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Eccentric muscle damage: mechanisms of early reduction of force

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ABSTRACT

Pain and weakness are prominent symptoms which occur after a delay in muscles which have been stretched during contraction (eccentric contraction). These symptoms are particularly severe when the exercise is unaccustomed and when the stretch occurs in muscles on the descending limb of the force–length relation, i.e. at long muscle lengths. It is known that sarcomeres are potentially unstable on the descending limb and it has been proposed by Morgan that uncontrolled elongation of some sarcomeres occurs during eccentric contractions on the descending limb. In this article, the evidence that this mechanism leads to the reduced force is considered. If overextended sarcomeres persist after the eccentric exercise it will cause a shift in the peak of the force–length curve. There is also evidence that in some types of muscle, excitation–contraction coupling is impaired and contributes to the muscle weakness. Cytoskeletal proteins stabilize the sarcomeric structure and may be injured either by the overextended sarcomeres or by activation of proteases. The potential of these mechanisms to contribute to the effects of muscle training and to the symptoms of muscle disease, such as muscular dystrophy, is considered.

Keywords cytoskeletal proteins, eccentric contraction, muscle damage, reduced force, sarcomere disorganization, skeletal muscle.

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Pain and weakness are common symptoms of muscle use, damage and disease. During intense muscle activity pain and weakness are present during the activity but generally recover rapidly when the activity ceases; this is the familiar muscle fatigue which has been intensively studied but is not considered in the present article (for review see Fitts 1994, Allen *et al.* 1995). However, when the exercise includes a substantial eccentric component (stretching of contracting muscles) the pain and weakness are minor during the activity but develop thereafter. It is important to stress that such eccentric contractions are part of normal activities, for instance, walking downstairs or lowering a heavy weight. More generally in any movement of a joint controlled by an agonist/antagonist muscle pair, one will be shortening and the other will be lengthening. In exercise in which the eccentric component is substantial, repeated and unaccustomed, the weakness is apparent during and immediately after the muscle activity but the pain, tenderness, swelling and stiffness develop more slowly and are most prominent on the first and several subsequent days after the causative exercise. For this reason, this constellation of symptoms is often called 'delayed onset muscle soreness' (DOMS). The timing of these changes suggests that the muscle weakness might be a primary consequence of the muscle damage while the pain and other features may be secondary manifestations of acute inflammation triggered by some aspect of the muscle damage (Armstrong *et al.* 1991, Smith 1991). For this reason and because studies on isolated muscle can analyse force production but not pain or tenderness, the focus of this article is on the causes of early muscle weakness following eccentric muscle contractions.

EARLY STUDIES ON ECCENTRIC MUSCLE DAMAGE

In a remarkably prescient study, Hough (1902) first described delayed muscle pain and distinguished it from the pain which occurred in muscles during activity. His subjects repeatedly contracted a finger against a spring so that the contraction was concentric but an eccentric component would occur during relaxation. He noted that, after repeated daily training session for some weeks, the delayed muscle pain was absent. However,

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after a 6-month rest, the first training session after the rest was pain-free but pain started to develop some hours later. On the next day the pain was severe and the muscle was weaker. Hough suggested that the pain of muscle fatigue was caused by the accumulation of metabolites whereas the delayed muscle pain was caused by 'some sort of rupture within the muscle'.

An important study by Katz (1939) examined the force–velocity relation in isolated frog muscles. He first showed that there was a discontinuity in the force–velocity relation such that proportionately greater force was required for a given rate of stretch than for the same rate of shortening [Fig. 1b; note region (ii) has a greater slope than region (i)]. He also showed that when the force applied to the muscle was greater than 1.8 P_o (where P_o is the isometric force) then the muscle suddenly yielded and was stretched at a very high velocity [Fig. 1b; region (iii)]. Both these results are important to the understanding of the stability of the muscle on the descending limb of the force–length curve (see later). Katz also noted that after such rapid stretching had occurred the muscle was permanently weaker, especially if the stretch exceeded the optimum length of the muscle. In addition he commented that in such cases the peak of the force–length curve was shifted to longer lengths [Fig. 1c; curve (i) is the control, curve (ii) illustrates the shift in peak which can occur when 20% of sarcomeres no longer contract and act as passive compliance]. These observations led Katz to suggest that during such rapid stretches some part of the muscle became permanently stretched and then acted as an additional element of elastic tissue in the muscle.

