Exercise Induced Myopathys

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Abstract
Muscle damage and myopathy-like changes can occur following strenuous and uncustomed exercise, particularly activity involving eccentric contractions. The associated strength loss can have a long lasting impact on performance. The question of how eccentric contraction induced injury effects muscle performance and what mechanism(s) account for the strength loss is addressed in this review by focusing attention on the changes in activation and mechanical properties of the muscle. We also describe an animal model developed in our laboratory that creates a standardized stretch injury in order to understand the more about the pathophysiology of the so-called ‘muscle strain injury’. Recent data question the exact role of inflammation and invading leukocytes in the repair process. Changes in single fiber mechanics and alteration in contractile protein expression may have implications for understanding the regulation of muscle injury and repair.

Key words: eccentric, excitation-contraction coupling, injury, skeletal muscle, strength.

In this chapter we will review the pathophysiology of repetitive eccentric contraction-induced injury as well as acute stretch injury to skeletal muscle. The understanding of both conditions has been improved by the development of animal models to isolate single factors and provide important mechanistic information about injury and recovery from injury.

Repetitive Eccentric-Contraction Injury

It is well known that certain pathophysiological conditions lead to destabilization of the muscle fiber’s internal environment resulting in myopathy-like conditions within the muscle. Similar morphological changes have also been observed following strenuous and uncustomed exercise, particularly activity involving eccentric muscle contractions [2, 9, 12, 15, 18]. These myopathic-like changes can occur in the presence of mechanical, neural and physiological alterations in the muscle.

Muscle strength can be decreased by 50% or more following performance of a relatively few high-force, eccentric contractions. The strength loss can be prolonged, taking a month or more for complete recovery. Some suggest that the initial strength loss is due solely to damage of the force-bearing structures within the muscle [33]. Others contend that most of the early strength loss results from a failure of excitation-contraction (E-C) coupling processes and that a slow loss of contractile proteins in the days following injury prolongs recovery time [16, 17, 32]. Regardless of the initial cause(s) for the loss in strength, there is a further decline in strength in the following days that is accompanied by inflammatory changes within the muscle [37, 49].

The cellular mechanism(s) explaining this strength loss remain controversial. Warren and colleagues found that force loss due to eccentric exercise could be recovered by using caffeine to potentiate Ca2+ release from the sarcoplasmic reticulum (SR) [51]. Studies of mouse muscle following eccentric contractions have shown that E-C failure can explain up to 75% of the decrement in P0 from 0 to 5 days post exercise [27, 28]. The question of what happens in the muscle following eccentric exercise injury and what comes first, E-C decoupling or sarcomere disruption is still open to debate.

Studies postulate that during repeated eccentric contractions the number of disrupted sarcomeres increases until a point where membrane damage as well as damage to the coupling mechanism occur [47]. On the other hand, Takekura et al. postulated that the first step in the damage process is t-tubule disruption [46]. It may be difficult to reconcile these different theories given that studies published to date have used a variety of stimulation and contraction protocols. Furthermore, this lack of
standardization and its resemblance to clinical injury may be questionable.

Changes in activation and mechanical properties of skeletal muscle following injury

Tension output of skeletal muscle is composed of contributions from the passive elements together with crossbridge cycling. The passive force-length relationship is approximately exponential, consequently tension rises in a steep fashion at longer muscle lengths. Passive tension has been attributed to the elastic filaments and Z-lines [24]. It has been shown that following eccentric exercise of the elbow flexors, resting muscle stiffness increases and the relaxed arm adopts a slightly flexed posture [31]. Howell et al. have shown that muscle stiffness doubles immediately following eccentric exercise and remains elevated for several days [25]. Chleboun and co-authors suggest that the increase in muscle stiffness following eccentric exercise is due to muscle swelling [11]. However, this is likely not a complete explanation since stiffness rises immediately post-exercise while swelling does not become significant until 24 hours later.

