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Loss of dystrophin and some dystrophin-associated proteins with concomitant signs of apoptosis in rat leg muscle overworked in extension

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Abstract This study investigated the basis for the high severity of damage to skeletal muscle due to eccentric exercise, i.e., to muscles generating force while lengthened. Fast and slow rat leg muscles maintained in an extended position were examined after 2–24 h of continuous stimulation. The treatment caused the injury to some regions of both muscles. Within the better preserved parts of the muscles, i.e., those without signs of necrotic processes, dystrophin, spectrin, and some of the dystrophin-associated proteins (β-dystroglycan, α-sarcoglycan, and γ-sarcoglycan) disappeared from sarcolemma of many fibers. The reduction or loss of dystrophin from the sarcolemma was more evident than that of other proteins examined, with sarcoglycans apparently being the most preserved. Several muscle fibers devoid of dystrophin contained apoptotic nuclei. Simultaneously, Bax, Bcl-2 and caspase-3 proteins appeared in many fibers. Our results indicate that a normal muscle overworking in an extended position undergoes the loss of several membrane skeletal proteins because of the excessive stress to the membrane cytoskeleton, which can lead to fiber death by either apoptosis or necrosis. This experimental model may represent a good model for mimicking the pathogenetic events in several muscular dystrophies.

Key words Skeletal muscle · Eccentric exercise · Apoptosis · Dystrophin · Dystrophin-associated proteins

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Introduction

During normal physical exercise skeletal muscle fibers transmit tension loads from contracting myofibrils to the cytoskeleton and eventually to the extracellular matrix via transmembrane structures at the sarcolemma [32]. In many cells, such structures are identified as the focal adhesion complexes (FAC), where transmembrane force transfer occurs through integrins with the recruitment of downstream signaling molecules, such as vinculin, talin, α-actinin and integrin-dependent kinases [9, 15, 40]. In skeletal muscle these structures have not been precisely identified, but the ordinate costameric distribution of dystrophin and associated proteins [25, 27, 35] suggests that these may represent the muscular correlate of FAC. Dystrophin and the tightly associated sub-complexes of proteins, dystroglycans, sarcoglycans and syntrophins, form at sarcolemma a membrane-skeleton structure comprising the mechanical link between actin peripheral cytoskeleton and the laminin extracellular matrix [11, 26, 31]. However, other proteins are known to be associated to the dystrophin complex: cytoskeletal proteins, such as spectrin, vinculin, talin, α-actinin, and integrins, and non-cytoskeletal proteins, e.g., those involved in the voltage-gated sodium channel, nNOS, and Grb2 [16, 23, 45, 46]. Interestingly, a direct interaction between sarcoglycans and integrins has been recently demonstrated [47]. Thus, the dystrophin complex represents both a membrane structure with mechanical functions [11, 33] and a scaffold to locate sarcolemma signaling proteins [45]. Consistent with the important role of the dystrophin complex at the sarcolemma of skeletal muscle, the loss of dystrophin and of some of the associated proteins, such as α- and γ-sarcoglycan, represents the primary pathogenetic event leading to severe muscular dystrophies [30, 36, 48].

Mechanical overload, commonly known to increases the mass of skeletal muscle, has been postulated to be dependent on contractile solicitation of cytoskeletal elements [19]. However, abnormal or excessive exercise may causes severe alterations to the muscle leading to immedi-

ate and prolonged reduction in the capacity to generate muscle force [12, 24]. For example, sustained eccentric exercise, i.e., repeated contractions during which a muscle is extended, is known to cause severe injury to the muscle, associated with profound disarrangement of the myofibrillar and cytoskeletal architectures [13, 20]. It is worth noting that excessive stimulation of cytoskeletal elements represents a stimulus sufficient to cause apoptosis in many cells [14]. Apoptosis is a physiological form of cell death by which organisms eliminate redundant, damaged or infected cells [8]. The death program is accomplished by activating specific machinery that includes pro-apoptotic and anti-apoptotic proteins, such as Bax and Bcl-2, respectively, and a family of cysteine proteases collectively known as caspases [1, 8].

The occurrence of apoptosis in skeletal muscle fibers is well established, even though the mechanism activating the programmed cell death has not been elucidated [17, 29, 34, 37, 42, 43]. However, morphological features of apoptosis are only rarely seen in mature skeletal muscle (A. Fidzianska, personal communication). Consistent with a possible role of the dystrophin complex in activating apoptosis in skeletal muscle, signs of apoptotic process have been detected in some human skeletal muscle dystrophies, such as Duchenne muscular dystrophy, α-sarcoglycan-deficient and merosin-deficient muscular dystrophies, and other limb-girdle muscular dystrophies [4, 38, 41].

Here we show that stimulation of muscles in extend positions represents a suitable experimental protocol for evaluating the sequential events involved in damage production. We confirm and extend previous observations proposing that the dystrophin complex is a sensor for the excessive abnormal contractile activity of skeletal muscle [6]. Moreover, we demonstrate that dystrophin disassembly is accompanied by that of β-dystroglycan and α-sarcoglycan. Finally, we show that fibers deprived of dystrophin undergoes apoptosis in parallel to an overexpression of Bax, Bcl-2 and caspase-3, which are key components of the apoptotic pathway.

Materials and methods

Three-month-old female albino Wistar rats were used. Soleus and extensor digitorum longus (EDL) muscles were set in an extended position by immobilization of the ankle joint in a plastic tube at an angle of 90 or 160°, respectively. Stimulating electrodes were implanted on the sciatic nerve, under pentobarbital and ether anesthesia, a few days before the experiment. The sciatic nerve was stimulated continuously for 2, 4, 6 or 24 h by pulses of 0.3 ms duration and 20 Hz frequency. Untreated muscles, muscles that were immobilized in extension but not stimulated, and muscles that were stimulated but without joint immobilization were used as controls. Each experiment was performed at least in triplicate, with a total of about 50 animals.

Immediately after decapitation, each muscle was excised (taking care to maintain it in the resting or experimental length), measured, attached to a plastic rod and frozen in isopentane cooled in liquid nitrogen. Muscles were then stored at –70°C. Histological examination was performed on transverse cryostat sections by staining with hematoxylin and eosin.

