Muscle Injury, Regeneration, and Repair

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Abstract: This article reviews relevant muscular anatomy and describes the metabolic, temperature, and mechanical hypotheses as possible mechanisms of muscle injury. It describes the four stages of muscle injury, regeneration, and repair: Ca^{2+}-overload, autolysis, phagocytosis, and regeneration/repair. The article concludes with some likely clinical implications for prevention and treatment of muscle injury.

Key Words: Muscle, Injury, Regeneration, Repair

A plethora of information is available in physical therapy literature on injury, regeneration, and repair of the connective tissues. This is understandable: after all, the different types of connective tissue (CT) make up a large part of the body. Knowledge of CT physiology and pathophysiology forms the basis for prevention and treatment of many injuries. In contrast, information on muscle injury, regeneration, and repair is not as readily available to physical therapists, yet muscle injury is common. It can be the result of mechanical forces; excessive tension may be generated during a passive stretch or a contraction, especially a lengthening or eccentric contraction[1-4]. Excessive mechanical force is also the reason for muscle injury due to contusions[2, 4-10] and lacerations[9, 10]. Muscle injury can result from thermal stress, such as extreme heat or cold[1, 6,7]. Myotoxic agents can cause muscle injury. The local anesthetics marcaine[6] and lidocaine in combination with epinephrine[10] have been shown to cause muscle fiber necrosis. Excessive doses of corticosteroids[10] and certain snake and bee venoms are also myotoxic[1]. Prolonged ischaemia also disrupts muscle; clinically this may be seen in compartment syndromes but also after the tourniquet application commonly used to create a bloodless field for surgical procedures[4, 6,9-11].

The goal of this article is to increase the therapist's understanding of muscle injury, regeneration, and repair. An increased understanding of the process of muscle injury and subsequent regeneration or repair can provide us with a theoretical basis for more appropriate prevention and treatment of muscle injuries. In this article relevant anatomy is reviewed. This will better allow for a discussion of possible causative mechanisms and the stages of muscle injury, regeneration, and repair. A review on the effectiveness of interventions to prevent or treat muscle injury is outside the scope of this article, but some possible clinical implications of the information will be discussed.

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Anatomy

The myofiber or muscle fiber is the fundamental cellular unit of skeletal muscle: it is a multi-nucleated syncytium formed by fusion of myoblastic cells[10, 12]. This myofiber contains the myofibrils, which are composed of sarcomeres, arranged in series[13-15]. A sarcomere is the smallest contractile unit of the muscle; it is composed of two types of proteins: contractile and structural proteins[15] (Figure 1).

The contractile proteins make up the thick and thin filaments. The thin filament consists of two intertwined filamentary or F-actin chains. Tropomyosin and troponin-C, -I, and -T are located in the groove between these two chains. Myosin is the main protein of the thick filament; it consists of two myosin heavy chains (MHC) and four myosin light chains (MLC). The thick filament is formed by aggregation of these myosin molecules[14, 15.] One way to distinguish between the different fiber types is by identifying the different isoforms of MHC and MLC that they contain[15].

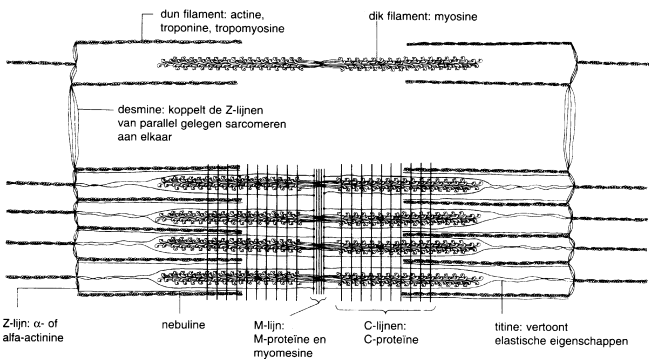

Fig. 1: *Sarcomere. In: Huijbregts PA, Clarijs JP. Krachttraining in revalidatie en sport. Utrecht: De Tijdstroom BV, 1995.*

Cross-striation of skeletal muscles is the result of an orderly arrangement within and between sarcomeres and myofibrils. Maintaining this orderly arrangement is the role of the structural proteins. The myofibrils are aligned parallel to the long axis of the muscle fiber; the thin filaments are anchored on either side of the sarcomere to a transverse structure called the Z-disc. In the Z-disc, each thin filament is connected to four thin filaments from the adjacent sarcomere by a protein called alpha-actinin[13-15]. In turn, alpha-actinin is thought to be anchored in the Z-disc by the proteins zeelin-1 and zeelin-2[1]. The Z-disc contains the proteins desmin, filamin, synemin, and zeugmatin at its circumference[10]. Desmin is called an intermediate filament: its 8-12 nm diameter places it in size between a smaller and a larger group of myofibrillar proteins[3]. It maintains inter-myofibrillar orientation by linking the Z-discs of adjacent myofibrils, and it stabilizes mitochondria and nuclei to the sarcolemma[3, 9.] The Z-disc structure of the slow-twitch (ST) fibers is more complex and thicker than that of the fast-twitch (FT) fibers[10, 13-15]; this structure may play a role in the selective FT fiber injury observed after eccentric exercise[3]. The M-line is another transverse structure, located in the middle of the thick filament. In this M-line, M-protein runs perpendicular to the myosin molecules and maintains their spatial orientation. Myomesin anchors the protein titin to the M-line[13-15]. Intermediate filaments bridge the axis of the muscle fiber connecting adjacent M-lines[3]. C-lines are transverse structures composed of C-protein: this protein again stabilizes the thick filament arrangement[13]. Nebulin and titin are two proteins oriented parallel to the thick and thin filaments. Titin runs from the Z-disc to the M-line and is hypothesized to have a role in restoring acto-myosin contact when no overlap exists after excessive stretch; it also keeps the thick filament centered in the sarcomere[13].