To estimate how long E-C coupling failure contributes to the strength loss after injury, Ingalls et al. measured force production by the EDL during exposure to caffeine or 4 chloro-m-cresol (i.e., pharmacological agents acting to promote SR Ca2+ release) at 1, 2, 3, 5 and 14 d following injury [27, 28]. Muscle force production while under the influence of both agents was moderately (i.e., 11-21%) reduced compared to a 51% reduction in Po at 0-5 days. By 14 days, the caffeine group elicited normal force levels while the 4 chloro-m-cresol group remained significantly different from that of the contralateral control. Based on the disproportionate reduction in force production elicited by the pharmacological agents compared to the reduction in isometric strength, it was concluded that E-C coupling failure could account for up to 75% of the strength deficit within the first 5 days, with the remainder of the strength deficit being accounted for by the physical disruption and/ or alteration of the force bearing structures.

E-C coupling is typically defined as the sequence of events that begins with release of acetylcholine from the neuromuscular junction and finishes with Ca2+ release from the SR. A number of studies have attempted to identify the exact site of failure in contraction-injured muscle [49, 50, 51]. Two logical sites of failure (the plasmalemma and the T-tubular membrane system) have been the subject of ongoing debate. Warren and colleagues noted a mild reduction in the capacity of contraction-injured muscle fibers to conduct action potentials along the plasmalemma [50]. Isometric torque, EMG root mean square (RMS) and M-wave mean and median frequencies were measured before, immediately after, and at 1, 3, 5 and 14 days. In parallel experiments, nicotinic acetylcholine receptor (AChR) concentration was measured in TA muscles to determine whether excitation failure elicited a denervation-like response [50]. Immediately after the eccentric contraction protocol, torque was reduced by 47-89%, while RMS was reduced by 9-21%. However, the RMS decrement was not different from that observed for the concentric contraction protocol, which did not elicit large torque deficits. One day later, both the eccentric and concentric RMS had returned to baseline values and did not change over the following 2 weeks. However, torque production by the eccentric group showed a slow recovery over the same interval and was still depressed up to 30% after 2 weeks. M-wave mean and median frequencies were not affected by either protocol. AChR concentration was elevated by 79 and 368% at 3 and 5 days, respectively, with a gradual return to control levels over the next 14 days. At the time of peak AChR concentration in the eccentric protocol muscles (i.e. 5 days), AChR concentrations in concentric muscles were not different from controls. In conclusion, these data were the first to show that there is no major role for impaired plasmalemmal action potential conduction in the E-C coupling failure induced by eccentric contractions. In addition, a muscle injured by eccentric contractions shows a time course in AChR levels similar to a transiently denervated muscle [50].

Concerning T-tubular membrane function, Ingalls et al. have attempted to determine the contribution of E-C coupling failure to the decrement in maximal isometric tetanic force (Po) [27, 28]. The left anterior crural muscles of female mice were injured in vivo with 150 eccentric contractions. Po, caffeine-, 4-chloro-m-cresol-, and K+ induced contracture forces, sarcoplasmic reticulum (SR) Ca2+ release and uptake rates, and intracellular Ca2+ concentration [Ca2+]i were then measured in vitro in injured and contralateral control muscles. The results of this study estimated that E-C coupling failure can explain 57-75% of the Po decrement from 0 to 5 days post-injury. Comparable reductions in Po and K+-induced force (51%), and minor reductions (0-6%) in the maximal SR Ca2+ release rate suggest that the E-C coupling defect site is located at the T-tubule-SR interface. Confocal laser scanning microscopy indicated that resting [Ca2+]i were elevated and peak tetanic [Ca2+]i were reduced, whereas peak 4-chloro-m-cresol-induced [Ca2+]i were unchanged immediately after injury. By 3 days post-injury, 4-chloro-m-cresol-induced [Ca2+]i were depressed, probably because of decreased SR Ca2+ release and uptake rates (17-31%). These data indicated that the decrease in Po during the first several days after injury primarily stems from a failure in T-tubular membrane function.

