12, 34), a rise in passive tension (32, 33), and a fall in tetanic force. The drop in force is due to three causes. One, if force is measured at the original optimum length, it is less because of the shift in optimum. Second, during repeated eccentric contractions there will be some metabolic fatigue. Force recovery from fatigue will be complete within 1–2 h as is seen after concentric exercise, which is not accompanied by any muscle damage (20, 21). The persisting force drop after eccentric exercise that takes a week or more for full recovery is due to damage to muscle fibers (1, 19, 22). The additional damage indicator studied in these experiments is the change in LFD.

The main finding of this study was that LFD resulting from a series of eccentric contractions, and measured from tension traces during “frequency-ramp” stimulation, could be partially reversed by adjusting the muscle to its new optimum length (Fig. 2). So one contributing factor to LFD after eccentric exercise is the effect of muscle length. There are numerous reports of LFD after eccentric exercise (2, 13, 19, 25), but in these studies the length of the muscle was not adjusted to take into account the change in optimum length after the eccentric contractions. So it remained unclear whether the increase in LFD was simply an expression of the change in optimum length or whether other factors were operating as well.

Our working hypothesis at the start of these experiments had been that any change in LFD after the eccentric contractions was simply a result of the change in whole muscle optimum length. That is, LFD increased entirely as a result of a reduced sarcomere length after the increase in series compliance (12, 34), given the well-known length dependence of the stimulation frequency-tension curve (24). So adjusting muscle length to the new optimum was predicted to fully recover the value of LFD measured at the original optimum. Figures 4 and 5 show that this was not the case; there was only a partial reversal, implying that other factors were contributing.

Our data confirm that LFD is also induced by fatigue (Fig. 6). After 50 concentric contractions, which did not produce any evidence of damage typical of that seen after eccentric contractions, as shown by the absence of any shift in optimum length, LFD had increased by $\sim 0.2$. When this was taken into account, the relationship between change in muscle optimum length and LFD after the eccentric contractions had only half the slope of the relationship measured without taking fatigue into account. These considerations led to the conclusion that the combined effects of length and fatigue were more than enough to account for the changes seen after eccentric contractions.

How could this be reconciled with the findings illustrated in Figs. 4 and 5? The only way would be to postulate that the length dependence of LFD measured before the eccentric contractions was greater than that after the eccentric contractions. That is, one previously unrecognized consequence of the eccentric contractions was a reduction of the length dependence of LFD. Such a conclusion would explain why LFD measured at the new optimum was not fully reversed back to its pre eccentric value.

Why might the length dependence of LFD become less after a series of eccentric contractions? It is possible that in a muscle with fibers containing disrupted sarcomeres from a series of eccentric contractions, low-frequency stimulation leads to more internal motion (14) than in an undamaged muscle and this results in a disproportionately low tension. This would not be reversed by stretching the muscle to its new optimum length. According to this hypothesis, the length dependence of LFD in an undamaged muscle, especially at longer muscle lengths is, at least in part, the result of the increase in tendon stiffness at the higher tensions (23). Adding the extra compliance of disrupted sarcomeres after the eccentric contractions would therefore increase LFD, especially at long lengths.

Both of these postulated explanations assume that the length dependence of myofibrillar Ca$^{2+}$ sensitivity observed in skinned fibers (10, 27) is a fundamental property that remains unaltered by eccentric contractions. One possible factor attributable to eccentric contractions that may modify this relationship is an increase in dispersion of sarcomere lengths in muscle fibers (29). If, as a result of the presence of overextended and disrupted sarcomeres after the eccentric contractions, the av-
verage length of still functioning sarcomeres falls, this will reduce the myofibrillar Ca$^{2+}$ affinity.

Direct measurements of Ca$^{2+}$ in the myoplasm of mouse muscle fibers after a series of eccentric contractions have shown a fall in tetanic Ca$^{2+}$ concentration and a rise in resting Ca$^{2+}$ concentration (2). However, similar experiments in frog fibers, using a more rapidly responding dye, did not show any reduction in the size of myoplasmic Ca pulses, in response to stimulation, but still a clear shift in optimum length (18). There are two fatigue-related components to the reduced Ca$^{2+}$ release during stimulation, a metabolic component and a component dependent on elevation of the sarcoplasmic Ca$^{2+}$-time integral (7). It is the latter that is thought to be primarily responsible for low-frequency fatigue. If eccentric contractions lead to reduced length dependence of Ca$^{2+}$ release, this would reduce any effect on LF from the change in optimum length. It is known that changes in Ca$^{2+}$ release are rather complex (18).

In a recent study, it has been reported that the stimulation frequency-tension curve measured after eccentric contractions remained unchanged when muscle length was adjusted to its new optimum (36). We do not have any simple explanation to account for this result. These authors also reported that isometric contractions had no effect on the curve, suggesting that the preparation used, single fibers from mouse limb muscles, was substantially fatigue resistant. The eccentric protocol used was a little more severe, 40% stretches compared with an 18% reduction in the size of myoplasmic Ca pulses, in response to stimulation, but still a clear shift in optimum length (18). There are two fatigue-related components to the reduced Ca$^{2+}$ release during stimulation, a metabolic component and a component dependent on elevation of the sarcoplasmic Ca$^{2+}$-time integral (7). It is the latter that is thought to be primarily responsible for low-frequency fatigue. If eccentric contractions lead to reduced length dependence of Ca$^{2+}$ release, this would reduce any effect on LF from the change in optimum length. It is known that changes in Ca$^{2+}$ release are rather complex (18).

The use of the frequency ramp for stimulation has both advantages and disadvantages. The obvious disadvantage is that the measurements are not made in a true steady state. Thus the high-frequency asymptote is extrapolated from the shape of the curve, not measured directly (Fig. 3). Of course this applies once again, to reduce its impact on motor control.

Second, we have found that the length dependence of LF decreases less after a series of eccentric contractions. This is a new observation. Its understanding is likely to provide further insight into the damage process. The direction of the effect is, once again, to reduce its impact on motor control.

GRANTS

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