Sarcoplasmatic reticulum (SR) is an intracellullar structure that sequesters and releases the Ca^{2+}-ions needed for acto-myosin interaction. In FT fibers, the SR embraces every individual myofibril; in ST fibers, it may contain multiple myofibrils. The ATP-dependent enzyme calcium-ATP-ase plays an important role in pumping Ca^{2+}-ions back from the muscle fiber cytoplasm (or sarcoplasm) into the SR. ATP-depletion and subsequent decreased calcium-ATP-ase function may play a role in the Ca^{2+}-induced autolysis discussed below. The transverse tubular (TT) system consists of invaginations of the muscle fiber membrane closely associated with the SR: action potentials are rapidly propagated throughout the muscle fiber by way of this TT-system[15].

The muscle fiber membrane (or sarcolemma) is a lipid bilayer structure[16]. Small invaginations or caveolae allow for stretch of the sarcolemma[15]. The protein dystrophin, lacking in patients with Duchenne muscular dystrophy, plays an essential role in mechanical strength and stability of the sarcolemma[1]. Extensive membrane infolding and interdigitation with the extracellular CT increase the surface area of the myotendinous junction (MTJ), on average, with a factor of 10 to 20. Infolding at the MTJ of ST fibers is even more extensive, increasing surface area about 50-fold[16]. The MTJ has visco-elastic mechanical characteristics; the increased ST fiber infolding may be in response to the creep experienced by the MTJ of these fibers during prolonged low-load contractions. The difference in MTJ surface area may be yet another explanation for selective FT fiber injury as a result of tensile forces[16]. The membrane infolding also places the membrane and the interface between membrane and extracellular CT at a very low angle (near zero) relative to the tensile force developed by the myofibrils (Figure 2). The sarcolemma appears to be more resistant to the resultant shear forces than to the tensile forces that would be produced if the sarcolemma were oriented more perpendicular to the myofibril. The interface between sarcolemma and extracellular CT also seems more resistant to shear forces. Muscle atrophy increases the angle of the membrane-terminal filament interface exposing it to more tensile forces; this may explain failure of the MTJ after immobilization-induced disuse atrophy[16]. Despite these adaptations, the MTJ is very vulnerable to tensile failure[4, 9,16,17]:

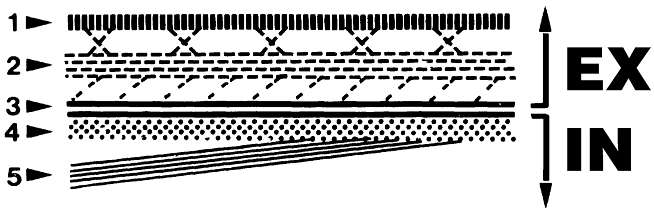

Fig. 2: Schema of the structures involved in force transmission between tendon and contractile proteins of muscle cell. Extracelluar components (EX) include tendon collagen fibers (1) and basement membrane (2). The junctional plasma membrane (3) separates extracelluar (EX) and intracelluar (IN) force-transmitting structures. Within the cell, thin actin filaments (5) are attached to the cell membrane by dense, subsarcolemmal material (4). Copyright 1987 American Academy of Orthopaedic Surgeons. Reprinted from Garrett W, Tidball J. Myotendinous Junction: Structure, Function, and Failure. In: Woo SLY, Buckwalter JA. Injury and repair of the musculoskeletal soft tissues. AAOS, Rosemont, IL, 1987.

a predilection for a tear near the MTJ has been reported in the biceps and triceps brachii, the rotator cuff muscles, the flexor pollicis longus, the peroneus longus, the medial head of the gastrocnemius, the rectus femoris, the adductor longus, the iliopsoas, the pectoralis major, the semimembranosus, and the whole hamstrings group[2, 18].