Immunohistochemistry

An immunofluorescence study was performed on 8-μm cryostat transverse sections of the muscle as previously described [6]. The sections were incubated for 1 h at room temperature with monoclonal antibodies specific for dystrophin (1:300), β-dystroglycan (1:50), α-sarcoglycan (1:20), spectrin (1:50), γ-sarcoglycan (1:50) (all obtained from Novocastra Laboratories, Newcastle-upon-Tyne, UK) and with rabbit polyclonal antibodies against caspase-3 (H-277), Bcl-2 (C-21) and Bax (M.-20) (all from Santa Cruz Biotechnology, Santa Cruz, Calif.) diluted 1:50. Frozen transverse sections were collected on polylysine-coated glass slides. The sections were incubated with 1% BSA (or 5% normal goat serum) in PBS for 30 min. The slides were then incubated 1 h with primary antibodies diluted in PBS. After two 5-min washings with PBS, the slides were incubated with the secondary antibody. Monoclonal antibody-treated samples were incubated for 1 h at 37°C with anti-mouse rhodamine conjugated antibody (Dako, 1:100), whereas the polyclonal antibody-treated samples were incubated with a biotin-conjugated goat anti-rabbit Ig (1:250 diluted in 1% BSA) for 1 h at 37°C, followed by 1 h in streptavidin-Cy3 (1:250 diluted in 1% BSA). After three 10-min washing steps, slides were mounted in Elvanol and photographed on a Zeiss Axiophot epifluorescence microscope. Negative controls were performed by omitting the primary antibody.

Double labeling with antibodies and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)

Double labeling with anti-dystrophin or anti-α-sarcoglycan antibodies was performed before TUNEL labeling. Cryostat sections were first processed with the selected monoclonal antibody followed by the secondary antibody (see above), then washed in PBS, fixed with 2% paraformaldehyde for 10 min and, after an additional 10-min washing in PBS, permeabilized by treatment with 0.1% Triton X-100 and 0.1% sodium citrate for 30 min. The in situ nick end-labeling of fragmented DNA (TUNEL) was performed using terminal deoxynucleotidyl transferase with fluorescein-conjugated nucleotides as described by the manufacturer (in situ Cell Death Detection Kit-POD, Roche), as previously described [6]. Quantification of TUNEL-positive myonuclei was performed as previously described [34].

Results

Soleus and EDL muscles were continuously stimulated in an extended position for 2–24 h. Several area from 6- to 24-h-stimulated muscles showed edema, variability of fiber dimension, and cell damage (Fig. 1). The effects of stimulation were less evident after 2–4 h, with variability in the response and a limited severity, so that these muscles were not considered in the study. After 24-h stimulation, fiber damage was extended to many regions of the muscle, showing the presence of clear signs of necrosis, as demonstrated by the presence of infiltrating mononuclear cells (Fig. 1D), consistently with previous findings [6]. These findings were the same in hematoxylin-eosin-stained muscles (Fig. 1), after immunostaining, and at the ultrastructural level [20, 21].

Within one experimental muscle, regions showing a severe damage, i.e., containing numerous necrotic fibers, were commonly observed together with regions where the fibers appeared well preserved. However, careful inspection of the normal-looking area of stimulated muscles

