Review

Biomechanical response of skeletal muscle to eccentric contractions

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Abstract

The forced lengthening of an activated skeletal muscle has been termed an eccentric contraction (EC). This review highlights the mechanically unique nature of the EC and focuses on the specific disruption of proteins within the cell known as cytoskeletal proteins. The major intermediate filament cytoskeletal protein, desmin, has been the focus of work in this area because changes to desmin occur within minutes of ECs and because desmin has been shown to play both a mechanical and biologic role in a muscle’s response to EC. It is hoped that these types of studies will assist in decreasing the incidence of muscle injury in athletes and facilitating the development of new therapies to treat muscle injuries.

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1. Introduction

Based on the classic force–velocity relationship in skeletal muscle,1 it is clear that skeletal muscle actions associated with lengthening (eccentric) contractions are associated with high muscle forces. Numerous investigators demonstrated that when eccentric exercise is performed, muscle damage and muscle soreness result. Eccentric contractions (ECs) are interesting to study, not only to understand how muscle is injured during intense exercise but also because there is evidence that these types of contractions produce high strengthening effects. This finding may be considered a 2-edged sword: ECs can produce strengthening but also injure the muscle. This review summarizes the mechanics and some biological aspects of muscle injury gleaned from animal models.

1.1. Mechanics of ECs

Evidence that ECs are unique is based on the observation that muscle behaves mechanically differently when shortening compared with lengthening. This finding shows up as a dramatic discontinuity of the force–velocity relationship for shortening compared with lengthening. For example, when a muscle shortens at about 1% of its maximal velocity, the maximum tetanic tension decreases to ~95% maximum tetanic tension (P0). However, when a muscle is forced to lengthen at the same slow velocity, that is, 1% of its maximal velocity, the tension increases precipitously to >125% P0 (Fig. 1)! It is becoming increasingly clear that the classic cross-bridge theory is really not able to explain a number of mechanical phenomena that are known to occur with muscle lengthening.2,3 Despite ECs being a normal part of the gait cycle experienced by many muscles in the body, relatively little is known about the physiology of ECs. Many investigators agree that ECs, if performed at a high intensity, can cause injury, EC physiology is an exciting area of research that promises new vistas in therapeutic and exercise treatment.

1.2. Human model of EC

One finding on which many agree is that, after an intense bout of eccentric exercise, muscle soreness is not immediately experienced but maximizes about 24–48 h later. This phenomenon has been termed delayed onset muscle soreness and is uniquely related to the EC and not to exercise itself. This property is easily demonstrated by comparing subjective impressions of soreness between individuals who have performed exercise involving ECs with those who have performed exercise involving isometric contractions (ICs). Unfortunately, it is difficult to quantify soreness; therefore
investigators have searched for other, more objective parameters to study eccentric exercise. One such parameter that is measurable in both animals and humans is the circulating level of creatine kinase (CK), an enzyme found in striated muscle that catalyzes the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) according to the reaction:

\[
\text{Creatine Phosphate} + \text{ADP} \rightarrow \text{Creatine} + \text{ATP}
\]

(In fact, ATP is so rapidly regenerated from ADP by CK that ATP levels remain almost unchanged during muscle contraction, even during very intense anaerobic exercise). CK is located inside muscle fibers and, under normal conditions, remains there. However, when exercise is extremely intense and a cell is injured, CK is released into the bloodstream where it can be detected. Therefore, CK is often used as an indirect biomarker of myofiber integrity and injury.

2. Serum CK levels after EC

In a seminal work on injury and training, Evans et al. measured serum CK levels after intense eccentric exercise in young college students. Subjects performed eccentric exercise consisting of a single 45-min bout of high-intensity work. They reported 2 major findings. First, they showed that CK levels did not immediately increase after eccentric exercise but were elevated a few days after the exercise bout, peaked 5 days after the exercise bout, and remained elevated for several days thereafter (untrained, Fig. 2). These data suggest that muscle fibers do not simply break in response to exercise and release their contents (like a popped water balloon). The CK data suggest that muscle fibers experience some type of injury that then initiates a cascade of events that includes a loss of intracellular muscle components. The cascade may involve events that continue for several days, as illustrated by the protracted elevated CK levels shown in Fig. 2.

Evans et al. also demonstrated that, when subjects had been trained by performing the same eccentric exercise training protocol before the eccentric exercise bout, the magnitude and duration of the increased CK levels were greatly attenuated (Fig. 2, filled circles). These trained subjects also had increased CK levels before the experimental exercise bout, suggesting that they were experiencing greater muscle fiber turnover, probably owing to the eccentric training. The study by Evans et al. presents 2 important results on which most investigators agree: (1) muscle damage and soreness owing to EC is delayed and prolonged and (2) prior eccentric training provides a protective effect against further muscle damage.

