

Cellular responses in exertion-induced skeletal muscle injury

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Abstract

Muscle injury is a common result of muscle exertion caused by overload and over-activity. In this presentation, an attempt was made to discuss models of muscle injury which involve exertion but not excessive strain, although most functional activities of the extremities require some eccentric muscle actions. Muscle injury is characterized by cellular and extracellular matrix responses which appear to be common to all types of muscle trauma – even in the absence of bleeding. Using tenotomy and functional over-load of the rat hindlimb muscles as examples, illustrations of several of these responses are presented and discussed. (*Mol Cell Biochem* **179**: 189–196, 1998).

Key words: skeletal muscle, injury, myofibers, extracellular matrix, exertion, rat

Introduction

Much attention has been focused on skeletal muscle injury due to strain or intense exercise. Exercise, especially those activities involving eccentric muscle actions (muscles are stretched while active), results in injury to skeletal muscle fibers and extracellular matrix in rats [1] and humans [2]. This injury can range from a disrupted sarcomere [3] or sarcolemma [4] to a broken myofiber [5]. The degree of injury and frequency of repeated injury determines the outcome – degeneration, regeneration or fibrosis.

Concentric muscle actions (muscles shorten while active) include diaphragmatic breathing, coughing, chewing without grinding, and exercises such as cycling, stair climbing, swimming, movements against hydraulic resistance (i.e. isokinetic) and weight lifting in which only the concentric muscle action is performed. Over-loading has been studied in a few of these activities. From those samples where myofiber morphology has been studied, the muscle response seems to follow a reproducible pattern seen in traumatic injuries such as crush or over-strain. It seems reasonable that muscle tissue can only undergo a limited number of responses. These responses include atrophy, hypertrophy, hyperplasia, fiber splitting, myosin transformation, cell infiltration, non-muscle cell

proliferation, satellite cell activation and cell necrosis. Regeneration of myofibers with extracellular matrix expansion and collagen deposition is a common feature of muscles which have been over-loaded – sometimes leading to fibrosis. Another type of muscle injury, due to over-use, can result from chronic activation of a fast muscle or fast fibers in a mixed muscle. Here the muscle is over-loaded in the sense that the energy supply can be depleted and some cells actually die and may be substituted by newly formed fibers with higher oxidative capacity [6]. In the over-loaded and over-use models, the processes of degeneration and regeneration [7] appear the same with differing time courses.

The muscle injuries described above fall under the major category of exertion-induced muscle injury. However, because the mechanisms of over-use are different, it is useful to subdivide them into two categories: over-load and over-activity. Over-load represents a condition where the force requirement of the muscle exceeds the habitual or functional requirements. Over-activity occurs when the firing rate of the motor neuron is increased beyond its physiological levels or duty cycles. While over-loading is common during growth and work-induced hypertrophy, over-activity is seen most often in disease states characterized by spasticity or tetany.

Effects of chronic over-load

Chronic over-load can be produced by tenotomy, ablation and denervation of agonist muscles which requires the remaining muscles to do more work. These chronic over-load models were instrumental in defining the mechanisms of work-induced muscle growth and hypertrophy [8]. For example, hypertrophy of muscle fibers was shown to be related to increased amino acid transport and protein synthesis [8]. It was generally agreed that the number of myofibers did not change with over-load. However, apparent increases in fiber number could be seen in some parts of the muscle. Satellite cells were implicated in the development of small fibers in the EDL muscles [9, 10]. However, further investigation of the development of small fibers in the soleus muscle revealed that the 'new' fibers were actually split fibers at the distal end of the soleus [11] – more likely a result of physical damage [11]. Thus, the surgically over-loaded muscle may be a better model for exertion-induced muscle damage than for muscle hypertrophy resulting from physiological over-loading (i.e. strength training).

Both myofibers and extracellular matrix can be involved in the response to chronic over-load. The most striking change following over-load is an increase in the number of fibers which demonstrate fast myosin isoforms in some but not all muscles. This is especially true for the slow twitch muscles such as the soleus muscle of the rat. The number of fast myosin fibers, hypertrophied fibers and the presence of small fibers are characteristic of these over-loaded muscles.

It is surprising to see increased numbers of fast twitch fibers in over-loaded soleus muscles. However, following heavy resistance exercise training, more fast fibers were found in deep region of the rat rectus femoris muscles [12]. In the soleus muscle, these fast fibers were apparently the same size as the slow fibers and would be expected to function normally. Since it is generally recognized that activity determines the myosin isoform (e.g. decreased activity, as occurs in hindlimb unloading or spinal cord transection [13], produces more fast fibers in the rat soleus muscles), the increase in fast fibers in over-loaded muscles supports the concept that both activity and load may regulate myosin isoforms. This observation has interesting functional implications if the motor neuron pool has also changed so that some of the soleus muscle behaves as if it were the gastrocnemius in its role as an ankle planter flexor. In contrast, repeated use of the rat plantaris, a fast muscle, leads to the development of more slow fibers [14] consistent with the role of activity in myosin isoform development.