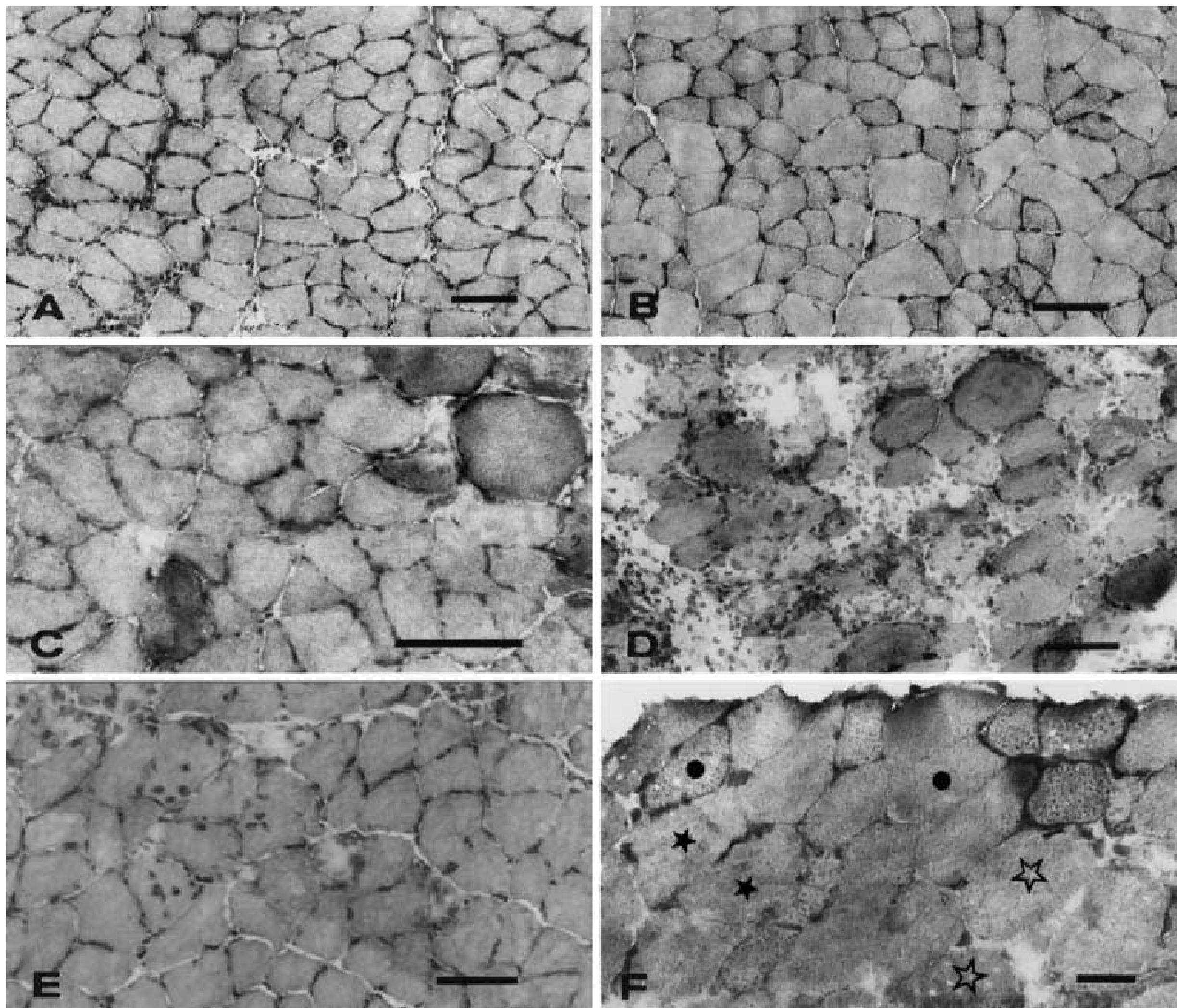

Fig. 1 Hematoxylin and eosin staining of cryostat sections from rat soleus (**A**, **C–E**) and EDL (**B**, **F**) muscles. Control soleus (**A**) and EDL muscles (**B**); soleus muscle stimulated during extension for 6 h (**C**) and for 24 h (**D**, **E**); EDL stimulated during extension for 24 h (**F**, this picture is a serial section of those shown in Fig. 3 **D–F**). Irregularity of dimension and shape of some of the fibers and diversity of intensity staining can be seen (**C–F**); the presence of damaged fibers and infiltration by mononucleated cells is also evident (**C–E**). *Bars* **A**, **B** 50 μm; **C–E** 25 μm

showed that many of the fibers did not contain dystrophin at the sarcolemma or that this protein was present only discontinuously (Figs. 2 A, 3 A, D). Analysis of serial sections from 6-h-stimulated soleus muscle revealed that β-dystroglycan (Fig. 2 B), spectrin (Fig. 2 D) and γ-sarcoglycan (Fig. 2 E) were also absent from the sarcolemma, even though these proteins appeared to be more preserved than dystrophin. In contrast, α-sarcoglycan (Fig. 2 C) was better preserved than the other proteins (in Fig. 2 compare the fiber indicated by the empty star).

In the 24-h-stimulated EDL muscle, within the preserved area of the muscle it was possible to identify regions in which dystrophin and β-dystroglycan were completely absent from sarcolemma, whereas α-sarcoglycan was still evident (Fig. 3 A–C). On the other hand, there were also regions with apparently normal fibers, i.e., expressing all three proteins at sarcolemma, together with fibers where only α-sarcoglycan was preserved, and fibers completely devoid of all three proteins (Fig. 3 D–F).

Thus, the disarrangement of the costameric dystrophin complex was commonly observed in both EDL and soleus muscles during the 4- to 24-h stimulation, whereas it was very rarely seen after 2-h stimulation (not shown). It is worth noting that fibers devoid of dystrophin at the sarcolemma, as well as of the other proteins under investigation, often showed a pale more intense staining of the cytoplasm (compare, e.g., in Fig. 2 A the cytoplasm of fibers with an without dystrophin at the sarcolemma). This also appears to be a common observation in dystrophic muscles (A. Fidianska, personal communication).

Fibers lacking dystrophin at the sarcolemma, or any of the other associated proteins, were never observed in control soleus and EDL muscle (Fig. 2 F), and, especially, in control muscles maintained in extended position without stimulation (not shown). Conversely, stimulation of muscles kept in a neutral position only rarely caused the occurrence of some damage and, very rarely, the appearance

