

Early events in stretch-induced muscle damage

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Morgan, D. L., and D. G. Allen. Early events in stretch-induced muscle damage. *J. Appl. Physiol.* 87(6): 2007–2015, 1999.—Unaccustomed exercise involving stretch of active muscle at long length causes an immediate loss of tension-generating capacity, a shift of optimum length, and changes in excitation-contraction coupling. Eventually, fiber damage may be observed, resulting in pain and tenderness. The subject of this review is the early stage in this process, particularly the cause of the immediate drop in tension. There is strong evidence pointing to sarcomere length instabilities and nonuniformities as important contributors to these changes. The evidence includes the influence of initial length, electron microscopy of rapidly fixed active fibers, the shift in optimum length in single fibers, and the effects of training on sarcomere numbers. Experiments using Ca²⁺-sensitive dyes clearly show changes in excitation-contraction coupling, but cross-species comparisons indicate that these are not always able to explain the consequences seen. We conclude that sarcomere length instabilities provide the most comprehensive explanation of the early consequences of eccentric exercise.

muscle damage; sarcomere length instability; eccentric exercise; excitation-contraction coupling

PHYSICALLY ACTIVE HUMANS are aware that after a bout of intensive and unaccustomed exercise, the muscles involved feel weaker, particularly after exercise that involves stretching contracting muscles, known as eccentric exercise (58). Over the next few days, the muscles become sore and tender, and these symptoms recover over the next week or so. It is also widely recognized that if the same exercise is repeated subsequently, the symptoms are reduced, i.e., there is a training effect.

This review concerns the cellular mechanisms underlying these symptoms, particularly focusing on the mechanism(s) of the early weakness and associated changes. The later muscle pain and tenderness have been reviewed by others (e.g., Ref. 62) and will not be considered here. Nor do we consider the more serious stretch-induced injuries such as muscle tears and disruption of the muscle-tendon junction (see Ref. 22 for review).

We use the term “muscle damage” to include all the changes that follow eccentric exercise, rather than the more limited definition of damage apparent by histology. Immediate changes are reductions in tension, more at low stimulation frequencies than at high, and increases in the optimum length for tension generation. Later changes include increases in plasma levels of muscle proteins, swelling, stiffness, inflammation, and soreness.

Possible Causes of Reduced Tension After Eccentric Activity

To understand the mechanisms by which muscles become weaker after activity, it is necessary to consider the normal pathway for muscle activation. Muscles are activated by the motor center in the brain sending a volley of action potentials through the spinal cord to the motoneurons, exciting the muscle cell membrane via the neuromuscular junction. Within the muscle cell, the action potential conducts along the surface membrane and down the t tubules. The voltage sensors detect depolarization and trigger Ca²⁺ release from the store in the sarcoplasmic reticulum (SR). Ca²⁺ then binds to troponin and initiates the cross-bridge cycle that leads to tension development and/or shortening. The number of tension-producing cross bridges in each sarcomere depends on the sarcomere length. If the external tension on the muscle exceeds the tension that would be developed at constant muscle length, then the muscle is stretched while activated [known as an eccentric contraction (58)]. Relaxation occurs by pumping Ca²⁺ back into the SR. In such a chain of events, failure at any stage leads to reduced tension, and our task is to identify the role of various components and, particularly, to ascertain why they are sensitive to eccentric exercise.

These ideas allow us to divide the possible causes of muscle weakness into a number of categories.

1) Changes in the central nervous system, motor nerve, or neuromuscular junction.

2) Inexcitable muscle cells, presumably due to gross cellular damage.

3) Failure or reduction of Ca^{2+} release.

4) Changes in the Ca^{2+} sensitivity of the contractile machinery.

5) Disorganization of the contractile machinery.

Many studies have produced eccentric damage in the intact animal and then measured the tension deficit after removal of the muscle by using direct muscle stimulation (19, 28), establishing that much of the damage is to the muscle itself. Thus, whereas the pain and weakness lead to changes in the way the brain drives muscle activity, central changes are not the only or main cause of eccentric muscle damage.

In whole muscles, an inherent difficulty is to determine the relative importance of fibers becoming inexcitable and fibers remaining excitable but producing less tension. It is clear from many histological studies (1, 46) that there are some terminally damaged fibers. However, the fact that reductions of tension have been observed in isolated single fibers that are excitable and are not obviously damaged (2, 51) clearly shows that eccentric damage can partially reduce fiber tension production.

