ACUTE EXERCISE-INDUCED MUSCLE INJURY

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ABSTRACT: While much research has recently been focussing on the chronic effects of overtraining, the acute damaging effects of individual eccentric exercise bouts on muscle remain of interest and underlie long-term training effects. Systemic markers of muscle damage are limited in terms of sensitivity and reliability. A clearer insight into the extent of the damage and mechanisms involved are being obtained from ultrastructural, functional and molecular examination of the muscle. There are currently indications that while the initial muscle damage may appear to have negative consequences in the short term, intense eccentric exercise appears to initiate a remodelling process and promote favourable adaptation of muscle following training, which has applications for promoting health, rehabilitation and sports performance.

Introduction

Early changes observed within the muscle after unaccustomed, eccentric (lengthening contractions) exercise include disruption of the sarcomeres [6,24,51], disruption of the cytoskeletal elements that are involved in force transmission [33,38], damage to the muscle cell membrane [39,48], impaired excitation-contraction coupling [70,72], loss of calcium homeostasis [16] and a subsequent loss of force production [46,72], swelling, stiffness and muscle soreness [11]. Although the precise time course and sequence of these events requires further research, this review will discuss the current information relating to the aetiology of acute exercise-induced muscle injury, the presentation of this muscle damage as well as the different markers that have been used to diagnose or characterise muscle injury.

Structural alterations: sarcomere, cytoskeletal and membrane damage

It is well accepted that muscle damage occurs in response to over-stretching of "weak" sarcomeres during eccentric contractions [57, 68]. This stretching of sarcomeres results in reduced myofilament overlap and a reduction in optimal tension generated according to the length-tension relationship [57]. Repeated overextension of these sarcomeres also leads to their disruption (Figure 1) and a shift in the length-tension relationship in those muscles that have both functioning sarcomeres and those overstretched and damaged by eccentric contractions. When the region of disruption is large enough, the muscle cell membrane, T-tubules, sarcoplasmic reticulum (SR) and other cytoskeletal elements are also damaged [57].

Ultrastructural changes have been observed 2-3 days [66,73], 7-8 days and as long as 10 days after eccentric exercise [43,54]. As mentioned above, early events include disruption to sarcomeres, cytoskeletal elements, specifically desmin and dystrophin (and associated proteins), the sarcoplasmic reticulum (SR), disrupted T-tubules and the cell membrane. These changes include A-band filament disturbances [24], disrupted I-bands [53], extracellular matrix disturbances [65], autophagic vacuoles [24], central nuclei [25], swollen or missing mitochondria [24], displaced organelles and randomly orientated myofilaments [51] and capillary disruption [65].
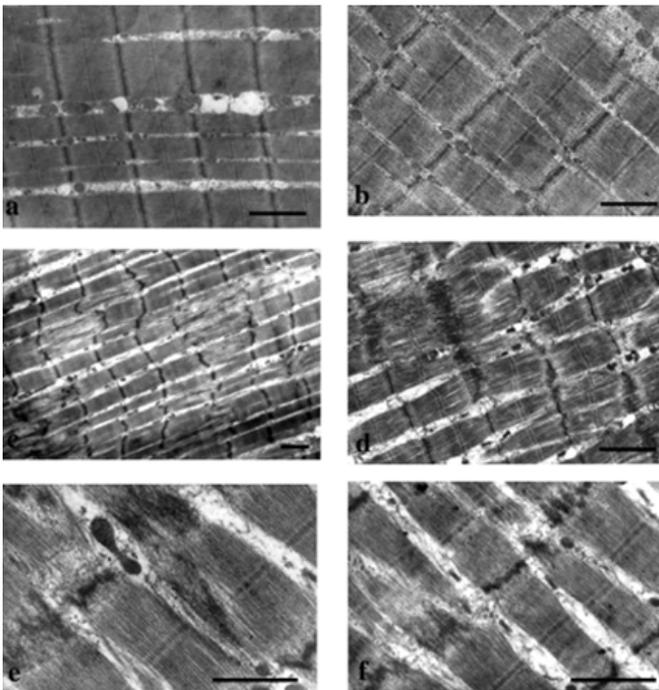
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membrane integrity. Desmin is a protein that forms part of the structural scaffolding and is thought to play an important role in maintaining the structure of Z-discs as well as helping to maintain proper alignment of sarcomeres within and between myofibrils. Research, predominantly in animal studies, has shown that muscle fibres lose "staining" (immunostaining of muscle cross-sections – provides information about the localization of specific proteins) for desmin within minutes after the initiation of eccentric contractions [38]. In addition, the number of fibres that lack desmin increases with time following an eccentric exercise bout and those fibres that lose desmin staining demonstrate accumulation of plasma fibronectin, indicating a loss of membrane integrity in these fibres [38].