Others studies have attempted to identify the relationship between skeletal muscle function and protein metabolism after initiation of eccentric contraction-induced injury [33]. Mouse anterior crural muscles were injured in vivo and studied for indexes of muscle function, injury, phagocyte infiltration, and protein metabolism. Animals were administered anti-polymorphonuclear cell and anti-macrophage antisera in an attempt to reduce phagocytic infiltration into injured muscle. Force production in the
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EDL was reduced 55% immediately following injury and did not recover significantly until 120 h post-injury (28% below baseline). However, rates of protein degradation were not elevated until 48 h post-injury (60% above normal) and were not correlated with changes in force production. Phagocytic infiltration was evident 24–120 h post-injury and was correlated with the increase in protein degradation. Rates of protein synthesis began to increase approximately 48 h following injury and were elevated by 83% at 5 days. By two weeks, muscle protein degradation and synthesis rates had returned to normal, as well as specific force production, and phagocytic infiltration. However, muscle mass, protein content, and absolute force production were lower than normal. Collectively, these data suggest that a rapid increase in degradation of force-bearing protein structures could contribute to the initial reduction in strength following injury.

In summary, there is no simple explanation from whole muscle experiments for the strength loss that occurs following eccentric contraction-induced skeletal muscle injury. The strength loss appears to result from a complex interaction of mechanisms. We acknowledge that our understanding of this strength loss comes predominantly from studies of mouse muscle in which the contractions have been induced through maximal electrical stimulation. It is thus very much possible that the strength loss induced by these models may differ qualitatively from that elicited by submaximal, voluntary eccentric contractions. Furthermore, the lack of a standardized protocol makes comparisons between the various studies somewhat difficult.

Changes in myofibrillar protein expression following repetitive eccentric contractions

It is well established that variable expression of myofibrillar contractile protein isoforms is a major determinant of contractile properties for a given muscle. For example, altered expression of myosin isoforms during development and following experimental manipulation correlate with differences in the rate of development, maximal shortening velocity, and power-load relationships [38]. Changes in the expression of troponin T and tropomyosin isoforms are associated with significant changes involving alterations in the expression of myosin heavy chain (MHC) and myosin light chain (MLC) isoforms, as well as desmin and titin [32].

In order to further elucidate potential mechanisms contributing to the strength loss associated with repeated eccentric contractions, Ingalls et al. examined the changes in myosin heavy chain (MHC) and actin composition together with the decrease in maximum isometric force [27, 28]. Po and protein contents were measured in injured and contralateral control EDL muscles for up to 28 days following an exercise bout. Po was reduced by approximately 40%, while MHC and actin contents were unchanged from 0 to 3 days after injury. Whereas Po partially recovered between 3 and 5 days, MHC and actin contents in the injured muscles declined by 19 and 20%, respectively. Decrement in Po were similar to the reductions in MHC and actin contents at 14 (approximately 24%) and 28 (approximately 11%) days. Evaluation of myofibrillar and soluble protein fractions indicated significant reductions in the content of major proteins at 5 and 14 days. Immunoblots of heat shock protein 72 revealed elevations starting at 0.25 days, peaking during 1–3 days, and declining after 5 days. It was concluded that decreased contractile protein content was not related to the initial decrease in Po. However, decreased MHC and actin contents could account for 58% of the Po reduction at 5 days, and for nearly all the decrements in Po from 14 to 28 days. Warren et al. (1994) have previously reported that eccentric contractions performed in vitro result in reduced maximal Ca²⁺-activated force in skinned EDL muscle fibers. The decrease in economy following eccentric exercise-inducing injury was attributed to two main factors. First, in skinned fibers isolated from the injured muscles, the ratio of maximal actomyosin adenosinetriphosphatase activity to force production was up by 37.5%, suggesting uncoupling of ATP hydrolysis from force production. Second, increased reliance on anaerobic metabolism along with the fluorescent microscopic study of mitochondrial membrane potential and histochemical study of ATP synthesis suggested an uncoupling of oxidative phosphorylation in the injured muscles.