The terminal sarcomeres near the MTJs are shorter than the more centrally located sarcomeres: this allows for increased speed of contraction but decreased force[16]. It has been hypothesized that the increased contraction speed allows for pre-loading of the MTJ before the other sarcomeres reach peak tension[16]. Muscle contraction (and thus possibly MTJ pre-loading) results in greater force and energy absorbed prior to failure[18]. The distance of these terminal sarcomeres to the neuromuscular junction is greater, meaning that an action potential has to travel further along the sarcolemma and the TT-system. Shorter terminal sarcomere length may allow for a more uniform tension development along the whole length of the muscle fiber rather than earlier tension development[15]. Hypotheses with regards to the role of the shorter terminal sarcomeres in causing muscle fiber injury are discussed below. Filamentous actin connects the Z-discs of the terminal sarcomeres to sub-sarcolemmal densities at the MTJ. The proteins vinculin and talin connect these actin filaments to the sarcolemma of the MTJ. The terminal Z-discs are also directly structurally continuous with these densities[16].

The basement membrane is a loose matrix of glycoproteins and collagen fibers located outside the sarcolemma[14]. At the MTJ, the large transmembrane glycoprotein integrin may link the proteins vinculin and talin (discussed above) to the basement membrane[16]. The basement membrane consists of a basal and a reticular lamina. The basal lamina is located closest to the cell and consists of an inner lamina rara and an outer lamina densa[10]; the reticular lamina merges with the endomysium along the length of the fiber; at the MTJ, it merges with the collagen fibers of the tendon[16]. The protein laminin, the glycoprotein fibronectin, and type IV collagen have been hypothesized to play a role in this basement membrane-tendon interaction[16]. The basement membrane has an important mechanical function: it dissipates up to 77% of the energy lost during a stretch-shortening cycle of the muscle[1]. It may seem illogical to introduce an energy-dissipating structure such as the basement membrane in a chain of structures whose function is to transmit force. However, a perfectly elastic system may suffer oscillation-induced tensile strain; the basement membrane provides for functionally necessary viscous energy loss and thus protects the muscle fiber and tendon[16].

A CT sheath called the endomysium encloses individual muscle fibers; groups of fibers are enclosed by the epimysium. The perimysium encloses the whole muscle and is directly continuous with the deep fascia[10, 15]. Beyond the physiological length of the muscle, actin-myosin overlap is lost: here these CT sheaths (together with some of the structural proteins and the basement membrane) are responsible for resisting tensile force[18]. Damage to these sheaths is more likely in multi-joint muscles, which may be exposed to greater stretch. Intact CT sheaths may also act to bypass tension past dysfunctional or scarred zones of the muscle fiber[9]. The endomysium is less well-developed in FT fibers, possibly again making them more susceptible to stretch-induced injury[17].

Blood vessels supplying the muscle run in these CT sheaths between muscle fiber bundles. They course at right or oblique angles through the perimysium. In the epimysium, they form a capillary network around the individual fibers. The capillaries have a tortuous course when the muscle is contracted, and they straighten out when the muscle is extended[10]

As noted earlier, muscle fibers are a multi-nucleated syncytium formed by the fusion of mono-nucleated myoblasts: the muscle fiber nuclei are located peripherally in the muscle fiber just below the sarcolemma[15]. During myogenesis, a subpopulation of myoblasts is not incorporated into this syncytial structure. These myoblasts become the satellite cells and are located outside of the sarcolemma, but inside the basement membrane. Through mitosis, these cells provide the myonuclei needed for postnatal growth. At maturation, the satellite cells become mitotically quiescent. The important role satellite cells have as stem cells providing the myoblasts needed for muscle regeneration is discussed below. FT fibers have fewer satellite cells than do ST fibers[12]. With age, the number of satellite cells decreases; the effect of these factors on the ability for full regeneration after muscle injury is unknown[15].

Muscle injury mechanisms

The introduction notes that muscle injury may result from mechanical stress, thermal stress, myotoxic agents, and ischaemia. The next section discusses the probable central event in muscle injury, the loss of calcium homeostasis[1]. There are three groups of hypotheses regarding the initial event causing this loss of calcium homeostasis: metabolically induced, temperature induced, or mechanically induced[1,19].

Metabolic hypotheses

Metabolic hypotheses concentrate on two mechanisms: ATP-depletion and the production of oxygen free radicals. Depletion of ATP might result from increased metabolic demands due to exhaustive exercise or ischaemia[11]. Lowered intracellular ATP-concentrations will decrease calcium-ATP-ase activity and cause SR dysfunction[1]. A progressive depression of Ca^{2+}-uptake has been shown with increased duration of low-intensity exercise, but also with short duration high-intensity exercise[20]. The inability to sequester calcium at an appropriate speed will slow muscle fiber relaxation, and slowed relaxation will affect mechanical properties of the myofibrils resulting in a functional rigor. If this rigor is confined to only some myofibers or myofibrils, the resultant shear forces between adjacent muscle fibers and myofibrils during continued muscle contraction and relaxation may cause mechanical interfiber and interfibrillar disruption, respectively[9,17].