Dystrophin is a large cytoskeletal protein associated with the cell membrane and is thought to help maintain the integrity of the membrane during the repeated mechanical loading that muscle cells experience through everyday contractions. At six hours after eccentric exercise, dystrophin staining is completely missing in some fibres, and this has been shown to accompany the loss of desmin as well as another structural protein, alpha-actinin. The number of affected fibres has been shown to increase for up to 2 days after exercise, indicating progression of damage over time [34]. To compound the loss of membrane stability, other members of the complex of proteins associated with dystrophin, specifically beta-spectrin and alpha-sarcoglycan, show signs of damage early following eccentric contractions [19]. It has been suggested that this rapid loss of membrane-stabilizing function may render the cell membranes more susceptible to damage by further contractions [32].

Plasma proteins to diagnose muscle damage

Earlier and more recent studies of exercise-induced muscle injury have used increases in plasma levels of intracellular muscle proteins (e.g. creatine kinase (CK), myoglobin, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), myosin heavy chain (MHC) [32], alpha-actin (α-actin) [44]) as markers of injury. The increased plasma levels of muscle proteins have been taken to indicate disruption of the cell membranes of muscle fibres from which the intracellular proteins leak into the blood. A problem with using blood proteins is that the concentration is a reflection of what is released by the tissue and what is cleared from the circulation [32].

The popular use of blood CK concentration as a marker of muscle damage after eccentric exercise has a number of limitations. The time course of CK activity depends on the type of exercise used to induce muscle damage [11]. The two most commonly used modalities to induce muscle damage are downhill running and high-force eccentric exercise that involves forced lengthening of the muscle using weights or dynamometers. While plasma CK activity peaks approximately 12-24 h after downhill running (reaching only 300-600 IU) [8], CK after high-force eccentric exercise does not increase until 24-48 h after exercise, reaching much higher levels ($\geq$2500 IU) [12]. The use of blood CK is also further complicated due to the large inter-subject variability in similar exercised

FIG. 1. ELECTRON MICROGRAPHS OF LONGITUDINAL SECTIONS ILLUSTRATING MUSCLE DAMAGE FOLLOWING ECCENTRIC EXERCISE IN HUMAN SKELETAL MUSCLE.

Note: (a), (b) – Normal ultrastructural profiles. (d), (e), (f) – Myofibrillar disruption (Z-line streaming) 1 day following eccentric exercise (Féasson et al., J Physiol 2002; 543: 297-306.). Reprinted with permission

Direct evidence of disturbances in myofibrillar organization due to eccentric contraction was first provided in 1981 [23]. Using light microscopy, these authors reported that focal disturbances to the muscle two days after subjects repeatedly descended stairs were three-fold larger than in control muscle and muscle obtained seven days after exercise. These findings laid the foundation for more in-depth investigations into muscle damage characteristics after eccentric contractions.

Using electron microscopy, Newham et al. [51] demonstrated focal myofibrillar damage in muscle immediately following a bout of eccentric contractions. This damage increased over time for up to three days after bouts of eccentric cycling and stepping. There was evidence of single disrupted sarcomeres, or half-sarcomeres, surrounded by normal-appearing sarcomeres [51], while disruption of Z-line architecture was the principal abnormality, with evidence of "streaming" or widening of the Z-lines. These structures also appeared to be completely absent in some cases. Interestingly, Costa et al. [14] did however not confirm these findings and therefore challenge the premise that muscle membrane damage occurs following eccentric exercise [14]. It is yet to be elucidated whether methodological differences may however explain these conflicting results.