Warren et al. have compared the reaction of slow soleus and fast extensor digitorum longus (EDL) fibers to eccentric exercise-induced injury [47]. In addition, they studied the effect of altering the muscle’s daily loading pattern through hindlimb suspension (HS). Decrements in contractile performance and lactate dehydrogenase (LDH) release were measured at 15-min intervals over 1 h. Immediately following the eccentric contraction protocol, markedly greater decrements in Po occurred in the normal EDL than in the normal soleus. LDH release was 2.7-fold greater in the EDL than in the soleus muscles. To investigate the role of recent muscle loading on injury, EDL and soleus tissues subjected to HS for 14 days then performed the eccentric contraction protocol. Hindlimb suspension resulted in greater decrements in contractile performance (Po) for the soleus muscles compared with the EDL. For the same purpose, Macpherson et al. tested the susceptibility to contraction-induced injury in single permeabilized muscle fiber segments from fast EDL and slow soleus muscles of rats [34]. Following single stretches of varying strains and under three conditions of Ca²⁺ activation (none, submaximum, and maximum), greater force deficits were produced in fast than slow fibers. The authors concluded that although there were differences in the strain required to produce injury in fast and slow muscle fibers, the ultrastructural damage was strikingly similar for a given force deficit.

Muscle Stretch Injury

We have developed an animal model to study the biomechanics and pathophysiology of stretch injury to skeletal muscle [4]. The system consists of a geared
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electric motor, a loading frame, a torque sensor, an angular position sensor, a closed loop control system, and a custom designed nerve stimulator. The tibialis anterior (TA) is stimulated by peroneal nerve activation. At muscle tetany, the ankle is plantar flexed within the ankle’s physiological range of motion to create an injury at the myotendinous junction (Figure 1).

A key event in the early stages of muscle repair is the inflammatory response producing both polymorphonuclear and mononucleated cellular infiltration of the injury site. The process begins with cytokine and growth factor release from injured fibers that produce a chemotactic signal to surrounding inflammatory cells. The neutrophil is the first cell to appear following muscle stretch injury [43]. Neutrophil invasion of damaged muscle occurs as early as 1hr following toxin exposure [39] and similar findings have been noted in studies of eccentric exercise [14]. A primary function of neutrophils is phagocytosis of necrotic muscle fibers and cellular debris [1]. In addition, invading neutrophils may serve as a source of pro-inflammatory cytokines such as IL-8 [1] and TNFα [13]. These cytokines may play a role in the upregulation of inflammation and provide a signal for monocyte invasion of the injured tissue.

Recent observations have suggested that leukocytes may also contribute to oxidant generation following muscle stretch injury [3, 8]. Neutrophils contain a number of enzymes such as myeloperoxidase and NADPH oxidase that are capable of generating free radicals, such as superoxide (O$_{2}^{-}$), hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO$^\bullet$), and hydroxyl radicals (HO$^\bullet$). The exact role neutrophil-derived oxidants play in muscle inflammation and repair is yet unknown. It is possible that these highly reactive molecules cause direct cell damage, including modification of nucleic acids, proteins, and lipids that would therefore suggest a negative effect on the repair process. The presence of neutrophils has been correlated with structural damage and tissue injury in a rabbit model of myocardial ischemia [52]. Our laboratory has recently demonstrated that neutrophils peak 24h post-injury [43]. This peak correlated with peak oxidant production and as well as an extension of the initial amount of myofiber damage [43]. On the other hand, it is possible that oxidants act to amplify the host’s inflammatory response by the upregulation of pro-inflammatory cytokines such as NF-κB [14].