SR dysfunction will increase cytosolic Ca^{2+}-concentrations. Even physiological concentrations have been shown to result in autolytic degradation. Duration of increased Ca^{2+}-concentrations and biological availability to activate autolytic enzymes may explain why these physiological concentrations do not initiate autolysis in vivo: Ca^{2+}-concentrations are transient due to continuous binding to troponin-C and subsequent re-uptake in the SR[1]. However, the Ca^{2+}-pool in the SR has been shown to be sufficient for initiating autolytic breakdown, even in the absence of sarcolemmal disruption[20]. SR dysfunction initiating autolysis resulting in delayed loss of sarcolemmal integrity may explain why blood levels of muscle enzymes indicating sarcolemmal damage usually only increase a minimum of 24 hours after a muscle injury inducing exercise bout[21].

Arguing against this ATP-depletion hypothesis is the fact that global cytosolic ATP-concentrations remain near resting level despite exhaustive exercise. However, this does not exclude compartmental decreases in ATP-concentration. The lower metabolic cost of eccentric exercise when compared to concentric exercise makes the ATP-depletion hypothesis an unlikely explanation for muscle injury induced by eccentric strength training regimens[1]. Teague and Schwane[22] argued against a metabolic explanation for this type of injury: they had subjects perform ten repetitions of eccentric elbow flexion at 60% of their maximal isometric force. Subjects were given no rest, 15 seconds, 5 minutes, or 10 minutes between repetitions, but all had symptoms of post-exercise muscle-injury favoring an explanation of cumulative mechanical strain rather than a metabolic cause. The ATP-depletion hypothesis may, however, play a role in explaining the predilection for FT fiber damage with high-repetition, low load eccentric endurance-type regimens as observed by Friden et al[23]. It may also explain the selective FT fiber damage resulting from ischaemia seen by Lieber et al[11]. With this type of eccentric exercise (or tourniquet-induced ischaemia), the lower oxidative capacity of FT fibers may result in more rapid substrate depletion, subsequent functional rigor, and mechanical damage[3], or alternatively loss of calcium homeostasis and autolysis without mechanical damage.

A second metabolic hypothesis concentrates on an increased production of free oxygen radicals. Superoxide anion and hydrogen peroxide are free oxygen radicals produced as a common metabolic intermediate in highly active tissues[20]. Free radicals can oxidize phospholipids, carbohydrates, and proteins[1,20]. Membrane lipid peroxidation may result in sarcolemmal disruption and subsequent rapid calcium influx disturbing the calcium homeostasis[1]. The pump function of calcium-ATP-ase is impaired by free radical oxidation of its sulfhydryl groups[20]. Free oxygen radical production increases during exercise[19]. The lower metabolic cost of eccentric exercise has been hypothesized to cause a relative overperfusion with increased sarcoplasmic pO_2 favoring the production of free oxygen radicals[1]. Mechanical disruption of the cytoskeleton stabilizing the organelles in the muscle fiber has also been hypothesized to alter the position of the mitochondria and physically disrupt the association of the components of mitochondrial respiration resulting in increased free radical production in the mitochondrial electron transport system[1]. Arguing against this free radical hypothesis as an explanation for eccentrically induced injury is the fact that the metabolic cost for eccentric exercise is lower than that of concentric exercise. Also, overperfusion during eccentric exercise has not been demonstrated. Free radicals may, however, play a role in other types of muscle injury.

Temperature hypotheses

Increased muscle temperature is usually associated with decreased injury potential: it reduces gamma fiber activity and sensitivity of muscle spindles to stretch, enhances enzymatic function, decreases viscosity, and increases CT extensibility[18]. However, increased temperature may also increase the rate of unwanted structural lipid and protein degradation. Increased sarcolemmal viscosity may bring the enzyme phospholipase A_2 into contact with its phospholipid substrate in the plasma membrane resulting in sarcolemmal degradation[1]. Increased temperature may negatively affect calcium-ATP-ase function[20]. Eccentric muscle activity may result in increased temperature, as some of the energy absorbed by the muscle is dissipated by heat. Relatively lower heat removal (due to a lack of vasodilation) may raise intramuscular temperature[1]. The temperature hypothesis may also play a role in thermal muscle damage and muscle injury due to concentric exercise.

Mechanical hypotheses

A muscle fiber will fail if the tensile or shear stress induced in a structural component exceeds its yield strength. This stress may be the result of a single mechanical event or of cumulative, repetitive, mechanical events[1]. Multijoint muscles can be subject to greater stress and are, therefore, at greater risk for mechanically induced failure[1]. In addition, the thinner and less complex Z-disc, the smaller MTJ surface area, and the less well-developed endomysium put FT fibers at greater risk for tensile failure. FT fibers are capable of higher speed and force production than ST fibers resulting in higher tensile stress[1,15]. Forces produced during eccentric muscle action may exceed maximal isometric force by 50-100%[1]. There is some evidence that FT fibers are preferentially recruited for eccentric muscle action[24]. As discussed earlier, their lower oxidative capacity may put the FT fibers at further risk for damage. Eccentric exercise has been reported to selectively damage FT fibers[3,23]. However, eccentric exercise may also cause ST fiber injury: Mair et al[25] found increased blood levels of a MHC isoform found in ST fibers after 7 sets of 10 repetitions of eccentric quadriceps activity at 150% of maximal voluntary strength.