Studies of serum CK levels have provided information regarding the types of exercise that cause injury (those that are biased toward ECs) and the protective effects of prior eccentric training (as described elsewhere in this article). A dramatic demonstration of the muscle adaptations that occur with repeated exercise bouts was provided by Newham et al. They studied the effects of eccentric exercise of elbow flexor muscles performed 3 different times separated by 2 weeks. After the first bout, as expected, the maximum strength dropped precipitously (Fig. 3A) and CK levels began to increase dramatically (Fig. 3B). However, 2 and 4 weeks later, a different result was observed. A significant decrease in maximum strength again occurred (Fig. 3A, arrows), but there was no corresponding change in serum CK levels (Fig. 3B). These data indicated that some type of muscle remodeling occurred owing to the initial EC and that remodeling persisted even 2 weeks later. This striking result made the muscle physiology community think long and hard about both the nature of muscle adaptation and the stimuli that cause the adaptations to occur. More is said about this elsewhere in this article in the context of cytoskeletal adaptation.

Using serum CK measurements such as those provided herein, investigators have made advances in our understanding...
of eccentric exercise, demonstrating, for example, that (1) CK levels are significantly elevated after eccentric exercise,4,6 (2) the perception of soreness after eccentric exercise is out of phase with serum CK levels,7 and (3) a protective effect of previous training can be demonstrated by comparing CK levels with untrained control values.4 Unfortunately, it is often assumed that the level of CK activity is somehow related to the magnitude of muscle injury, although this idea has not been tested explicitly.8,9 There is a great need in the muscle exercise community for some type of serum marker of muscle injury that provides an estimate of the magnitude of muscle injury, similar to the serum assay for troponin I that provides a rough estimate of the amount of heart muscle involved in a myocardial infarction.10

White rabbits subjected to noninvasive eccentric exercise of the ankle dorsiflexors by rotating the ankle 30° in dorsiflexion while directly stimulating the muscles via the peroneal nerve. This pattern was repeated every 2 s for 30 min resulting in 900 ECs of the dorsiflexors, simulating a single, but intense, eccentric exercise bout.11 After 1, 2, 7, 14, or 28 days, the maximum dorsiflexion torque was measured directly by activating the peroneal nerve transcutaneously and measuring dorsiflexion torque. In addition, a blood sample was collected and immediately centrifuged for measurement of serum CK activity.

The lack of an association between dorsiflexion torque and serum CK revealed that serum CK levels provided poor predictive power in estimating skeletal muscle function (Fig. 4). No correlation was observed between CK activity and torque with the regression relationship calculated describing only ~8% of the experimental variability.12 It may not be surprising that this relationship was relatively poor because a muscle fiber’s permeability to intramuscular enzymes may or may not be correlated with cellular contractile function. For example, in a separate study, it was demonstrated that numerous muscle fibers subjected to eccentric exercise that retained their ability to exclude plasma fibronectin demonstrated significant structural abnormalities, such as loss of intracellular desmin, myofibrillar disruption, and Z-disk disintegration.11 These fibers would be considered injured, but would not contribute to the total pool of serum CK. Thus, although serum CK levels may provide a gross indication that skeletal muscle injury has occurred, the precise CK level does not provide an indicator of muscle injury magnitude. This finding is seen in the data when CK levels and dorsiflexion torque measured 1 day after eccentric exercise are compared with those measured 7 days after exercise (Fig. 4). After 1 day, CK levels (3846 IU/L) were >6 times more than those obtained for the samples obtained after 7 days (Fig. 4, arrows), even though the dorsiflexion torque for the 1-day samples was only 10% less than the torque

![Graph](image-url)
measured 7 days after eccentric exercise. For these data, a significant difference between CK activities was accompanied by no difference between torque values. Therefore, investigators are encouraged to use caution when attempting to infer muscle functional properties based on serum enzyme levels.