The first clear evidence of the morphological changes in eccentric muscle damage was provided by Fridén *et al.* (1981). They examined muscle biopsies from humans who had run down many flights of stairs and had developed DOMS. There were no signs of fibre necrosis or rupture by light microscopy but electron microscopy showed characteristic changes in the Z lines including broadening, spreading of Z-line material through the sarcomere, and the normal uniform registration of Z lines across the fibre was disturbed. On one or both sides of the abnormal Z lines, the contractile proteins often showed supercontraction or disorganization. These changes could be limited to a single myofibril or could involve many adjacent myofibrils and were scattered throughout the affected muscle biopsies.

CHARACTERISTICS OF ECCENTRIC MUSCLE DAMAGE

A simple example of eccentric muscle damage is that produced by box stepping in human subjects. Box stepping involves stepping onto a box (300–500 mm

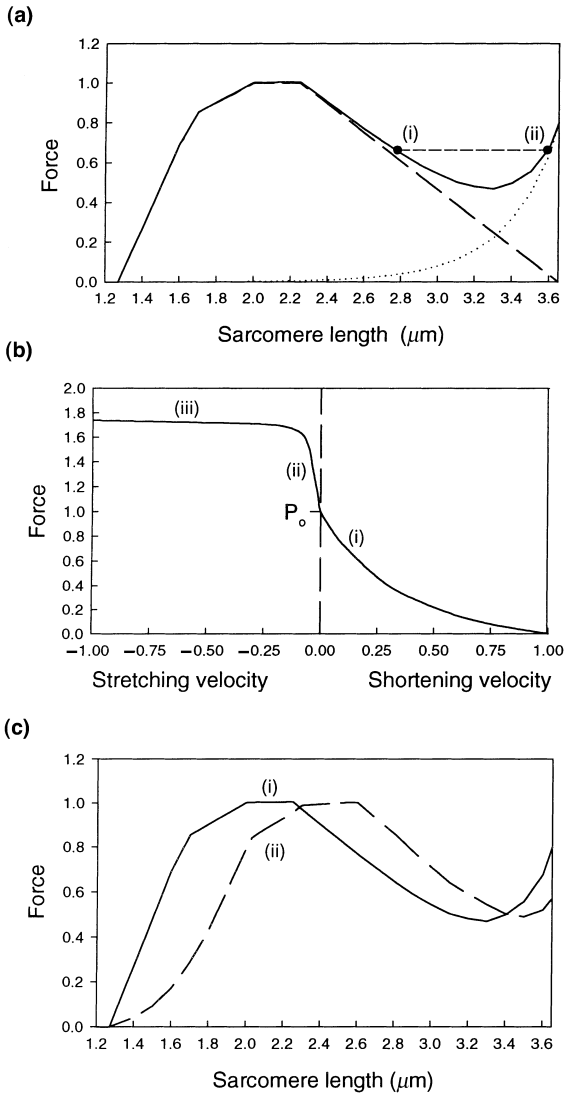

Figure 1 Representative force–length and force–velocity relations of skeletal muscle. (a) Force–length relation (dashed line) after Gordon *et al.* (1966). Dotted line shows the resting force; dashed line shows the active force; continuous line shows the total force. The relation shown is for a muscle with relatively little resting force so that the total force has a prominent region of negative slope. Point (i) represents the force of a particular sarcomere; if it were to extend the force would fall allowing further extension. This process of extension ends at point (ii) where the total force is again equal to that at point (i). Force is shown in units of P_o, the peak isometric force. (b) Force–velocity relation for both shortening and stretching (after Woledge *et al.* 1985). Velocity is shown in units of V_{max}, the maximum unloaded shortening velocity. Note the discontinuity of slope at zero velocity, so that the slope at region (ii) is greater than at region (i). When the applied force exceeds about 1.8 P_o the muscle exhibits a 'yield point' and at higher forces its lengthening velocity increases very rapidly (region (iii)). (c) Curve (i) shows total force as a function of length as in Panel a. Curve (ii) shows the effect on the curve if 20% of sarcomeres are assumed to have been damaged and to produce only passive force (after Morgan & Allen (1999) which has further details).

