Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components

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ABSTRACT

Exercise involving lengthening of an activated muscle can cause injury. Recent reports documented the mechanics of exercise-induced muscle injury as well as physiological and cellular events and manifestations of injury. Loss of the cytoskeletal protein desmin and loss of cellular integrity as evidenced by sarcolemmal damage occur early during heavy eccentric exercise. These studies indicate that the earliest events in muscle injury are mechanical in nature, while later events indicate that it may be more appropriate to conclude that intense exercise initiates a muscle remodeling process. We conclude that muscle injury after eccentric exercise is differently severe in muscles with different architecture, is fibre type-specific, primarily because of fibre strain in the acute phase, and is exacerbated by inflammation after the initial injury.

Keywords cytoskeleton, delayed-onset muscle soreness, muscle injury, muscle morphology, muscle strength.

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Delayed-onset muscle soreness (DOMS) is the sensation of muscular discomfort and pain during active contractions that peaks 24–48 h after strenuous exercise. The initial symptoms are most evident at the proximal and distal muscle tendon junctions and spreading throughout the entire muscle. Skeletal muscle soreness and injury are associated with intense exercise but are more pronounced if the individual is unaccustomed to the exercise performed. Thus, even individuals in excellent athletic condition may experience muscle soreness and damage when performing exercise which is new to them. Although loss of strength has been observed very soon (within minutes) after exercise, the underlying causes of the relation between DOMS and the loss of muscle strength has yet to be explicitly proven.

ECCENTRIC MUSCULAR ACTIVITY

Erling Asmussen established in the 1950s that DOMS is directly associated with the eccentric component of exercise (Asmussen 1952, 1956). During an eccentric action, a contracting muscle is forced to lengthen while producing tension. During concentric action the muscle produces tension while shortening. The intermediate, isometric contraction, produces tension while the muscle remains essentially at the same length. All three actions are common components of daily movement. The tension generated during eccentric action is higher than that for either of the other actions (Fig. 1) (Katz 1939, Singh & Karpovich 1966, Komi & Rusko 1974, Stauber 1989) although fewer motor units are recruited (Bigland-Ritchie & Woods 1976). In addition to relative differences in tension between actions, Fig. 1 also demonstrates variations in tension as a function of contraction velocity. During concentric activity the tension/velocity relationship is explicitly formulated as described by the Hill equation (Hill 1938) and is attributed to the alignment of actin and myosin optimizing cross-bridge formation (Huxley 1969). Because of the nature of an eccentric action, the tension generating mechanism can be expressed in two phases. First, the muscle contracts to generate tension and then the muscle is passively stretched by an external force while generating additional tension (Katz 1939, Stauber 1989). The passive stretching has led investigators to suspect elastic components of the intact muscle-tendon system as possible contributors to the additional tension (reviewed by Chapman 1985). Recent studies imply that muscle passive tension is determined by the particular titin isoform expressed and that it is variable between different striated muscles, even within the muscle.
same species (Wang et al. 1993, Labeit & Kolmerer 1995). Other investigators attribute, in theory, the energetics of cross-bridge formation during the contraction/stretching phase as the reason for the higher tension for eccentric activity (Stauber 1989). Recently, Linari and coworkers demonstrated that during lengthening the cross-bridge number increases while the average degree of cross-bridge strain is similar to that induced by the force-generating process in isometric conditions (Linari et al. 2000).

OBJECTIVE FINDINGS ASSOCIATED WITH DELAYED ONSET MUSCLE SORENESS

The general findings when examining an individual with DOMS symptoms include prolonged strength loss, a reduced range of motion, and often elevated levels of serum creatine kinase (CK). A multitude of studies have demonstrated that eccentric exercise results in increase in serum CK levels 24–48 h after the exercise bout and may persist for 3–6 days. Creatine kinase is an intramuscular enzyme responsible for maintaining adequate ATP levels during muscle contraction. Its appearance in blood is interpreted as an increased permeability or breakdown of the membrane surrounding the muscle cell.

The perception of soreness after eccentric exercise is out of phase with serum CK levels (Newham et al. 1988). It is also important to note that the substantial muscle injuries and necrosis demonstrated in animal exercise experimentation cannot be reproduced with eccentric exercise protocols in humans. Investigators have demonstrated a protective effect of previous training, comparing CK levels to untrained control values (Evans et al. 1985). It is often assumed that the level of CK activity is somehow related to the magnitude of muscle injury, although this potential correlation has not been explicitly proven. In a preliminary study of 26 rabbits performing eccentric exercise with their ankle dorsiflexors we found a poor correlation between serum CK levels and depressed skeletal muscle force generating capacity after eccentric exercise (Fig. 2) (Fridén & Lieber 2000). It may not be surprising that this relationship is relatively poor because a muscle fibre’s permeability to intramuscular enzymes may or may not be correlated with cellular contractile function. For example, we previously demonstrated that numerous muscle fibres subjected to eccentric exercise that retained their ability to exclude plasma fibronectin demonstrated significant structural abnormalities such as loss of intracellular desmin, myofibrillar disruption, and Z-disk disintegration.

