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Literature Review

Exercise-Induced Muscle Damage in Humans

ABSTRACT

Clarkson PM, Hubal MJ: Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 2002;81(Suppl):S52–S69.

Exercise-induced muscle injury in humans frequently occurs after unaccustomed exercise, particularly if the exercise involves a large amount of eccentric (muscle lengthening) contractions. Direct measures of exercise-induced muscle damage include cellular and subcellular disturbances, particularly Z-line streaming. Several indirectly assessed markers of muscle damage after exercise include increases in T2 signal intensity via magnetic resonance imaging techniques, prolonged decreases in force production measured during both voluntary and electrically stimulated contractions (particularly at low stimulation frequencies), increases in inflammatory markers both within the injured muscle and in the blood, increased appearance of muscle proteins in the blood, and muscular soreness. Although the exact mechanisms to explain these changes have not been delineated, the initial injury is ascribed to mechanical disruption of the fiber, and subsequent damage is linked to inflammatory processes and to changes in excitation-contraction coupling within the muscle. Performance of one bout of eccentric exercise induces an adaptation such that the muscle is less vulnerable to a subsequent bout of eccentric exercise. Although several theories have been proposed to explain this "repeated bout effect," including altered motor unit recruitment, an increase in sarcomeres in series, a blunted inflammatory response, and a reduction in stress-susceptible fibers, there is no general agreement as to its cause. In addition, there is controversy concerning the presence of sex differences in the response of muscle to damage-inducing exercise. In contrast to the animal literature, which clearly shows that females experience less damage than males, research using human studies suggests that there is either no difference between men and women or that women are more prone to exercise-induced muscle damage than are men.

Key Words: Eccentric Exercise, Skeletal Muscle, Creatine Kinase, Magnetic Resonance Imaging, Repeated Bout, Inflammation, Muscle Weakness

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One of the earliest studies of exercise-induced muscle damage was published in 1902, in which Hough[1] described delayed onset muscle soreness and suggested that soreness was the result of microtears in the muscle. In the next 90 yr, the study of muscle soreness and damage lay almost dormant, such that between 1970 and 1980 only about five studies of muscle soreness in humans are available. This number increased to about 42 between 1981 and 1990. However, in the past 10 yr, the interest in exercise-induced muscle damage has grown, and more than 125 studies of exercise-induced muscle damage in humans have been published.

Because it is well documented that eccentric (muscle lengthening) actions result in damage, studies of muscle damage have used exercises that use eccentric actions of isolated muscle groups (e.g., elbow flexors) or exercises that are biased toward eccentric actions (e.g., downhill running or stair descents). This article reviews the pertinent studies that describe the changes observed in muscle after damaging exercise and provides insight into the mechanisms that explain these changes. Also, the rapid adaptations to damage (repeated bout effect) and possible sex differences are discussed.

INDICATORS OF DAMAGE

Direct assessment of damage in human muscle is difficult because it is only possible through analysis of muscle biopsies or through magnetic resonance imaging (MRI). The problems inherent in muscle biopsy analysis are obvious in that such a small sample is used to estimate damage in an entire muscle. Moreover, because the damage is not ubiquitous in the muscle but focalized, it is possible to overestimate or underestimate damage. Imaging techniques have been used to assess damage (edema) in whole muscle. Although this is a non-invasive technique, it is still not clear what the changes in the images indicate.

Because of the invasive nature and inherent errors in using the biopsy technique and the lack of knowledge of exactly what MRI assessments are telling us, several investigators have used indirect measures to assess damage. In a recent review of the literature, Warren et al.[2] calculated that the three most commonly used indirect damage markers in reviewed studies using human subjects were subjectively determined muscle soreness (63% of studies), blood protein assessment (52% of studies), and maximal voluntary contraction force (50% of studies). An overview of the time course of several changes that occur in response to muscle damage is found in Figure 1.

Cellular and Subcellular

In 1981, Fridén et al.[3] provided some of the first evidence of muscle fiber damage in humans after exercise. Subjects performed repeated stair descents, and biopsies of the soleus muscle were taken 2 and 7 days later. The biopsy analysis showed myofibrillar disturbances and Z-line streaming. In a follow-up study, Fridén et al.[4] examined muscle samples taken about 1 hr, 3 days, and 6 days after a backwards cycling exercise and found that 32%, 52%, and 12%, respectively, of the observed fibers showed evidence of focal disturbance. The percentages corresponded to 1.6%, 2.4%, and 0.6% of the fiber area. Changes in ultrastructural integrity were noted as Z-line streaming, Z-lines out of register, loss in thick myofilaments, loss in mitochondria in areas that showed abnormalities, and disturbed arrangement of filaments at the A-band (Fig. 2). Disturbances predominantly occurred in type II fibers. Findings of Newham et al.[5] concurred with those of Fridén et al.[4] in that biopsy samples taken 24–48 hr after exercise showed greater damage than those taken immediately after exercise.

Recently, Roth et al.[6] suggested that hypercontracted fibers and evidence of ultrastructural damage

	SOR	CK	T2	STR	Acute (Inflammation)	Chronic (Inflammation)
Exercise Stimulus				↓↓↓	↑	
1-12 h post-exercise				↓↓↓	↑↑↑	
24 h post-exercise	↑↑↑	↑	↑	↓↓↓	↑	
48 h post-exercise	↑↑↑	↑	↑	↓↓		↑
3-5 d post-exercise	↑	↑↑	↑↑	↓		↑↑
5-7 d post-exercise	↑	↑↑↑	↑↑↑	↓		↑↑↑
7+ d post-exercise		↑↑	↑↑	↓		↑↑

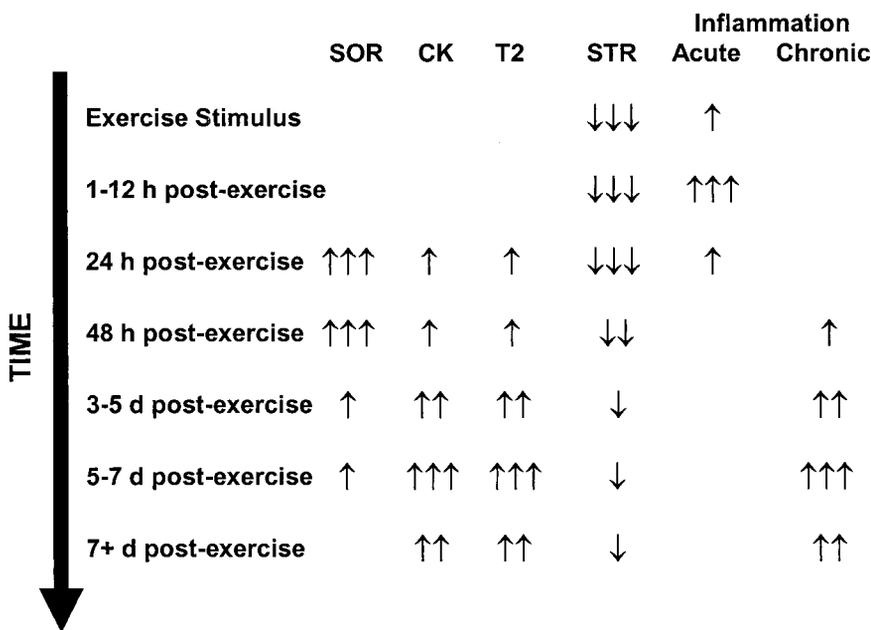*TIME* (downward arrow along left side)