height) at a constant rate (15–20 min^{-1}) continued for 15–60 min (Davies & White 1981, Newham *et al.* 1983a). During the step up the quadriceps contracts and shortens (concentric contraction) while during the step down the quadriceps contracts but is stretched by the weight of the body (eccentric contraction). If the subject is instructed to always step up with one leg and down with the other then the two muscles are exercised entirely concentrically or eccentrically. At the end of this procedure maximum voluntary force was significantly reduced only in the eccentrically exercised muscle (Newham *et al.* 1983a). Stimulated muscle force at high frequencies (50 Hz) was reduced by about 30% in the eccentrically contracted muscle and recovered over 24 h, whereas in the concentrically exercised muscle the reduction in force was only about 8% and only apparent for a few hours (Davies & White 1981). The ratio of force at a low frequency of stimulation (10 Hz) to that at a high frequency (50 Hz) was also reduced more in the eccentrically exercised muscle (Newham *et al.* 1983b) and this can be an indicator of impairment of excitation–contraction coupling (Edwards *et al.* 1977, Westerblad *et al.* 1993). Pain and tenderness developed over some hours in the eccentrically exercised muscle and then persisted for several days. Muscle biopsies showed no detectable change in the concentrically contracted muscle but in the eccentrically contracted muscle there were morphological abnormalities both immediately after the exercise and these were more pronounced after 30 h (Newham *et al.* 1983a). These included disorganized myofilaments, Z-line material distributed throughout the sarcomere and loss of Z lines; these changes could either be focal or more widespread. The appearance of intracellular muscle enzymes (creatine kinase) in the plasma is an indicator of muscle cell damage and showed a variable and slow increase (peak at 4–5 days) (Newham *et al.* 1983c).

An important issue in all studies of eccentric muscle damage is to distinguish between the reduction in force caused by fatigue and that caused by the eccentric contractions. For this reason, it is important to compare the eccentric exercise against an isometric (or concentric) control and to design the study so that the reduction in force after the isometric series is minimal. It is generally accepted that an isometric (or concentric) contraction uses substantially more energy than the equivalent eccentric contraction (Bigland-Ritchie & Woods 1976, Woledge *et al.* 1985). This means that provided fatigue is minimized in the isometric or concentric control it is likely to be small in the eccentric case.

Factors which determine the magnitude of eccentric muscle damage

Early studies of these sorts in humans have now been repeated in intact animals, isolated muscles and single muscle fibres. The fact that similar reductions in force can be observed in all of these preparations is strong evidence that the primary site of the damage is within the muscle itself. The use of these simplified preparations has allowed the factors which induce eccentric damage to be analysed and the key features of eccentric damage have also been investigated in greater detail. In the next section are described some of the factors which influence the development of eccentric muscle damage.

There is widespread agreement that damage increases with the number of eccentric contractions (McCully & Faulkner 1986, Warren *et al.* 1993a) and with the length of the stretch (Lieber & Fridén 1993, Brooks *et al.* 1995). In contrast shortening over the same range generally produces little damage (McCully & Faulkner 1985, Balnave *et al.* 1997), stretching of relaxed muscle produces little damage (Newham *et al.* 1988, Jones *et al.* 1989) and the velocity of the stretch is not critical (McCully & Faulkner 1986, Warren *et al.* 1993a). It is also generally accepted that there is a large training effect, so that the damage caused by a given amount of eccentric exercise declines with repetition and returns after a substantial period of inactivity (Newham *et al.* 1987, Balnave & Thompson 1993).