Increased velocity of eccentric lengthening may decrease the number of acto-myosin cross-bridges available to resist the external force. This increases the force per active cross-bridge predisposing the contractile proteins to mechanical disruption[1]. Metabolically induced functional rigor may cause non-homogenous lengthening of adjacent sarcomeres or myofibrils: shear stresses can damage desmin and other intermediate interfibrillar proteins causing a disruption of myofibrillar arrangement[1,3]. Improper interdigitation of thick and thin filaments after overstretching a sarcomere weakens the sarcomere and may add stress on other sarcomeres or the adjacent SR and sarcolemma; the sarcolemma has been shown to bear most of the passive tension at sarcomere lengths over 140-150% of resting length[1]. Excessive sarcomere lengthening may also damage the longitudinally oriented proteins titin and nebulin, explaining the observed axial misalignment of thick filaments after eccentric exercise[3].

The apparent predilection of muscle injury for the MTJ occurs regardless of muscle architecture, direction of force, and whether the muscle was contracted or passively stretched[4,9]. Differences in sarcomere length affect contractile speed and force[3,15]; this results in different forces being transmitted to the Z-disc where shorter and longer sarcomeres are located next to each other in series. The undue directional stress may mechanically disrupt the Z-disc. MTJ injury may be a result of the shorter terminal sarcomeres joining up with the longer more centrally located sarcomeres[3].

Stages of muscle injury, regeneration, and repair

Independent of injury mechanism, the pathophysiological events in muscle tissue regeneration and repair are very similar[8]. The four stages of this process are Ca^{2+}-overload, autolysis, phagocytosis, and regeneration/repair[1].

Ca^{2+}-overload

As discussed earlier, loss of intracellular calcium homeostasis is central to muscle injury, regardless of whether the injury mechanism is metabolic, thermal, mechanical, or, as is most likely, a combination of these injury mechanisms. The importance of maintaining cytosolic free Ca^{2+}-concentrations within narrow margins is indicated by the large number of mechanisms the muscle fiber has for transporting Ca^{2+} out of the sarcoplasmic compartment[1]. There are multiple ways in which cytosolic calcium concentration can increase. The extracellular free Ca^{2+}-concentration is 2-3 millimol/l versus the intracellular concentration of 0.1 micromol/l of a resting muscle fiber. Disruption of the sarcolemma would allow Ca^{2+} entry into the muscle fiber down its electrochemical gradient[1]. Dysfunction of the SR, whether metabolically, mechanically, or thermally induced, may also increase cytosolic calcium concentrations, sufficient for initiating autolysis[1,20]. Voltage-dependent Ca^{2+}-channels are located in the TT-membranes; stretch-sensitive Ca^{2+}-channels may be mechanically opened during lengthening contractions. Calcium channel blockers have been shown to attenuate exercise-induced muscle injury, but the importance of these channels is considered minimal[1,19]. Several muscle fiber receptors act to release Ca^{2+} from internal stores or allow for interstitial influx; some of these receptors respond to histamine and bradykinin[1]. In the Ca^{2+}-overload phase, the calcium-transport and buffering mechanisms are overwhelmed, resulting in increased intracellular Ca^{2+}-concentrations and subsequent autolysis[1].

Autolysis

Autolytic mechanisms are initiated if the sarcoplasmic Ca^{2+}-concentration is sufficiently elevated for a sufficient period of time. Armstrong et al[1] call this stage the autogenetic stage. Though not a proteolytic event, increased Ca^{2+}-concentrations may cause uncontrolled contraction of sarcomeres in the affected region; this serves to wall off the injured area during subsequent stages, but it also increases tensile forces and may increase damage. Mitochondria can buffer elevations in cytosolic calcium levels. The mitochondria of ST fibers buffer Ca^{2+} at a rate two to three times that of FT fiber mitochondria. Intramitochondrial accumulation in the nanomolar range actually increases function, but accumulation in the micromolar range depresses mitochondrial function. Mitochondrial Ca^{2+}-overload may result in ATP-depletion[1]. Calpains are Ca^{2+}-dependent intramuscular proteases, which, when activated by elevated calcium concentrations, can cleave myosin, alpha-actinin, talin, and vinculin[1,7,20]. The primary form of the enzyme phospholipase A_2 (PLA_2) is Ca^{2+}-dependent[1,19]. PLA_2 is located in the sarcolemma, organelle membranes, sarcoplasm, and intracellular lysosomes. It damages the cell membrane by using membrane phospholipids as a substrate for the production of arachidonic acid and subsequently prostaglandins, leukotrienes, and thromboxanes[1]. PLA_2 is the main active ingredient in snake and bee venom[1]. Prostaglandin E_2 (PGE_2) is one of the products of PLA_2. PGE_2 stimulates the activity of proteolytic enzymes contained in intacellular lysosomes; these lysosomal proteases may play a role in degrading myofibrillar proteins[1]. The amount of intramuscular lipofuscin granules increases after eccentric exercise. Lipofuscin is an indigestible residue of lysosomal degradation, supporting a role for these proteolytic enzymes in exercise-induced muscle damage[3].