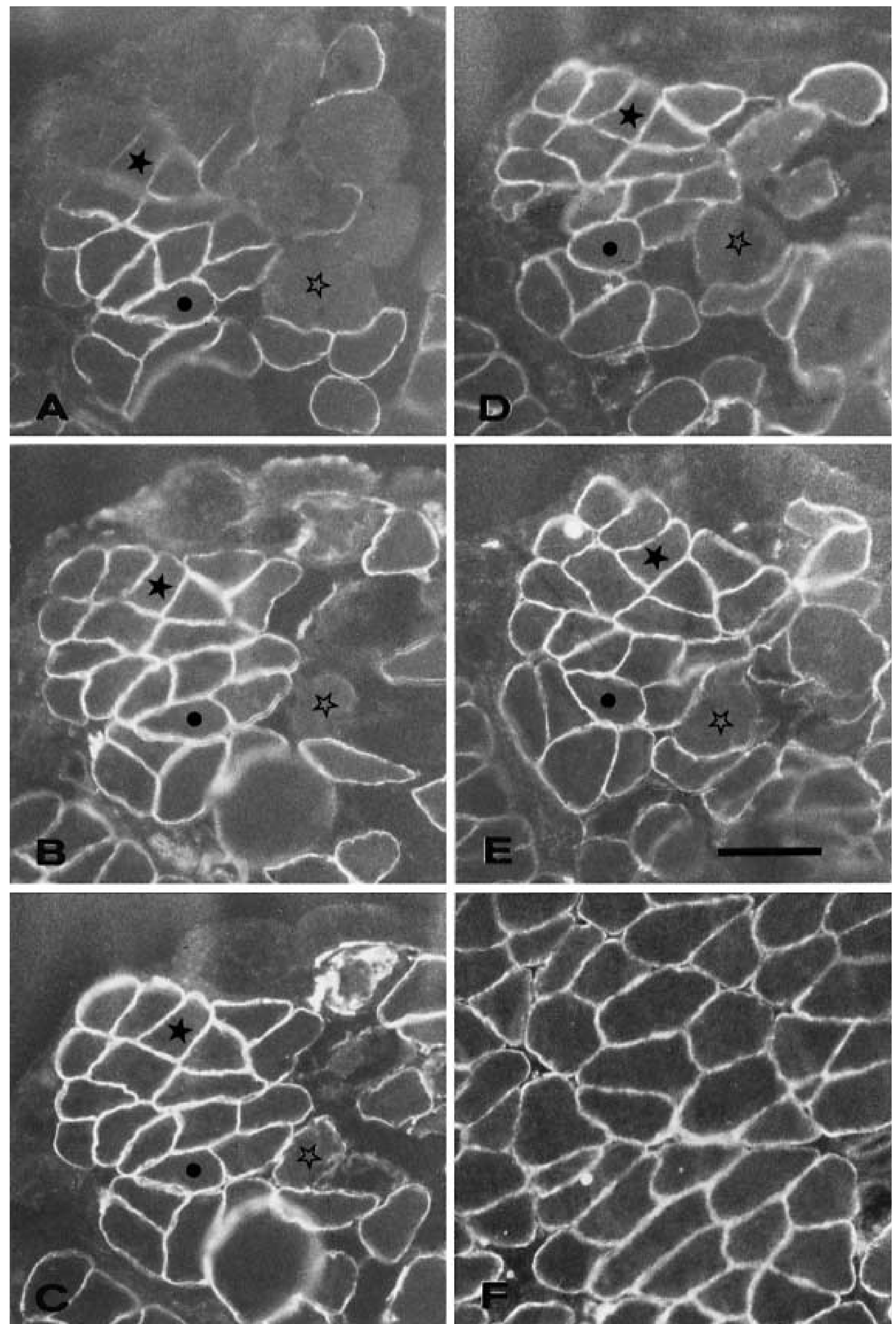

Fig. 2 Immunofluorescence analysis of sarcolemma proteins in normal soleus (**F**) muscle and after 6 h of eccentric exercise (**A**–**E**). Serial cryostat sections of soleus muscle after eccentric exercise were stained with antibodies specific for dystrophin (**A**), β-dystroglycan (**B**), α-sarcoglycan (**C**), spectrin (**D**) and γ-sarcoglycan (**E**). For comparison, normal soleus muscle was stained with the anti-dystrophin antibody (**F**). In the stimulated muscle, several fibers present a normal continuous sarcolemmal staining with all of the antibodies used (one such fiber is indicated by a *black circle* which represents the same fiber in each panel). In contrast, some fibers are not stained by the anti-dystrophin antibody (**A**), whereas they are stained by the other antibodies (**B**–**E** *black star*). Moreover, some fibers are not stained by any of the antibodies except anti-α-sarcoglycan (**C**, *open star*). *Bar* **A**–**E** 25 μm; **F** 20 μm

of both fibers with discontinuous sarcolemmal dystrophin staining and infiltrating inflammatory cells (not shown).

The possible occurrence of apoptosis in muscles stimulated during extension was evaluate in both soleus and EDL muscles by TUNEL. The number of TUNEL-positive nuclei increased from the very low level of 50–80/ mm^3 found in normal untreated muscles to 800–1,600/ mm^3 in stimulated muscles (Fig. 4). These values correspond to about 0.8–1.7% of total nuclei of the experimental muscle.

To evaluate whether the occurrence of apoptosis was related to the disarrangement of the dystrophin complex, we double stained the stimulated muscles with anti-dystrophin or anti-α-sarcoglycan antibodies and TUNEL. In the fairly well-preserved area of muscle fibers stimulated while in an extended position, double staining with anti-dystrophin and TUNEL revealed that many fibers deprived of dystrophin at the sarcolemma also contained TUNEL-positive nuclei (Fig. 4 B–D). Interestingly, these fibers always showed a substantial cytoplasmic staining with the anti-dystrophin antibody. In addition, fibers with absent or reduced expression of α-sarcoglycan at the sarcolemma were also found to have TUNEL-positive nuclei (Fig. 4 F).