There has been more debate about whether damage correlates better with the force developed during the stretch or with some characteristic of the length change such as starting length or final length. Many studies have shown a correlation between the degree of damage and the maximum force during the stretch (McCully & Faulkner 1986, Warren *et al.* 1993a). Warren *et al.* (1993a) performed a multiple regression analysis of a variety of mechanical parameters and concluded that maximum force achieved during the stretch showed the best correlation with the damage. This led them to the conclusion that the damage is caused by rupture or failure of some element in the muscle associated with the high force. However, such data are difficult to interpret because in many muscles the total force (resting plus active) increases with length over the whole range of lengths. This means that both the isometric force and the eccentric force are closely correlated with muscle length. To distinguish the importance of these variables, it is best to use a muscle which has a pronounced fall in total force above L_o, so that there is a range of lengths over which force decreases but length increases [e.g. Fig. 1a]. Talbot & Morgan (1998) used toad sartorius muscle for this reason and varied starting length, stretch size and velocity. Damage was assessed by reduction in force and shift in the peak of the force–length curve. The results showed strong correlations between damage and initial length and amplitude of stretch and weak or neglible correlations with velocity,

force before stretch and peak force during stretch. This result is supported by other animal studies (Lieber & Fridén 1993) and by humans studies in which the extent of DOMS depended on the starting length (Newham *et al.* 1988).

MECHANISMS OF ECCENTRIC MUSCLE DAMAGE

Sarcomere inhomogeneity

It has been recognized for many years that sarcomeres are fundamentally unstable on the descending limb of the force–length curve. If one sarcomere is slightly shorter or stronger than its neighbour, it will shorten at the expense of its neighbour which makes it stronger and its neighbour weaker and thereby tends to increase the disparity in sarcomere lengths and in strength. Such potential instability is reduced by the presence of resting force, which reduces or eliminates the region of negative slope, and by the fact, noted above, that the force produced during stretching is rather greater than the force produced in a shortening sarcomere (Katz 1939). Nevertheless inhomogeneities of sarcomere spacing develop slowly and can be detected in isometric contractions on the descending limb of the force–length curve (Gordon *et al.* 1966). An important insight by Morgan (1990) was that when contracting muscles are stretched on the descending limb a new phenomenon can occur. He pointed out that when the velocity of stretch exceeds about 0.1–0.2 of the maximum shortening velocity (V_{max}), then sarcomeres which exceed their yield point will start to elongate very rapidly [Fig. 1b; region (iii)]. Inevitably this will happen to the weakest sarcomere first and the rapid extension of this sarcomere will then be the main source of elongation in the muscle. This proposal is known as the popping sarcomere theory. Elongation of such a sarcomere will only cease when the total force is again equal to the other sarcomeres in series at which point there may be very little or no filament overlap [Fig. 1a; illustrated by one sarcomere moving from point (i)–(ii)]. As the weakest sarcomeres will be distributed throughout the muscle, this type of localized overextension of sarcomeres would show a similar distribution. The theory so far describes what will occur during a single contraction. When the muscle relaxes these overstretched sarcomeres presumably mostly reinterdigitate and are undamaged because the function is usually little affected by a single eccentric contraction. However, after repeated eccentric contractions it is likely that in some sarcomeres the thick and thin filaments fail to reinterdigitate correctly. Such sarcomeres might either remain extended or produce less force and therefore extend rapidly in a new contraction.

Morgan showed that this proposal could account for a number of puzzling muscle properties (Morgan 1994) and can account for many of the features of eccentric muscle damage (for details see Morgan & Allen 1999). Key points are that the damage only occurs when the stretch extends beyond the peak of the force–length relation. This follows from the sarcomere instability only arising on the descending limb of the force–length relation. The absence of effect of stretch on a relaxed muscle similarly arises because the force–length relation of relaxed muscle only has a positive slope [Fig. 1a; dotted line shows the resting force]. Shortening has no such effect because it does not lead to overextended sarcomeres.

But what is the experimental evidence to support this theory? As noted above the key feature is the rapid elongation of the weakest sarcomeres during an eccentric contraction. Optical techniques on live fibres do not have the resolution to detect such changes, so detection of such abnormally stretched sarcomeres required the development of rapid fixation techniques so that a fibre could be fixed during a single tetanus and studied by electron microscopy (Brown & Hill 1991). This study showed that following stretches during contraction, occasional overstretched sarcomeres could be observed surrounded by normal sarcomeres. Talbot & Morgan (1996) developed this approach by counting the number of overextended sarcomeres in a muscle fixed during an eccentric contraction and showed that these sarcomeres could account for about half of the overall stretch. Such overstretched sarcomeres were much fewer in isometric contractions on the descending limb and in shortening contractions. An important additional observation was that after relaxation most overstretched sarcomeres disappeared indicating that the myofibrils were capable of reinterdigitating. Nevertheless a few abnormal sarcomeres remained.