Figure 1: Time course of changes after maximal eccentric exercise. *One arrow*, minor increase/decrease; *two arrows*, moderate increase/decrease; *three arrows*, large increase/decrease. *SOR*, soreness; *CK*, creatine kinase; STR, strength.
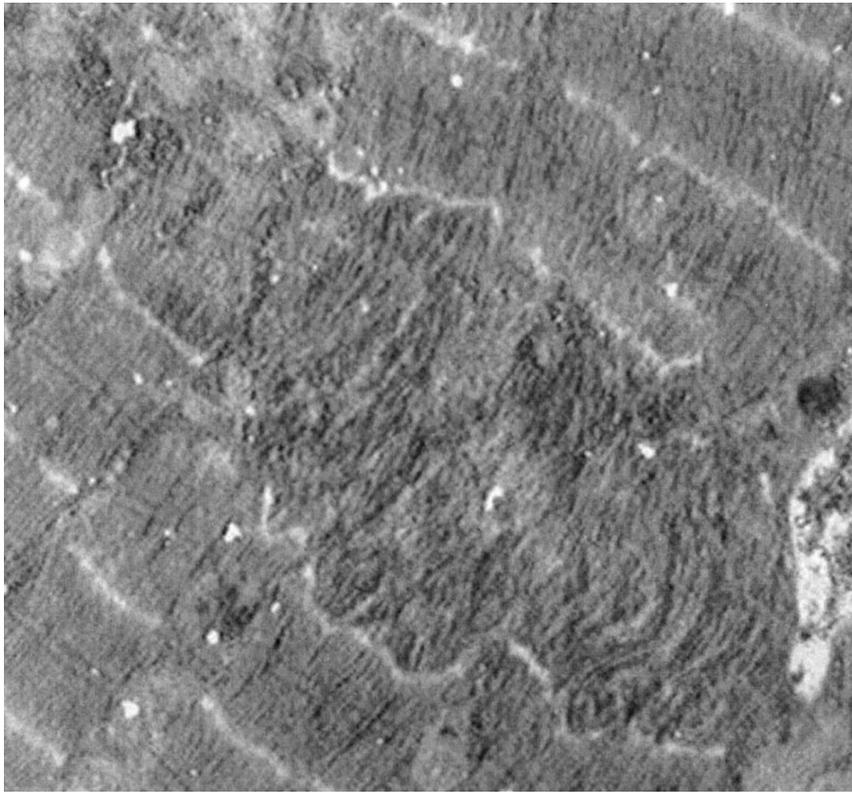
Figure 2: Electron micrograph illustrating muscle damage of the vastus lateralis. Muscle sample taken 48 hr after the subject performed 300 eccentric contractions. Original magnification, ×12,000; JOEL transmission electron microscope. Photograph courtesy of Douglas Mahoney and Mark Tarnopolsky, McMaster University, Hamilton, Ontario, Canada.

might be caused by the biopsy procedure itself, including the mincing of the tissue. Malm et al.[7] examined the effects of multiple (seven) biopsies taken over a period of 7 days in control subjects and in subjects who performed eccentric cycling exercise and found that both conditions resulted in similar changes in infiltrating neutrophils and macrophages. Thus, the biopsy procedure itself can produce some changes mistakenly attributed to exercise-induced damage. Moreover, the small size of the tissue sample in comparison with a whole muscle makes the quantitation of damage problematic because a damaged area could be missed or over-represented in the sample.

Because of the characteristic Z-line streaming found with exercise-induced damage, Fridén et al.[8] proposed that the Z-lines were the "weak link" in the myofibrillar chain. They examined the possibility that the cytoskeletal protein desmin, which links Z-lines together, may be susceptible to exercise-induced damage. Biopsies from subjects who performed eccentric exercise (backwards cycling) were examined by indirect immunofluorescence microscopy using an antibody specific to desmin. The pattern of desmin staining was disrupted in samples taken 3 days postexercise. Also, granules beneath the sarcolemma were found in the exercised muscle, which were thought to be lipofuscin granules indicative of lysosomal activity.

Stauber et al.[9] studied biopsy samples from the biceps brachii taken 48 hr after subjects performed an eccentric exercise to examine changes in the extracellular matrix. Mast-cell degranulation was noted in the perimysial area primarily near blood vessels, and mononuclear cells were observed in the perimysial and endomysial regions. When stained for extracellular matrix proteoglycan components, it appeared that the extracellular matrix was pulled away from the fibers into a widened interstitial space. Fibrinogen and albumin, normally found in capillaries, were also found in the perimysial and endomysial spaces. Results of this study demonstrated damage to the extracellular matrix and possibly damage or disturbance of the capillaries.

From these data on humans, it seems that the initial insult of the exercise creates an insult to fibers, which results in damage to the ultrastructure, extracellular matrix, and possibly to capillaries. These disturbances activate an inflammatory response, which may serve as part of the repair and regeneration process. The inflammatory response will be discussed in a subsequent section.

MRI

MRI has been a powerful tool to gain understanding of what is occurring in the entire muscle. Shellock et al.[10] were among the first investigators to examine signal intensity (T2 relaxation time) changes after eccentric exercise and reported a prolonged change in T2 after exercise. This finding was unique because prior studies using fatiguing concentric exercise (that did not result in muscle damage) found short-lasting increases in T2 immediately after exercise. Because the increase in signal intensity is thought to reflect increases in water, the prolonged changes after eccentric exercise are considered to indicate edema in the exercise-damaged muscle. Changes in MRI after eccentric exercise are presented in Figure 3.

Mair et al.[11] examined the relationship between changes in MRI and changes in other indices of muscle damage (strength loss and increases in blood creatine kinase (CK) activity, myoglobin concentration, and myo-
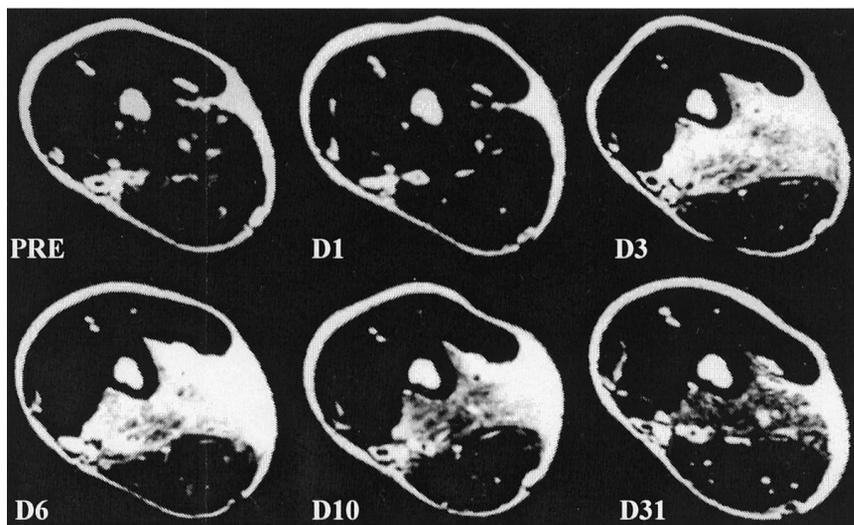
Figure 3: Time course of change in magnetic resonance images (before [*PRE*] and days [*D*] 1, 3, 6, 10, and 31 after exercise) of a subject who performed 24 maximal eccentric exercises of the elbow flexors. In this particular case, the brachialis muscle is predominantly affected by the exercise. Image courtesy of Dr. Kazunori Nosaka, Yokohama City University, Yokohama, Japan.

sin heavy chain fragment concentration) after eccentric contractions of the knee extensors. The T2 relaxation time, assessed at 3, 6, and 9 days after exercise, showed peak increases at 6 days postexercise, which seemed to coincide with the peak CK responses, but occurred later than peak soreness development.

These data showed that strength was being restored and soreness was dissipating while the signal intensity was increasing. Nurenberg et al.[12] found that ultrastructural damage assessed from MRI-guided biopsies correlated significantly with increased MRI signal intensity 48 hr after downhill running.