MUSCLE INJURY

The immediate loss of strength after eccentric exercise has been referred to as overstretch of sarcomeres, resulting in a non-optimal overlap between actin and myosin filaments length (Faulkner et al. 1993) as well as changes in excitation-contraction coupling (Balnave & Allen 1995, Morgan & Allen 1999). Faulkner and coworkers suggested that during eccentric exercise some sarcomeres are stretched beyond overlap and thereby injured while others maintain their length. It is well known that eccentric exercise induces greater changes than comparable contractions of either isometric or concentric activity (Warren et al. 1993). Despite the greater force loss after eccentric contractions, the resting membrane potential of isolated muscles fatigued by either eccentric or isometric contractions were similar (Warren et al. 1993). Because
Hypercontraction

Fibre lesion

Figure 3 Schematic drawing of longitudinal section of muscle fibre with segmental damage surrounded by two normal fibres. Bilateral to the central necrotic zone are hypercontraction zones noted. In the region of necrosis, phagocytes are observed both inside and outside the partially damaged muscle fibre membrane. The hypercontraction zones displace adjacent fibres at the level of lesion.

There is no evidence of excitation failure the ability to produce action potentials is expected to be unaffected. Regardless of exact mechanism, it is generally agreed that initial force decline is caused by mechanical injury. A number of mechanical factors such as muscle length, force and velocity seem to play a role in the consequences of eccentric contractions. Newham et al. (1988) found more pronounced strength loss after eccentric exercise at a long muscle length compared with a short muscle length. Although the nature of morphological injury to the muscle has been well documented the mechanism of the injury is not fully understood. Although muscle tissue is extremely plastic (Pette 1990) destructive changes of muscle ultrastructure may occur in response to unusual demands (Hoppeler 1986). Morphological abnormalities after various types of exercise have been reported in both animal (Armstrong et al. 1983, Kuipers et al. 1983, McCully & Faulkner 1985, Lieber & Fridén 1988) and human models (Fridén et al. 1981, 1983, 1984, Newham et al. 1983). Different types and locations of cellular lesions after heavy exercise are shown in Table 1.

The vast majority of morphological studies indicate that the Z disk is the most vulnerable structure to eccentric exercise induced injury. Damage has also been found in the sarcolemma, T tubules, myofibrils and the cytoskeletal system. In the mild type of damage, immediately after exercise, single or very few sarcomeres throughout the muscle may be affected, usually showing Z-disk streaming (wavy appearance) with myofibrillar disarray. In more severe cases, extensive streaming (wavy appearance and widening of Z-disk stain) and smearing (dispersion of Z-disk material into neighbouring sarcomeres) of the Z disk, focal loss of the Z disk and displacement of Z-disk material may occur. Both type 1 and type 2 fibres seem to be affected although the majority of studies demonstrate type 2 biased damage (Lieber & Fridén 1988). It has been shown that sarcoplasmic reticulum channel proteins may play a role in the aetiology of muscle injury (Gilchrist et al. 1992). Several studies indicate that disturbances in calcium metabolism may be associated with muscle changes and weakness after heavy exercise (Reid et al. 1994). More recent studies have suggested that the early cytoskeletal changes (Lieber et al. 1996) may be attributed to cytoskeletal proteins located at the level of the Z disk (α-actinin, vimentin and desmin).

The most striking change observed after eccentric rabbit dorsiflexion exercise was the loss of desmin staining in a significant portion of muscle fibres across the muscle (Lieber et al. 1996). Three days following eccentric exercise, over 30% of the EDL fibres had lost desmin staining. This percentage rapidly decreased, so that by 7 days post-injury the percentage was about 10% for the EDL. By 28 days post-injury, less than a fraction of a percentage of desmin negative fibres could be found. Interestingly, most but not all desmin negative fibres were also fibronectin positive, indicating loss of cellular integrity accompanying cytoskeletal disruption. It should be noted that desmin staining was lost although the muscle fibre still maintained contractile and oxidative enzymes.