The above data establish that within a single eccentric contraction, overextended sarcomeres can be observed and confirm the popping sarcomere hypothesis. However, eccentric damage is normally observed in isometric contractions following multiple eccentric contractions so the key question becomes how the individual overextended sarcomeres, which normally reinterdigitate at rest, lead to the kind of extensive damage observed after many eccentric contractions. These include substantial regions of sarcomere disorganization which spread over all or part of the fibre cross-section. Morgan proposed that during relaxation, most overextended sarcomeres return to normal but a few do not (Talbot & Morgan 1996). With repeated eccentric contractions, the number of permanently weakened or overstretched sarcomeres gradually increases. To illustrate the possible mechanisms

involved Fig. 2a illustrates four normal sarcomeres and indicates diagrammatically the disposition of the cytoskeletal proteins desmin (d) (dashed lines) and titin (t) (dotted lines). Figure 2b shows the effect of a 30% stretch which is uniform for the lower two sarcomeres but the upper right sarcomere is overextended and compensated by the upper left sarcomere which remains at its original length. Note that under these conditions the desmin that joins the Z discs between the upper and lower pairs of sarcomeres is greatly stretched and may be liable to damage (indicated by line with dot in Fig. 2b). Another possibility is that the desmin connections remain intact but cause the lower right sarcomere to become stretched and it is apparent that overstretched sarcomeres could propagate laterally by this kind of mechanism. Note that the right-hand titin molecule in upper right sarcomere (indicated by arrow in Fig. 2b) is greatly overextended and may also be liable to damage. Alternatively the overstretched titin molecules may tend to stretch the next sarcomere in series allowing damage to propagate longitudinally.

In understanding the consequences of overstretched sarcomeres, it is important to recall that in a simple series of sarcomeres the force recorded at the end is not dependent on the number of sarcomeres in series and that loss of force production from some sarcomeres does not necessarily affect the developed force. Instead the damaged sarcomeres increase the series compliance so that the peak of the force–length curve is shifted to a longer length but not necessarily reduced in amplitude (Fig. 1c; for details see Morgan & Allen 1999). This shift in the force–length curve to longer lengths following eccentric damage was first noted by Katz (1939) and has now been observed in isolated muscles (Talbot & Morgan 1998), in intact muscles (Lynn *et al.* 1998) and in humans (Saxton & Donnelly 1996).

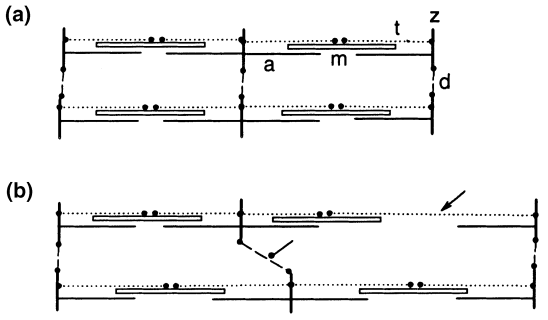

Figure 2 Schematic diagram of four neighbouring sarcomeres. (a) Normal arrangement near L_{max} (the length giving maximum force). 'a' indicates thin filaments, 'm' indicates thick filaments, 'z' indicates the Z disc, 't' indicates titin (dotted line, the large dots show the points of attachment) and 'd' indicates desmin (dashed line). (b) Possible arrangement after 30% stretch. Lower sarcomeres show uniform 30% stretch. Upper sarcomeres show one overextended sarcomere on the right and one sarcomere showing no stretch on the left. Arrow indicates overstretched titin molecule, line with dot indicates overstretched desmin filament.