In addition to neutrophil invasion of the injury site, monocytes appear that eventually mature and differentiate into macrophages. Pulse labelling with tritiated thymidine at the time of muscle injury results in fewer labeled cells compared with animals labeled 2.5 days prior to tissue damage [5]. Adhesion molecules such as E-selectin and P-selectin appear critical for the influx of leukocytes (neutrophils and monocytes) to the injured tissue. Neutrophils can stimulate the proliferation of resident macrophages, of which two subtypes are now recognized to play a role in muscle injury and repair. The first subpopulation expresses the ED1$^+$ antigen and is capable of invading damaged muscle fiber to phagocytose cellular debris and damaged myofibrillar material [21, 22]. The ED2$^+$ macrophages are resident macrophages present throughout the regenerative process, however their exact role remains unknown. ED2$^+$ macrophages do not appear to invade damaged tissue, rather their primary function may be to serve as sources of growth factors and cytokines such as IGF-1 [5], IL-6 [23], and PDGF [44] that may regulate myoblast proliferation and/or differentiation.

Cytokines and Muscle Damage

Cytokines are a diverse family of intercellular signaling proteins that influence the movement, proliferation, differentiation, and metabolism of target cells [10]. These actions can occur by direct interaction of the cytokine with its particular receptor or by the ability of

![Figure 1. Hematoma at the myotendinous junction.](image)

![Figure 2. Force-pCa relationship in skeletal muscle.](image)
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one cytokine to induce synthesis of other cytokines and hormones. Stimuli such as mechanical damage, oxidants, and stress hormones may modulate or induce cytokine activity. Some generalizations about their role in soft tissue injury and repair appear possible. First, IL-1β and TNFα are often associated with promotion of muscle inflammation. On the other hand, cytokines such as transforming growth factor-β (TGFβ), IL-4, and IL-10 assist in down regulation of the host inflammatory response. Cytokines appear to also play an important role in muscle repair through satellite cell chemotaxis. For example, FGF-2 and TGFβ promote the migration of muscle precursor cells in vitro [20].

Muscle Fiber Regeneration

Following removal of damaged cellular products by infiltrating inflammatory cells, a series of events occurs that involves two competing events; muscle fiber regeneration and collagen synthesis. The satellite cell which resides in the periphery of the muscle fiber between the basal lamina and the sarcolemma is the primordial cell for muscle regeneration [35, 45]. An intact basal lamina appears essential to satellite cell activation and is capable of expressing various extracellular matrix components necessary for regeneration [6]. If the basal lamina of the damaged fiber remains intact following injury, satellite cells form myogenic cells that fuse with existing fibers or with each other to form myotubes. In vitro experiments have shown satellite cell proliferation can occur within hours of injury [6]. It also appears that the sarcolemma can exert a negative control on the satellite cell to prevent its proliferation and differentiation [7].

A delicate balance of several factors exists that determine both the speed and the extent of myofiber regeneration following injury. Growth factors such as basic Fibroblast Growth Factor (bFGF), Insulin-like growth factor (IGF-I), and Transforming Growth Factor (TGF-β) have received the most attention to date [53]. These growth factors are made available to the injured tissue in a variety of ways including local availability, disruption of the extracellular matrix, local synthesis, and blood-borne arrival. Studies have shown an increased expression of IGF-1 in satellite cells, myoblasts, and myotubes during skeletal muscle regeneration [30]. IGF-1 levels under the influence of growth hormone have been shown to increase several fold following muscle injury [30, 54]. Furthermore, an acute bout of eccentric exercise increases IGF-1 levels in muscle tissue sections for up to 4 days post-exercise although the exact significance of this increase is yet unknown [54].

Collagen synthesis

Adequate connective tissue production is necessary for normal force transmission to occur and for the muscle’s resistance to tensile loading to be preserved. On the other hand, excessive collagen synthesis can lead to fibrosis that can potentially alter muscle stiffness and joint performance.