Exactly what produces the increase in signal intensity is not clear. Takahashi et al.[13] found that changes in T2 relaxation time after knee extension eccentric exercise paralleled changes in cross-sectional area, both peaking about 12–24 hr postexercise. This finding was taken to indicate an accumulation of water in the damaged muscle, either due to an injury to connective tissue, an increase in capillary permeability, a degradation of proteins in the muscle cells, or combination of factors. To investi-

gate whether changes in muscle T2 relaxation time were due to increased intracellular or extracellular fluid volume, Ploutz-Snyder et al.[14] used a leg negative pressure device to create edema by increasing extracellular fluid volume and compared this with exercise-induced changes. They analyzed three components of T2, with the premise that the intermediate and long T2 component corresponded to the bulk of intracellular fluid and extracellular fluid, respectively. The T2 components differed between the leg negative pressure *vs.* the exercise condition. The authors concluded that the T2 change during fatiguing (nondamaging) concentric exercise is most likely from changes inside the muscle cells. This does not necessarily mean that the long-lasting changes after eccentric exercise are due predominantly to changes in the intracellular environment, as components of T2 have not been analyzed after eccentric exercise, but such analysis seems warranted.

In a careful study of the time course of muscle volume changes (assessed with MRI), T2 changes, pain, and serum CK activity, Foley et al.[15] found that pain and muscle vol-

ume peaked at 48 hr postexercise, followed by a rise in CK activity and increase in T2. All measures, except muscle volume, returned to baseline by 14 days. At 14 days postexercise, muscle volume had decreased below baseline by about 7–10%, and this decrease was still evident 8 wk later. The authors concluded that the loss of muscle volume reflected a loss in stress-susceptible fibers that were damaged irreparably by the exercise.

From the studies cited above, the changes in T2 after strenuous eccentric exercise are evident long after most other direct and indirect indicators of muscle damage have been resolved. Shellock et al.[10] noted elevated T2 as long as 75 days after exercise of the elbow flexors, and Nosaka and Clarkson[16] found increased T2 up to 31 days postexercise in some subjects. Currently, there is no explanation for this long-lasting effect.

MRI has been useful in assessing which muscles have been damaged after the exercise. For example, after eccentric forearm flexion exercise, subjects differed in the extent of damage in synergistic muscles, with some subjects showing increased signal intensity in the biceps, others showing increases in the brachialis, and still others showing increases in both muscles.[16] After an eccentric exercise designed to damage the quadriceps muscle, prolonged changes in T2 were found for the vastus lateralis, the intermedius and the medialis but not the rectus femoris.[13] Similarly, examination of cross-sectional area showed that only the rectus femoris did not show delayed swelling. LeBlanc et al.[17] demonstrated variability among sections of the same muscle after eccentric exercise. In images of the thigh taken 2.5 days after knee flexion and extension exercise, the more distal slice showed increased signal intensity in the semitendinosus and biceps femoris short head, and the more proximal slice showed increased signal intensity in the

semitendinosus and gracilis. It should be pointed out that changes in T2 may not reflect differences in recruitment after eccentric exercise, since Rodenburg et al.[18] reported that changes in the inorganic phosphate/phosphocreatine ratio, indicating energy use in the muscle, were homogeneously distributed over the elbow flexor muscles, whereas T2 increases occurred to different extents at different sites after the eccentric forearm flexor exercise.

Force Loss

Prolonged strength loss after eccentric exercise is considered to be one of the most valid and reliable indirect measures of muscle damage in humans.[2] Decrements in force immediately after exercise that does not produce damage (e.g., concentric contractions) are restored in the next few hours and are generally considered to be to due to metabolic or neural fatigue.[19] Concentric protocols are typically associated with strength losses of 10–30% immediately after exercise, with strength returning to baseline within hours after exercise.[20,21] Eccentric-biased downhill running protocols that produce minimal damage typically generate approximately 10–30% force loss immediately after exercise, with a recovery period longer (up to 24 hr postexercise) than that associated with concentric protocols.[22–24] The highest degrees of strength loss and the most prolonged recovery times are associated with high-force eccentric exercise.[25] High-force eccentric exercise (exercise consisting of maximal eccentric actions) can often generate up to 50–65% loss of force-generating capacity when compared with preexercise values.[26–28] Research using animal models has shown that this greater strength loss after eccentric exercise is likely due to damage generated from higher strains placed on the muscle.[29,30] Prolonged force loss in the days after eccentric exercise can typically last 1 and 2 wk.[26]

This prolonged recovery time results from the initial damage during the exercise and additional damage during the regeneration process.

Strain. Experimental data demonstrate that mechanical strain on muscle is one of the primary causes of exercise-induced muscle damage. From animal data, it has been demonstrated that lengthening a muscle beyond 140% of its optimum length during eccentric contractions places an unaccustomed mechanical strain on the muscle, both decreasing force production capacity after exercise and increasing muscle injury.[31]

Animal studies have demonstrated that the extent of force loss after eccentric exercise was related to the initial length of the muscle in that more force loss occurred when contractions were performed at longer muscle lengths.[32–34] Studies have investigated this relationship in humans[21,35,36] and reported similar results in that more force was lost after subjects exercised with muscle initially set at a longer length. For example, Newham et al.[35] had subjects perform an exercise consisting of eccentric contractions of the elbow flexors at a short length (at which the contraction started at full elbow flexion and ended at 60 degrees of extension) or at a long length (at which the contraction started at 45 degrees of flexion and ended at full extension). They reported a 10% static force decrement after exercise at the shorter muscle length and a 30% decrement after exercise at the longer muscle length. These results confirm data assembled from animal studies indicating that exercise at longer initial muscle lengths creates a larger amount of strain on muscle fibers. This stretching of the muscle causes a linear deformation (i.e., displacement or strain) of certain sarcomeres within the muscle beyond their normal length. Moreover, although exercise at longer muscle lengths generates less total force per contraction

than exercise at shorter lengths, it ultimately creates more exercise-induced muscle damage.[21,37]

Additional support for the idea of a deformation of sarcomeres after high-force exercise is found in data showing a disproportionate loss of strength at shorter muscle lengths in the days after eccentric exercise.[37,38] For example, Saxton and Donnelly et al.[38] had subjects perform 70 maximal eccentric contractions of the elbow flexors and then measured the force production capacity of the subjects at several arm angles (50, 90, and 160 degrees of elbow flexion) from 1 to 4 days and at 10 days postexercise. This study found that force production at all angles was significantly decreased at all time points after exercise. Force production at 50 and 90 degrees was significantly more impaired than force production at 160 degrees, both immediately postexercise and up to 4 days after exercise. Whereas the peak force preexercise was achieved at 90 degrees of elbow flexion, the postexercise peak force produced was at 160 degrees of elbow flexion until 10 days postexercise, when values returned to baseline. These data suggest a transient shift of the optimum length for peak force production toward longer lengths (and consequently onto the descending limb of the force-length curve) in the days after eccentric exercise.