Excitation–contraction coupling

An alternative or additional hypothesis for the fall of force following eccentric exercise is that it is caused by a failure of some step in excitation–contraction coupling. Warren *et al.* (1993b) provided evidence for this possibility in mouse soleus by measuring the force produced by caffeine which directly releases Ca^{2+} from the sarcoplasmic reticulum (SR) and bypasses some parts of normal excitation–contraction coupling. They showed that eccentric exercise caused a substantial fall in tetanic force but that the force produced by caffeine was unchanged by such exercise. The fact that the caffeine-induced force was unchanged by eccentric exercise suggested that mechanical factors were relatively unaffected and that it was changes in Ca^{2+} release which were the main cause of the reduced force under these circumstances. A similar conclusion was reached by Balnave & Allen (1995), who measured the $[Ca^{2+}]_i$ in single mouse fibres. Following a moderate eccentric protocol, the tetanic $[Ca^{2+}]_i$ was reduced and they showed that this could account for most of the reduction in force. Caffeine was able to restore the tetanic $[Ca^{2+}]_i$ and the force to normal, implying that structural damage was minimal. However, with larger stretches repeated a greater number of times there was evidence for both a structural component to reduced force (i.e. which could not be overcome by caffeine) and a reduction in Ca^{2+} release. A small increase in resting $[Ca^{2+}]_i$ was present following the eccentric damage and this may be important as a trigger for some of the subsequent stages of eccentric damage, for instance, the proteolysis and subsequent inflammation. The reduced tetanic $[Ca^{2+}]_i$ and increased resting $[Ca^{2+}]_i$ following eccentric exercise have been confirmed in further studies in mice (Ingalls *et al.* 1998, Lynch *et al.* 1997).

If the tetanic $[Ca^{2+}]_i$ is uniformly reduced at all frequencies of stimulation, this will have relatively little effect at high frequencies because the $[Ca^{2+}]_i$ approaches the level which saturates the contractile proteins. Conversely at low stimulus frequencies, the tetanic $[Ca^{2+}]_i$ lies on the steep part of the $[Ca^{2+}]_i$ – force relation and a reduction can lead to a relatively large fall in force (Westerblad *et al.* 1993). Thus, reduced SR Ca^{2+} release offers a simple explanation for the fractionally larger reductions of force at low frequencies of stimulation (Davies & White 1981, Newham *et al.* 1983b). However, the increased compliance as a consequence of weakened or overstretched sarcomeres also tends to slow the rate of rise of force and will reduce force at low frequencies more than at high frequencies.

Thus, the appearance of fractionally larger reductions in low frequency force should not necessarily be regarded as evidence of excitation–contraction coupling failure following eccentric exercise.

These results from different laboratories are in agreement that in mouse muscle changes in excitation–contraction coupling can be a major contributor to the fall in force. However, in amphibian muscle, Morgan *et al.* (1996) found that the reduced force caused by eccentric damage could not be accounted for by changes in Ca^{2+} release. We have recently confirmed this result in cane toads (E.W. Yeung, C.D. Balnave & D.G. Allen, unpublished observation), so there appears to be a difference between mammals and amphibia in this respect.

What is the mechanism of these changes in Ca^{2+} handling and why are they observed in some species and not others? The membrane structures involved in excitation–contraction coupling, the T tubules and the SR, are, respectively, perpendicular and parallel to the long axis of the fibre. In overstretched sarcomeres, the SR could be damaged or its connection with the T tubules might be impaired in the same way that the longitudinally oriented titin is overstretched in Fig. 2b. Likewise, the T tubules, which pass across the fibre, are likely to be placed under great stress where one myofibril is stretched but its neighbour is not. Exactly how these distortions would affect function is a matter for conjecture at present. One possibility is that T tubules might be sheared off between myofibrils leading to leakage of extracellular Ca^{2+} into the intracellular space. Presumably such damage might seal over, leaving a residual conduction problem for the T tubule. Alternatively, changes in the membrane potential secondary to stretch-induced damage might underlie some of these changes. Evidence for this possibility is equivocal with one study showing no changes in resting membrane potential following eccentric exercise (Warren *et al.* 1993b), while a more recent study found a depolarization of ~10 mV which was blocked by stretch activated channel inhibitors (McBride *et al.* 2000).