Effects of chronic over-activity

Increased activity can result from spasticity, chronic electrical stimulation, tetanus toxin administration, and some types of exercise training (e.g. running). Spasticity in

rats [15] and cerebral palsy in humans [16] were characterized by an increase in the number of slow muscle fibers with myofiber hypertrophy observed in humans [16] from apparent over-use.

In rats treated with tetanus toxin, unique myopathic changes have been reported [17]. Tetanus toxin produces muscles which are continually active but also held in a shortened, immobilized state because the antagonist muscles are unaffected. The muscle fibers atrophy and contain spheroid cytoplasmic bodies which consist of granular material forming a dense central core with radiating filaments [17]. This was particularly true for the rat plantaris muscle. The radiating filaments consisted of granular material of the same density as the Z-band material and stained for both desmin and actin. Thus, some reorganization of the intermediate filament system may result from chronic over-activity in the shortened position.

Perhaps the best studied model of chronic over-activity is the use of electrical nerve stimulation [18] which has long been used by physiologists to study activity-related adaptation in skeletal muscles. Here there is a clearly defined time course of adaptation beginning with the sarcoplasmic reticulum [19], followed by changes in muscle mitochondria and capillary growth [20], and finally altered sarcomere chemistry [21] and morphology [22]. Although there have been reports of fast-fiber degeneration [6], the myosin transformations can be observed within existing myofibers [23].

Evidence of injury can be seen in samples from chronically overloaded muscles [6] including necrotic and regenerating fibers. If muscles can become depleted of energy or large breaks occur in the plasma membrane, then altered calcium homeostasis can lead to cell death [24]. Subsequently, the necrotic tissue is removed by phagocytic cells, primarily macrophages. Then, activated satellite cells migrate to the injured area and become myoblasts. Together with other myoblasts, they fuse to become small myotubes and eventually fully functional fibers [7]. Apparently these regenerated fibers become innervated and functional. It is interesting to note that the reinnervation process has not been studied in injured muscles.

Cellular response to injury

Release of intracellular proteins such as creatine kinase [25] and myoglobin [26] and the entry of extracellular proteins such as albumin [4] have been useful in monitoring myofiber damage resulting from cell membrane damage. However, differences in time course of injury and appearance of these markers can make interpretation of myofiber injury difficult [25]. In addition, small membrane breaks sufficient to alter calcium homeostasis could exist and not lead to translocation of proteins but still result in calcium over-load. The use of small probes such as procion yellow [27] can localize damaged myofibers where calcium entry can occur. The use of the muscle specific protein, dystro-

phin, to localize ruptures in the muscle membrane has proven difficult except when large perturbations are present [28].

Membrane damage could involve the transverse tubules or sarcoplasmic reticulum. In either case, excitation-contraction coupling would be altered. In studies with mice, the electrical properties of the sarcolemma were not altered after myofiber strain injury [29]. However, a force deficit was still observed. Using caffeine to release calcium from the sarcoplasmic reticulum (SR), the deficit was not observed. Thus, some step between action potential generation and calcium release from the SR was altered by strain injury. The most likely anatomical site would be the junction between the t-tubule and the sarcoplasmic reticulum. Damage to the t-tubule was documented by the redistribution of cell membrane probes and the increase of free calcium concentration following injury [30]. However, these membrane lesions have proven difficult to resolve with great certainty.

In contrast, intracellular damage in response to injury is easily identified by altered sarcomere structure. The sarcomere may be pulled apart or lack a well-defined Z disc [5]. Some of the alterations in sarcomere structure are the result of disrupted intermediate filaments due to proteolytic attack or mechanical damage. Both transverse and longitudinal structures, which serve to hold the sarcomeres and filaments in alignment, are altered as both desmin [31] and titin [32] staining are abnormal following injury.

The functional outcome of muscle damage is a force deficit which does not recover with rest sufficient to restore function after fatigue but requires several days [33]. The altered sarcomere structure and rupture of elastic filaments could lead to the decrease in force generation [32]. The time required for force recovery [33] is consistent with the hypothesis that new proteins must be synthesized and organized into functional sarcomeres or membranes (i.e. repair or regeneration).