3. Muscle ultrastructure after EC

What ultrastructural changes occur in skeletal muscle after intense ECs? Fridén et al. performed a classic study in which he quantified the extent and type of muscle injury that occurred in humans following model ECs. Subjects were asked to pedal against a motor-driven ergometer that was moving in the opposite direction to their applied force (Fig. 5). Subjects generated extremely high-power levels for 30 min and exercised at 80%–100% of their maximal oxygen consumption. Immediately upon completion of the exercise bout and 3 and 6 days thereafter, small biopsies were taken from their vastus lateralis muscles. The most consistent change observed in the experimental muscle biopsies was that the normal periodic myofibrillar pattern (Fig. 6A) became highly disorganized, especially at the Z-disk (Fig. 6B). The nature of the disruption was focal, often extending only a few sarcomeres. This myofibrillar disruption was accompanied by breakage of the myofibrillar cytoskeleton as evidenced by significant redistribution of proteins associated with the cytoskeleton, such as vimentin, laminin, and desmin. Fridén et al. demonstrated that the ultrastructural disruption was only observed after EC; again, the magnitude of the disruption was greatly attenuated if the subjects had been previously trained by performing the same eccentric exercise. When isokinetic strength was measured from subjects after eccentric exercise, a small but significant decrease in isokinetic torque was observed at high angular velocities, even at 6 days after the exercise bout. These investigators cautiously interpreted their data as indicating that preferential damage to the fast muscle fibers had occurred.

Fig. 5. Experimental method for inducing eccentric muscle contractions in human quadriceps femoris muscles. The individual pushes against the pedal, which is being driven in the reverse direction by a torque motor. Adapted from Fridén et al. with permission.

4. Eccentric exercise of isolated muscles

McCully and Faulkner were the first to report the results of ECs imposed directly in isolated animal muscles to investigate muscle function and the cellular response to injury. They attached the distal portion of the mouse extensor digitorum longus (EDL) muscle to a specially designed motor that could forcibly lengthen the muscle by a controlled amount. Using this apparatus, they exercised animals eccentrically, isometrically, and concentrically and compared the muscular response.

McCully and Faulkner found that, after a single 30-min bout of exercise, the Po decreased the most when the exercise was eccentric compared with isometric or isotonic (Fig. 7). The decrease in Po after EC was greatest 5 days after the exercise bout and recovered to control levels after about 30 days. During this time period, significant cellular infiltration was observed—again only in the eccentrically exercised muscles. Although all groups decreased in Po, only the eccentric group showed dramatic signs of inflammation.

This type of experiment was repeated to investigate the fiber type-specific effects of EC. Again, 3 types of contractions were used—EC, IC, and passive stretch (PS)—among the groups of experimental animals to determine whether the stretch alone damaged the muscle. Muscles were exercised in 1 of the 3 modes for 30 min, and muscle contractile properties were then measured.

As expected, we found that, after 30 min of EC, IC, or PS exercise, the Po decreased to the greatest extent in the EC group compared with either the IC or PS group (Fig. 8). In addition, the tension decrease was accompanied by a muscle slowing, as evidenced by a significant decrease in twitch and tetanic rate of rise of tension.

4.1. Time course of tension change during exercise

For all 3 treatments, the peak tension decreased monotonically with time (Fig. 9). No abrupt decrease in tension was observed for the EC treatment, which would have suggested that the damage had occurred as a discrete, coordinated event or that a tear had occurred along the length and width of the muscle. Instead, the EC time course showed an interesting phenomenon that was elucidated based on an indirect calculation. The logic of this indirect calculation was that an EC could be viewed as a simultaneous IC and a PS. If an EC were no more than these 2 phenomena occurring at the same time and in parallel, then the EC force would just be the sum of the PS force plus the IC force. Therefore, the added component of tension was defined owing to EC (Fig. 9, solid circles) as the difference between the peak tension during EC and the sum of the IC and PS tensions during the same time period.
EC tension level \((P_{EC})\) and the sum of the peak IC tension \((P_{IC})\) and peak PS tension \((P_{PS})\). That is, addition tension \((P_{ADD}) = P_{EC} - (P_{IC} + P_{PS})\). Notice that early in the treatment period, the \(P_{ADD}\) was \(>500\) g, but decayed relatively rapidly and became 0 after about 7 min. Thus, early in the treatment period the muscle experienced 500 g more tension than it would have experienced as the simple algebraic sum of IC and PS. The significance of this finding is addressed elsewhere in this article as the damage mechanism is discussed. The important point to note about these data is that the action associated with EC happens relatively rapidly and early in the exercise bout.

4.2. Muscle morphologic changes after eccentric exercise

In an effort to understand the basis for the contractile results, the morphology of the muscles was observed at both the light microscopic and electron microscopic levels. Although the morphology of samples from the PS and IC groups seemed to be normal, the most obvious result was that the eccentrically exercised muscles exhibited increased portions of abnormal fibers when viewed in cross-section. These fibers appeared rounded, more lightly stained on hematoxylin and eosin, and approximately 4 times the normal size (Fig. 10A). Only fibers from muscles in the EC group demonstrated this abnormal appearance and they were always depleted of glycogen, confirming that they had been activated. What fiber type were these enlarged fibers? Fiber type was determined by staining serial sections for myofibrillar adenosine triphosphatase activity, succinate dehydrogenase activity, and \(\alpha\)-glycerophosphate dehydrogenase activity. It was found...
that all enlarged fibers were of the fast glycolytic (FG) fiber type (Fig. 10D). This observation provided insights into the damage mechanism as described elsewhere in this article.