One prediction of these ideas is that the distribution of elevated resting $[Ca^{2+}]_i$ or reduced tetanic $[Ca^{2+}]_i$ release might have a similar distribution to the distribution of overstretched sarcomeres. However, tests of this idea have failed to identify focal regions of elevated resting $[Ca^{2+}]_i$ or reduced tetanic $[Ca^{2+}]_i$ as predicted although this may simply reflect inadequate resolution of the techniques used (Balnave *et al.* 1997, Ingalls *et al.* 1998).

The reason for these differences between mammals and amphibia is unclear. One difference is the location of the T tubule at the Z line in amphibia and at the junction of actin and myosin filaments in mammals; this might lead to differences in the mechanical consequences of overstretched sarcomeres. There are also differences in the linkage of the T tubule to the SR with the result that coordinated release by multiple SR Ca release channels (Ca^{2+} sparks) seem to be absent in mammalian species but present in amphibia (Shirokova *et al.* 1998). While the mechanisms remain unclear, these differences in Ca^{2+} release between species illustrate the point that different eccentric protocols and different muscles can exhibit different patterns of eccentric damage.

Cytoskeletal elements

Cytoskeletal proteins such as titin, nebulin, desmin and dystrophin have roles in stabilizing the sarcomeric structure and transmission of forces laterally across the fibre and from fibre to fibre (for review see Patel & Lieber 1997). In a series of studies Lieber and Fridén have explored possible changes in these cytoskeletal proteins using antibody staining techniques to visualize the proteins. Initially they showed that loss of desmin staining of fibres was a feature of eccentric damage and could occur in cells whose membrane was intact and whose contractile proteins appeared normal (Lieber *et al.* 1994). Subsequently, they showed that desmin loss could appear as early as 5–15 min after the eccentric exercise suggesting that it might be an early feature of eccentric damage. Many of these desmin negative cells later showed an increase in titin staining and eventually degenerated (Lieber *et al.* 1996). These ideas have led to the following hypotheses (Lieber & Fridén 1999): (i) that overextended sarcomeres cause a local rise in $[Ca^{2+}]_i$ by one of the mechanisms discussed above; (ii) elevated $[Ca^{2+}]_i$ causes activation of proteases such as calpain which hydrolyses desmin; (iii) this loss of the structural support of desmin contributes to the development of the sarcomere disorder. A difficulty for this hypothesis is that while increases in resting $[Ca^{2+}]_i$ have been observed (see above), they are small and it is not clear that they would activate calpain sufficiently rapidly so as to cause desmin disruption in 5–10 min. It is also important to note that eccentric damage can be observed immediately after a single very large stretch making it unlikely that proteolysis of cytoskeletal proteins is a major contributor (Brooks *et al.* 1995). Note that there are at least two possibilities for the loss of desmin staining by antibodies; one is that desmin filaments damaged by overstretched sarcomeres no longer bind antibody normally, the other is that proteolysis of desmin impairs antibody binding. It is also difficult to determine whether loss of desmin staining is a cause or a consequence of sarcomere disruption. It is also intriguing that titin staining apparently increases in eccentrically damage fibres and, again, the functional significance of this observation is unclear (Lieber *et al.* 1996).

SOME CONSEQUENCES OF ECCENTRIC MUSCLE DAMAGE

Role in muscle training

Eccentric muscle damage shows a pronounced training effect so that after a number of eccentric training periods the weakness, pain, tenderness, etc. are all much less pronounced (Newham *et al.* 1987, Balnave & Thompson 1993). Interestingly, concentric training does not produce the same protective effect (Schwane & Armstrong 1983). It is known that exercised muscles can synthesize new sarcomeres and Morgan (1990) proposed that this might contribute to the training effect by shortening the average sarcomere length. Consequently, a given stretch would be more likely to occur on the ascending limb of the force–length curve and eccentric damage would therefore be avoided. This idea was tested first by showing that rats trained on a declining treadmill (eccentric exercise) averaged 12% more sarcomeres than rats trained on an inclined treadmill (Lynn & Morgan 1994). Subsequently, it was also shown that the peak of the torque/joint angle curve was shifted to longer lengths in the eccentrically trained rats and that they exhibited less damage after a period of eccentric exercise (Lynn *et al.* 1998).