It is also necessary to distinguish reductions in tension associated with activity in general (fatigue) from those specifically associated with eccentric contractions. For this purpose, most studies in the literature use isometric contractions with the same stimulation pattern as controls. It is obviously desirable to use a protocol in which the component due to fatigue is relatively small. It is established that muscles stretched during contraction generally consume less energy than during an equivalent isometric contraction (12, 13, 68). Thus an equivalent series of isometric tetani used as a control would be expected to show an equivalent or even greater decline of tension caused by the metabolic consequences of activity. Because eccentric damage can be observed after a single contraction, provided the eccentric stretch is of sufficient size (6), it is clear that the contribution due to fatigue can be very small if the stimulation paradigm is chosen appropriately.

The above arguments suggest that *categories 1 and 2* are unable to account for the reduced tension under all conditions. Of the remaining categories, there is some evidence for changes in Ca^{2+} sensitivity (2). However, changes in organization of the sarcomere structure and changes in excitation-contraction coupling (*categories 5 and 3*) appear to be the main contributors to the early reduction in tension. Much of the remainder of the review explores these issues in greater detail. Both of these categories affect tension in a way that is dependent on muscle length, and we need, therefore, to consider how the reductions in tension are affected by the muscle length at which they are measured.

MUSCLE PROPERTIES AFTER ECCENTRIC STRETCH

Shift of Optimum Length After Eccentric Contraction

The length dependence of tension is of considerable practical importance as it determines the joint angle for optimum torque. It is also of theoretical importance as part of the experimental basis for the sliding-filament theory (24). Thus the discovery by Katz (34) that the length-tension relationship changed after active stretches (eccentric contractions) was of great importance, although it was largely ignored until recently. Katz showed that after eccentric contractions there was a shift in the length-tension curve to longer lengths, but only if the stretches extended beyond optimum length. Katz also noted other changes, all consistent with the "partial transformation of active contractile into passive elastic tissue." In terms of modern understanding, this is most naturally interpreted as the failure of some sarcomeres to produce active tension (see Fig. 1 and later discussion).

The shift in optimum length reported by Katz (34) has now been confirmed in several preparations. Frog single fibers (51) showed a shift in optimum mean sarcomere length ranging from 0.1 to 0.2 μm /sarcomere after eccentric contractions with stretches beginning at 2.2 μm . In most fibers, the optimum tension fell.

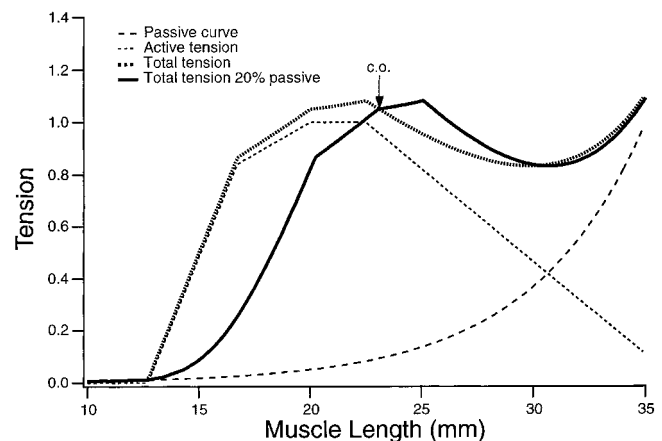


Fig. 1. Model of a muscle with some sarcomeres disrupted. Length-tension curves for a simulated muscle of 10,000 sarcomeres/fiber. "Active tension" curve is derived from that of Gordon et al. (24) for frog muscle. "Passive curve" is taken as an exponential relation for a sarcomere, scaled appropriately. Passive curves vary substantially between species and muscle, and the curve shown is near the center of observed range. "Total tension" curve is formed from these 2 by adding tension at each length; i.e., assuming a parallel connection. "Total tension, 20% passive" curve was constructed by assuming that 80% of sarcomeres showed the total curve while 20% were disrupted and showed the passive curve and requiring that the tension in all sarcomeres be the same, i.e., assuming a series connection. Note that the peak tension is unaffected but that at short lengths passive sarcomeres reduced tension, whereas at longer lengths they caused increased tension; i.e., there was a crossover (c.o.) of the total tension and the total tension, 20% passive curves. Fatigue or damage to some fibers in a multifiber preparation will cause the disrupted curve to be reduced in amplitude, abolishing the crossover. Addition of an inextensible tendon will shift all curves equally to the right, with shift at any tension being the same for all curves.