Infiltrating cells

It has generally been accepted that tissue damage including skeletal muscle leads to some cellular degeneration and infiltration by mononuclear cells to remove cellular debris [7]. Although macrophages are present in injured muscles, they do not account for the majority of the cells seen. Using specific antibodies, other mononuclear cells such as lymphocytes have been localized in regions of muscle injury [34]. Taken together, macrophages, monocytes and lymphoid cells still represent only a small fraction of the mononuclear cells present. However, these cells would produce a wide variety of important cytokines responsible for inflammation, cell proliferation, cell migration, and regeneration. One example is the ability of macrophages to regulate proliferation and differentiation of satellite cells *in vitro* [35]. However, even in the absence of macrophages, the response of muscles to strain injury was unaltered [36].

When specific markers for mesenchymal cells such as vimentin were used, the majority of the cells in the regions, which demonstrated cellular proliferation or infiltration, stained positively. Thus, they were either fibroblasts, satellite cells or myoblasts. Using double labelling techniques, small mononuclear cells can be identified as myoblasts by the presence of both desmin and vimentin. This combination of these two intermediate filament proteins has been used as a marker for regeneration of muscle fibers [37]. The localization of vimentin within normal myofibers also reveals activated satellite cells [37]. Thus, the small cells in the extracellular matrix which stained for both desmin and vimentin were satellite cells, myoblasts or myotubes and those that stained for both desmin and dystrophin [28] were myoblasts, myotubes or myofibers. Cells which stained only for vimentin were fibroblasts. Thus, the components of the cytoskeleton of skeletal muscle cells serve as useful markers for muscle-specific cells.

Satellite cells and myogenesis

Satellite cells are activated following injury and may account for the increased number of myonuclei seen in muscles following over-loading [38] and injury [34]. Satellite cells can enter into mitosis after injury due to the action of specific growth factors [39] or proliferate to form additional satellite cells [40]. However, the majority of satellite cells, which participate in the hypertrophied muscle, are committed before the injury is applied [40]. Many, but not all, of the processes involved in myogenesis are active following injury. Thus, injury must activate the myogenic regulatory factors [41]. Although bromodeoxyuridine is present in cell nuclei [40] indicative of increased nucleic acid synthesis, it is curious that evidence of cell division is rarely, if ever, seen.

Regeneration versus myofiber splitting

In standard biopsy samples, it is often difficult to distinguish between: (1) regeneration which produces new small myofibers and (2) fiber branching (splitting) which results in small fibers derived from an existing myofiber. Branching fibers can be identified by nitric acid digestion [38] which removes the connective tissue but leaves the myofibers intact. Branched fibers were observed in muscles from surgically over-loaded [11] and exercise-trained [42] rats. In muscle samples which are viewed in cross-section, branching fibers can be seen grouped together in the space occupied by the parent fiber [42] whereas regenerating fibers can exist as individual fibers at some distance from each other.

If each new fiber, which has split from its parent, has an extracellular matrix of normal dimensions and content surrounding it, then the entire muscle has more connective

tissue to resist further injury. The possibility that fiber branching or splitting reduces strain at sites along the myofiber where the strain is concentrated would be a unique adaptation for these over-loaded muscles.

Extracellular matrix (ECM) expansion and composition

One of the cellular responses to injury is a change in the composition and quantity of the extracellular matrix surrounding cells in injured muscles [1, 34, 43]. This sequence following injury is similar to that seen during embryogenesis [44]. First a loss of chondroitin-6-sulfate proteoglycan (PG) due to action of hydrolytic enzymes indicating matrix degeneration. Then, chondroitin-6-sulfate PG reappears 4 days later around small fibers indicating that they are post-fusion myotubes [45]. In contrast, heparan sulfate PG remains after injury and serves as a tube into which the newly constructed myofiber can grow [1].

In chronically over-loaded muscles, the matrix expands and surrounds small fibers as well as existing myofibers [46]. In extreme cases, fibrosis can result [46]. Fibrosis results from a deposition of collagen Type I around the myofibers. Fibrosis is a normal response to repeated cell injury and muscle is no exception [46].

Response to chronic injury

Chronic injuries are more difficult to study because skeletal muscle adapts well to stress. However a few models are available such as repeated toxin injections [47] and repeated strains in animals [48] and post-polio syndrome in humans [49]. In each of these, repeated injury is the causative agent in muscle dysfunction with time.

Recently, we have used methods of over-loading in the rat which would mimic exertion-induced injury and minimize the strain over-load (eccentric muscle actions). Two such models are presented here: (1) the tenotomized plantar flexor model where the functional demand (load) on the soleus and plantaris muscles is increased by removal of the action of the gastrocnemius; (2) the adaptive over-load model where one limb has to compensate for the immobilization of the other foot caused by tetanus toxin injection. Tenotomy is produced by cutting the tendon of the gastrocnemius and removing a small length of the distal portion. This surgical procedure removes about 80% of the overall force output of the plantar flexor muscles of the rat. The soleus and plantaris muscles must compensate for the loss of the gastrocnemius but are also stretched by the powerful antagonists. This combination of over-load and stretch may produce different results than other types of over-loading.