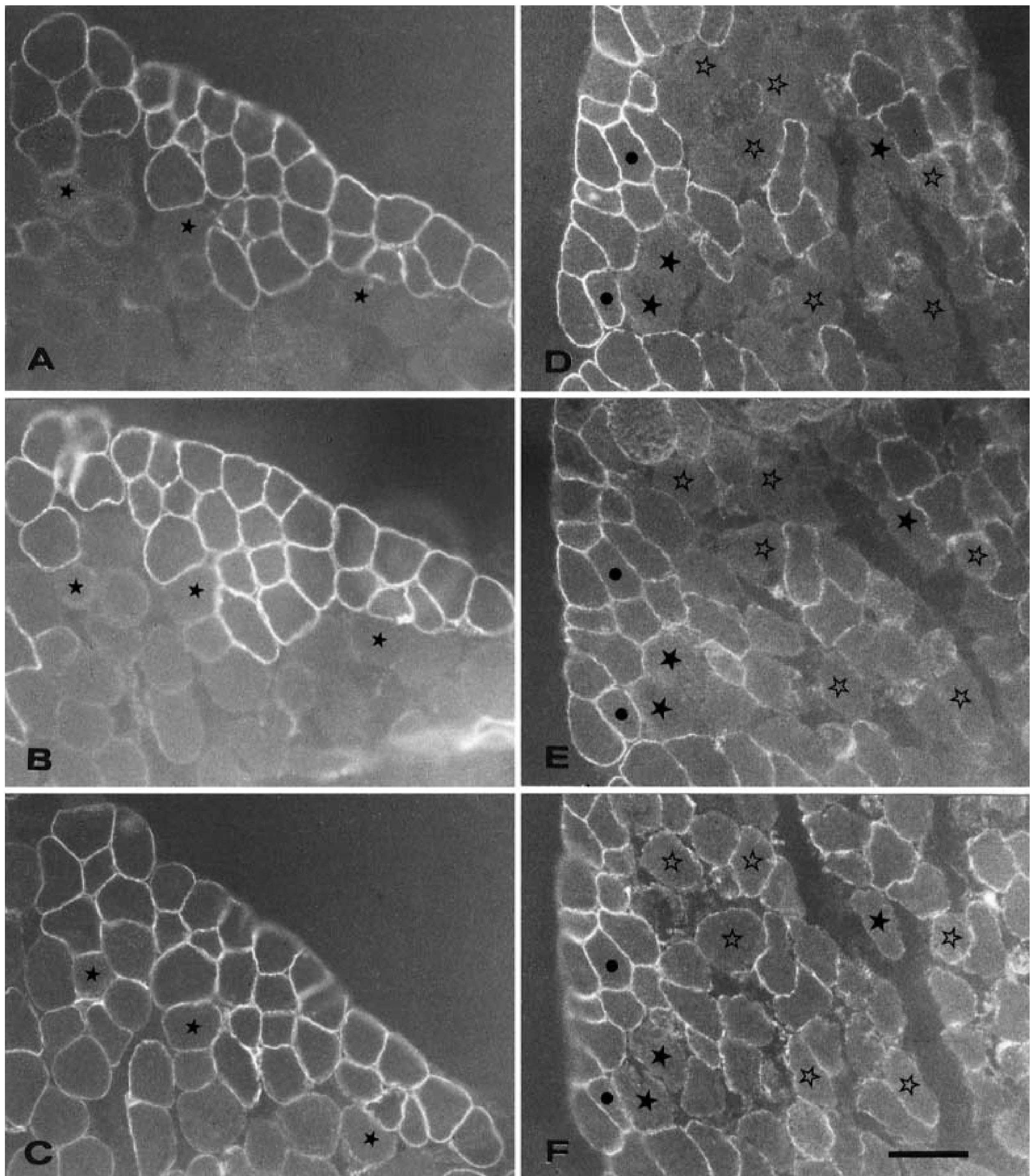

Fig. 3 A–F Immunofluorescence analysis of sarcolemma proteins from EDL muscle after 24 h of eccentric exercise. Two different serial cryostat sections were stained with antibodies specific for: dystrophin (**A**, **D**), β-dystroglycan (**B**, **E**), and α-sarcoglycan (**C**, **F**). Immunostaining of serial sections (**A**–**C**) reveals that a group of fibers are not stained at the sarcolemma by the anti-dystrophin (**A**) and anti-β dystroglycan (**B**) antibodies, while almost all the fibers are stained by the anti-α-sarcoglycan antibody (**C**). It is worth noting, however, that the intensity of the anti-α-sarcoglycan staining is heterogeneous. Analysis of cryostat sections presented in **D**–**F**, serials of that in Fig. 1 F, shows the presence of fibers stained by the three antibodies *(black circles)* but also of fibers not stained *(black stars)*. Many fibers are not stained by anti-dystrophin (**D**) and anti-β dystroglycan (**E**), while they are stained by the anti-α-sarcoglycan antibody (**F**, *empty stars*), even though the intensity of the latter antibody is very variable. *Bar* 25 μm

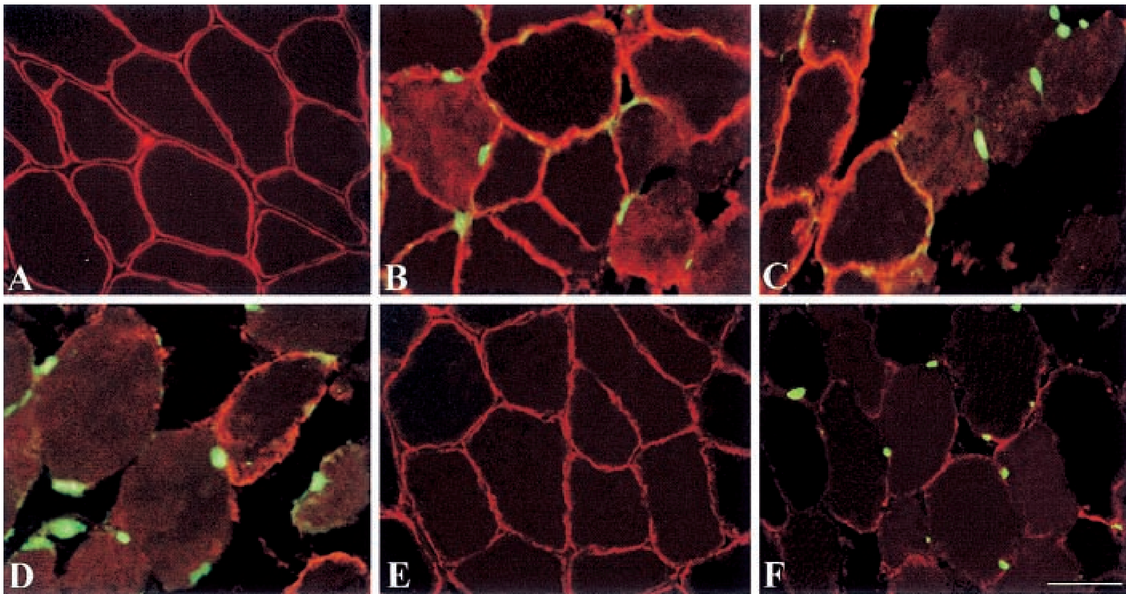

Fig. 4 Double staining with anti-dystrophin (**A**–**D**), or anti-α-sarcoglycan antibodies (**E**, **F**) and TUNEL. Cryostat sections were from control soleus muscle (**A**), soleus muscle stimulated for 6 h while under extension (**B**) and from EDL muscles stimulated for 24 h while under extension (**C**–**F**); **E** represents a very well-preserved area. TUNEL-positive nuclei are evident inside fibers lacking dystrophin or α-sarcoglycan staining (**B**–**D**, **F**, respectively). In contrast, TUNEL-positive nuclei are never detected in fibers with homogeneous dystrophin or α-sarcoglycan sarcolemma staining (**A**–**E**). The stimulated soleus muscle (**B**) contained about 1,600 TUNEL-positive nuclei/mm^3 (i.e., about 1.12% of the total nuclei); the stimulated EDL muscle (**C**, **D**, **F**) contained about 1,200 TUNEL-positive nuclei/mm^3 (i.e., about 1.7% of the total nuclei). *Bar* 8 µm