However, in one fiber, the optimum tension was almost unchanged, so that the length-tension curves before and after the eccentric contractions showed a "cross-over," as reported by Katz (34) (see Fig. 1). This is an important observation because it shows that the shift cannot always be explained by a reduced level of activation. A similar shift in the optimum of the length-tension curves has been demonstrated in whole toad sartorius muscles (64, 69). The passive tension becomes significant only at very long lengths in these muscles, so that the stretches occurred over lengths where passive force was small and total tension fell with increasing length. Furthermore, passive tension was unchanged by the eccentric contractions, eliminating the possibility of tendon slippage. Equivalent observations have also been made in rat vastus intermedius muscles (41) and human forearm flexors (60).

However, much of the literature continues to ignore this possibility, measuring tension before and after exercise at the same muscle length or joint angle. Under these circumstances, the reported tension reduction will include an unknown component due to a shift in the length-tension curve.

Recovery of optimum length. Human triceps surae has been eccentrically exercised by walking backward downhill on a treadmill (31) and demonstrated an immediate shift in optimum angle. The time course of recovery varied between a few hours and several days, but was clearly more rapid than the recovery of tension, which took about a week. Toad muscles also showed a shift in the length-tension relationship, which reversed between 3 and 5 h, whereas the decrement of tension did not reverse during this time (64). In contrast, the shift reported by Saxton and Donnelly (60) persisted almost as long as the tension decrease.

Twitch-Tetanus Ratios and the Frequency-Tension Relationship

Included among the observations of Katz (34) was a reduced twitch-to-tetanus ratio, which he interpreted as being due to increased series compliance. In terms of the then-current Hill model (26), a contractile component having a fixed duration of active state will develop less tension when faced with a greater compliance, in accord with observation. With cross-bridge theories, the reduced tension with more series compliance arises from a combination of the cross-bridge distribution being shifted to lower average extension (and, hence, tension per cross bridge) and of reduced numbers of cross bridges at the peak of the twitch, as more have shortened to the point of detachment before peak twitch is reached. For an unfused tetanus, similar effects occur, with increased compliance causing more internal movement, in turn abbreviating the twitches and reducing the degree of fusion and, hence, tension.

In recent human experiments, attention has been focused on tension as a function of stimulus rate. In particular, it has been found that the mean tension at low stimulation rates falls more than the tension at higher rates (14, 32, 45, 53). Similar observations have been made in animal models (45), isolated muscles (30),

and in single fibers (2). The changes in tension as a function of stimulation frequency have generally been interpreted as changes in activation rather than in series compliance (see *Excitation-Contraction Coupling*). However, it is quite possible that series compliance is a significant contributor. An important consequence is that the animal is likely to perceive the muscle as weak, since a higher than normal degree of effort (i.e., firing rate in the motor nerve) will be required to achieve a given tension.

Excitation-Contraction Coupling

The first experimental evidence that the tension deficit after eccentric exercise might have its origins in changes in excitation-contraction coupling came from studies of caffeine contractures in isolated soleus muscles (67). The key finding was that, whereas eccentric exercise reduced isometric tetanic tension at the midrange length by 43%, compared with 4% after control isometric contractions, the caffeine contracture tension was the same after both types of exercise. This result could arise either because Ca^{2+} release is reduced after eccentric exercise or because a proportion of fibers in the muscle was inexcitable to electrical stimulation but still capable of responding to caffeine. The latter possibility was investigated by measuring resting membrane potential of 25 surface fibers from each of 8 muscles, which were found on average to be normal, suggesting that electrical properties were unaffected. Thus Warren et al. (67) concluded that much of the early deficit of tension was attributable to reduced Ca^{2+} release. Another possibility is that the stimulus rate used was not sufficient to achieve a maximal tetanus without potentiators at the shorter length of the active sarcomeres (59).

A contribution from impaired excitation-contraction coupling to reduced tension after eccentric contraction has been supported by further studies on mammalian muscles (2, 28). Balnave and Allen (3) measured tension and intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in single mouse muscle fibers subjected to eccentric stretches. When the eccentric activity was moderate (10 tetani with stretches of 10%), tetanic $[\text{Ca}^{2+}]_i$ was reduced by 6%, and this could explain the reduced tension, which was particularly evident at low stimulus frequencies. Tetani at 100 Hz showed only a moderate decrement of tension (25%), which could be completely overcome by caffeine potentiation. Thus, under these circumstances, reduced Ca^{2+} release could explain the reduced performance. These conclusions have been confirmed in a subsequent study (28). In contrast, a more severe eccentric regime produced larger effects on tension, some of which were attributable to reduced Ca^{2+} release, but there was also a reduction in maximum tension and in Ca^{2+} sensitivity, which presumably reflect disruption of the sarcomere structure. These studies also demonstrated a small increase in resting Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and a reduced pumping rate of the SR Ca^{2+} pump, each of which might contribute to Ca^{2+} -induced changes at a later stage.