The use of tetanus toxin to produce an immobilized hindlimb [17] that must be supported by the untreated limb is a unique model of functional over-load, although not

unlike taping the leg to the body in a flexed position [50]. Both require the animal to ambulate with three legs but the tetanus treated animal has a greater load due to the extended hindlimb. The dose of tetanus is selected so that it only effects the injected limb and tetany lasts for about 4 weeks. Although eccentric muscle actions were minimized in this model, the soleus and plantaris would be expected to perform some activities in which lengthening under load occurred.

All of the cellular responses described above can be seen to some degree in animals subjected to either technique of over-loading. Some specific examples will be highlighted.

Myofibers

Over-loading the soleus by removal of the action of the plantaris and gastrocnemius muscles for two weeks produced a typical injury response (Fig. 1). First, the apparent number of fibers staining for fast myosin isoforms increased. Second, a common pattern with over-load injury was the presence of large (Fig. 1C, 4635 μm^2 , $n = 5$) and small myofibers (Fig. 1C, arrow) along with apparent degenerating myofibers (i.e. fibers with weak myosin stain but which lack laminin, Fig. 1 asterisk). In the groups of small fibers, a heterogeneity in myosin isoform was noted indicating that differential expression of myosin resulted from branching of the same fiber (Fig. 1D, arrowhead). Other areas with grouping of small fibers revealed homogeneity of stain (Fig. 1D, arrow). However, without a cell boundary marker (e.g. laminin), this group of fibers could be mistaken for a single fiber. Thus, it is evident that fiber branching and the development of new, very small myofibers can co-exist in over-loaded muscles.

The distal location of branching fibers in the soleus muscle from tenotomized animals as in Fig. 1 was hypothesized to result from the reattachment of the gastrocnemius muscle onto the proximal end of the soleus and reducing strain in that area [11]. However, in the functionally over-loaded limb, the distal end of the soleus also had more fibers even though the gastrocnemius remained intact. It is possible that the distal end of the muscle is susceptible to greater injury for all types of over-load.

Proliferation of cells in over-loaded muscles can be seen in Figs 2 and 3. A common feature of these muscle samples is an increase in desmin staining in small cells and along the border of large cells (Fig. 2B, arrow) some of which were hypertrophied. Central nuclei were a common feature in these muscles with some nuclei surrounded by radiating material (Fig. 2C).

Extracellular matrix

In regions with marked increases in extracellular material following injury, fibronectin was present (Fig. 3D). Since