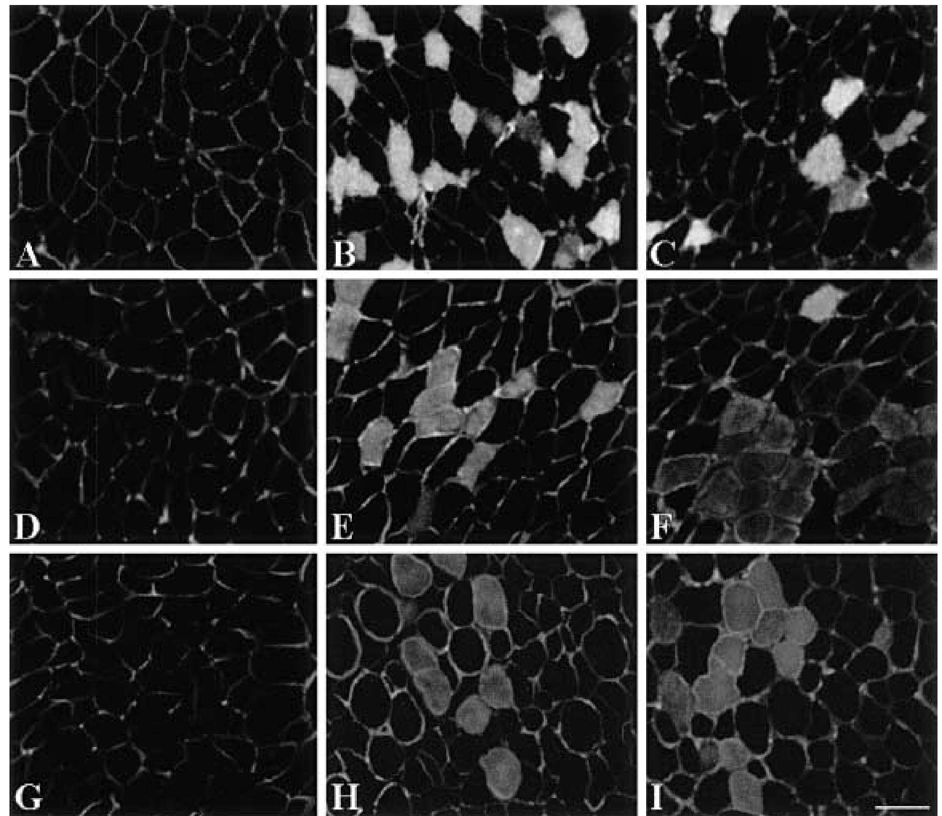

Fig. 5 Cryostat sections from control soleus muscle (**A**, **D**, **G**), 6-h- (**B**, **E**, **H**) and 24-h-stimulated (**C**, **F**, **I**) soleus muscle. Sections were stained with anti-Bax (**A**–**C**), anti-Bcl-2 (**D**–**F**) and anti-caspase-3 (**G**–**I**) antibodies. Control muscle was not stained by the three antibodies, whereas after 6 and 24 h of eccentric exercise some fibers in the apparently preserved areas of the muscle were stained by the antibodies. *Bar* 40 µm

Because of the presence of TUNEL-positive nuclei in muscles stimulated during extension, the expression of some apoptotic proteins was investigated using antibodies specific for Bax, Bcl-2 and caspase-3. Staining of cryostat sections from the well-preserved regions of soleus muscles stimulated for 24 h while in an extended position demonstrated that some fibers expressed substantial levels of Bax (Fig. 5 B, C). Consistent with Bax activation, some fibers from the 6- and 24-h-stimulated soleus muscles also showed overexpression of Bcl-2 (Fig. 5 E, F) and caspase-3 (Fig. 5 H, I). It is worth noting that some of the fibers expressing detectable levels of caspase-3 showed a round shape (Fig. 5 H), also observed in some dystrophin deficient TUNEL-positive fibers (Fig. 4 D).

Discussion

In the present study we have shown that eccentric exercise, i.e., a condition in which repeated contractions are applied to an extended muscle, produces profound modifications in skeletal muscle. Histologically, occasional small areas showing a severe muscle fiber injury associated to the presence of necrosis were evident, as also observed previously [2, 6, 21]. However, even within the apparently well-preserved regions of the muscle dramatic alterations occurred. We have recently shown that within these areas there was a substantial reduction of dystrophin at sarcolemma in some fibers. Concomitantly, in the same areas, we demonstrated the occurrence of significant muscle fiber apoptosis [6]. Here we confirm and extend these preliminary observations. We have demonstrated that continuous eccentric exercise causes the progressive disassembly of the dystrophin-based membrane skeleton within the well-preserved muscle regions of fast- and slow-twitch rat muscles. Since fibers lacking elements of the dystrophin complex also showed some apoptotic nuclei, substantial alterations of the dystrophin complex appears to represent an important signal for the activation of programmed cell death. Consistent with activation of apoptosis in the apparently spared areas, we demonstrated an elevated expression of Bcl-2, Bax and caspase-3 in many muscle fibers.

Force produced by individual muscle fibers during normal contractile activity is transmitted to the extracellular matrix and to the neighboring muscle fibers via transmembrane structures at the sarcolemma [32]. The main structure accomplishing this function is the dystrophin complex, which comprises three distinct sub-complexes of proteins: dystroglycans, sarcoglycans and syntrophins [26]. Besides the dystrophin complex, other cytoskeletal proteins, like spectrin, vinculin, talin, α-actinin and integrins, also appear to be involved in supporting the sarcolemma [23, 35, 39, 47]. At the sarcolemma, those proteins form a complex cytoskeletal structure organized with a costameric distribution, aligned to the Z- and M-lines [25, 27, 35, 45]. Thus, costameres represent the membrane-associated cytoskeletal structure that provides the link between the contractile apparatus and the extracellular matrix, protecting the sarcolemma from mechanical stresses and transmitting the force of contraction extracellularly [11, 31, 33]. In addition, costameres constitutes a scaffold for locating several important signaling molecules at the sarcolemma [16, 45].

The disassembly of the dystrophin complex as a consequence of eccentric exercise, as demonstrated by our results (Figs. 2, 3), seems to follow a precise sequence of events through which dystrophin deprivation occurs initially, possibly associated with that of β-dystroglycan, and this is followed by that of sarcoglycans (Figs. 2, 3). Spectrin, known to be an important element of the costamere, also disappears from sarcolemma (Fig. 2). Among the four sarcoglycans, α- and γ-sarcoglycan appear to be distinct components of the complex, because it has been recently demonstrated that β-, γ-, and δ-sarcoglycan are tightly associated each other, whereas α-sarcoglycan acts as separate unit [10, 18]. Consistent with a distinct function of α-sarcoglycan is our recent findings showing that this protein is an ecto-ATPase [5]. Interestingly, the present data show that α-sarcoglycan is apparently more protected than γ-sarcoglycan from the effects of eccentric exercise. The lower sensibility to mechanical stress of α-sarcoglycan compared to dystrophin and β-dystroglycan could be ascribed to the fact that dystrophin and β-dystroglycan are directly involved in the mechanical link from the cytoskeletal elements to the extracellular matrix [11, 31, 33]. Moreover, since dystrophin is a very long and flexible molecule [22], it could be more sensitive to mechanical tension than the transmembrane α-sarcoglycan. Dystrophin has been found to be highly exposed to the action of proteolytic enzymes [3], whereas α-sarcoglycan seems more resistant to damage in necrotic muscles after toxin injury [44].