Phagocytosis

The autolytic activity continues into the phagocytic phase[1]. Ca^{2+}-induced hypercontraction of disrupted and retracted myofibrils isolates the injured area[27]. By the second day after the injury, this hypercontracted band makes place for a membranous structure, the demarcation membrane, which then walls off the necrotic area[5]. Neutrophil leucocytes, macrophages, and T-lymphocytes invade the injured area. However, phagocytic cells are the prominent feature of this stage: leucocyte concentrations decline within 24 hours and only 30% of the T-lymphocytes present in the wound area are actually activated[7]. Armstrong et al[1] reported that the phagocytic phase starts within two to six hours. Garrett[2] reported an invasion of inflammatory cells by one to two days. Russell et al[6] noted macrophage infiltration within 24 hours, and Reid[4] stated that phagocytosis is ongoing by 48 hours after injury. Macrophage infiltration only occurs when the necrotic area is (re) vascularized[10]; this may explain the discrepancies noted with regards to the start of the phagoytic stage. We can expect that injuries compromising circulation, such as ischaemic injuries, take longer to enter this phagocytic phase.

The inflammatory and phagocytic cells may be the result of activation and mitosis of quiescent cells already present in the muscles[7]. St. Pierre and Tidball[26] described the presence of macrophages in the tendon of rats near the MTJ, which increased their synthetic activity in response to altered mechanical demands on the MTJ. However, the majority of cells probably result from chemotaxis out of the circulatory system[7]. The substance providing the stimulus for this chemotaxis is unknown. Injured muscle releases a number of substances into the extracellular space; basic fibroblast growth factor (bFGF), transferrin, and an uncharacterized mitogen all have mitogenic functions on myogenic cells. Platelet-derived growth factor (PDGF) is also released by injured muscle. PDGF is a mitogen and chemo-attractant for both inflammatory cells and fibroblasts. However, PDGF has a very short half-life. The delay between injury and arrival of inflammatory cells makes it likely that the substances released serve to activate the resident macrophages and fibroblasts. These macrophages, already present in the muscle, then secrete transforming growth factor beta (TGF-beta), interleukin-1 (IL-1), and PDGF, all chemo-attractants to inflammatory cells[7].

In addition to removal of cellular debris and phagocytosis, the inflammatory cells regulate the start of muscle regeneration[7]. Macrophages secrete insulin-like growth factor (IGF), bFGF, and TGF-beta[6,27]. These substances activate both satellite cell proliferation and myoblast differentiation, and cause the expression of embryonic MHC[7,27], the MHC-isoform seen initially in regenerating muscle[26,28]. NSAIDs are commonly used after injury, including muscle injury; however, these medications may be contra-indicated as a result of their inhibition of macrophage and neutrophil function. Tidball[7] reported that using NSAIDs resulted in slower regeneration and weaker MTJs. Damage to the basement membrane may also play a role in the activation of satellite cells by exposing these cells to the mitogen-rich extracellular matrix[28].

Regeneration and repair

There are two distinct ways in which a muscle can respond to injury: regeneration and repair. Regeneration involves complete restoration of the pre-injury structure. With repair, the production of a CT scar may restore muscle continuity but at the same time inhibit complete regeneration. There is the mistaken notion, even in some recent medical literature, that skeletal muscle does not have the potential to regenerate[8,10].

After activation by factors excreted by the macrophages and/or exposure to the mitogen-rich extracellular environment as a result of a lesion to the basement membrane, satellite cells start to proliferate and differentiate into myoblasts[5]. There is no satellite cell recruitment from adjacent muscles[12]. Schultz[12] noted that satellite cells migrate to the injury site from all along the muscle fiber. In contrast, Hurme and Kalimo[27] stated that migration of satellite cells plays a minor role; most myoblasts may be produced near or at the injury site. By three days post-injury, these myoblasts fuse and form a syncytial myotube[5]. Myotubes are characterized by a centrally located chain of enlarged nuclei, prominent nucleoli, and abundant polyribosomes upon which muscle proteins are synthesized[10]. The myosin produced initially contains an embryonic MHC-isoform, which is replaced later by adult isoforms[26,28]. The contractile proteins are first assembled into well-organized bundles of thick and thin filaments at the periphery of the myotube. Later myofilaments are added successively towards the center of the myotube. Construction of the TT-system and SR occur simultaneously with the assembly of the myofilaments. Breaking up the central chain of nuclei and subsequent migration of these nuclei to a position near the sarcolemma marks the transition from myotube to muscle fiber[10]. There are two theories with regards to the formation of growth extensions at the end of the damaged muscle fibers. The continuous theory holds that muscle is repaired by outgrowth of the damaged myoplasm with migration of myonuclei towards the damaged muscle fiber stumps. The discontinuous theory states that myoblasts fuse, form myotubes, and then fuse with the muscle fiber stumps. Robertson et al[29] found evidence supporting the latter theory in their experiments in mice.