Although no ultrastructural abnormalities were observed in any of the muscles from the IC or PS groups, a significant portion of the fibers in the EC group displayed various degrees of disorganization of the sarcomeric band pattern like the one that Fridén et al. observed after eccentric exercise in human subjects. Streaming and smearing of the Z-disk material, focal loss of Z-disks, and extension of Z-disks into adjacent A-bands were commonly seen. The Z-disk smearing was always found in specific locations within the same fiber and never extended across the whole fiber (and infrequently over >3 sarcomeres).

4.3. Putative mechanisms of EC-induced damage

The fact that only FG fibers demonstrated histologic abnormalities suggested that fiber oxidative capacity was important in determining the extent of fiber damage that occurs immediately after EC. Based on the hypothesis that muscle fiber oxidative capacity was a determining factor in fiber damage, we hypothesized a damage scheme that predicted that muscle injury during EC occurred as follows:

1) Early in the exercise period (i.e., within the first 10 min), type FG muscle fibers fatigue.
2) Based on their inability to regenerate ATP, they enter a rigor or high-stiffness state.
3) Subsequent stretch of stiff fibers mechanically disrupts the fibers, resulting in the observed cytoskeletal and myofibrillar damage.

This hypothesis was appealing for several reasons. First, it explained the well-known protective effect of endurance training on EC-induced damage, which was mentioned elsewhere in this article. Endurance training is known to result in an increased muscle oxidative capacity and, therefore, FG to fast oxidative-glycolytic fiber subtype conversion. Because fast oxidative-glycolytic fibers do not fatigue and enter rigor as readily as FG fibers, EC-induced damage would be expected to be less after endurance training. An appeal of this hypothesis is that it made a testable prediction. A second damage mechanism that could depend on fiber oxidative capacity relates to the other
4.5. Mechanical factors causing muscle injury

We could again hypothesize a damage scheme that occurs as follows:

1) Early in the exercise period, FG fibers fatigue.
2) Based on their inability to regenerate ATP, mitochondria lose their calcium-buffering capacity.
3) Increased intracellular calcium results in activation of the calcium-activated neutral proteases (which were active in denervation), lysosomal proteases, and other cellular processes that are calcium mediated.

4.4. Test of oxidative capacity as a causative factor in muscle injury

As a test of the idea that low oxidative capacity predisposes a muscle to EC-induced injury, we artificially increased muscle oxidative capacity using a chronic electrical stimulation protocol. Because FG fiber oxidative capacity is extremely low and because training can dramatically increase FG fiber oxidative capacity, actually converting it to the fast oxidative-glycolytic fiber type, we hypothesized that the protective effect of training was to increase fiber oxidative capacity. This hypothesis was directly tested by pretreating rabbit anterior compartment muscles with electrical stimulation for 30 min per day for 5 days per week for 3 weeks. It was important to stimulate the muscles enough to cause the oxidative capacity change but not so much as to transform fiber type or cause dramatic muscle fiber size changes like the ones observed with chronic stimulation patterns.

Inspection revealed that electrical stimulation dramatically changed the blood content of the muscles, because the stimulated muscles became much redder than the control muscles. To quantify the oxidative changes owing to electrical stimulation, muscle fiber oxidative enzyme activity (as indicated by the citrate synthase enzyme) and muscle capillary density and geometry were measured. As anticipated, we measured a significant increase in EDL oxidative activity and capillary density with smaller changes in the tibialis anterior. Note that the normal EDL and tibialis anterior muscles have differing oxidative capacities (Fig. 11A, filled bars), but after 3 weeks of stimulation their oxidative functions are nearly identical (Fig. 11A, open bars). This finding illustrates the fact that an oxidative enzyme activity significantly increased with stimulation, but the increase was more dramatic for EDL. Photographs on bars are of TA muscles subjected to the treatment shown. Note the deep red color of the stimulated TA compared with the control TA. (B) Maximum tetanic tension activity of rabbit TA and EDL muscles before and after 3 weeks of low-frequency stimulation. Horizontal red bars above graphs represent normal TA or EDL maximum tetanic tension. EDL = extensor digitorum longus; TA = tibialis anterior. Experimental data from Patel et al. and comparative normal data in part (B) from Lieber et al. with permission.