Cytoskeletal elements involved in transmitting force from the sarcomeres through the membrane are also disrupted early following eccentric contractions. Disrupted elements include those important for maintaining Z-disc structure, sarcomeric organization, and cell

individuals [12]. Research has reported CK activity after high-force eccentric exercise ranging from 236 to 34,500 IU [51,52]. Age, gender, race, muscle mass, activity levels and genetic differences (polymorphisms) [41] have all been examined in attempts to explain the differences. Presence of total CK in plasma does also not faithfully reflect the extent of damage as it is composed of different isoenzymes (CK-MM, CK-MB, CK-BB) of different origin [5]. The lack of sensitivity and reproducibility of this marker has thus resulted in a prudent attitude towards its value and findings are hence viewed with scepticism. The use of α-actin as a marker of skeletal muscle damage has on the other hand been shown to have higher sensitivity (63-100%), and it represents more than 20% of all muscle cell proteins. α-Actin is also detected within 1 hour after the onset of muscle damage and can be detected in the serum for up to 72 hours after its release, indicating greater stability over time [25].

Other circulating markers of exercise-induced muscle damage include skeletal troponin I (STnI), which has been shown to increase and peak in parallel with CK and stay elevated for at least 1-2 days after exercise [63], and fatty acid binding protein [64], which together with myoglobin has been found to increase and decrease more rapidly than CK [68]. It has been suggested that they are possibly more useful than CK for the early detection and monitoring of exercise-induced muscle damage [63]. In order to differentiate between skeletal and cardiac muscle damage, measurement of the serum activity of cardiac troponin-T (cTnT) has also been suggested as it is regarded as a reliable marker of cardiac, but not skeletal muscle damage [63].

Interpretation of elevated levels of circulating muscle proteins and their correlation with tissue damage is thus complex. Although they are however sometimes still used to detect skeletal muscle injury, they are associated with large variability and more sensitive and precise methods have been developed to detect membrane damage.

Immunohistochemical techniques to diagnose muscle damage

Using immunohistochemical techniques, extracellular proteins and dyes appearing in the intracellular space of muscle fibres have been used as markers of membrane damage following skeletal muscle injury. The principle behind these methods is that membrane damage would allow the entry of macromolecules that are normally excluded from the intracellular space by the cell membrane. These methods allow determination of the specific muscle fibres that have incurred membrane damage and the time of wounding following eccentric contractions. Using downhill running to induce muscle damage in rats, McNeil and Khakee [48] demonstrated that immediately following the exercise bout, approximately 20% of muscle fibres in the medial head of the triceps brachii muscle stained positively for serum albumin. Typically, albumin is confined to the extracellular space, and therefore the result suggested that albumin leaked into these muscles through membrane disruption. In this study, the appearance of albumin within the muscle fibres was also associated with an increased plasma level of CK [48]. Together these markers indicated muscle cell membrane damage immediately after down-

hill running. In addition, the presence of Evans blue dye within muscle fibres has been used as a marker of membrane disruption [26]. When this dye is injected into the circulation it binds to serum albumin. Research has demonstrated that dye-positive fibres can be observed within minutes following eccentric contractions, whereas muscle not exposed to exercise does not demonstrate uptake of dye [26].

Strength loss: disruption of excitation-contraction coupling

Large deficits in force production immediately following eccentric-induced injury have been demonstrated. Strength loss and recovery is commonly used as an indicator of exercise-induced muscle damage and is considered one of the most valid and reliable indirect markers of exercise-induced muscle damage [69]. Downhill running or eccentric cycling results in immediate strength losses of 10-30%, with recovery at 24 h. Moderately intensive eccentric exercise results in strength losses of 30-50%, while intense protocols reduce strength by 50-70%, with recovery times of 7-10 days, extending to several weeks [60].