The exact mechanism by which force is lost after eccentric exercise has not been positively established. One theory holds that sarcomeres lengthen in a nonuniform manner during lengthening contractions, and this idea is at the center of the "popping-sarcomere" hypothesis, put forth by Morgan in 1990.[39] When muscle is lengthened slowly, sarcomere lengths uniformly stretch with increasing tension. However, when this tension is sudden or of high force, Morgan theorized that a nonuniform stretching takes place in the sarcomeres and that some sarco-

meres reach a point at which they are stretched too far, generating damage. Morgan proposed that as the muscle is lengthened, weak sarcomeres are stretched to a length at which they are only supported by passive elements and are popped.[40] Also, the transient force loss after eccentric exercise could be due to damage within tendon attachments or the series elastic elements of the muscle.[38]

Low-Frequency Fatigue. Another hallmark manifestation of muscular force impairment in the period after exercise is the phenomenon of low frequency fatigue (LFF). After damage-inducing exercise, there is a decreased ability to generate force at lower stimulation frequencies, which can take up to a week after exercise to recover.[21,26,41–43] For example, the force loss measured from a tetanic electrical stimulation of the muscle at 10 or 20 Hz from preexercise to postexercise is exacerbated when compared with the force loss measured from a stimulation at 50 or 100 Hz.[26,43,44] Edwards et al.[19] were the first to report a disproportionate loss of force-production capabilities in human muscle at lower stimulation frequencies. These authors also provide data to refute either high-energy phosphate loss (recovery of muscle phosphagens measured from muscle biopsies in this study outpaced recovery of force production at low frequencies) or central fatigue (as LFF occurs even when muscle is externally stimulated) as a cause of LFF. Edwards et al.[19] suggest that LFF is due to a reduction in contractile activation or, in other words, failure within the excitation-contraction coupling process. Animal studies implicate a reduction in the amount of calcium from the sarcoplasmic reticulum after damaging exercise as the primary cause of LFF[45] and offer evidence that the inability of muscle to produce maximal forces after eccentric exercise results from a disruption of the excitation-contraction coupling process.[29,46]

Further data in support of excitation-contraction coupling as a cause of LFF can be found in human studies. Deschenes et al.[47] reported a significant decrease in neuromuscular efficiency (torque/integrated electromyogram) through 10 days postexercise. Neuromuscular efficiency is a measure of the ability of the contractile element to respond to neural input.[47] As torque in this study returned to baseline after 7 days, the authors ascribe these continued disturbances in neuromuscular efficiency to dysfunction in excitation-contraction coupling. Hill et al.[43] found that, along with a 33% decrement in knee extensor maximal voluntary contraction strength after exercise, there was a significant decrease in torque production at low stimulation frequencies, and this was significantly correlated with a decrease in calcium release.

Jones[42] suggested that, in addition to reduced calcium release, LFF may be the direct result of damage to the myofibrils. Jones noted that one of the unexplained aspects of the force-frequency relationship of muscle is that it is length dependent such that at short muscle lengths there is a shift in the curve to the right. If the popping-sarcomere hypothesis is correct, then the overall length of the fiber would remain the same but the sarcomeres at the end would be shortened and the more central sarcomeres elongated and popped. The force-frequency curve would then be shifted to the right in the same way as would occur in a shortened position.[42] Indeed, Saxton and Donnelly[38] found that eccentric exercise resulted in a shift to the right in the length-tension relationship such that force loss was greatest when force was tested when the muscle was at the shortest length.

Bimodal Strength Reduction. Evidence from animal studies has demonstrated a bimodal reduction in force-generating capacity after eccentric exercise, seen both in caffeine-induced force production[48] and electrically stimulated force production.[49] In short, two phases of force loss are seen in this pattern, with the first (and larger) force loss occurring immediately after exercise. This first phase is followed by a period of force recovery several hours after exercise and then a second period of force loss occurring several hours after exercise. MacIntyre et al.[50] reported a bimodal reduction in eccentric torque after 300 eccentric contractions of the quadriceps in a group of ten young adult women. They observed a first decrement in torque immediately after exercise, improvement in torque production 2–4 hr after exercise, and then a second (smaller) drop in torque between 20 and 24 hr. Although MacIntyre et al.[50] were the first to report bimodal functional losses in humans, it must be pointed out that most studies in this area do not measure strength between 0 and 24 hr after exercise and use static force measures rather than dynamic torque measures.

MacIntyre et al.[50] suggest that the second drop in torque could be related to either a secondary injury resulting from the inflammatory response or to inhibition from muscle soreness. However, given that the greatest force loss occurs immediately after exercise, when there is no soreness, it is unlikely that soreness or pain play a role in the initial decrease. Also, Newham et al.[26] provided evidence contrary to the idea that subjects were unable to fully activate muscles due to pain. Moreover, Newham et al.[26] showed that superimposition of electrical stimulation during a maximum voluntary contraction did not lead to any further production of force, indicating that, although they were sore, the subjects were able to fully activate their muscles.

CK Activity in the Blood

Many studies have assessed the appearance of muscle proteins in the blood after eccentric exercise to pro-

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vide indirect evidence of muscle damage. The muscle enzymes lactate dehydrogenase, aspartate aminotransferase, carbonic anhydrase isoenzyme II, and CK have been assessed.[51] Other muscle proteins have also been used as indicators of damage, including myoglobin, heart fatty acid binding protein, troponin, and myosin heavy chain.[51] Although all of these have been shown to increase after damage-inducing exercise, CK has received the most attention, perhaps because the magnitude of increase is so great relative to other proteins, and the cost of the assay is comparatively modest. There has been no systematic study of all of these markers together, so we know little about how they all relate to one another in time course or extent of appearance in the blood. This is further complicated by the fact that the two types of exercise predominantly used to study muscle damage, downhill running and high-force muscular contractions, show very different CK responses. For example, after downhill running, CK peaks about 12–24 hr postexercise, with increases ranging from 100 to 600 IU,[52,53] whereas after high-force eccentric exercise (e.g., maximal contractions of the elbow flexors), the increase does not begin until about 48 hr postexercise, with peak activity (generally 2,000–10,000 IU) occurring about 4 to 6 days postexercise (Fig. 4).[54]

The use of any muscle protein in the blood as a marker of muscle damage, however, is problematic, because blood concentration is a function of what is being produced in the muscle and what is being cleared from the blood. Concentric exercise performed over several days after an eccentric exercise can result in increased levels of CK in the blood but no evidence of damage using other measures, such as MRI.[55] Sorichter et al.[55] suggested that the increased CK activity was due to a massaging out of the CK from damaged muscle. However, Saxton and Donnelly[56] found that performance of a light exercise in the days after a bout of high-force eccentric exercise resulted in less of an increase in serum CK activity than performance of the eccentric exercise alone. They interpreted this finding as not only representing a decrease in release from the damaged tissue, but also a faster clearance of CK from the blood. Nosaka and Clarkson[57] examined the effect of performing a second bout of eccentric exercise when blood CK levels were elevated from a performance of a previous exercise (using the opposite limb). They found that the amount of increase in CK was less than expected when the baseline values were already high, suggesting that clearance was activated by the increase in CK from the first bout, thereby resulting in more rapid clearance after the second bout.

CK is released by muscle into the lymphatic system where it is transported to the thoracic duct and enters the blood stream. Havas et al.[58] examined whether the increase in CK activity after exercise was affected by changes in lymph flow. After an 18-km run, subjects were placed either in a bed-rest group (to reduce lymph flow) or a normal activity group. They found that the increase in CK activity was significantly less in the bed-rest group. Similar results were reported by Sayers et al.,[59] who immobilized the arm for 4 days after subjects performed an elbow flexion eccentric exercise and found a smaller increase in serum CK activity compared with subjects who performed the same exercise but were not immobilized.

Perhaps the biggest problem with using CK (or any other muscle protein in the blood) as a marker of muscle damage is the large intersubject variability in the response, with a range of 236 to 25,244 IU/liter cited in one study.[60] Generally, those individuals with the highest CK activity in the blood roughly have the greatest damage assessed from MRI, but this is not a perfect relationship.[60] Also, Newham et al.[61] found a significant correlation between plasma CK and uptake of technetium-99m pyrophosphate in sore muscles, and the time-course of the responses was similar.