We suggest that the response of a normal muscle contracting under unusual conditions parallels that of the dystrophic muscle. In this respect, the dystrophin complex of proteins at the sarcolemma appears to represent the sensor of excessive contractile activity. Further, we suggest that, in dystrophic muscles, the lack of dystrophin or other components of the complex produces during the normal physiological activity a mechanical stress to the fiber, which the costameric structure is not able to attenuate and redistribute. Thus, even normal activity leads to muscle fiber injury. As a consequence, the threshold for any exercise-induced muscle damage is expected to be much lower in the dystrophic than in the control muscle, as it has been demonstrated in the mdx mice [34, 37], while the mechanism causing muscle damage appears to be the same.

The presence of TUNEL-positive nuclei in fibers from muscles stimulated in an extended position (Fig. 4) demonstrates the activation of the apoptotic processes within those muscles. This is confirmed by the increased levels of Bax, Bcl-2 and caspase-3 proteins (Fig. 5), important members of the apoptotic pathway. Increased levels of caspase-3 have been recently demonstrated in myocytes after myocardial ischemia and reperfusion in vivo [7]. Similarly, Bcl-2, the anti-apoptotic protein, and Bax,

the pro-apoptotic protein, were shown to be elevated in ventricular myocytes from human hearts with myocardial infarction [28]. Importantly, in skeletal muscle the Bcl-2 levels increase both in Duchenne and limb-girdle muscle dystrophies [41] and in experimentally denervated and reinnervated muscles [42]. Our results demonstrate that apoptosis is not limited to pathological muscles but that it may occur also in normal mature skeletal muscle fibers. Removal of muscle fiber segments or of individual nuclei may represent a physiological mechanism to adapt skeletal muscle structure and dimension to some changed functional demands. Furthermore, the present data demonstrate that relevant alterations of the costameric structure, such as loss of dystrophin and some of the associated proteins, may represent a sufficient signal to switch on the apoptotic process. In addition, perturbation of signaling proteins associated to the dystrophin complex [16, 45] may contribute to the activation of apoptosis.

In conclusion we propose that: (1) experimental eccentric exercise may represent a convenient model for the study of pathogenetic events leading to muscle fiber injury, especially considering that it appears to mimic that degenerative processes occurring in the dystrophic muscle, and (2) apoptosis is likely a physiological mechanism adopted by skeletal muscle to adapt its structure and dimension to changed functional demands.

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References

1. Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 281 : 1322–1326
2. Armstrong RB, Ogilvie RW, Schwane JA (1983) Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol 54 : 80–93
3. Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE, Knowlton KU (1999) Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. Nat Med 5 : 320–326
4. Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LVB, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS, Lefranc G (1999) Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkBα/NF-kB pathway in limb-girdle muscular dystrophy type 2A. Nat Med 5 : 503–511
5. Betto R, Senter L, Ceoldo S, Tarricone E, Biral D, Salviati G (1999) Ecto-ATPase activity of α-sarcoglycan (adhalin). J Biol Chem 274 : 7907–7912
6. Biral D, Sandri M, Jakubiec-Puka A (1998) The fate of dystrophin and some signs of apoptosis in the skeletal muscle work-overloaded in extension. Basic Appl Myol 8 : 205–210
7. Black SC, Huang JQ, Rezaiefar P, Radinovic S, Eberhart A, Nicholson DW, Rodger IW (1998) Co-localization of the cysteine protease caspase-3 with apoptotic myocytes after in vivo myocardial ischemia and reperfusion in the rat. J Mol Cell Cardiol 30 : 733–742
8. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15 : 269–290
9. Burridge K, Chrzanowska-Wodnicka M (1996) Focal adhesions, contractility, and signaling. Annu Cell Dev Biol 12 : 463–519
10. Chan Y, Bonnemann CG, Lidov HGW, Kunkel LM (1998) Molecular organization of sarcoglycan complex in mouse myotubes culture. J Cell Biol 143 : 2033–2044
11. Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol 122 : 809–823
12. Faulkner JA, Brooks SV (1994) An in situ single skeletal muscle model of contraction-induced injury: mechanistic interpretations. Basic Appl Myol 4 : 17–23
13. Friden J, Lieber RL (1998) Segmental muscle fiber lesions after repetitive eccentric contractions. Cell Tissue Res 293 : 165–171
14. Frisch SM, Ruoslahti E (1997) Integrins and anoikis. Curr Opin Cell Biol 9 : 701–706
15. Giancotti FG (1997) Integrin signaling: specificity and control of cell survival and cell cycle progression. Curr Opin Cell Biol 9 : 691–700
16. Gossrau R (1998) Nitric oxide synthase I (NOS I) is a costameric enzyme in rat skeletal muscle. Acta Histochem 100 : 451–462
17. Hack AA, Ly CT, Jiang F, Clendenin CJ, Sigrist KS, Wollmann RL, McNally EM (1998) γ-Sarcoglycan deficiency leads to muscle membrane defects and apoptosis independent of dystrophin. J Cell Biol 142 : 1279–1287
18. Holt KH, Campbell KP (1998) Assembly of the sarcoglycan complex. J Biol Chem 273 : 34 667–34 670
19. Ingber DE (1997) Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol 59 : 575–599
20. Jakubiec-Puka A, Carraro U (1989) Ultrastructure of the striated muscle electrostimulated in extension. Proceedings of the 3rd Vienna International Workshop on Functional Electrostimulation. Basic, Technology and Application. Baden/Vienna, September 17–20 1989, Department of Biomedical Engineering, University of Vienna, Vienna, pp 63–87
21. Jakubiec-Puka A, Szczepanowska J, Carraro U (1995) Ultrastructure of the skeletal muscle overworked in extension. In: Frey M, Giovanoli P (eds) Proceedings of the 4th International Muscle Symposium, 1995 Zurich, Universitätsspital Zurich, Zurich, pp 117–120
22. Koenig M, Monaco AP, Kunkel LM (1988) The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. Cell 53 : 219–228
23. Lakonishok M, Muschler J, Horwitz AF (1992) The α5β1 integrin associates with a dystrophin-containing lattice during muscle development. Dev Biol 152 : 209–220
24. Lieber RL, Friden J (1994) Biomechanical basis of muscle cellular damage. Basic Appl Myol 4 : 25–33
25. Masuda T, Fujimaki N, Ozawa E, Ishikawa H (1992) Confocal laser microscopy of dystrophin localization in guinea pig skeletal muscle fibers. J Cell Biol 119 : 543–548
26. Michalak M, Opas M (1997) Functions of dystrophin and dystrophin associated proteins Curr Opin Neurol 10 : 436–442
27. Minetti C, Beltrame F, Marcenaro G, Bonilla E (1992) Dystrophin at the plasma membrane of human muscle fibres shows a costameric localization. Neuromuscul Disord 2 : 99–109
28. Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H (1996) Expression of bcl-2 protein, an inhibitor of apoptosis, and bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. Circulation 94 : 1506–1512
29. Mukasa T, Momoi T, Momoi MY (1999) Activation of caspase-3 apoptotic pathways in skeletal muscle fibers in laminin α2-deficient mice. Biochem Biophys Res Commun 260 : 139–142