The effects of eccentric stretches on Ca^{2+} regulation have also been reported in frog single fibers (51) by using a dye that recorded individual Ca^{2+} transients, even during a high-rate tetanus. The stretch protocol in this study was relatively mild and produced an average of 11% decrement in the maximum tension and 0.1–0.25 μm /sarcomere shifts in the peak of the length-tension relationship. Under these circumstances, the peak amplitude of twitch and tetanic $[\text{Ca}^{2+}]$ transients showed little consistent change, although increases in the duration of the transient and of resting $[\text{Ca}^{2+}]_i$ were detected. The authors concluded that reduced peak $[\text{Ca}^{2+}]_i$ was not the cause of the shift in peak of the length-tension relation.

It is clear then that eccentric damage involves disturbance to Ca^{2+} dynamics, but it appears that the net result on average $[\text{Ca}^{2+}]$ is not consistent. There were many differences between these preparations, including dye, temperature, species, and twitch-to-tetanus ratio. These observations suggest that eccentric exercise may have more than one effect on excitation-contraction coupling, possibly affecting both release and uptake of Ca^{2+} . These multiple effects may differ in different species and with different regimes of stretching, leading to inconsistent overall effects.

Structural Changes

Most studies of muscle structure are made hours or days after the eccentric contractions are complete (1, 15, 19–21, 39, 45, 46, 55). They show regions of damaged sarcomeres, ranging in size from a single sarcomere of a single myofibril to many sarcomeres across a whole fiber. Here, we are particularly concerned with studies made in muscles fixed during or immediately after the first contraction during which the muscle was stretched. Brown and Hill (8) showed that, in fibers stretched during contraction to sarcomere lengths longer than the optimum (i.e., on the descending limb of the tension-length curve), there were occasional sarcomeres in which the thin filaments were either partially or completely pulled out of the thick-filament array in either one or both halves of the sarcomere. These disorganized sarcomeres coexisted with apparently normal sarcomeres. Brown and Hill pointed out that the normal sarcomeres were necessarily shorter than expected for the overall length of the muscle, and since they were on the descending limb of the length-tension curve, they would therefore produce more tension than expected for the overall length of the muscle (see Fig. 1). It is known that after active stretch during a tetanus, the tension during the remainder of the tetanus is unexpectedly increased (16, 17). Thus the observation of Brown and Hill (8) provided at least a partial explanation for this phenomenon (see also Ref. 50).

The rapid-fixation techniques developed by Brown and Hill (8) were used by Talbot and Morgan (63) on the surface fibers of whole toad sartorius muscle. Fibers from a muscle that was fixed while still active after a single stretch of 10% of rest length, confined to the descending limb of the length-tension curve, showed

$8.4 \pm 0.7\%$ of sarcomeres with myofibrils not overlapping in one-half of the sarcomere. The overstretched sarcomeres were estimated to account for 64% of the stretch applied to the fibers. Another muscle, fixed during an isometric contraction at the final length of the first muscle, showed no overlengthened sarcomeres. A third muscle was fixed under conditions that were identical in all respects to the first, except length, which was on the ascending limb. Loss of overlap was seen in 3.1% of sarcomeres. A fourth muscle was fixed immediately after relaxation from a single eccentric contraction identical to that undertaken by the first. A few (<1%) noninterdigitating half-sarcomeres were seen, clearly showing that the majority of overstretched sarcomeres reinterdigitate during relaxation after a single contraction. Thus sarcomeres are preferentially disrupted during a stretch on the descending limb of the length-tension curve but mainly recover on relaxation, and multiple contractions are needed to produce the structural changes that have been observed in relaxed fibers.

Degradation of Cytoskeletal Proteins

In addition to the contractile proteins, muscles contain a range of cytoskeletal proteins that stabilize the contractile proteins and allow for the transmission of tension both longitudinally and laterally (see Ref. 56 for review). The role of cytoskeletal proteins in stabilizing the sarcomere structure suggests that they may have a potential role in the prevention or development of eccentric damage. Here, we briefly discuss three cytoskeletal proteins with suggested roles in eccentric contraction damage.