We sought to measure mechanical factors that cause injury. As a result of the force–velocity relationship shown in Fig. 1, ECs are associated with high force and result in muscle damage, which has typically led to the presumption that the high muscle forces imposed on the muscle caused the muscle injury. Although this supposition is attractive in theory, there is only anecdotal experimental evidence in support of such a claim. Therefore, we designed an experiment in which muscle stress and strain were altered systematically and muscle strength was measured after stretch, the force achieved during the stretch could be altered relative to the muscle deformation timing was altered relative to the muscle deformation to achieve altered stresses at identical strains (Fig. 12A) at 2 different strain magnitudes. Note that by mechanically delaying the muscle stretch and permitting the muscles to develop force before stretch, the force achieved during the stretch could be increased. Then, we eccentrically exercised muscles at high strain (25% muscle fiber length) and low strain (12.5% muscle fiber length) and at high force and low force at each of these strains. Maximum tetanic tension was the main parameter analyzed. We...
used stepwise regression to partition the effect of stress and strain on the magnitude of decrease in tetanic tension. We found that the magnitude of injury was more closely related to the magnitude of the muscle strain than to the stress imposed on the fibers (Fig. 12B). This result may not apply to other muscles.

5. Sarcomere “popping” as the cause of injury

As stated elsewhere in this article, the mechanics of stretched muscle are very unique. This property has been known for some time and yet the uniqueness of these mechanical events has been difficult to explain. In a creative and insightful answer to this question, the Australian electrical engineer Dr. David Morgan proposed a dramatic phenomenon to explain muscle injury with EC. Instead of proposing muscle injury to individual sarcomeres, Morgan proposed that it is the interaction between sarcomeres that results in the injury. Briefly, the theory proposed that small strength differences along the length of the myofibril (owing to small sarcomere length differences or even cross-sectional area variations) means that sarcomeres may be pulling against each other with slightly different forces during contraction. Sarcomere length differences could result in different sarcomere strengths because, at longer sarcomere lengths on the descending limb of the length—tension curve, sarcomeres have less filament overlap and thus generate lesser forces compared with their shorter counterparts in series. This difference in sarcomere strength can permit a differential length change of sarcomeres during stretch, whereby the longer sarcomeres are stretched more than the shorter ones and thus take up a disproportionate fraction of the length change. This differential length change continues during a stretch until finally, Morgan proposes, the lengthening sarcomeres get so long that the myofilaments no longer overlap and then pop to a very long length.

Although such a phenomenon sounds strange and definitely caught the muscle mechanics and muscle injury community by surprise, the theory makes testable predictions. Morgan’s popping sarcomere hypothesis suggests that muscle adaptation after eccentric exercise occurs to keep sarcomeres from attaining the longer sarcomere lengths that render them vulnerable to the instability described elsewhere in this article. The most obvious adaptation is for the muscle to increase serial sarcomere number and, therefore, decrease the magnitude of the stretch absorbed by each sarcomere population.

The proposed adaptation mechanisms were tested in a number of ways. In one experiment, Lynn and Morgan trained rats by downhill running, which induced ECs of the quadriceps, especially of the vastus intermedius. The rats were exercised for 30 min per day for 5 days. Three days after the 5-day training period, muscles were harvested and detailed architectural studies were performed to determine the serial sarcomere number within the fibers. The number of sarcomeres in series within the vastus intermedius fibers was shown to increase owing to downhill running (Fig. 13A), which was amazing. This experiment was the first time that serial sarcomere number changes associated with a particular mode of exercise had been seen. By training another group of rats to run uphill, the investigators also tested whether running training alone could induce such a sarcomere number change. The uphill-trained rats showed no sarcomere number increase, and even a slight decrease, providing support for increase in sarcomere number only owing to eccentric training. One appealing feature of this hypothesis is that it provides a mechanistic explanation for muscle adaptation observed after eccentric training—sarcomere number changes. Such changes have about the right...
time course to cause the protection and are readily accomplished by muscle.

A second test of the hypothesis was to rapidly chemically fix eccentrically contracting muscles and look for the popped sarcomeres. Lynn and Morgan used the appropriate random sampling methods that would avoid bias, as well as a reasonable statistical approach to analyzing their data and provided evidence for the presence of popped sarcomeres (Fig. 13B). A final prediction based on this hypothesis is that the popped sarcomeres, because they are only bearing the load with their passive elastic properties, cause an increase in muscle series compliance. Interestingly, in both animal and human studies of ECs, a shift in the length–tension curve (or torque–joint angle curve in humans) to longer lengths has been observed (Fig. 13C). It has been demonstrated that a major fraction of the injury can be accounted for by the increase in series muscle compliance. Although these data are controversial, the results and implications are intriguing. Do muscles get injured by popping sarcomeres? At this point, there is some theoretical and experimental evidence for this theory.