30. Noguchi S, McNally EM, Othmane KB, Hagiwara Y, Mizuno Y, Yoshida M, Yamamoto H, Bonnemann CG, Gussoni E, Denton PH, Kyriakides T, Middleton L, Hentati F, Hamida MB, Nonaka I, Vance JM, Kunkel LM, Ozawa E (1995) Mutations in the dystrophin-associated protein γ-sarcoglycan in chromosome 13 muscular dystrophy. Science 270 : 819–822
31. Pasternak C, Wong S, Elson EL (1995) Mechanical function of dystrophin in muscle cells. J Cell Biol 128 : 355–361
32. Patel TJ, Lieber RL (1997) Force transmission in skeletal muscle: from actomyosin to external tendons. Exerc Sport Sci Rev 24 : 321–363
33. Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL (1993) Dystrophin protects the sarcolemma from stresses developed during muscle contraction. Proc Natl Acad Sci USA 90 : 3710–3714
34. Podhorska-Okolow M, Sandri M, Zampieri S, Brun B, Rossini K, Carraro U (1998) Apoptosis of myofibres and satellite cells: exercise-induced damage in skeletal muscle of the mouse. Neuropathol Appl Neurobiol 24 : 518–531
35. Porter GA, Dmytrenko GM, Winkelmann JC, Bloch RJ (1992) Dystrophin colocalizes with β-spectrin in distinct subsarcolemmal domains in mammalian skeletal muscle. J Cell Biol 117 : 997–1005
36. Roberds SL, Leturcq F, Allamand V, Piccolo F, Jeanpierre M, Anderson RD, Lim LE, Lee JC, Tome FMS, Romero NB, Fardeau M, Beckmann JS, Kaplan JC, Campbell KP (1994) Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. Cell 78 : 625–633
37. Sandri M, Podhorska-Okolov M, Geromel V, Rizzi C, Arslan P, Franceschi C, Carraro U (1997) Exercise induces myonuclear ubiquitination and apoptosis in dystrophin-deficient muscle of mice. J Neuropathol Exp Neurol 56 : 45–57
38. Sandri M, Minetti C, Pedemonte M, Carraro U (1998) Apoptotic myonuclei in human Duchenne muscular dystrophy, Lab Invest 78 : 1005–1016
39. Senter L, Luise M, Presotto C, Betto R, Teresi A, Ceoldo S, Salviati G (1993) Interaction of dystrophin with cytoskeletal proteins. Binding to talin and actin. Biochem Biophys Res Commun 192 : 899–904
40. Shyy JY-J, Chien S (1997) Role of integrins in cellular responses to mechanical stress and adhesion. Curr Opin Cell Biol 9 : 707–713
41. Tews DS, Goebel HH (1997) DNA-fragmentation and expression of apoptosis-related proteins in muscular dystrophies. Neuropathol Appl Neurobiol 23 : 331–338
42. Tews DS, Goebel HH, Schneider I, Gunkel A, Stennert E, Neiss WF (1997) DNA-fragmentation and expression of apoptosis-related proteins in experimentally denervated and re-innervated rat facial muscle. Neuropathol Appl Neurobiol 23 : 141–149
43. Tidball JG, Albrecht DE, Lokensgard BE, Spencer MJ (1995) Apoptosis precedes necrosis of dystrophin-deficient muscle. J Cell Sci 108 : 2197–2204
44. Vater R, Harris JB, Anderson LVB, Roberds SL, Campbell KP, Cullen MJ (1995) The expression of dystrophin-associated glycoproteins during skeletal muscle degeneration and regeneration. An immunofluorescence study. J Neuropathol Exp Neurol 54 : 557–569
45. Williams MW, Bloch RJ (1999) Extensive but coordinated reorganization of the membrane skeleton in myofibers of dystrophic (mdx) mice. J Cell Biol 14 : 1259–1270
46. Yang B, Jung D, Motto D, Meyer J, Koretzky G, Campbell KP (1995) SH3 domain-mediated interaction of dystroglycan and Grb2. J Biol Chem 270 : 11 711–11 714
47. Yoshida T, Pan Y, Hanada H, Iwata Y, Shigekawa M (1998) Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. J Biol Chem 273 : 1583–1590
48. Zubrycka-Gaarn EE, Bulman DE, Karpati G, Burghes AHM, Belfall B, Klamut HJ, Talbot J, Hodges RS, Ray PN, Worton RG (1988) The Duchenne muscular dystrophy gene product is localized in sarcolemma of human skeletal muscle. Nature 333 : 466–469