The small volume of tissue damage immediately after eccentric exercise does not account for the large reduction in strength [60]. This lack of association has led to the idea that much of the early loss of function following eccentric contractions results from impairment of excitation-contraction coupling [32]. This impairment could occur at any point in the chain of events between depolarization of the muscle cell membrane and the release of calcium from the sarcoplasmic reticulum that is required for force production in the myofibrils [32]. Research has demonstrated that calcium release from the sarcoplasmic reticulum is impaired in injured muscles. Eccentric contractions have also been shown to alter the structure of T-tubules. Both of these changes would be responsible for the impairment of excitation-contraction coupling [32].

Altered calcium homeostasis promotes muscle damage

A higher concentration of free calcium is found in the extracellular space compared to the sarcoplasm inside muscle cells. However, disruptions of the muscle cell membrane due to eccentric contractions would allow calcium to move down its concentration gradients, with the potential for greatly increasing calcium concentrations in the muscle cell [3]. In addition, within the SR, calcium concentrations are also higher than the sarcoplasm. Therefore, any disruption of the SR would also result in an influx of calcium into the cell [27]. Research has demonstrated that calcium concentrations are increased in skeletal muscle cells following eccentric contractions [16]. Damage to the sarcolemma and/or the SR leads to increased sarcoplasmic calcium at rest as well as to impaired calcium release upon electrical stimulation [32].

Increased calcium concentrations within the sarcoplasm have the potential for activating many different molecular pathways in skeletal muscle, including the phospholipase-prostaglandin pathway and the calpain proteolytic pathway. Membrane disruption, initially caused by eccentric contractions, allows for calcium influx into the
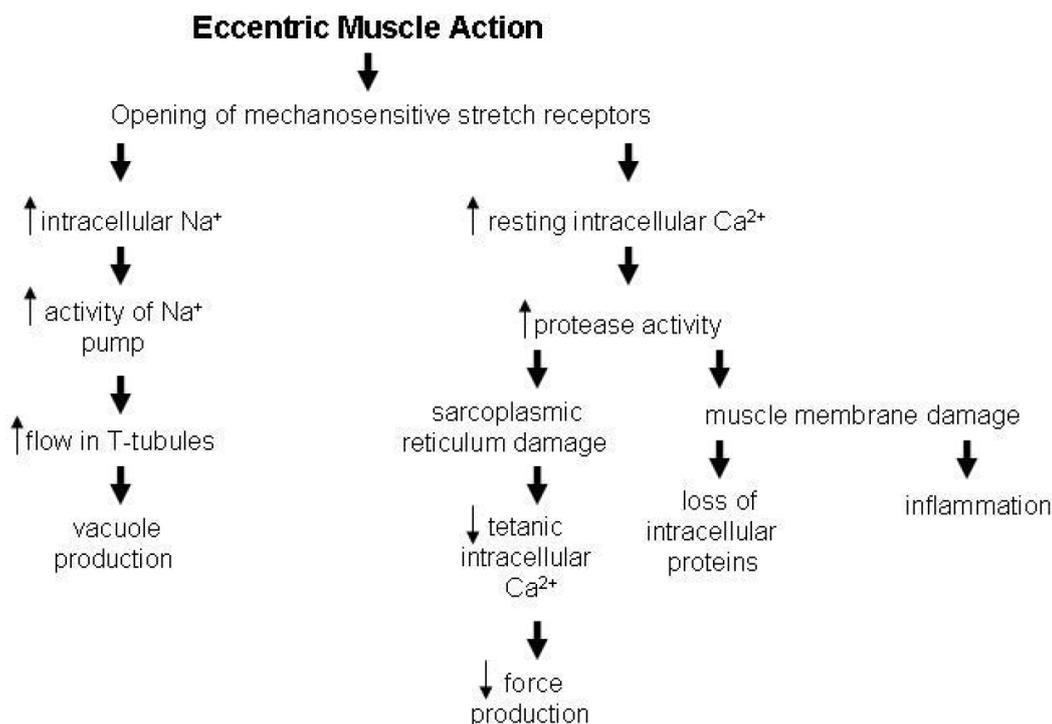
Eccentric Muscle Action