6. Rapid cytoskeletal disruption after eccentric exercise

There is an extensive network of intermediate filaments in skeletal muscle that interconnects adjacent myofibrils radially and longitudinally. In skeletal muscle, the intermediate filament protein is desmin. In light of the rapid changes in muscle mechanical property with eccentric exercise (Fig. 9), we stained muscle tissue at time periods ranging from 5 min to several days after eccentric exercise for desmin. Surprisingly, there was a significant and widespread loss of desmin after eccentric exercise (Fig. 14). This finding is a spectacular when one considers that the frozen section shown in Fig. 14 is about 8 μm thick and will thus be a stack of 3–4 sarcomeres and all of their associated proteins. In some cases, desmin loss happened as early as 5 min into the EC exercise bout. This finding is the earliest documented structural change observed in muscle after EC. The rapidity of the effect points to a specific type of enzymatic hydrolysis as a likely mechanism rather than gene regulation, which would require much more time. An attractive candidate for the proteolytic mechanism is the calcium-activated protease Calpain, which is present in skeletal muscle and for which desmin is a substrate. The mechanism of action of Calpain requires an increased intracellular calcium ion concentration ([Ca^{2+}]).

6.1. Muscle injury in a desmin knockout model

Based on the putative central role of desmin in mediating muscle injury, ECs were performed on a knockout model developed by Capetanaki, a developmental biologist. This model was created in mice in which the desmin gene had been deleted by homologous recombination and thus had no desmin intermediate filaments interconnecting the myofibrils within
the muscle cell. Capetanaki’s group\textsuperscript{30,31} had previously shown that desmin could play an important role in muscle development and they were interested in how the muscle would develop in the absence of desmin. They used modern genetic engineering to replace the desmin gene with a desmin null gene and found that the mice, while living, had heart muscles that demonstrated abnormalities reminiscent of many heart diseases.\textsuperscript{32,33} It was hypothesized that, in the absence of the intermediate filament network, the muscle would be more mechanically fragile, analogous to the experiments performed by applying ECs to mdx mice.\textsuperscript{34}

The experiments were performed on the mouse EDL. This very fast muscle (>98\% fast fibers) has a small size that made it convenient for this experiment. Each muscle underwent a series of 10 ECs, one every 3 min. Note that this number is much smaller than the one used in many of the studies described herein. This is because the initial injury had been shown to be primarily mechanical in nature; thus, experimental protocols were modified to minimize the influence of fatigue on muscle injury. For each EC, the muscle was first activated isometrically until tension stabilized (~200 ms), then a 15\% fiber change was imposed, resulting in a rapid increase in tension (Fig. 15). This protocol was used to have a contraction-by-contraction estimate of the force-generating ability of each muscle. During the first 200 ms, when the muscle was activated isometrically, we obtained an estimate of the muscle’s ability to generate tension in the absence of the complicating factors associated with muscle lengthening. As shown, tension during the lengthening phase increased in 2 phases, a reflection of the muscle’s short-range stiffness. Muscle length was held fixed, during which tension declined owing to active stress relaxation. Stimulation was then ceased and the muscle length was returned to its starting value.

Measurement of the initial muscle properties before any EC revealed that the knockout muscles generated lower isometric stress compared with the normal wild-type (WT) muscles. However, the most surprising result was that, after 10 ECs, the WT muscles had decreased in isometric stress capability by ~25\%, whereas the knockouts had dropped only by ~9\% (Fig. 16, filled symbols). In other words, the muscle that was missing its intermediate filaments was injured less compared with WT muscle that contained desmin. There is experimental evidence supporting the idea that stress can be a major cause of muscle injury,\textsuperscript{35} so perhaps the knockouts were injured less because they bore less stress. This argument caused concern because it represented a confounding interpretation of the results. This issue was addressed directly by altering the stress-generating capability muscles from WT and knockout mice. One way of achieving this is by aging the WT animals ~40 weeks, because it has been shown that active muscle stress decreases with age. After aging the WT mice ~40 weeks, isometric stress between the young knockouts and the old WT muscles was almost the same (Fig. 16, filled squares and open circles). This fortuitous result permitted us to compare directly the stress effect with the knockout effect. All 4 datasets obtained

![Image](image_url)