Nosaka and Sakamoto[62] injected bupivacaine, a local anesthetic known to induce myonecrosis, into the biceps brachii. Injections of 2, 10, and 20 ml in two subjects resulted in proportional changes in plasma CK activity, peaking at 12–24 hr at about 200, 400, and 800 IU/liter, respectively. These changes related to those noted by MRI. The time-course and
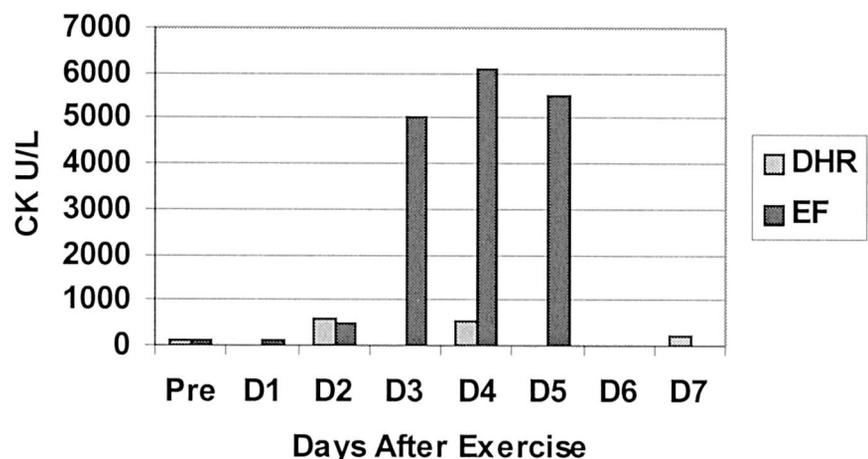

Figure 4: Plasma creatine kinase (*CK*) activity after downhill running (data modified from Eston et al.[22]) and elbow flexion maximal eccentric contractions (data modified from Nosaka et al.[27]).

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amount of CK activity increase in this study are more similar to results found after downhill running. After high-force eccentric exercise, there is a delayed (peaking 4–6 days postexercise), larger increase in CK activity (up to 20,000 IU/liter), which the authors suggest must be due to a different mechanism than the myonecrosis found in this study.

The large variability in CK response to exercise is not understood and does not seem to be related to sex, muscle mass, or activity level of the subjects. A recent study showed that serum CK activity was related to serum glutathione activity, and the authors suggested that glutathione served as a CK preserving agent during the lifetime of the enzyme in circulation.[63] Whether this or other factors will ultimately explain the large variability in the CK response to exercise remains to be determined. However, it is clear that blood CK activity provides only an indirect qualitative marker of muscle damage and may be influenced by several factors other than damage, including possible genetic factors.

Muscle Soreness

Muscle soreness appears many hours after performance of the damage-inducing exercise and peaks 24–48 hr postexercise.[20,54,64] The degree of soreness differs from one type of exercise to another, roughly depending on the amount of damage induced. For example, exercises that do not produce profound muscle damage, such as downhill running or isokinetic eccentric knee extension, produce soreness values of about 4 or 5 on a scale of 1 (no soreness) to 10 (very sore), whereas maximal eccentric contractions of the elbow flexors produce soreness values of about 7–8. The differences in soreness values are consistent with the differences in prolonged force loss and increases in blood CK activity in that the maximal eccentric contraction exercise of the elbow flexors produces

greater and more prolonged force loss and higher CK activity than the other exercises. Although the extent of soreness differs after downhill running vs. high-force eccentric exercise, the time-course is similar.

It is possible that soreness results from swelling and pressure in the muscle. Fridén et al.[65] examined muscle fiber size and intramuscular pressure after subjects performed an eccentric exercise of the tibialis anterior muscle. Forty-eight hours after the exercise, muscle fibers analyzed from muscle biopsy samples were larger and intramuscular pressure was greater. The larger the increase in muscle fiber area, the longer it took for the tissue fluid pressure to return to normal. Crenshaw et al.[66] confirmed fiber swelling and increased intramuscular fluid pressure in knee extensor muscles made sore from eccentric exercise.

Although swelling is evident after eccentric exercise,[67] MRI changes indicating edema are not coincident in time with soreness.[18] After eccentric elbow flexion exercise, swelling begins, starting gradually at about 48 hr and peaking up to 10 days postexercise.[54] Clearly, soreness peaks long before peak swelling occurs. However, Nosaka and Clarkson[16] noted that the swelling seemed to be located within the muscle tissue up to 5 days postexercise and then moved to the subcutaneous area. It is possible that swelling in the muscle fibers activates free nerve endings in muscle, contributing to the sensation of soreness.

Noxious chemicals such as histamines, bradykinins, and prostaglandins have been implicated in producing the sensation of soreness. They are released when muscle tissue is damaged and activate type III and type IV nerve afferents that carry messages of pain from the muscle to the central nervous system.[68] Injection of bradykinin, prostaglandin E2, or histamine into muscle results in hyperalgesia.[69–71] Although these

chemical substances are considered to produce muscle soreness, there is no direct evidence for this. The excitatory action of these chemicals can sensitize the muscle nociceptors, which can lower the threshold for stimulation. In this way, the nociceptors may be activated by other stimuli, such as mechanical deformation or swelling. Howell et al.[72] suggested that the time-course of soreness reflected the presence of chemical mediators, but pressure would be the physical stimulus for the sensation of pain.

FACTORS THAT CAUSE OR EXACERBATE DAMAGE

Eccentric contractions clearly cause more muscle damage than either concentric or static contractions. Greater damage with eccentric contractions is not related to metabolic fatigue but due to a mechanical insult.[73] This has been attributed to the fact that, as muscle lengthens, the ability to generate tension increases and a higher load is distributed among the same number of fibers, resulting in a higher load per fiber ratio.[74,75] However, the fact that soreness peaks 24–48 hr postexercise and swelling becomes pronounced several days after exercise suggests that damage may be exacerbated in the days after exercise. The inflammation occurring after the initial insult is likely responsible for the continued damage and may also function in the regeneration process.

Initial Insult. Electromyography (EMG) data demonstrate that activation patterns during eccentric muscle actions are different than those controlling static or concentric muscle actions.[74,76,77] Although maximal eccentric muscle actions can produce greater output forces than both maximal concentric or static muscle actions, they produce lower EMG activity. Some data from studies using indwelling EMG electrodes support

preferential recruitment of larger, fast-twitch (fatigable) motor units during eccentric actions[75,76] in contrast to the typical recruitment pattern associated with the Henneman size principle (in which small, non-fatigable motor units are preferentially recruited and larger motor units are recruited sequentially as the intensity of the contraction increases[78]). However, studies using surface EMG techniques have not found evidence of altered recruitment and suggest that preferential recruitment of larger motor units is an inconsistent occurrence associated with noise within the biological system and not a general strategy during eccentric contractions.[79–81]

Other neuromuscular differences between eccentric contractions and concentric or static contractions include a decreased motor unit firing rate when subjects switched from concentric to eccentric contractions, increased motor unit synchronization during eccentric *vs.* concentric contractions,[82] and increased variability in the modulation of activity of motor units (i.e., discharge rate and patterns) during lengthening contractions *vs.* both static and concentric contractions.[83] These differences suggest unique command signals from the nervous system during eccentric contractions that, together, can predispose muscle undergoing eccentric contractions to greater degrees of strain and damage after exercise.

Jones and Round[84] noted that reperfusion injury after vascular surgery produced a rise in plasma CK activity and histologic changes in biopsy samples that were similar to those found after exercise-induced damage. Reperfusion injury was due to a restriction of blood flow followed by increased blood flow. This increase in blood flow is thought to set off a burst of respiration and increased free radicals leading to muscle damage. However, Jones and Round[84] suggested that the muscle damage

with reperfusion injury might also be caused by damage to capillaries. Since the damage after reperfusion is so similar to eccentric exercise–induced damage, they proposed that the mechanical trauma to muscle by the eccentric contractions would likely result in damage to the capillary endothelium. The damaged capillaries could then contribute to edema and a compromised blood flow, leading to more fiber damage. Indeed, Hellsten et al.[85] found increased levels of xanthine oxidase, a source of oxygen radicals, mainly in the microvasculature endothelial cells of eccentrically exercised vastus lateralis. Crenshaw et al.[86] found that after an ultramarathon race, where muscles were sore and there was ultrastructural fiber damage in the biopsy analysis, the majority of capillaries exhibited disturbed endothelial cells.