The myoblasts also produce a highly hydrated extracellular matrix. Initially, hyaluronic acid is the main constituent of this matrix, but this is later replaced by a muscle-specific chondroitin-sulphate proteoglycan (M-CSPG). Anchored at the cell surface, M-CSPG ensures sufficient space for subsequent increases in muscle fiber girth by increasing volume through binding water electrostatically. M-CSPG is finally replaced by heparan-sulphate proteoglycans; these insert into the cell membrane and form part of the new basement membrane[10]. By day 7, a new basement membrane forms within the old one[5].

Myotubes may release chemo-attractants for vascular endothelial cells, thus promoting adequate revascularization[10]. Complete rupture of a myofiber disconnects part of the fiber from the neuromuscular junction. Regeneration occurs independently of innervation. Final differentiation into the different muscle fiber types does, however, depend on reinnervation of the regenerating denervated portion of the muscle fiber[9].

Optimal regeneration is facilitated by an intact basement membrane, an uninterrupted vascular supply, and a functional nerve[17]. An intact basement membrane keeps out fibroblasts and a majority of newly forming collagen fibers[5]. Re-growth of damaged muscle fibers is obstructed by excessive, interposed CT[5]. Collagen deposition in the injury zone may prevent longitudinal fusion and result in split muscle fibers sometimes found after injury[29]. With contusion, laceration and strain CT sheaths may be disrupted along with muscle fibers[5]. Bleeding occurs after this type of injury; this bleeding may escape through the perimysium and fascia and dissipate into the subcutaneous space, but it may also remain contained within the muscle substance[2]. This intramuscular haematoma is replaced by proliferating granulation tissue, which may mature into a CT scar[5]. Extensive CT proliferation is found especially in crush injuries[27]. Tensile forces introduced in the injured fiber prematurely may re-injure the fiber, cause renewed bleeding, and result in excessive CT deposition and repair rather than regeneration. Jaervinen and Lehto[8] caused a partial rupture in rat gastrocnemius muscle. Immediate mobilization resulted in increased bleeding, more abundant inflammatory cell infiltration, a more parallel orientation of the regenerating myofibers, increased capillarization, but also more extensive proliferation of CT in the injured area. Immobilization led to a more irregular orientation of muscle fibers, but less CT proliferation. Immobilization also greatly decreased force to failure, elongation at failure, elastic stiffness, and energy absorption capacity. Mobilization, on the other hand, quickly restored these parameters to control values. Short immobilization times decreased CT proliferation as compared to immediate mobilization. After two days of immobilization, remobilization resulted in re-rupture; no rupture was noted after five days of immobilization. Two days of immobilization resulted in similar elastic stiffness values as in muscles immediately mobilized.

Implications

An in-depth review of the effectiveness of the interventions used for prevention and treatment of muscle injury is outside the scope of this article. However, it is possible to derive some theoretical implications from the material presented. Atrophy predisposes the MTJ to failure: careful progressive resistance exercise after immobilization resulting in disuse atrophy may restore the sarcolemma-tendon angle and prevent injury associated with more aggressive remobilization. Excessive stretch as may occur especially in multi-joint muscles has been associated with tensile muscle failure. A stretching regimen may adapt the length of these multi-joint muscles to the excursion required during sport and ADL tasks. A warm-up may also prevent tensile injury by increasing CT and muscle extensibility, by decreasing unwanted tension increases as a result of overactive stretch reflexes, and by increasing enzymatic and thus muscle function. High muscle temperatures may initiate muscle injury. Athletes should probably not work out in extremely high temperatures. Cardiovascular exercise increases capillarization and may allow for increased dissipation of excess heat production. Endurance-type exercise may also play a role in prevention of muscle injury as a result of high-repetition low load eccentric exercise: fiber type conversion can increase muscle fiber oxidative capacity and allows the muscle fibers to better deal with high metabolic demands. Of course, technique training and the resultant improved neuromuscular coordination may prevent muscles and CT from ever being stressed to the point of failure.

Theoretically, muscle regeneration restores contractile characteristics to a greater extent than does muscle repair. Immediate mobilization rapidly restores mechanical characteristics but also results in greater CT proliferation. A short period of post-injury mobilization temporarily depresses tensile characteristics but results in less CT deposition and more complete regeneration. However, short-term immobilization may predispose the muscle to re-rupture early in the remobilization period. Tensile forces should be reintroduced carefully. NSAIDs depress inflammatory cell function and may slow regeneration and negatively affect eventual MTJ strength. Repair is more likely to occur in injuries such as contusions and lacerations, where excessive bleeding and disruption of the basement membrane impair complete regeneration.