It is clear that eccentric exercise leads to a measure of protection against eccentric damage and it follows that in the training of athletes, whose activity involves a significant eccentric component, it is important to include some eccentric training. This issue has been reinforced by the recent observation that purely concentric training actually exacerbates the magnitude of eccentric damage (Whitehead *et al.* 1998). An interesting issue is whether eccentric or concentric training leads to greater strength when measured in an isometric contraction. This is of interest because the component of activity which is the stimulus to hypertrophy and increased strength remains uncertain. Because eccentric contractions produce more force but consume less energy than the equivalent concentric contractions they allow a test of these possibilities. Experimentally, the answer is equivocal with some studies which find no difference between eccentric and concentric contractions in strength training (Jones & Rutherford 1987), while others find that eccentric training is less effective (Mayhew *et al.* 1995) or more effective (Colliander & Tesch 1990).

Role in disease

As eccentric contraction can cause weakness, pain and cell damage in normal muscles, it is of interest to consider whether this might be one of the mechanisms contributing to degenerative muscle diseases. Humans with Duchenne muscular dystrophy and *mdx* mice lack dystrophin, a protein thought to have a structural role at the surface membrane. In the absence of this protein, muscles fibres degenerate more rapidly than normal and also fail to regenerate. Several groups have shown that muscles from the *mdx* mouse are particularly susceptible to eccentric damage and suggest that this contributes to the degeneration which underlies the disease (Head *et al.* 1992, Moens *et al.* 1993). This finding could reflect the importance of dystrophin as a structural protein helping to transmit forces from the intracellular cytoskeletal protein network to the extracellular structural proteins. Alternatively, it could be that the bizzare branching and variations in cross-sectional area of *mdx* fibres (Head *et al.* 1992) lead to weakened regions in fibres which become the site of excessive stretch.

CONCLUSIONS

Understanding of the mechanisms of force reduction following eccentric exercise has made considerable progress in the last decade. It is clear that some individual sarcomeres elongate excessively during the stretch but that most of these sarcomeres return to normal during relaxation with the thick and thin filaments reinterdigitated. With repeated stretch it is probable that these sarcomeres gradually become damaged and then fail to reinterdigitate. It seems likely that the cytoskeletal elements and the T tubules and the SR also become damaged in these elongated sarcomeres. In addition, the region of damage propagates both longitudinally and laterally so that the regions of damage in fibres gradually involves many sarcomeres. Thus, in fibres damaged by repeated eccentric stretches there are at least four mechanisms of force reduction assuming that the experimental design eliminates fatigue as a contributor: (i) weakened or overstretched sarcomeres cause a shift in the peak of the force–length curve to longer lengths (Fig. 1c). Thus, if the isometric force is measured at the original length giving maximal force then force at this length will be reduced; (ii) changes in excitation–contraction coupling in mammalian muscles lead to reduced Ca^{2+} release and reduced force. In amphibian muscle this mechanism does not appear to contribute; (iii) in many whole muscles there are fibres which are clearly degenerating and would be inexcitable (McCully & Faulkner 1986); (iv) it seems likely that there are sarcomeres which are still close to their normal length but give less force, for instance, because their thick and thin filaments do not reinterdigitate. Surprisingly, this is a category of damage for which there is as yet little specific evidence. To establish this mechanism it is necessary to work with single fibres to eliminate (iii) above, measure $[Ca^{2+}]_i$ to eliminate (ii) and stretch the fibre to the new peak of the force–length curve to eliminate (i).

Many challenges remain for the future. A clear understanding of the factors which cause the overstretched sarcomeres to fail to reinterdigitate and for the region of damage to propagate is needed. Understanding of the changes in excitation–contraction are rudimentary and it will be important to identify the relative importance and functional consequences of damage to the surface membrane, the T tubules and the SR. We need to identify the extent to which raised resting $[Ca^{2+}]_i$ contributes to many of the subsequent phases, such as proteolysis, inflamation, regeneration, etc. The role of the various cytoskeletal proteins is likely to be critical to the process and we need more details of when, how and why these become disrupted and the consequences of this disruption for sarcomere stability and function. Improved understanding of the role of eccentric damage in muscle training and degenerative diseases offers the promise of better athletic training regimens and improved management of muscle diseases.

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