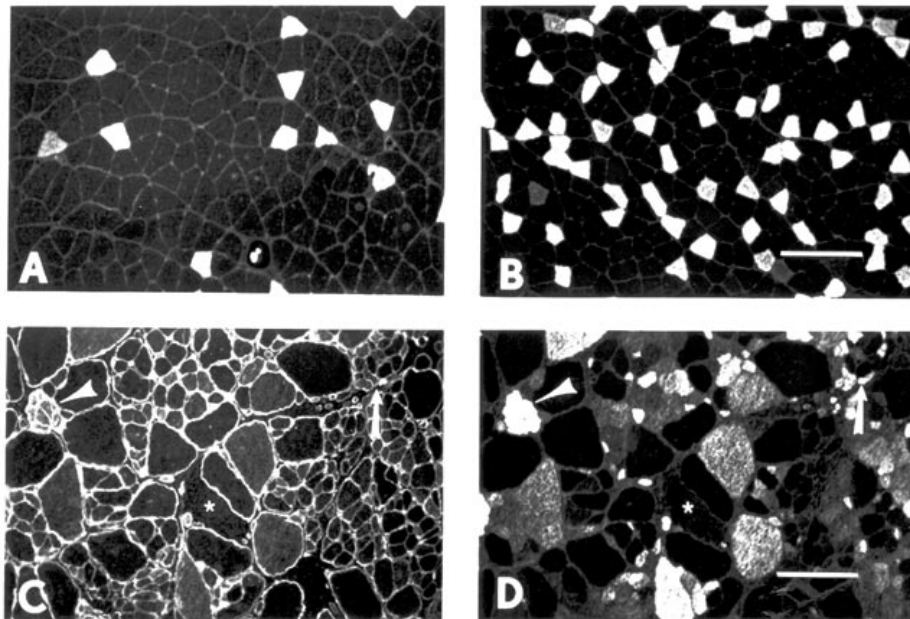


Fig. 1. Over-loaded soleus muscles from a rat with the tendon of one gastrocnemius severed (tenotomy). (A) Immunohistochemistry of fast myosin; Control soleus. (B) Immunohistochemistry of fast myosin; Overloaded soleus. Bar = 200 μ m. (C) Immunohistochemistry of laminin; Overloaded soleus. Arrowhead = cluster of fibers with the same myosin isoform. Arrow = cluster of fibers with multiple myosin isoforms. (D) Immunohistochemistry of fast myosin; Over-loaded soleus. Arrowhead = cluster of fibers with the same myosin isoform. Arrow = cluster of fibers with multiple myosin isoforms. Bar = 100 μ m. Note: Asterisk represents a degenerating myofiber; C and D are double-labelled on one muscle section.

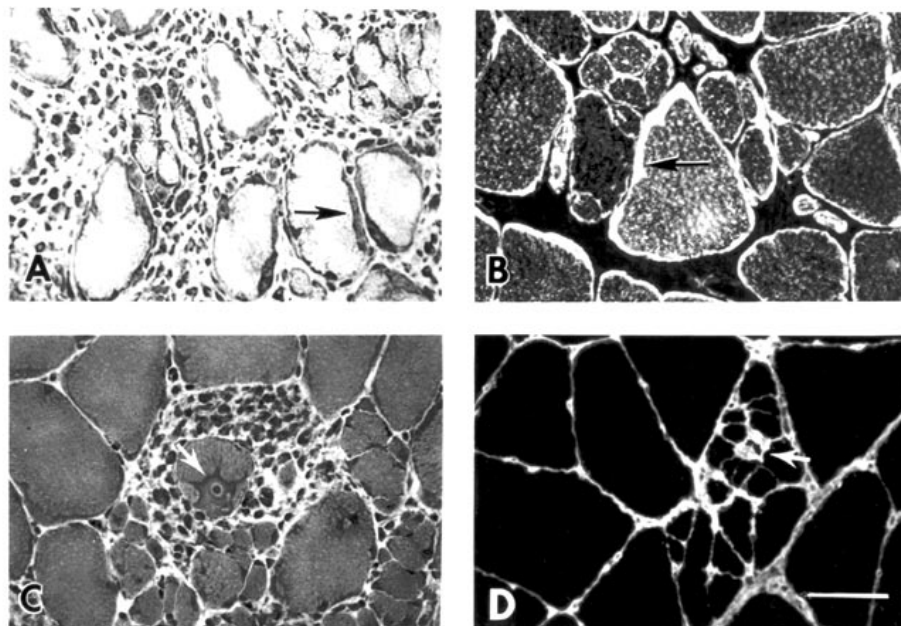


Fig. 2. Soleus muscles from a rat with the tendon of one gastrocnemius severed (tenotomy). (A) Azure A stain; Over-load, Arrow = cytoplasmic area of positive material. (B) Immunohistochemistry of desmin; Over-load, area of intense desmin positive material. (C) Azure A stain; Over-load, Arrow = nucleus with radiations. (D) Immunohistochemistry of fibronectin; Over-load, Arrow = central deposit of fibronectin with radiations. Bar = 50 μ m.