\textbf{Fig. 14.} (A) Immunohistochemical section of normal rabbit tibialis anterior muscle labeled with an anti-desmin antibody. Note that every fiber is filled with desmin. (B) Section of rabbit tibialis anterior muscle labeled with an anti-desmin antibody after a single bout of 30 eccentric contractions. Note that many fibers in the section do not label with the desmin antibody and are defined as being desmin negative. Calibration bar = 100 \( \mu \text{m} \). (C) Percentage of fibers that stain as desmin negative after various time periods of eccentric contraction and recovery. Note that a larger fraction of EDL (open bars) fibers are affected compared with TA (filled bars). The only change that is not statistically significant is the TA after 5 min. EDL = extensor digitorum longus; TA = tibialis anterior. Adapted from Lieber et al.\textsuperscript{11,27} with permission.
by this experiment are shown in Fig. 16. Independent of the presence of desmin, the aged animals generated less isometric stress compared with their younger counterparts. Also, independent of age, the desmin knockout animals generated less stress compared with their WT counterparts. Finally, after the EC protocol, the stress generated by the knockout and WT animals of the same age are, essentially, identical. This finding provides evidence for the role of desmin in normal muscle and provides support for the idea that, with EC exercise, desmin loss (in WT muscles) is part of the reason why force declines over time.

There is some preliminary support for such a hypothesis. First, morphologic analysis of the WT and knockout muscles demonstrated many more cytoskeletal ultrastructural abnormalities in WT compared with knockout muscles. One abnormality that was particularly striking was the significantly greater proportion of Z-disks that were slanted more than 30˚ in WT (10.4% ± 1.5%) compared with knockout muscles (5.8% ± 0.9%), which supports the concept of greater strained connections between adjacent myofibrils in WT compared with knockout muscles. In addition, we observed sliding of myofibrils adjacent to one another as a muscle was stretched in WT and desmin-deficient muscles.

6.2. Direct substitution of desmin into desmin knockout muscles

The experiments described herein in desmin knockout muscles demonstrate changes in muscles that had never expressed desmin. The results may be due to the lack of desmin itself or may be due to the lack of desmin in muscle during development. To directly test the role of desmin in muscle, DNA encoding a green fluorescent protein (GFP)-desmin fusion protein was introduced into adult muscles lacking desmin. This allowed for distinguishing desmin’s functional role in adult muscle from the developmental consequences of desmin deficiency and to more directly link desmin to events that occur in muscle when it is activated or deformed. Single fibers from WT and either untreated or transfected desmin null muscles were subjected to confocal imaging during linear deformation to quantify the degree of sarcomere alignment, nuclear deformation, and mechanical continuity under loading.

WT muscle showed high sarcomere matrix regularity and continuity under strain (Fig. 17A, A’, A’’), whereas desmin null muscles showed irregular sarcomeres and loss of spatial continuity (Fig. 17B, B’, B’’). Single fibers from transfected mutant muscles showed regional +GFP-desmin expression (Fig. 17C) and confocal microscopy of these transfected areas showed realigned myofibrils and restored spatial continuity during loading, demonstrating mechanically functional connections (Fig. 17D, D’, D’’). Displacement of adjacent myofibrillar Z-disks were quantified by Fourier analysis and expressed as the phase shift variance between adjacent myofibrils under stretch (Fig. 17E). The slope of this relationship indicated the degree of connectivity among myofibrils. The phase shift variance slope was not significantly different between the WT and +GFP-desmin—transfected desmin null muscles (+GFP-desmin; Fig. 17E; p > 0.7), but both were significantly lower than desmin null muscle (No Tx; Fig. 17E), desmin null muscles transfected with GFP alone (+GFP;
Fig. 17. Serial confocal images of the Z-disk striations in single muscle cells being stretched while imaging striation pattern. (A, B, and D) Three different cells at 3 comparable sarcomere lengths. (A, A’ , and A”) WT muscle cell. (B, B’, and B”) Desmin null muscle cell. (D, D’, and D”) Desmin null muscle cell transfected with +GFP-desmin imaged in a transfected region (region highlighted in C). All cells are labeled with α-actinin except the desmin null muscle cell transfected with +GFP-desmin. (E) Sample myofibrillar phase shift variance values as a function of sarcomere length obtained from each of the experimental groups. Note the similarity in slopes between the wild-type (0.00456 μm²) and the transfected desmin null muscles (+GFP-desmin, 0.00639 μm²) compared with the desmin null muscle, which is about 3 times greater (No Tx; 0.0158 μm²). (F) Quantification of the degree of connectivity across the fiber by the phase shift slope of the striation pattern, which is obtained as the fiber is stretched. The 2 treatments with the greatest connectivity are the wild-type and desmin null muscle transfected with +GFP-desmin, which are both significantly different from the other 3 treatment groups (*p < 0.0001). (Mean ± SE, n = 5–8 muscles per group.) EP = electroporation; GFP = green fluorescent protein. Adapted from Palmisano et al. with permission.
Fig. 17F), or desmin null muscles subjected to electroporation alone (EP; Fig. 17F; $p < 0.0001$). Thus, the properties of muscle that differ in desmin null mice are directly attributable to desmin, not to the indirect effect of lack of desmin during development or to the transfection procedure, because only +GFP-desmin realigned the myofibrils.