Titin is a very large-molecular-weight protein that connects the Z line to the myosin filaments. It is responsible for much of the resting tension in highly stretched fibers (44) and has an important role in locating the thick filaments in the center of the sarcomere. During prolonged contractions in skinned fibers at sarcomere lengths at which titin produces little tension, thick filaments gradually develop substantial misalignment, which is restored in the resting fiber. Such structural change is exaggerated when titin is selectively destroyed by irradiation (27). When muscles are stretched to a sarcomere length of approximately $>4 \mu\text{m}$, the attachments of titin to the myosin molecule appear to slip, recruiting more free titin (65). Thus titin has an important role in reinterdigitation of the sarcomere after stretch, and the gradual failure of reinterdigitation, which occurs after repeated eccentric stretches, could be related to stretch-induced titin damage. To our knowledge, there are no published data on this possibility.

Desmin is a structural protein mainly located in the Z disks and connecting adjacent Z disks and Z disks at the edge of the fiber to the costamere in the surface membrane. Thus it contributes to the alignment of Z disks across a fiber and will also transmit lateral tension. For instance, if a small number of sarcomeres in a myofibril were damaged, longitudinal transmission of tension could still occur by lateral transmission to

neighboring myofibrils that were still intact. Transgenic animals lacking desmin have been produced and show loss of myofibrillar regularity (47). The possible role of desmin in muscles damaged by eccentric contraction has been studied by using antibodies after histological sectioning (37). This study identified “desmin-negative” fibers and showed that, in the extensor digitorum longus, 23% of fibers were desmin negative at 1 day, 35% at 3 days, but only 10% were desmin negative at 7 days. Most fibers that were desmin negative were also fibronectin positive, indicating that the surface membrane integrity was damaged. However, particularly at *day 1*, there were desmin-negative fibers that were fibronectin negative, indicating that desmin breakdown could be initiated in intact cells. These experiments raise the question whether the desmin loss is part of the cell damage, perhaps initiated by activation of proteases, or whether damage to desmin is a very early and specific feature of eccentric damage that contributes to the sarcomeric disorganization (38).

Dystrophin is one of a number of proteins forming a glycoprotein complex located in the sarcolemma but with attachment to actin filaments. Absence or abnormality of dystrophin or other proteins in this complex leads to muscular dystrophy in which damage to fibers is a prominent feature. The precise function of dystrophin is unknown, but studies of the *mdx* mouse, which lacks dystrophin, have shown that skinned fibers from dystrophic fast muscles such as extensor digitorum longus are strikingly more susceptible to eccentric damage (25, 48, 57). These experiments suggest that eccentric muscle damage may have an important role in the development of some muscle diseases.

Factors Determining the Magnitude of Eccentric Damage

An important step in determining the mechanism of this damage is to find which parameters of the stretch are important in determining its extent. There is general agreement that longer stretches cause more damage (7, 36). In contrast, shortening over the same range of lengths does not cause any damage (45). Stretches in relaxed fibers generally cause no detectable damage (32, 54). A cumulative effect of extra eccentric contractions is also common, although the effect is usually nonlinear, with further increases in the number of eccentric contractions causing smaller increases in damage (46, 64). Similarly, there is general agreement that velocity is not a major determinant (46, 66). In situations where tension has been varied other than by changing length, there is general agreement that greater tension produces greater damage (46).

When length is changed, tension also changes, depending on where on the length-tension curve the muscle is operating. Many muscles work, and many experiments are conducted, mainly on the ascending limb of the curve of active tension against length. Furthermore, if total tension is considered, many whole muscles do not show a descending limb, so tension and length are highly correlated. Perhaps for this reason,

Warren et al. (66) found that the correlation of damage (tension reduction) with the total of active and passive tension was stronger than that with length. However, they only varied length over 5% of optimum length and, furthermore, did not measure optimum length for each muscle but took it as the center of the anatomic range. In contrast, Newham et al. (54) found greater damage in human elbow flexors at longer length, even though tension was reduced. However, they did vary the elbow angle, and changes in moment arm may have affected the amplitude of the stretch imposed on the muscle.

Using toad sartorius, Talbot and Morgan (64) showed a very strong dependence of the shift in optimum length on the range of sarcomere lengths involved in the stretch and clearly differentiated this from a tension dependence. This is possible in these muscles because of the clear descending limb of both active and total tension. Tension reduction was also measured and depended similarly on muscle length, although with greater variability. This has been confirmed in whole rat muscle (41) and is supported by the observation that eccentric damage is greatest in the regions of a skinned fiber segment that initially contained the longest sarcomeres (42).

So it now seems clear that sarcomere length is a major determinant, with damage increasing greatly as the stretches move onto the descending limb of the length-tension curve. As a consequence, it is important to establish the sarcomere length of the fibers in a muscle in the most reliable way, such as direct measurement of sarcomere length, or establish the peak of the tension-length curve with maximally activated tetani. Use of the optimum length for twitches is unreliable, since it can occur at a different sarcomere length to the tetanic optimum length (11).