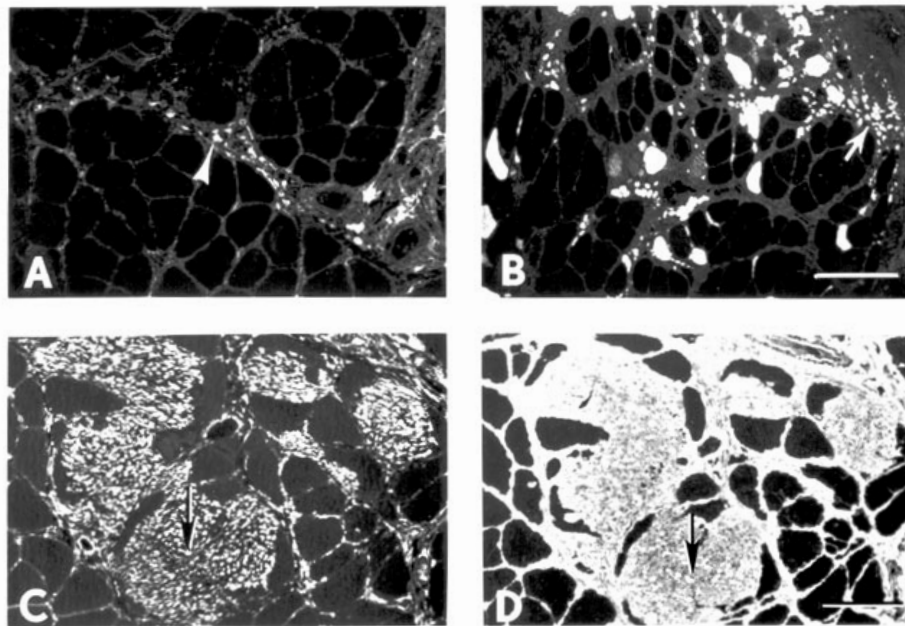


Fig. 3. Soleus muscle from a rat with the contralateral limb injected with tetanus toxin thus over-loading the opposite limb. (A) Immunohistochemistry of a macrophage cell-surface antigen; Over-loaded soleus, Arrowhead = points to macrophage in the extracellular matrix. (B) Immunohistochemistry of fast myosin; Over-loaded soleus, Arrow = points to small presumptive myoblasts or myotubes. Bar = 200 μ m (C) Immunohistochemistry of vimentin; Over-loaded soleus, Arrows indicate fibroblasts. (D) Immunohistochemistry of fibronectin; Over-loaded soleus, Arrows indicate fibroblasts. Bar = 100 μ m. C and D are double-labelled on one muscle section.

the antibody used to detect fibronectin does not distinguish between plasma fibronectin and tissue fibronectin [51, 52], the source of the fibronectin cannot be definitively identified. However, since no disruption of the vascular system occurred and small fibronectin positive cells can be seen (Fig. 3D, arrows) in the extracellular matrix which also stained for vimentin (Fig. 3C, arrow), secretion of fibronectin by fibroblasts seems the most probable source. However, some myofibers could be identified with intracellular localization of fibronectin (Figs 2D and 3C). Intracellular deposits of fibronectin have been reported earlier [53] but the significance of such an intracellular location remains unexplained. The similarity of the fibronectin staining with that of a nucleus with prominent radiations raises the possibility that the migration of the nucleus to a more central position precedes the formation of splits and that fibronectin is somehow involved.

In addition to fibronectin, the expanded extracellular matrix contains the usual constituents such as collagens I and III, proteoglycans, and glycoproteins. Some of these proteins are growth factors such as fibroblast growth factor [54] and some proteins are translocated from blood such as albumin and IgG [55]. The presence of both cells and matrix components supports the concept that the expansion of matrix following exertion-induced injury is not due to edema but to cell proliferation/migration and increased secretion of matrix material. Although some inflammatory cells such as macrophages are present (Fig. 3A, arrowhead), the majority

of cells are fibroblasts (Fig. 3C) and small myoblasts (Fig. 3B, arrow). The adaptive role of the expanded matrix and the fate of the small cells has not been adequately addressed in injured muscles. However, it is assumed that the process is the same as that described for muscle regeneration after mincing [7].

Summary

Skeletal muscle injury caused by over-load or over-activity can result in muscle degeneration and regeneration. Over-load, where the force exceeds the functional or habitual output of the muscles, is a common occurrence during work-induced hypertrophy. Over-activity is characterized by an increase in the firing rates of motor neurons beyond their physiological levels as seen in spasticity or spasm. The response to overexertion of either type is complex and often results in tissue injury. Cell proliferation or migration is a characteristic response to muscle injury but many of the mononuclear cells remain poorly defined. Some of the mononuclear cells arise from existing myofibers by fiber splitting or de novo by myogenesis and can be identified by the use of cell-specific markers and double labelling techniques. For example, myoblasts contain both desmin and vimentin and can be distinguished from fibroblasts which

stain for fibronectin and vimentin; no specific stain is unique to myofiber splitting. We have observed clusters of small fibers containing a single myosin isoform together with radiations of extracellular matrix material from a central nucleus which may identify myofiber splitting. However, the cellular processes responsible for myofiber splitting in response to muscle over-load and the functional outcome of fiber splitting remain unknown.