FIG. 2. ROLE OF STRETCH ACTIVATED CHANNELS AND ECCENTRIC MUSCLE ACTIONS (ADAPTED FROM ALLEN [2])

cell, which results in the activation of phospholipase A2. Activated phospholipase A2 promotes further muscle cell damage by contributing to the breakdown of the cell membrane, resulting in the subsequent loss of intracellular molecules [17]. As mentioned, calcium also activates calpains, which are proteolytic enzymes responsible for initiating the breakdown of myofibrils [19]. Calpains have also been shown to cleave many of the cytoskeletal elements reported to be damaged following eccentric contractions, including desmin, dystrophin and spectrin [32]. In addition, calpains may specifically degrade Z-discs in skeletal muscle, and may be responsible for Z-disc disruption following eccentric contractions [38]. The association between calpain activity and the extent of muscle damage is unknown [58].

Inflammation in skeletal muscle tissue

The mechanical trauma caused by eccentric exercise results in an inflammatory response. Fluid, plasma proteins, as well as inflammatory cell populations infiltrate the injured tissue, with the main aim of clearing debris from the injured area and preparing it for regeneration [11]. Neutrophils are the first cells to begin accumulating in the tissue at the injury site, destroying necrotic tissue through phagocytosis while working in conjunction with resident macrophages from the muscle tissue itself [11]. Research has demonstrated rapid infiltration of inflammatory cells (predominantly neutrophils) into skeletal muscle 45 min – 2 h after eccentric exercise [20,40]. In addition, macrophages have been shown to appear around 6 h, peak around 24 h after exercise and still be present in the muscle, together with T-lymphocytes, 9-14 days following eccentric exer-

cise [30]. It has been suggested that the extended stay of these cells is related to their involvement in the repair/remodelling process. Two subpopulations of macrophages have been shown to be involved in the removal of cellular debris (ED1+) as well as muscle repair (ED2+) through the activation of satellite cells [67].

Current thinking supports a mechanism whereby eccentric actions may activate stretch channels in the muscle, culminating in reduced force production and inflammation (Figure 2). Stretch-activated ion channels, also referred to as mechanosensitive channels, are embedded within cellular membranes of most tissues, including skeletal muscle, and are involved in a number of different activities [22,49]. McBride et al. [45] have proposed that activating these channels via eccentric actions may serve as a stimulus for muscle adaptation and hypertrophy.

Swelling

Swelling occurs within the muscle following exercise-induced injury [60]. Common methods that have been used to test for the presence of swelling after eccentric exercise include measuring muscle group circumference, ultrasonography (to measure muscle thickness) and magnetic resonance imaging (MRI). Increases in circumferences and ultrasound measurements have been shown to peak at 4-5 days after exercise [52]. Magnetic resonance imaging has demonstrated intracellular oedema in muscle tissue after eccentric exercise [56]. Specifically, the signal intensity of MRI (called the T2 relaxation time) is dependent on the amount of water in tissue and appears to reflect intracellular oedema [11]. Acute injury and an increase in oedema or haemorrhage will prolong T2 relaxation times [11]. Research has

demonstrated that changes in MRI, reflecting oedema, are associated with increased levels of muscle proteins (e.g. CK and myoglobin) in the extracellular space after eccentric exercise. Using MRI, researchers have demonstrated an acute phase of swelling with an initial increase 0-1 h after exercise followed by a gradual phase with increases in swelling from 1 to 6 days after exercise [21,56]. Changes in intramyocellular lipid levels have also been shown to follow eccentric exercise [28] and this accumulation may indirectly contribute to localised swelling.

Decreased range of motion

Research has demonstrated a decrease in the voluntary range of motion (ROM) in joints following eccentric exercise. The underlying mechanism responsible requires further research. However, it has been suggested that an increase in the number of contracted fibre segments related to either an increase in resting cytosol calcium levels or ultrastructural damage may be responsible [57]. In addition, the reduction in ROM may be partly attributed to a concomitant increase in fluid accumulation (swelling). A decrease in ROM has been reported to be one of the most valid and reliable indicators of exercise-induced muscle damage [69]. The range of motion decreases immediately following exercise and can be reduced by 20-45 degrees [12,29]. Whilst recovery begins within 24 h, full recovery is not achieved until 10 days after exercise [60].