Belcastro (1993) reported increased calpain activity in hindlimb muscles after treadmill running. He postulated that early mechanical events which initiate injury and which result in later inflammation require an intermediate event. A most likely intermediate event is an increase in intracellular calcium level (Balnave & Allen 1995). While cyclic calcium concentration changes are normal in the muscle contraction cycle,
chronically elevated intracellular calcium can activate endogenous proteases (e.g. calpain), causing muscle deterioration. As a result, increased intracellular calcium remains an attractive feature for most muscle injury models. Belcastro also demonstrated an increased affinity of calpain for calcium after exercise, suggesting that exercise itself increased the total amount of calpain available to destroy muscle tissue as well as its affinity for calcium. A given amount of damage could thus occur after exercise at a lower calcium concentration. Interestingly, the calpain is specific for cytoskeletal proteins, whereas actin and myosin do not appear to be affected. Two proteins associated with the intermediate filament system (\(\alpha\)-actinin and desmin) are highly affected.

MORPHOLOGICAL MANIFESTATIONS

In a number of previous animal muscle injury studies, fibres with abnormal shapes and sizes have been used as morphological indices of acute exercise-induced muscle injury (Armstrong et al. 1983, McCully & Faulkner 1985, Lieber & Fridén 1988, Fridén et al. 1991, Lieber et al. 1991). Particularly, very large fibres were demonstrated as a result of eccentric exercise. These fibres were always identified as the fast-twitch glycolytic (FG) fibre type. Furthermore, these large fibres frequently displayed intracellular deposits of plasma fibronectin indicating membrane lesion (Fridén et al. 1991). The large fibres that were found in histological sections following repetitive eccentric contractions represent segmental hypercontraction of the fibre (Fridén & Lieber 1998). This phenomenon occurs adjacent to muscle fibre plasma membrane lesions and necrosis and manifest itself as very small sarcomere lengths (Fig. 3). On both sides of a lesion, the fibre regained its contractile elements and myofibrils became increasingly densely packed. The large fibres appeared at variable distances from the bulging zones as fibres with normal thickness or again significantly wider than neighbouring ‘normal’ fibres. At the border region between wide and thin fibre portions, invading cells were found although consistently to a much lesser extent than in the thin region. In the thin region, the plasma membrane was only infrequently intact and numerous macrophages were always present at 3 days post-exercise. It is important to remember that these ‘extreme’ exercise models may not directly relate to the human DOMS models where much less damage have been demonstrated.

After an eccentric exercise protocol in the rabbit, ultrastructural analyses revealed that the typical A-band lattice was consistently lost, and Z-disk lattices and even Z-disk material were absent in the biopsies taken 1 h post-exercise (Fridén & Lieber 1996). Thin and thick filaments were severely disorganized and the remnants of the band pattern were wavy and consistently broken in multiple places. Sarcoplasmic reticulum and T tubules were either normal or dilated in the 1-h post-exercise biopsies while they appeared rounded and with an increased and often granular density in biopsies taken later after eccentric exercise.

The fibres of lower antidesmin density but negative antifibronectin staining presumably correspond with the previously reported immediate ultrastructural consequence of eccentric load, i.e. distortion of the alignment of the A and I bands, irregular Z disks and slippage of the thick filaments out of the thin filament lattice (Dix & Russell 1991, Lieber et al. 1991). It is reasonable to assume that the fibre as an entity is essentially in good structural condition unless the focal injuries appear multiple times in the same fibre. This is, however, not possible because once a fibre is disrupted the remaining intact fibres supposedly take up the tension put on the muscle. This would mean that eventually fewer fibres will be subjected to forced lengthening as has been proposed earlier (Fridén et al. 1983, Armstrong 1990). On the other hand, a focally damaged fibre may function normally, especially...
Hortobágyi et al. (1998) found that this recovery occurred independently of cell disruption and concluded that it was mediated by neural factors. Hortobágyi’s results were based on the fact that after a second bout of eccentric exercise EMG, patellar tendon reflex amplitude and CK level were normalized although 23% of the muscle tissue was damaged.

In summary, we believe that the following series of muscle fibre events are taking place after DOMS-causing exercise: cytoskeletal disruptions, loss of myofibrillar registry, i.e. Z-disk streaming and A-band disorganization, loss of cell integrity as manifested by intracellular plasma fibronectin stain, hypercontraction of injured fibre regions and invasion of inflammatory cells. Preventive and therapeutical agents can act on several of these levels, but so far there is no consensus on an existing treatment that fulfils the requirement of efficacy and no or minimal negative effect on the muscle injury-repair process.

REFERENCES