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References

1. Fritz VK, Stauber W: Characterization of muscles injured by forced lengthening. II. Proteoglycans. *Med Sci Sports Exerc* 20: 354–361, 1988
2. Stauber WT, Clarkson PM, Fritz VK, Evans WJ: Extracellular matrix disruption and pain after eccentric muscle action. *J Appl Physiol* 69: 868–874, 1990
3. Gibala MJ, MacDougall JD, Tarnopolsky MA, Stauber WT, Elorriaga A: Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. *J Appl Physiol* 78(2): 702–708, 1995
4. McNeil PL, Khakee R: Disruptions of muscle fiber plasma membranes. *Am J Pathol* 140: 1097–1109, 1992
5. Ogilvie RW, Armstrong RB, Baird KE, Bottoms CL: Lesions in the rat soleus muscle following eccentrically biased exercise. *Am J Anat* 182: 335–346, 1988
6. Maier A, Gambke B, Pette D: Degeneration-regeneration as a mechanism contributing to the fast to slow conversion of chronically stimulated fast-twitch rabbit muscle. *Cell Tissue Res* 244: 635–643, 1986
7. Carlson BM, Faulkner JA: The regeneration of skeletal muscle fibers following injury: A review. *Med Sci Sports Exerc* 15: 187–198, 1983
8. Goldspink D: Exercise-related changes in protein turnover in mammalian striated muscle. *J Exp Biol* 160: 127–148, 1991
9. James NT: Compensatory hypertrophy in the extensor digitorum longus muscle of the rat. *J Anat* 116: 57–65, 1973
10. James NT: Compensatory hypertrophy in the extensor digitorum longus muscle of the mouse. *J Anat* 122: 121–131, 1976
11. Vaughan HS, Goldspink G: Fiber number and fiber size in a surgically over-loaded muscle. *J Anat* 129: 293–303, 1979
12. Yarasheski KE, Lemon PWR, Gilloteaux J: Effect of heavy-resistance exercise training on muscle fiber composition in young rats. *J Appl Physiol* 69: 434–437, 1990
13. Leiber RL, Friden JO, Hargens AR, Feringa ER: Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. II. Morphometric properties. *Exp Neurol* 91: 435–448, 1986
14. Degens H, Turek Z, Hoofd LJC, Van't Hof MA, Binkhorst RA: The relationship between capillarization and fiber types during compensatory hypertrophy of the plantaris muscle in the rat. *J Anat* 180: 455–463, 1992
15. Mano Y, Mano K, Mayer RF, Deshpande SS, Albuquerque EX: Effects of paraplegia produced by intrathecal 6-aminonicotinimide on motor units in the rat. *Exp Neurol* 65: 435–456, 1979
16. Castle ME, Reyman TA, Schneider M: Pathology of spastic muscle in cerebral palsy. *Clin Ortho* 142: 223–233, 1979
17. Chou SM, Mizuno Y: Induction of spheroid cytoplasmic bodies in a rat muscle by local tetanus. *Muscle Nerve* 9: 455–464, 1986
18. Pette D: Activity-induced fast to slow transitions in mammalian muscle. *Med Sci Sports Exerc* 16: 517–528, 1984
19. Heilmann C, Pette D: Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation. *Eur J Biochem* 93: 437–446, 1979
20. Reichmann H, Hoppeler H, Mathieu-Costello O, von Bergen F, Pette D: Biochemical and ultrastructural changes of skeletal muscle mitochondria after chronic electrical stimulation in rabbits. *Pflugers Arch* 404: 1–9, 1985
21. Brown WE, Salmons S, Whalen RG: Mechanisms underlying the asynchronous replacement of myosin light chain isoforms during stimulation-induced fiber-type transformations of skeletal muscle. *FEBS* 192: 235–237, 1985
22. Eisenberg BR, Salmons S: The reorganization of subcellular structure in muscle undergoing fast-to-slow fiber transformation. A stereological study. *Cell Tissue Res* 220: 449–471, 1981
23. Staron RS, Gohlsch B, Pette D: Myosin polymorphism in single fibers of chronically stimulated rabbit fast-twitch muscle. *Pflugers Arch* 408: 444–450, 1987
24. Wrogemann K, Penna SDJ: Mitochondrial calcium over-load: a general mechanism for cell-necrosis in muscle diseases. *Lancet* 1: 672–674, 1976
25. Komulainen J, Kytola J, Vihko V: Running-induced muscle injury and myocellular enzyme release in rats. *J Appl Physiol* 77: 2299–2304, 1994
26. Vuori J, Rasi S, Takala T, Vaananen K: Dual-label time-resolved fluoroimmunoassay for simultaneous detection of myoglobin and carbonic anhydrase III in serum. *Clin Chem* 37: 2087–2092, 1991
27. Bradley WG, Fulthorpe JJ: Studies of sarcolemmal integrity in myopathic muscle. *Neurology* 28: 670–677, 1976
28. Stauber WT, Miller GR, Gibala MJ, MacDougall JD: Use of double labelling and photo CD for morphometric analysis of injured skeletal muscle. *J Histochem Cytochem* 43: 1179–1184, 1995
29. Warren GL, Lowe DA, Hayes DH, Karwowski CJ, Prior BM, Armstrong RB: Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *J Physiol* 468: 487–499, 1993
30. Warren GL, Lowe DA, Hayes DH, Farmer MA, Armstrong RB: Redistribution of cell membranes probes following contraction-induced injury of mouse soleus muscle. *Cell Tissue Res* 282: 311–320, 1995
31. Lieber RL, Schmitz MC, Misha DK, Friden J: Contractile and cellular remodelling in rabbit muscle after cyclic eccentric contractions. *J Appl Physiol* 77: 1926–1934, 1994
32. Crenshaw AG, Thornell I-E, Friden J: Intramuscular pressure, torque and swelling for the exercise-induced sore vastus lateralis muscle. *Acta Physiol Scand* 152: 265–277, 1994
33. Faulkner JA, Jones DA, Round JM: Injury to skeletal muscles of mice by forced lengthening during contractions. *Quart J Exp Physiol* 74: 661–670, 1989
34. Stauber WT, Fritz VK, Vogelbach DW, Dahimann B: Characterization of muscles injured by forced lengthening. I. Cellular infiltrates. *Med Sci Sports Exerc* 20: 345–353, 1988
35. Cantini M, Massimino ML, Brusson A, Catani C, Libera LD, Carraro U: Macrophages regulate proliferation and differentiation of satellite cells. *Biochem Biophys Res Commun* 202: 1688–1696, 1994
36. Lowe DA, Warren GL, Ingalls CP, Boorstein DB, Armstrong RB: Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *J Appl Physiol* 79: 1260–1270, 1995
37. Bornemann A, Schmalbruch H: Desmin and vimentin in regenerating muscles. *Muscle Nerve* 15: 14–20, 1992
38. Reitsma W: Skeletal muscle hypertrophy after heavy exercise in rats with surgically reduced muscle function. *Am J Phys Med* 48: 237–258, 1969

