Effects of Physical Exercise on Skeletal Muscle Fiber: Ultrastructural and Molecular Aspects

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Abstract

This review briefly summarizes varies research studies attempting to examine the mechanisms of skeletal muscle adaptations to exercise. The study of adaptations of exercise training reveals a wide range of integrative approaches, from the systemic to the molecular level. Research has revealed that exercise can be effective at preventing and/or treating some of the most common chronic disease. Strenous exercise creates situations that subject structure and cell metabolism to significant stress, including those of muscle damage and oxidative stress. The cytoskeletal contractile myofilament apparatus adheres through subsarcolemmal and transmembrane molecules to the surronding extracellular matrix with integrin and dystrophin associated chains of molecules being the two main adhesion complexes. Immediately after exercise, especially when it involves eccentric contraction, some of the proteins belonging to these complexes decrease both quantitatively and qualitatively. This decrease is followed by a subsequent return to normal values after a few weeks. High-intensity exercise, aso induces ultrastructural damages and few weeks after the eccentric contraction, examination of muscle samples shows a normal ultrastructural myofibrillar profile, indicating the existence of a remodelling response after exercise-induced damage. Responses to exercise are often highly variable among individuals, however that response may be mediated by variation in genes. At the present time, most of the mechanisms underlying the adaptation of human skeletal muscle to exercise still remain to be discovered.

Key words: Skeletal muscle**,** muscle injury**,** cytoskeletal proteins, cell-matrix interactions

Abbreviations: EC, eccentric contraction; DGC, dystrophin glycoprotein-complex; Hsp, heat-shock protection; SR, sarcoplasmatic reticulum; NO, nitric oxide; VEGF, vascular endothelial growth factor

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Skeletal muscle is a complex organ composed of particular cells, multinucleate syncytia gathered in a connective network that peripherally continues with the tendinous structures necessary to transmit contractile force of muscle fibers on bone. Skeletal muscle is composed of heterogeneous fiber types that vary in contraction velocity, endurance capability and metabolic enzyme profile. Both vascular and nervous factors are particularly important for motor performance. The management of the contractile machinery gathered inside myofibers is strictly linked to the activity of the myonuclei, mitochondria, and the system of T tubules and sarcoplasmic reticulum (SR). The way structural elements of myofibers are gathered strictly depends on the cytoskeletric system peripherally associated with the sarcolemma because of specific membrane proteins whose physiological role is to provide mechanical stability to the surface membrane during normal contraction as well as to carry out important receptor functions [13].

Skeletal muscle is a highly plastic tissue that adapts to changing functional demands by altering its constituent proteins. It is well established that strength training increases muscle strength and this increase is due to both neural and muscular factors [8]. Chronic contractile activity in muscle, not otherwise subjected to such a stimulus, promotes changes in the complement of contractile and metabolic proteins that optimize muscle function for this new type of activity [19]. Such remodelling involves altered patterns of both protein synthesis and protein degradation [4], thus making muscle tissue capable of enduring subsequent periods of exercise. Therefore, correct physical exercise involves several adaptations improving structural organization of

both muscle and contractile performance. It is also true that strenuous exercise and particularly eccentric contraction (EC) determines the muscle damage reported in literature [17].

Effects of exercise on skeletal muscle

Previously reported postexercise degenerative response includes an increase in fibrous connective tissue fiber necrosis, damage of contractile components, and disruption of membranous components, such as mitochondria [7, 16, 23]. After exercise, the cytoskeletal network is damaged as a result of sarcolemmal disruption, occuring even before the alteration of the contractile components, thus representing one of the first targets hit by overload or total load absence. Such damage can be explained through alteration of the cytoskeletal anchoring system to the sarcolemma. This system involves different proteic complexes and particularly, the integrin and dystrophin-glycoprotein complexes (DGC) that, as mentioned above, – mechanically match extra- and intracellular areas and work as membrane receptors activating a number of signalling systems towards the nuclei [13]. Such systems are important because their absence or hypoexpression brings about serious muscular pathologies known as dystrophies.

Immediately after exercise, some of the proteins belonging to these complexes decrease both quantitatively and qualitatively. This decrease is followed by a subsequent return to normal values after a few weeks and may provide a structural explanation for the protective effects towards a further series of exercises. It has been hypothesized that a repetition of eccentric exercise makes the extracellular matrix, myofibrils, cytoskeleton, and cell membranes more resistant, providing a morphological mechanism for rapid adaptation [6].

Statistical analyses of gene-exercise interactions and laboratory studies of cellular function have also revealed genes that are directly altered by exercise. Nevertheless, the mechanisms by which these alterations in gene expression subsequent to exercise occur have not been well studied.