Inflammation. Inflammation after muscle injury occurs to clear debris from the injured area in preparation for regeneration. This inflammation response is thought to be activated by the initial mechanical trauma and is characterized by infiltration of fluid and plasma proteins into the injured tissue and increases in inflammatory cell populations.[87–89] The proliferation of inflammatory cells is thought to amplify the initial muscle injury through increased release of reactive oxygen species and activation of phospholipases and proteases in the tissue at the injury site.[87]

The actual time-course of the inflammation response after exercise is variable, dependent on several factors such as exercise mode, intensity or duration, and the muscle groups utilized.[87,90] The inflammatory response in humans can be measured via several experimental methods, including serum counts of white blood cells or inflammatory proteins (such as the cytokines), directly from muscle biopsy tissue through bioassay, or from in vivo muscle using radionuclide

imaging techniques (Fig. 5). In vivo radionuclide imaging of inflammatory cells, however, is so far restricted to quantifying only certain types of cells (i.e., white blood cells can be counted but not differentiated using this method). The time-course during which markers of inflammation are found is also dependent on whether the measures are made directly (from muscle biopsy or imaging) or indirectly (from blood or urine).

Data from Direct Measurement of Muscle. Neutrophils (a ubiquitous granulocyte) 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. Elevated levels of neutrophils in muscle release proteolytic enzymes and oxygen radicals that degrade tissue and increase membrane permeability (allowing for a greater efflux of muscle enzymes such as CK into the blood).[91,92] Neutrophil presence has been documented in muscle after various types of eccentric exercise.[50,93,94] Fielding et al.[93] found that neutrophils were increased significantly in muscle tissue as soon as 45 min after a downhill running exercise, and this increase persisted at 5 days postexercise. In that study, biopsy samples were taken before, 45 min after, and 5 days after exercise. Although not identified specifically as neutrophils, MacIntyre et al.[50] found an increase in leukocytes in muscle after eccentric exercise of the quadriceps using in vivo radiolabeling of white blood cells with technetium-99m. The increase in leukocyte count in exercised muscle began at approximately 5 hr and continued to 24 hr after exercise. A follow-up to this study[94] showed increased leukocyte counts in both men and women at two and 4 hr after 300 eccentric contractions of the right quadriceps.
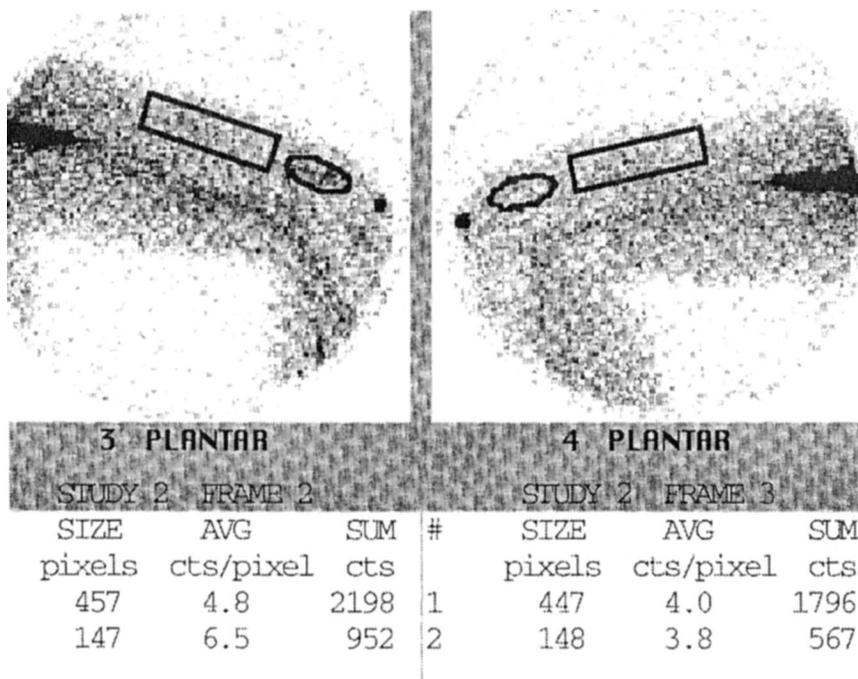
Thus, it seems that eccentric ex-

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SIZE pixels	AVG cts/pixel	SUM cts	#	SIZE pixels	AVG cts/pixel	SUM cts
457	4.8	2198	1	447	4.0	1796
147	6.5	952	2	148	3.8	567

3 PLANTAR — STUDY 2 FRAME 2

4 PLANTAR — STUDY 2 FRAME 3

Figure 5: A radionuclide white blood image, taken 4 hr after a subject performed eccentric exercise, of the lateral view of the thighs in the region of interest near the patella. The *left image* is the exercised thigh and the *right image* is the a nonexercised thigh. Courtesy of Dr. Donna MacIntyre, University of British Columbia, Canada.

ercise results in a migration of neutrophils into the muscle quite early, a process that peaks in the hours after exercise, even though neutrophilia can be detected in muscle tissue several days after exercise. This accumulation of neutrophils acts as a catalyst for further inflammation in the muscle via release of chemotactic agents that signal other inflammatory cells and begin the process of digesting surrounding necrotic tissue.

After the acute leukocyte/neutrophil aggregation, there is a subsequent increase in monocyte levels within the muscle. Subsequently, cytokine production by monocytes and lymphocytes in muscle tissue is followed by intramuscular degradation, further neutrophil and monocyte chemotaxis, and eventual resolution and healing within the tissue. Cytokines are released at the injury site and act as mediators by either facilitating or retarding the influx of inflammatory cells into the injured tissue. The subsequent course of the inflammation process depends on the relative balance between increases in proinflammatory cytokines (such as interleukin [IL]-1, IL-6, tumor necrosis factor, and interferon-α) and antiinflammatory cytokines (such as IL-10) to produce a sufficient inflammatory response to adequately repair injured tissue without creating an excessive response.[95,96]

Significantly increased IL-13 levels after downhill running have been reported. Cannon et al.[97] reported increased IL-13 in muscle up to 5 days after downhill running, and Fielding et al.[93] reported a 135% increase in IL-13 45 min after and a 250% increase in IL-13 5 days after a bout of downhill running. In multiple muscle biopsy samples taken before, after, and up to 7 days after eccentric cycling exercise, Malm et al.[7] reported no changes in IL-1 after exercise but a significant increase in IL-13 both postexercise and at 6 hr after exercise, with a return to baseline levels by 24 hr after exercise. Malm et al.[7] also found increased expression of T-cells (inflammatory mediators) at 6 hr, macrophages (phagocytic cells) at 48 hr, and natural killer cells (nonspecific immune system cells) from 6 hr to 7 days after exercise. However, some of these changes (i.e., neutrophil and macrophage infiltration and IL-1β activity) were similar in the nonexercised serial biopsy samples.

Although these studies[93,97] indicate a temporal progression of some acute inflammatory mediators migrating to the injury site in the hours or days after damaging exercise, the study by Malm et al.[7] casts doubt on the findings when no controls are used. Thus, some of the increases observed could be attributed to the biopsy procedure itself. However, it should be noted that even studies in which no biopsies were used still showed increases in neutrophils in the exercised muscle with techniques such as radiolabeling of neutrophils.[50]

Xanthine oxidase activity and indicators of inflammation were examined in subjects who performed eccentric exercise.[85] Xanthine oxidase uses molecular oxygen as an electron acceptor and generates reactive oxygen species, which can cause muscle damage.[98] Hellsten et al.[85] found an increased expression of xanthine oxidase in the microvasculature endothelial cells and an invasion of leukocytes, which may contain xanthine oxidase, over 4 days postexercise. They suggested that a secondary inflammatory process that induces xanthine oxidase might contribute to the generation of reactive oxygen species in the days after eccentric exercise and aggravate existing damage from the mechanical insult. This would explain the increase in ultrastructural damage noted in the days after eccentric exercise.