Transfected muscle morphology also revealed a role for desmin in force transmission from the fiber exterior to the myofibrillar nuclei based on the behavior of nuclei in single fibers. Nuclei in WT muscle subjected to stretch increased their length/width ratio (i.e., aspect ratio) in parallel with increased sarcomere length (Fig. 18A), whereas nuclei in desmin null muscles were significantly less deformed by stretch (Fig. 18B). The deformation pattern of nuclei within desmin null muscles transfected with +GFP-desmin showed restored resting shape as well as restored deformation in response to stretch (Fig. 18C). Desmin null muscles subjected to electroporation alone or transfection with GFP alone showed the same nuclear deformation pattern observed in untreated desmin null muscles. This observation was confirmed quantitatively. Nuclear deformation was quantified by linear regression as the change in the normalized nuclear aspect ratio as a function of sarcomere length (Fig. 18D). The change in the nuclear aspect ratio with sarcomere length was not significantly different between WT and +GFP-desmin–transfected desmin null muscles (Fig. 18E; $p > 0.5$), but both were significantly greater than all control groups (Fig. 18E; $p < 0.005$), demonstrating a rescued nuclear phenotype only in response to +GFP-desmin transfection.

The +GFP-desmin transfection of desmin null muscles also restored the muscle’s normal mechanical response to high stress. As shown in Fig. 16, WT muscles subjected to...
The sarcomere matrix was defined, and both passive and active viscoelastic forces were modeled to predict force generated by the muscle as a function of desmin content based on a previously defined, linear relationship between muscle isometric stress and percent force decline. The model predicted the observed nonlinear relationship between desmin content and force decline only when desmin was preferentially localized to the subsarcolemmal region (Fig. 19B, dashed line is the model prediction—note that it is not a simple curve fit). When desmin was modeled as either being localized centrally within the fiber or randomly distributed across the fiber, less or no effect on force production was seen. The data and modeling strongly support a force-transmitting role for desmin and further suggest that desmin’s functional properties may be region specific within the muscle cell.

Together these data reveal a central role for desmin being involved in both mechanical stress transmission and stress transduction in muscle. We demonstrated that desmin is specifically involved in myofibrillar alignment, nuclear integration within the myofibrillar matrix, and mechanical response to high stress. These results illustrate that the Z-disk, and particularly its associated desmin cytoskeleton, play a central role in both muscle force transmission and a muscle’s response to EC. Future studies will reveal the details of the molecular interactions among desmin and the rest of the sarcomere proteins, as well as the significance of desmin’s role in transducing ECs.

7. Conclusion

ECs represent a mechanically unique type of contraction in skeletal muscle. Although it is clear that EC is associated with injury and elicits a training effect, the details of these phenomena remain unclear. Tissue-level experiments demonstrated rapid changes in the desmin cytoskeletal network that set the stage for future mechanistic studies. It is hoped that these types of studies will assist in decreasing the incidence of muscle injury in athletes and facilitate development of new therapies to treat muscle injuries.

Competing interests

The author declares that he has no competing interests.
Fig. 20. The finite element muscle fiber array (A) and free-body diagram of 2 sarcomeres linked at a Z-disk (B). Viscoelastic sarcomere elements are linked longitudinally at Z-disk nodes to form myofibrils and laterally by desmin elastic filaments (blue) to form a 2-dimensional fiber. Extracellular matrix and sarcolemma composite (ECM) elements form the upper and lower bounds of the array, whereas tendon elements define each end. The location of each element is indexed by its row \((i)\) and column \((j)\). The array continues to extend in the direction of increasing \(i\) and \(j\) until it meets another ECM/tendon junction. The contractile element \((CE)\), the series elastic element \((SE)\), the parallel viscoelastic element \((PVE)\), the sarcomere length \((SL)\), and the series spring extension \((E)\) are labeled in the bold sarcomere. \(F = \) force; \(PT = \) passive tension. Adapted from Meyer et al.\(^{40}\) with permission.

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