What then of tension as such? A decrease in tension by moving down the descending limb of the length-tension curve does not decrease damage but increases it. Conversely, an increase in the strength of voluntary contraction can be expected to recruit more fibers, resulting in damage to more fibers and so more pain and more loss of maximal tension capability. So the effect of tension depends on how the tension is varied.

There have been several suggestions that damage depends on fiber type. When running rats on treadmills, Armstrong et al. (1) found that slow muscles were principally damaged. When stretching tetanically stimulated mixed muscles, Lieber and Fridén (35) found that fast fibers were preferentially damaged. In skinned fiber segments (43), the relationships between tension deficit and strain for extensor digitorum longus and soleus muscles had different intercepts and slopes. Clearly, the damage will depend on which muscles are activated and, as we have seen, on the sarcomere length range over which each fiber is stretched, which may vary with fiber type in a mixed muscle. Studies in which fast and slow fibers are stretched over closely equivalent parts of their length-tension curves are needed to resolve this. If the dependence of fiber type remains under these conditions, then explanations

based on fiber diameter (42) as well as protein differences should be considered.

Training Effects

Although it is not part of the immediate events, the training effect of eccentric exercise is very striking and needs to be accounted for by any comprehensive theory. The observation is that repetition of a bout of exercise after the cessation of soreness produces less severe signs and symptoms of damage than the initial bout (5, 10, 61). The speed of this training effect is more rapid than strength training or fiber-type conversion, which require weeks or months for a substantial effect (52).

THEORETICAL IDEAS

Popping-Sarcomere Hypothesis

The concept of sudden, nonuniform sarcomere extension when muscles are stretched on the descending limb was expounded by Morgan in 1990 (49), although it grew out of earlier work, especially by Julian and Morgan (33). This hypothesis is known as the “popping-sarcomere hypothesis,” and its rationale is as follows. At long length, the tension that can be generated by a half-sarcomere decreases with increasing length, as the filaments overlap, and so the opportunity for cross-bridge formation, decreases. Series connection of such sarcomeres makes for an unstable distribution of half-sarcomere lengths, as the weakest half-sarcomere will be preferentially lengthened and so become even weaker. Under most conditions, this instability is heavily damped by the tension-velocity curve, which shows that even slowly lengthening sarcomeres generate substantially more tension than isometric or shortening sarcomeres at the same length. During rapid lengthening, however, the tension-velocity curve asymptotes or yields (34), so that lengthening more rapidly does not result in more tension. This means that the instability becomes undamped or instantaneous under these conditions, leading to the hypothesis that “rapid lengthening of muscle at long length does not involve uniform lengthening of sarcomeres, but consists primarily of instantaneous, uncontrolled extension of individual half-sarcomeres in each myofibril, one at a time, in order from the weakest towards the strongest.” “Rapid lengthening” means fast enough to reach the yield point of the tension-velocity curve (>0.1 – 0.2 maximal velocity). “Long” means past the optimum length for tension generation. Each sarcomere will be stretched until its passive tension is able to support the tension. If there is no discernable distribution of weakest sarcomeres, the popped half-sarcomeres are expected to be randomly scattered throughout the three-dimensional lattice of the fiber.

This hypothesis has been used to explain many physiological results from single lengthening contractions (50). It can be extended to damage from eccentric exercise by considering what happens on relaxation. At least in frog single fibers, overextended sarcomeres are expected to stretch beyond filament overlap. Apparently, most of them return to normal on relaxation, as a

single stretch rarely produces significant damage. The hypothesis proposes then that “most sarcomeres return to normal, but that a small fraction fail to do so and either remain overextended or return to their normal length but are unable to develop tension in a subsequent contraction.” This may be a random failure to reinterdigitate or it may be due to rupture of titin. On a subsequent contraction, these “disrupted” sarcomeres will not generate active tension, giving a specific mechanism for Katz’s conversion of active to passive tissues.

The extension of disrupted sarcomeres is further postulated to put extra load on lateral neighbor sarcomeres through transverse connections (including desmin) between myofibrils, causing them to be more likely to pop during a subsequent contraction and so more likely to become disrupted. This is postulated to lead to a growing region of disrupted sarcomeres, which can eventually lead to tearing of membranes, either sarcolemma, transverse tubules, or SR, causing loss of Ca^{2+} homeostasis in the cell. This progression from disruption of filament overlap in a single myofibril to tearing of membranes is likely to depend on the distribution of weak sarcomeres in a fiber. If they are all in a particular region, then accumulation of disruption and tearing of membranes are more likely to occur than in a more uniform fiber where the weakest sarcomeres will be more uniformly distributed. The exact mechanism by which popped sarcomeres become damaged areas needs further investigation.