39. Clegg CH, Linhart A, Olwin BB, Hauschka SD: Growth factor control of skeletal muscle differentiation occurs in G1 phase and is repressed by fibroblast growth factor. *J Cell Biol* 105: 949–956, 1987
40. Rantanen J, Hurme T, Lukka R, Heino J, Kalimo H: Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: Evidence for two different populations of satellite cells. *Lab Invest* 72: 341–347, 1995
41. Eppley ZA, Kim J, Russell B: A myogenic regulatory gene, *gmfl*, is expressed by adult myonuclei after injury. *Am J Physiol* 265: C397–C405, 1993
42. Tamaki T, Uchiyama S, Nakano S: A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. *Med Sci Sports Exerc* 24: 881–886, 1992
43. Stauber WT, Fritz VK, Dahimann B: Extracellular matrix changes following blunt trauma to rat skeletal muscles. *Exp Mol Pathol* 52: 69–86, 1990
44. Gulati AK, Reddi AH, Salewski AA: Changes in the basement membrane zone components during skeletal muscle fiber degeneration and regeneration. *J Cell Biol* 97: 957–962, 1983
45. Hutchison CJ, Yasin R: Developmental changes in sulphation of chondroitin sulphate proteoglycan during myogenesis of human muscle cultures. *Dev Biol* 115: 78–83, 1986
46. Stauber WT, Knack KK, Miller GR, Grimmer JG: Fibrosis and intercellular collagen connections from four weeks of muscle strains. *Muscle Nerve* 19: 423–430, 1996
47. Sadeh M, Czyzewski K, Stern LZ: Chronic myopathy induced by repeated bupivacaine injections. *J Neurol Sci* 67: 229–237, 1985
48. Stauber WT, Miller GR, Grimmer JG, Knack KK: Adaptation of rat soleus muscle to 4 week of intermittent strain. *J Appl Physiol* 77: 58–62, 1994
49. Perry J, Barnes G, Gronley JK: The postpolio syndrome. An overuse phenomenon. *Clin Ortho* 233: 145–162, 1988
50. Jee WSS, Li XJ, Schaffler MB: Adaptation of diaphyseal structure with aging and increased mechanical usage in the adult rat: A histomorphometrical and biomechanical study. *Anat Rec* 230: 332–337, 1991
51. Gulati AK, Reddi AH, Zalewski AA: Distribution of fibronectin in normal and regenerating skeletal muscle. *Anat Rec* 204: 175–183, 1982
52. Kleiman HK, Klebe RJ, Martin GR: Role of collagenous matrices in the adhesion and growth of cells. *J Cell Biol* 88: 473–485, 1981
53. Friden J, Lieber RL, Thornell L-E: Subtle indications of muscle damage following eccentric contractions. *Acta Physiol Scand* 142: 523–524, 1991
54. Yamada S, Buffinger N, DiMario J, Strohman RC: Fibroblast growth factor is stored in fiber extracellular matrix and plays a role in regulating muscle hypertrophy. *Med Sci Sports Exerc* 21: S173–S180, 1989
55. Stauber WT, Ong SH: Extravascular localization of albumin and IgG in normal tonic and phasic muscles of the rat. *Histochem J* 13: 1009–1015, 1981