A better understanding of the injury mechanisms and processes involved in muscle, injury, regeneration, and repair allow us to derive theoretical implications for prevention and treatment. Research is needed to determine the effectiveness of the interventions suggested.

REFERENCES

1. Armstrong RB, Warren GL, Warren JA. Mechanisms of exercise-induced muscle fibre injury. Sports Med 1991;12:184-207.
2. Garrett WE. Muscle strain injuries: Clinical and basic aspects. Med Sci Sports Exerc 1990;22:436-43.
3. Friden J, Lieber RL. Structural and mechanical basis of exercise-induced muscle injury. Med Sci Sports Exerc 1992;24:521-30.
4. Reid DC. Sports Injury Assessment and Rehabilitation. New York, NY: Churchill Livingstone, 1992.
5. Hurme T, Kalimo H, Lehto M, Jaervinen M. Healing of skeletal muscle injury: An ultrastructural and immunohistochemical study. Med Sci Sports Exerc 1991;23:801-10.
6. Russell B, Dix DJ, Haller DL, Jacobs-El J. Repair of injured skeletal muscle: A molecular approach. Med Sci Sports Exerc 1992;24:189-96.
7. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc 1995;27:1022-32.
8. Jaervinen MJ, Lehto MUK. The effects of early mobilisation and immobilisation on the healing process following muscle injuries. Sports Med 1993;15:78-89.
9. Griffin LY. Orthopaedic Knowledge Update Sports Medicine. Rosemont, IL: AAOS, 1994;17-33.
10. Caplan A, Carlson B, Faulkner J, Fischman D, Garrett W. Skeletal Muscle. In: Woo SLY, Buckwalter JA, eds. Injury and Repair of the Musculoskeletal Soft Tissues. Rosemont, IL: AAOS, 1988.
11. Lieber RL, Pedowitz RA, Friden J, Gershuni DH. Decreased muscle speed, strength and fatiguability following two hours of tourniquet-induced ischaemia. Scand J Plast Reconstr Hand Surg 1992;26:127-32.
12. Schultz E. Satellite cell behavior during skeletal muscle growth and regeneration. Med Sci Sports Exerc 1989;21:S181-6.
13. Billeter R, Hoppeler H. Muscular basis of strength. In: Komi PV, ed. Strength and Power in Sport. Oxford: Blackwell Scientific Publications, 1992.
14. Jones D, Round D. Skeletal Muscle in Health and Disease. Manchester: Manchester University Press, 1990.
15. Huijbregts PA, Clarijs JP. Krachttraining in Revalidatie en Sport. Utrecht: De Tijdstroom BV, 1995.
16. Garrett W, Tidball J. Myotendinous junction: Structure, function, and failure. In: Woo SLY, Buckwalter JA, eds. Injury and Repair of the Musculoskeletal Soft Tissues. Rosemont, IL: AAOS, 1988.
17. Stauber WT. Eccentric action of muscles: Physiology, injury, and adaptation. In: Pandolf K, ed. Exercise and Sports Sciences Reviews, Vol. 17. Baltimore, MD: Williams & Wilkins, 1989.
18. Safran MR, Seaber AV, Garrett WE. Warm-up and muscular injury prevention: An update. Sports Med 1989;8:239-49.
19. Armstrong RB. Initial events in exercise-induced muscular injury. Med Sci Sports Exerc 1990;22:429-35.
20. Byrd SK. Alterations in the sarcoplasmatic reticulum: A possible link to exercise-induced muscle damage. Med Sci Sports Exerc 1992;24:531-6.
21. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. Med Sci Sports Exerc 1992;24:512-20.
22. Teague BN, Schwane JA. Effect of intermittent eccentric contractions on symptoms of muscle microinjury. Med Sci Sports Exerc 1995;27:1378-84.
23. Friden J, Sjoestroem M, Ekblom B. Myofibrillar damage following intense eccentric exercise in man. Int J Sports Med 1983; 4:170-6.
24. Nardone A, Romano C, Schieppati M. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. J Physiol 1989;409:451-71.
25. Mair J, Mayr M, Mueller E, Koller A, Haid C, Artner-Dworzak E, Calzolari C, Larue C, Puschendorf B. Rapid adaptation to eccentric exercise induced muscle damage. Int J Sports Med 1995; 16:352-6.
26. St. Pierre BA, Tidball JG. Macrophage activation and muscle remodeling at the myotendinous junction after modifications in muscle loading. Am J Pathol 1994;145:1463-71.
27. Hurme T, Kalimo H. Activation of myogenic precursor cells after muscle injury. Med Sci Sports Exerc 1992;24:197-205.
28. Zhang J, Dhoot G. Localized and limited changes in the expression of myosin heavy chains in injured skeletal muscle fibers being repaired. Muscle & Nerve 1998;21:469-81.
29. Robertson TA, Papadimitriou JM, Grounds MD. Fusion of myogenic cells to the newly sealed region of damaged myofibres in skeletal muscle regeneration. Neuropathology and Applied Neurobiology 1993;19:350-8.