Muscle soreness

Delayed-onset muscle soreness (DOMS) is a hallmark manifestation of exercise-induced muscle damage [60]. This is typically determined subjectively using a numerical scale called a visual analogue scale (VAS). Individuals are asked to mark on a fixed-length written scale the level of soreness felt in the muscle according to verbal instructions from the investigator [50]. Delayed onset muscle soreness develops 24-48 h after exercise, peaks at 24-72 h, and by 5-7 days after exercise has abated, with pain/soreness levels returning to baseline [12,13]. Regarding the localization of DOMS, some studies have reported that soreness is felt in the entire muscle [51] while other studies have demonstrated soreness at the distal portion of the muscle at the myotendinous junction [18]. The dull, aching pain that signals DOMS has been attributed to the type III and IV afferent neurons. Mechanical nociceptors (pain receptors) are typically type III fibres while type I fibres are polymodal (responding to mechanical, thermal and chemical signals) [60]. The primary mechanisms underlying the DOMS sensations include swelling and subsequent increased pressure within the muscle that activates resident mechanoreceptors (nociceptors). Chemical changes within the muscle have also been suggested to be responsible for DOMS. These chemical changes include increased histamine, bradykinins and prostaglandins that follow the infiltration of inflammatory cells and the breakdown of myocellular components. These substances activate the polymodal nociceptors that are sensitive to chemical signals [60].

Why are type II muscle fibres more susceptible to damage?

Research has demonstrated that fast glycolytic (type II) muscle fibres are more susceptible to eccentric contraction-induced injury than slow oxidative (type I) fibres [24]. This result has been demonstrated in both human and animal studies. Research performed on individuals who performed eccentric bicycle exercise revealed signs of injury predominantly in fast glycolytic muscle fibres of the quadriceps muscle group 18 to 72 h after exercise [24]. Similar findings have also been demonstrated in elbow flexors as well as foot plantar flexors [30]. Although it was initially thought that metabolic differences between type II and type I fibres could explain the increased susceptibility [55], it is now thought that type I fibres contain higher levels of certain cytoskeletal proteins that provide structural support for the sarcomeres and the cell membrane. These cytoskeletal proteins maintain the integrity of these structures in the face of repeated eccentric mechanical loading, and therefore demonstrated lower injury levels. Muscle fibre myosin heavy chain (MHC) content is one 'component' of the cytoskeleton that has been implicated in influencing susceptibility to damage; differences between MHC content in type I and type IIa/IIx fibres have been documented [10]. It has also been suggested that type I fibres have increased levels of other protective molecules, including a family of "stress proteins", specifically heat shock proteins, that protect the muscle from mechanical stresses experienced during eccentric contractions and enhance recovery/adaptation in the muscle [31].

Damage versus remodelling

An interesting perspective on cytoskeletal damage in the muscle in response to unaccustomed eccentric exercise has been suggested by Yu and colleagues [76]. These authors argue that the changes provide evidence for myofibrillar remodelling and adaptation in humans. Evidence to support their argument stems from research indicating that there is no loss of desmin staining in human muscles (as opposed to animal studies) 1 h, 2-3 days, or 7-8 days following eccentric exercise [76]. In fact, there is a focal increase in desmin staining, with desmin strands extending from 1 Z-line to another [76]. This finding has been interpreted as indicating that cytoskeletal damage is not the initiating damaging event in the muscle, but rather that desmin acts as a mechanical linkage in repair and remodelling of the sarcomere. Support for the remodelling hypothesis has also been provided by research demonstrating supernumerary sarcomeres (additional sarcomeres) and increased staining of actin and other cytoskeletal proteins involved in sarcomerogenesis (alpha-actinin, titin and nebulin) in some myofibrils after eccentric exercise [74,75].