The chronic inflammatory phase can extend histologic findings of accumulated mononuclear cells from several days to weeks after the initial insult. Samples from the biceps taken between 4 and 8 days after eccentric exercise showed little change from preexercise levels. However, samples taken 9–14 days after exercise

showed mononuclear cell infiltration with high acid phosphatase activity and some evidence of small regenerating fibers.[99] Cells with high acid phosphatase activity could be macrophages. Samples taken from the calf muscles 12 days postexercise showed massive infiltration of mononuclear cells with high acid phosphatase activity compared with earlier samples. By 20 days postexercise, there were few mononuclear cells observed but many smaller regenerating cells. Round et al.[100] found increased mononuclear cells in the muscle tissue from 9 to 14 days posteccentric exercise, with the majority of these cells being macrophages and T lymphocytes. Increased levels of monocytes and macrophages associated with the chronic inflammatory phase usually return to baseline within 3–4 wk after exercise.[87]

Data from Indirect Measures (Plasma and Urine). Indirect measures of the acute inflammatory response from either blood or urine samples have yielded varied results using an assortment of exercise protocols ranging from strenuous endurance exercise to short-duration/high-force eccentric exercise. Data indicate that neutrophil activation and mobilization are dependent on both the type and intensity of exercise. Several studies[101–104] have reported periods of acute neutrophil elevation in the blood lasting several hours after intense endurance exercise (such as downhill running). For example, Pyne et al.[103] found elevated plasma levels of neutrophils in response to downhill running, but a decrease in circulating neutrophils after uphill or level running. In response to eccentric exercise, Malm et al.[105] also found increased numbers of neutrophils 6 hr after exercise, but not at 24 or 48 hr after exercise. This suggests that an increase in circulating neutrophils precedes the same phenomenon in the muscle tissue, indicating a peripheral increase in inflammatory cells that eventually leads to increased neutrophil migration into the injured muscle.

The monocyte response in the blood has a similar time-course to that of circulating neutrophils and also precedes the accumulation of monocytes found in muscle tissue. Malm et al.[105] found increased monocytes at 6 hr after eccentric exercise but not at 24 or 48 hr after exercise. A follow-up study by this group found a biphasic increase in monocytes in the blood, with an initial rise related to oxygen consumption during eccentric bicycle exercise and a second rise 4 days after exercise.[7]

Significant changes in the blood after strenuous endurance exercise or short-duration/high-force eccentric exercise have been demonstrated for some, but not all, of the cytokines. The majority of published studies have reported increased levels of IL-6 in the blood after both prolonged endurance and eccentric exercise.[85,96,106–108] Changes in other cytokines after strenuous exercise have been less clear, given the variety of different exercise conditions used to induce damage. In a review of exercise and cytokines, Pedersen[95] describes ascending responses of several plasma cytokine levels (including IL-6, IL-8 and tumor necrosis factor-α) as exercise becomes more strenuous. In response to eccentric exercise, Smith et al.[96] found only modest increases in some plasma cytokines (IL-10 and macrophage colony stimulating factor) and a decrease in others (P-selectin and IL-13). Because few studies have examined changes in cytokines after eccentric exercise, the exact relationship between exercise intensity, level of muscle damage, and the relative production of cytokines and other inflammatory mediators in the tissue has not been clearly delineated.

Compromised Energy Availability. In 1987, O'Reilly et al.[109] first published data showing that there was a delay in glycogen resynthesis after eccentric exercise. They examined glycogen concentration from biopsies of the vastus lateralis muscle after subjects performed eccentric (backwards) cycling exercise. Immediately after the exercise, glycogen concentration was 39% of the initial values, and at 10 days postexercise, the glycogen values were only 56% of the initial values. Costill et al.[110] and Doyle et al.[111] also reported delays in glycogen resynthesis after knee extension eccentric exercise. To what extent this impairment affects muscle recovery is not known, yet understanding these changes may shed light on the challenges that the muscle faces during recovery from eccentric exercise injury.

Kirwan et al.[112] examined whether eccentric exercise produced an insulin resistance to explain the slow recovery of glycogen stores. Euglycemic-hyperinsulinemic clamps were performed 48 hr after subjects completed a downhill running exercise or a concentric cycle ergometry exercise, and results were compared with controls who did no exercise. Glucose disposal rates were significantly reduced after the eccentric exercise (3.47 mg/kg/min) compared with concentric exercise (5.55 mg/kg/min/) and controls (5.48 mg/kg/min/). King et al.[113] reported that plasma insulin response during the early phase of a hyperglycemic clamp was significantly higher in subjects who performed eccentric exercise than in those who performed concentric exercise. Taken together, the reduced glycogen resynthesis, in the face of possible higher circulating insulin and a decrease in glucose disposal, points to a deficiency in the ability to transport glucose into the muscle.

Several studies have identified the transport proteins that take glucose into the cell, and the predominant one in skeletal muscle is GLUT4. Insulin seems to stimulate translocation of GLUT4 from the intracellular

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pool to the muscle membrane. Asp et al.[114] examined GLUT4 concentration from biopsies of the vastus lateralis 1, 2, 4, and 7 days after eccentric knee extension exercise and compared these with the unexercised leg. There was a 68% and 64% lower concentration of GLUT4 in the exercised leg at 1 and 2 days postexercise, respectively, but GLUT4 levels were similar to the control muscle at 4 and 7 days postexercise. Glycogen concentration showed similar changes, with lower values for the exercised leg on days 1 and 2 and similar values on days 4 and 7 postexercise.

Asp et al.[115] also assessed whether the decrease in GLUT4 content in muscle after eccentric exercise was accompanied by changes in insulin action. Using a euglycemic clamp technique, they found that the glucose infusion rate necessary to maintain euglycemia during the clamp was lower after eccentric exercise compared with the control condition. However, maximal (but not submaximal) insulin-mediated glucose uptake in muscle was only marginally lower (11.9%) in the eccentric exercise condition. This was accompanied by 39% lower GLUT4 protein content and lower glycogen level in the muscle. GLUT4 protein content is not the only determinant of muscle glucose uptake. The authors suggested that the local muscle effect at maximal insulin stimulation was not sufficient to explain the overall insulin resistance, and thus, there must be release of some factor that induces systemic insulin resistance. Moreover, the fact that glucose uptake did not decrease at submaximal insulin concentrations suggests that glucose transport deficiency is likely not the only cause of the inability to restore muscle glycogen. The authors point to a decreased activation of glycogen synthase or increased glycogenolysis as contributing factors.

Insulin signals the insulin receptor through insulin receptor substrate-1 phosphorylation, which activates phosphatidylinositol 3-kinase, thereby activating Akt-kinase, finally leading to GLUT4 translocation and glucose uptake. To explain intracellular mechanisms for changes in insulin resistance associated with muscle damage, Del Aguila et al.[116] examined insulin signal transduction at the level of the insulin receptor substrate-1, phosphatidylinositol 3-kinase, and Akt-kinase in subjects who performed downhill running compared with controls. Biopsies were taken before and 24 hr postexercise during a hyperinsulinemic-euglycemic clamp. Insulin-stimulated insulin receptor substrate-1 phosphorylation was 45% lower after the downhill running compared with the control. Also, insulin-stimulated phosphatidylinositol 3-kinase, Akt serine phosphorylation, and Akt activities were 34%, 65%, and 20% lower, respectively. These data show that exercise-induced muscle damage causes a defect in insulin signal transduction and may explain, in part, the observed insulin resistance.

REPEATED BOUT EFFECT

Performance of only one bout of eccentric exercise that produces muscle damage results in an adaptation such that there is less evidence of damage when the exercise bout is repeated after a week and even up to 6 mo (with no intervening exercise between the bouts).[54,117] For example, there is significantly less muscle soreness and recovery of strength is faster after the second bout of exercise compared with the first bout. The most dramatic response is in the change in blood CK activity in which, after the first bout, the increase is dramatic, but after the second bout, there is virtually no change.[118,119] If the second bout of exercise was performed 2 to 6 days after the first bout (when the muscle was not yet fully recovered), recovery time from the first bout was unaffected.[120,121] In other words, the second bout did not result in a setback in recovery.