Types I and III collagen constitute the major types within skeletal muscle. Type I collagen, present primarily in the muscle fiber’s epimysium and perimysium, possesses relatively high tensile strength and stiffness and is therefore suitable for transmission of muscle-tendon forces. Type III collagen is located mainly within the perimysium and the endomysium. Its structure is similar to that of Type I collagen, but it forms thinner and more elastic fibers. In response to skeletal muscle injury, both Type I and III collagen are produced. Type III collagen synthesis increases before mature fibroblasts can be detected, with primitive, multipotent cells the presumed source. Later in the repair process, there is marked production of Type I collagen and restoration of the normal I:III ratio [26]. Several studies have demonstrated the presence of a high proportion of Type III collagen, in some cases for up to 6 wks, following injury [26, 29]. Recent studies have suggested that Types IV and V collagen may also be involved in the repair of damaged skeletal muscle [26, 29, 41].

Figure 3. Injured TA fiber. a. Pre-activated injured TA fiber (pCa 9.0 solution) SL = 2.40 µm. b. Maximally activated injured TA fiber (pCa 4.5 solution) SL = not determined.
Mechanical Properties of Stretch-Injured Single Fibers

We have examined maximum Ca\(^{2+}\)-activated tension, the Ca\(^{2+}\) sensitivity of tension and the rate of force redevelopment (k\(_{\text{tr}}\)) of single skinned skeletal muscle fibers from stretch-injured and sham control TA muscles 3 days following injury (Figure 2). Compared to the tension-pCa relationship in non-injured skeletal fibers, injured fibers demonstrate a rightward shift in the tension-pCa relation (non-injured pCa\(_{50} = 5.89\); injured pCa\(_{50} = 5.70\)) indicating that injured fibers exhibit a marked decrease in the Ca\(^{2+}\) sensitivity of tension. In addition, there is a significant reduction in the apparent cooperativity of tension development in the injured fibers, as indicated by the reduced slope of the tension-pCa relationship. The rate of tension redevelopment is significantly affected by muscle stretch injury as k\(_{\text{tr}}\) decreased from ~7.0 s\(^{-1}\) in non-injured fibers to ~2.5 s\(^{-1}\) in injured fibers.

Recent experimental evidence from skinned skeletal muscle fibers indicates that variable expression of tropomyosin isoforms results were associated with significant changes in the tension-pCa relationship, as manifested by significant alterations in the Ca\(^{2+}\) sensitivity of tension and steepness of the tension-pCa relation [19, 40, 42]. Furthermore, it is well established that the transition to the force-generating state (i.e., k\(_{\text{tr}}\)) is influenced by the type of myosin heavy chain isofrom present [36]. A representative single skinned fiber from an injured TA muscle is presented below (Figure 3; fiber width = 54 µm, sarcomere length = 2.35 µm). The panel on the left shows the fiber as it appeared in relaxing solution of pCa 9.0 (Figure 3a). The right panel shows the same fiber during maximal activation in a solution of pCa 4.5 (Figure 3b). It is noteworthy that in control, non-injured fibers sarcomere striation pattern is maintained throughout both submaximal and maximal activations (data not shown). Even under these conditions, there is minimal degradation in the striation pattern in control, non-injured fibers. However, the striation pattern is clearly lost during the first maximal activation of the injured fiber with distinct regions of sarcomere inhomogeneity. At this point the exact significance of this loss in sarcomere registry is unknown, however it is likely that intermediate filament proteins such as desmin and titin may be altered.

Summary

Intense eccentric muscle activity and stretch injury can both result in myopathy-like alterations of skeletal muscle. Our understanding of both conditions and the regulation of injury and repair continue to improve based upon observations of human exercise as well as animal models. Future work will undoubtedly incorporate molecular and cellular work to further our understanding of mechanisms of injury and repair of skeletal muscle following eccentric activity and injury.

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