Gene expression

Naturally this remodelling requires increased protein synthesis, a process regulated by gene expression. The role of genes in influencing susceptibility to muscle damage and the remodelling thereof is an aspect that is receiving more attention within the literature. A number of genes have been shown to be upregulated fairly rap-

idly following eccentric exercise [4,71] and it has also been shown that genetic background plays an important role in differential skeletal muscle remodelling [35]. Genes involved in stress management, structural integrity, protein synthesis, stem or satellite cell activation, inflammation, muscle repair, membrane repair and cell death have been shown to be up-regulated by exercise-induced muscle damage [41,42]. Gene array studies have resulted in the development of various hypotheses relating to recovery and adaptation following muscle damage. Barash and colleagues [4] proposed that specific genes are up-regulated in response to mechanical stretch or structural damage, whilst Chen et al. [9] proposed that eccentric contractions up-regulate the stress responsive gene p53 that promotes repair or apoptosis pathways. In addition, Mahoney et al. [41,42] have proposed that eccentric exercise activates genes that promote membrane synthesis. The damage and subsequent reparation process may also be influenced by gender, with Sonobe et al. [62] recently showing that intracellular Ca^{2+} homeostasis is maintained in female rats following eccentric exercise and not in their male counterparts.

Application to sports medicine and science

Investigating the mechanism(s) responsible for adaptation to acute exercise-induced muscle damage, particularly to eccentric exercise, is important for understanding how eccentric training can be applied in sports medicine/science for health promotion, orthopaedic and chronic disease rehabilitation, and performance enhancement. Chronic use of eccentric contractions results in pronounced protective adaptation within the muscle termed the "repeated-bout effect" [47]. Specifically, a repeated bout of the same or similar eccentrically biased exercise results in remarkably reduced symptoms of damage compared to the initial bout. This protective adaptation of a single bout of eccentric exercise has been demonstrated in human and animal models and may last for several weeks and up to 6 months [47]. Several mechanisms, including neural, mechanical and cellular adaptations, have been proposed to explain the repeated bout effect [47]. The load of the initial bout can be lower than the repeated bout, with research demonstrating that as few as 2-10 maximal eccentric contractions of the elbow flexors provide protection for a subsequent bout of 50 maximal contractions [7]. While the repeated bout effect is specific to the exercised muscle group(s), the mode of eccentric exercise used to promote the protective effect can differ, e.g. eccentric isokinetics of quads vs downhill running [7]. Importantly, for promoting sports performance, chronic eccentric training has been shown to increase muscle stiffness properties and jumping performance [59], as well as to increase isometric strength and muscle fibre (composites of type I and II fibres) area in both young and old individuals, compared to controls performing isolated concentric work or typical weight-lifting exercise. Specifically, following 8 to 11 weeks of chronic (2-3 times per week) eccentric resistance training, strength increased by approximately 40% (young) and 120% (old), while the vastus lateralis muscle fibre area increased by approximately 50% to 60% [36,37]. Finally, eccentric contractions and/or eccentrically biased exercise are effective in reducing sports-associated hamstring muscle strains [61], reversing or reducing muscle atrophy/sarcopenia in the elderly [36], treating tendinopathies [1] and reducing inflammation in chronic disease [14].

In conclusion, research on the aetiology and presentation of skeletal muscle damage in response to an acute bout of unaccustomed or strenuous eccentric exercise is important for understanding the mechanisms underlying muscle injury, healing and adaptation. Understanding these mechanisms will result in the development of better strategies for reducing the severity of muscle injuries as well as for improved approaches for expediting the healing or adaptive process. This of course has implications for sportsmen and women, but a greater understanding of the muscle damage and healing process also has important therapeutic implications for patients living with disease that directly or indirectly alters normal muscle function. Recently, there has been a major shift towards using molecular techniques to examine the expression or down-regulation of genes in response to exercise-induced damage [41]. This information will ultimately be used to explain the responses to exercise-induced muscle damage discussed in this review.

Conflict of interest declaration

There was no conflict of interest in preparing this manuscript.

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