Ultrastructural Changes

Acute and chronic exercise is associated with ultrastructural muscle damage that is mainly centered on the Z disk that anchors thin filaments and several intermediate filaments within the sarcomere. Most of the evidence pointing to Z-disk involvement comes from electron microscopic studies that show eccentric damaged sarcomeres, particularly sarcomeres out of register with one another, these disorganized sarcomeres coexisting with normal sarcomeres. Z disks appear to widen or disintegrate, and it is also possible to see regional disorganization of the myofilament and t-tubule damage. Disturbances of the mitochondria, sarcoplasmic reticulum, A band, and extracellular matrix have also been reported [6]. A common feature of after-training muscle fibers is the separation of myofibrils. It has been postulated that this separation may be indicative of damage to intermediate filament proteins such as desmin [18] .

The ultrastructural disruptions of muscle fibers, such as the breakage of exosarcomeric cytoskeleton proteins or the distortion of the alignment of the A and I band in the absence of fibre degeneration, may affect the relationship between the basal lamina and satellite cells and induce the release of some growth factors known to affect satellite cell activation and proliferation [8], such as fibroblast growth factor or insulin growth factor. These cells, first identified by Mauro [15], are uniformly distributed throughout the length of the muscle and located between the basal lamina and sarcolemma. It has been demonstrated that short or long-term strength training can induce their activation.

A few weeks after EC, examination of muscle samples shows a normal ultrastructural myofibrillar profile, indicating the existence of a remodelling response after exercise-induced damage. This observation shows that the contractile machinery first damaged by exercise can recover and adapt to further overload.

Cytoskeletal Proteins

In addition to contractile proteins, muscle contains cytoskeletal proteins that stabilize the contractile proteins and allow for transmission of tension both longitudinally and laterally. These cytoskeleton proteins may have a role in the prevention or development of eccentric damage. Besides these, desmin, a protein of cell-matrix connection system, could be involved in the sarcomere disruption following eccentric exercise. Desmin is a structural protein located in the Z disks, connecting adjacent Z disks and Z disks at the edge of the fiber to the costamere in the surface membrane. Thus it contributes to the alignment of Z disks across the fibers and also transmits lateral tension. Previous studies showed a loss of desmin immediately after EC [10]. Such loss resulted from raised resting $[Ca^{2+}]_i$ but preceded damage to contractile proteins. Many desmin negative fibers showed normal contractile filaments so this may reflect different speeds of propagation of desmin loss and contractile filament disruption along the fibers [1]. The mechanism by which loss of desmin staining occurs is not known; however, modifications in this staining could not be mediated by mechanisms that rely on gene regulation, but instead by mechanisms involving muscle fiber membrane disruption and subsequent proteolysis or conformational change of the cytoskeletal network [11]. Finally, after the intermediate filaments are disrupted by an increase in $[Ca^{2+}]_i$, that in turn activates proteasis $[Ca^{2+}]_i$ dependent such as calpain, the myofibrillar apparatus is disrupted and is unable to develop normal tension. The rapid loss of desmin, immediately after a single bout of eccentric contraction, recovers after 3-4 days, and such increase

represents a remodelling of the intermediate filament system. Newly synthesized desmin could serve to reinforce existing sarcomeres or be added to newly synthesized sarcomeres [12]. There is evidence suggesting the involvement of the small heat shock proteins (Hsp) in the process of remodelling, particularly in the assembly and maintenance of the intermediate filament network [21]. Hsp are important components of the cellular protective response against reactive oxygen species (ROS) and recent data indicate an increase in the expression of numerous Hsps in response to exercise and endurance training [14].

Previous studies report that cytoskeleton disruption occurred as fast as 5 min. after initiation of EC; such results suggest that direct mechanical or biochemical events are responsible for this disruption, rather than events that require gene regulation.

The main system of the cellular-matrix interaction systems is represented by two receptor complexes: dystrophin associated glycoproteins and integrins associated to talin and vinculin. Recently, a study carried out on human skeletal muscle undergoing EC showed changes in the dystrophin-glycoprotein complex, in particular discontinuous staining for dystrophin and reduced expression of α-sarcoglycan immediately after EC. Thus, this loss may destabilize the sarcolemma leading to modifications of membrane permeability. The decrease in the level of a-sarcoglycan may also regulate the intracellular calcium concentration and therefore, lead to the persistent activation of P2X receptors, resulting in intracellular $[Ca^{2+}]_i$ overloaded in muscle fibres [2] that in turn may activate $[Ca^{2+}]_i$ -dependent proteolitic pathways. This loss occurs before the loss of staining of the cytoskeletal protein desmin or before the staining of disorganized actin and may have an origin in the disruption of the plasma membrane [10]. The subsequent recovery of these proteic complexes after a few weeks may be involved in the subsequent remodelling of myofibrillar structure and this response may limit the extent of muscle damage upon a subsequent mechanical stress [5].