Consequences of the Popping-Sarcomere Hypothesis

The hypothesis provides a mechanism for the active-to-passive conversion that Katz (34) postulated and, hence, for all the observations that he made. The decline in active stiffness (34, 51) arises because fewer sarcomeres are active. The observation that damage only occurs if the stretch proceeds past optimum length follows naturally from the idea that instability of sarcomere lengths only occurs on the descending limb. Passive fibers have no descending limb, and this is why they are not normally damaged by stretch. The progressive development with repeated contractions is accounted for by the near-random disruption of a small number of the sarcomeres popped in each contraction. The possibility of different fibers progressing differently from disrupted myofilaments to loss of Ca^{2+} homeostasis is provided by variable distribution of the weakest sarcomeres. For example, a fiber may have a variable cross section, especially during hibernation or atrophy, with all of its weakest sarcomeres in one region, making tearing of membranes more likely after a given number of contractions. The small patches of disrupted sarcomeres observed in electron microscopy, down to single sarcomeres in single myofibrils, are central to the hypothesis. The failure to visualize sarcomere-pattern disturbances in the optical microscope is accommodated by the idea of individual disrupted half-sarcomeres in individual myofibrils, scattered throughout a near-uniform fiber.

The mechanical determinants are also generally compatible. Larger stretches will pop more sarcomeres.

More stretches will produce more opportunities for popping to progress to disruption. Velocity increases beyond the yield point will leave the pattern of popping, and hence the damage, unchanged. Recruitment of extra fibers will cause popping, and hence the possibility of damage, to be present in more fibers.

The reduction in the sarcomere length of the active sarcomeres at a fixed muscle length has a number of consequences. It is known that the activation curve of muscle shifts to higher $[Ca^{2+}]_i$ at shorter sarcomere lengths (18), possibly explaining the apparent reduced Ca^{2+} sensitivity after eccentric exercise (3). Correspondingly, the optimum length shifts to longer length at partial activation, possibly explaining the lesser rate of damage at partial activation. Another aspect of the same phenomenon is the increase in stimulation rate required to maximally activate at short sarcomere lengths (59), possibly explaining some of the tension fall at the original optimum muscle length and the greater tension loss at low stimulation frequencies.

The reversal of the shift in the length-tension curve could result from spontaneous reinterdigitation of filaments. Electron microscopy of conventionally fixed toad muscles immediately and 5 h after a series of eccentric contractions (29) found a decreased number of small disrupted areas and no increase in large areas, strongly suggesting that small areas of disruption are able to spontaneously reverse. If the fibers containing the most disrupted sarcomeres progressed to damage and loss of excitability, that would also lead to a reversal of the shift.

Morgan (49) proposed that training could take the form of growing extra sarcomeres in series, so reducing the sarcomere length for a given muscle length. This would avoid operation of sarcomeres on their descending limb. Such growth has now been shown in rat vastus intermedius muscles by estimating sarcomeres in fixed fibers (40) and by measuring optimum knee angle for generation of tension (41). This will reduce tension capability at short length unless tendon length decreases as well (41).

Contribution of Excitation-Contraction Coupling Changes

Changes in excitation-contraction coupling can produce an additional contribution to the immediate decline of tension after eccentric exercise. It has long been suspected that eccentric contractions, because of localized regions of overstretched sarcomeres, would cause damage to either the t tubules or the SR. Simplistically, damage to t tubules might be expected to produce initially localized regions of elevated resting $[Ca^{2+}]_i$ due to inward leak of extracellular Ca^{2+} . Later, these leaks might seal over, and the increased resting Ca^{2+} should be very limited, as one would expect the SR and the mitochondria to take up most of the additional Ca^{2+} . Large increases in mitochondrial Ca^{2+} have been reported after eccentric damage (15). Alternatively, if the SR were damaged, one might expect to observe raised resting $[Ca^{2+}]_i$ coupled with reduced Ca^{2+} release during a tetanus, and increased mitochondrial uptake

might also be a feature. The reduced Ca^{2+} release ought to be localized to the damaged regions. However, attempts to measure the distribution of elevated resting or reduced tetanic $[Ca^{2+}]_i$ have not so far detected any localized changes (4, 28), although this may simply reflect the lack of the resolution of the methods used. Another possibility is that the voltage sensor/SR release channel region may be especially sensitive to stretch (9), and if this were coupled to damage to the SR Ca^{2+} pump (2, 28) it could explain the $[Ca^{2+}]_i$ observations in mammalian muscle.