A single bout of moderate eccentric exercise leads to profound adaptations in human skeletal muscle; the specific mechanisms involved in this response have yet to be determined. However, from proteolytic response data, the differential expression of structural proteins and the induction of molecular chaperons appear to be involved in the damage-repair process.

Excitation-Contraction Coupling

Eccentric exercise may have effects on excitation-contraction coupling, possibly affecting both the release and uptake of $[Ca^{2+}]_i$ by the sarcoplasmic reticulum. It has been shown that downhill running exercise results in changes in the organization of the membrane system involved in E-C coupling in skeletal muscle fibers. The arrangement of the t-tubule network and the disposition of the triads changed following downhill running exercise. Several authors have shown that intracellular $[Ca^{2+}]_i$ accumulation causes muscle damage and since membrane depolarization is associated with $[Ca^{2+}]_i$ release from the SR, it is possible that repeated muscle contractions combined with impaired $[Ca^{2+}]_i$ uptake by the SR lead to muscle degeneration [22].

Conclusions

It is clear that eccentric exercise causes a number of ultrastructural, biochemical and metabolic alterations that at first glance appear to be devastating to the integrity of the muscle. Indeed, when physical exercise is occasional and intense, especially in sedentary and elderly subjects, muscle integrity is compromised. The mechanical damage induced by moderate and continuous exercise – specific to regular training – turns out to be a positive condition in that it can bring about structural and metabolic remodelling capable of increasing endurance towards subsequent mechanical stress. This capacity of adaptation is also associated with numerous changes in gene expression, upregulation of cellular protective mechanisms, and remodelling of muscle structure. Several factors can determine modifications of the gene expression and many of the signalling systems that can induce such changes are well known (autocrine, paracrine, hormonal, neural, growth factors, intermediate metabolite flows, etc.). Most of these signalling systems have myonuclei as targets because they can modify their processes of translation and transcription of mRNA. It has been recently suggested that the mechanical forces which develop during training can also directly influence the function of myonuclei through the stimulation of the membrane proteins associated with the cytoskeleton [13].

Depending on the intensity and amount of the applied stimulus, striated muscle can undergo 1) remodelling of its contractile machinery, 2) changes of neuromuscular junction, 3) changes of $[Ca^{2+}]_i$ release and in the $[Ca^{2+}]_i$ sensitivity of the contractile machinery; 4) upregulation of the mitochondrial system, 5) possible myonuclei increase through activation of satellite cells, 6) increases in its capillarity and blood flow/oxygen utilization capacity.

Many of these changes can be ascribed to the release of important regulation factors among which nitric oxide (NO) plays a key role. Its production in muscle is increased by exercise as a result of the chronic inflammation caused by training. Such free radicals affect multiple biological processes in muscle. In addition to being a potential modulator of blood flow, skeletal muscle-derived NO is an important regulator of muscle contraction and metabolism. In particular recent human data indicate that NO modulates muscle glucose uptake during exercise. Exercise training in healthy individuals promotes adaptations in various NO systems, which can increase its bioavailability, through

a variety of mechanisms, including increased NOS (nitric oxyde synthase) enzyme expression and activity. Such adaptations likely contribute to increased exercise capacity and protection from cardiovascular risks [9].

It has also been shown that physical exercise can activate the expression of so-called heat shock proteins whose production represents a protection factor present in all cell types [20]. This may explain the reason why the response to physical exercise in fact involves different organs and tissues. Among the effects of morpho-functional modifications following physical exercise it is important to consider the increased vascularization in the microcirculatory system induced by the production of angiogenetic factors such as vascular endothelial growth factor (VEGF), which causes a subsequent increase in local blood flow [3].

In conclusion, physical exercise has the ability to influence the genetic function of the muscle fibres, modifying their structure and metabolism and promoting the release of growth factors and other signalling molecules such as nitric oxide which work through the paracrine system to activate the satellite cells. The further investigation of these mechanisms and the intracellular signalling pathways that not only modify the muscle fibre phenotype but also exert a profound influence on the metabolism of the entire organism surely represents an important field for basic research in the field of Sports Medicine, targeted not only at improving athletic performance, but also at the prevention and treatment of major metabolic disorders by laying the foundations for the correct management of motor activity.

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