Note, however, that when tension actually increases at long lengths, as has been observed in amphibian muscles (34, 51), then uniform reductions in excitation-contraction coupling cannot be substantial.

Alternative Theories

Within the general structure of the sarcomere instability and nonuniformity hypothesis, there are some alternative possibilities. An alternative to failure of filaments to reinterdigitate could be tearing and possibly resealing of t tubules in individual fibrils, leading to failure of activation of those sarcomeres. This would provide a mechanism for both the transverse and the longitudinal spread of disruption, as inactive sarcomeres were stretched to long length, straining and tearing the t tubules of neighboring sarcomeres. Mechanically, these sarcomeres would be indistinguishable from the disrupted sarcomeres postulated by Morgan (49, 50), giving the same explanations of mechanical changes. It would be in accord with those experiments in which Ca^{2+} release was reduced but not with the frog single fibers, where no reduction was seen. The observations of lengthened sarcomeres in relaxed fibers are somewhat difficult to accommodate, as such inactivated sarcomeres might be expected to appear normal in relaxed muscle.

Lieber et al. (38) saw loss of desmin in 2.5–7.5% of fibers after only 5–15 min of eccentric contractions and suggested that this resulted from raised resting $[Ca^{2+}]_i$ but preceded damage to contractile proteins. However, the peak torque had fallen to <50% by this time, and the muscle had undergone 150 contractions, making it unlikely that desmin loss preceded tension loss. The fact that many desmin-negative fibers showed normal contractile filaments may reflect different speeds of propagation of desmin loss and contractile filament disruption along the fibers.

The idea that damage may occur in the tendon or myotendinous junctions has been suggested (23, 60) as an alternative to disrupted sarcomeres to explain Katz's increased series compliance (34). The appearance of shift up to 10% of fiber length in single intact frog fibers (51) makes this unlikely, as the effective tendon length is only a few percentage points of the fiber length, and the myotendinous junction is clearly visible. The shift in active but not passive curves (69) is also strongly counterindicative.

Mechanisms of Reduced Tension

We have identified four mechanisms of force reduction after eccentric exercise. 1) Fatigue, which can be identified and eliminated by control experiments in which muscles undergo the same stimulation pattern but without stretch. 2) Death or electrical inexcitability of some fibers in a whole muscle; avoidable by using single fibers in which it is clear whether the preparation is responding to stimuli. 3) Shift in the length-tension curve; accounted for by readjusting to the new optimum length. 4) Reduced excitation-contraction coupling; avoidable by using potentiators, such as caffeine, or by using frog fibers, where it apparently does not occur. It is unclear whether these four factors are sufficient to account for the observed reduction in tension; as yet, no reported single-fiber experiments have accounted for both *mechanisms 1* and *3*. The four factors are expected to have different time courses of recovery, so that the proportions due to each are likely to be quite variable.

Conclusions

We believe that the evidence is now strong that the earliest changes in eccentric stretches are overstretched sarcomeres randomly distributed throughout the muscle. Many of these overstretched sarcomeres will reinterdigitate spontaneously during or after relaxation and function normally again. Others will become disrupted and so become overstretched during a subsequent contraction. The resulting areas of overstretched sarcomeres can propagate, leaving larger damaged areas. The reduction in tension is a consequence of nonfunctional sarcomeres, a shift in the tension-length curve, and changes in excitation-contraction coupling, in different proportions under different circumstances.

Obvious challenges for the future are to define how and why reinterdigitation occurs and the mechanisms by which small areas of damage appear to spread. A better understanding of the changes in excitation-contraction coupling are needed as well as clearer evidence of the way in which changes in Ca^{2+} handling affect the longer term aspects, i.e., inflammation, cellular damage, and resynthesis of proteins and sarcomeres. The role of the various cytoskeletal proteins is beginning to emerge, and no doubt they influence many of the above processes.

Muscle damage of this sort may have important role in the development of various muscle diseases, such as muscular dystrophy where muscle fibers are damaged in normal use. Training of muscles by using eccentric contractions is popular with athletes and bodybuilders, as they have found this to be an effective strategy for increasing muscular strength. Increasing understanding of the underlying mechanisms will allow us to begin to understand such complex processes at a deeper level.

Both authors are grateful to the National Health and Medical Research Council of Australia for research support.

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