The mechanism to explain the repeated bout effect is not fully known. As a general statement, it can be assumed that the damage produced in the first bout of exercise in some way produces an adaptation such that the muscle is more resistant to subsequent damaging exercise. Moreover, even though the first bout may produce evidence of only mild damage, there is still a prophylactic effect on a subsequent, more intense bout of damaging exercise.[118] Brown et al.[122] had subjects perform 10, 30, or 50 maximal eccentric contractions on the first bout, and then 3 wk later, each group performed the 50 maximal contractions exercise. Even though after the 10-contraction exercise there was little evidence of damage (e.g., no increase in blood CK activity), there was still a prophylactic effect on the second bout of more intense exercise such that there was no increase in CK activity.

Several investigators have suggested that the repeated bout effect may be explained by neural factors, such as more efficient recruitment patterns during the second bout.[120,123] As early as 1902, Hough[1] suggested that soreness was caused by rupture in the muscle fibers due to uneven activation of the stimulating neurons. He further proposed that muscles out of training would differ from muscles in training in their innervation mechanisms. Hortobágyi et al.[124] had 12 subjects perform two bouts of 100 eccentric contractions of the quadriceps muscle spaced 2 wk apart. After the first bout, knee extensor strength decreased by 37% and surface EMG activity decreased by 28% at 2 days postexercise. After bout two, there were no significant changes in force or EMG activity. However, reflex amplitude, assessed via patella tendon tap, increased, suggesting either central facilitation or enhanced Ia sensitivity or both. The authors concluded that neural factors

were involved, at least in part, in the adaptation process. Arguments against neural factors as an explanation for the repeated bout effect come from animal models in which the repeated bout effect is found when muscles undergo electrically stimulated contractions.[117] Furthermore, McHugh et al.[117] did not find a difference between repeated bouts in EMG/unit torque during the exercise, suggesting that recruitment was not different between the bouts.

Armstrong[29] first suggested that the muscle damage observed after eccentric exercise could be due to irreversible damage to stress-susceptible fibers, those fibers that may already be in a state of decline and therefore fragile. Foley et al.[15] examined whether damage to stress-susceptible fibers may contribute to muscle damage after bout one, and because they would be destroyed after bout one, a second bout of exercise performed 8 wk later would not elicit the same damage response. After bout one of an eccentric elbow flexion exercise, they found an increase in T2 (from MRI) of about 65% and an increase in elbow flexor volume of about 40%. The peak CK activity was 21,000 IU/liter, and peak soreness rating was 8 on a scale of 1 to 10. At 14 days postexercise, muscle volume decreased by about 10% from baseline. After the second bout performed 8 wk later, T2 increased by 27%, CK increased to 2,600 IU/liter, and soreness was rated a 5.6 on a scale of 1 to 10. These changes were significantly lower for the second bout. The fact that muscle area actually decreased below baseline after bout one was taken as evidence of a loss of muscle fibers due to irreversible damage, and the authors suggested that these fibers might have been the stress-susceptible fibers. Then, after the second bout, only moderate changes in indirect indicators of damage were found, which the authors suggested would be consistent with reversible fiber disruption. Moreover, there was no further decline in muscle area after the second bout.

The data from Foley et al.[15] may be taken as evidence that the repeated bout effect is due to a loss in fragile fibers from the first bout of exercise. This theory implies that stress-susceptible fibers develop over time as a natural phenomenon and then are vulnerable to mechanical strain of the eccentric exercise when they are irreparably damaged and lost. If the same exercise were repeated after more fibers became vulnerable, they too would be lost. Then, if this pattern were continued, it would represent a significant decline in muscle mass over time, which does not seem realistic. An alternate interpretation of the data are that parts of fibers are fragile, and these areas are destroyed by the first exercise bout, resulting in a loss in muscle volume. Over time, these fibers are repaired, making them more resistant to damage. There is evidence that it may take about 6 mo for fragile fiber areas to develop as a natural progression due to muscle fiber disuse. Nosaka et al.[27] found that when subjects performed the same bout of eccentric exercise of the elbow flexors 6 or 10 wk apart, on the second bout there was a smaller change in range of motion, strength, serum CK, and soreness. When subjects repeated the exercise 6 mo later, changes in serum CK activity were still lower than after the first bout, but not as low as that found after 6 or 10 wk.

Pizza et al.[125] suggested that the immune response to exercise-induced muscle damage is blunted, or more fine-tuned, after a repeated bout of exercise. In their study, subjects performed two bouts of eccentric exercise spaced 3 wk apart. After the second bout, there was a decrease in circulating neutrophils and a lower state of neutrophil and monocyte activation. Since neutrophils and monocytes may induce more damage as they enter the fiber, their lower activation could result in less damage. It is thought that cytokines, such as IL-6, are released from exercise-damaged muscle as a result of acute inflammation. Croisier et al.[108] examined plasma IL-6 levels after a knee extension eccentric exercise consisting of maximal contractions. Subjects then performed five training sessions of submaximal exercise over a period of 3 wk, followed by performance of the initial maximal contractions exercise. Serum myoglobin levels increased dramatically, and muscle soreness developed after the first bout but not after the second bout. However, the increase in plasma IL-6 was similar after each for the maximal contraction exercises. Either IL-6 is unrelated to acute inflammation, or inflammation is not blunted as part of the repeated bout effect.

Based on animal data, it was suggested that the first bout of eccentric exercise could induce an increase in the number of sarcomeres in series. Lynn and Morgan[126] found that exercising rats using a downhill running protocol resulted in an increase in sarcomere number in series. An increase in sarcomere number in series may make the muscle more resistant to damaging effects of lengthening. Whitehead et al.[127] provided indirect evidence to show that sarcomere length in humans may relate to muscle injury. They had subjects train the triceps surae of one leg with concentric contractions 30 min/day for 5 days. Subjects then performed eccentric contractions with the trained and nontrained muscles. The eccentric exercise produced a shift in the optimal angle for force production that was significantly greater in the trained leg. Moreover, the concentrically trained muscle seemed to be somewhat more susceptible to muscle damage. Ploutz-Snyder et al.[128] reported similar results, and Whitehead et al.[127] interpreted these data to mean that concentric training led to a reduction in sarcomere number, which made the muscle more vulner-

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able to eccentric exercise- induced damage. To extend these findings to the repeated bout effect, one might assume that adding sarcomeres might protect against injury.

An important question regarding the repeated bout effect and diseased muscle is whether diseased muscle can adequately recover and adapt to exercise. If diseased muscle has more fragile fibers, the muscle would incur greater damage after an exercise challenge, and the muscle may be unable to recover from this crisis. This information would have implications for treatment strategies for patients with neuromuscular diseases who may have a higher percentage of susceptible or fragile fibers.

SEX DIFFERENCES

Animal studies have consistently described sex differences in response to both endurance and resistance eccentric exercise protocols.[129–132] In general, female animals demonstrate attenuated responses to eccentric exercise, with less muscle damage as indicated by indirect markers such as CK,[129,130] histologic measures (myofibrillar disruption),[133] and inflammatory responses[131] to eccentric exercise.

Although it is well documented that women have lower resting blood CK activity when compared with men,[134,135] studies of sex differences in response to exercise damage are less clear.[136] Some human studies have found that women show a lower serum CK response to aerobic types of exercise,[137,138] which are unlikely to produce significant muscle damage. After 120 min of cycle ergometry at ~50% max VO$_2$, Shumate et al.[138] reported a 541 units/liter rise in CK for men vs. an 81 units/liter rise for women. Brooke et al.[137] reported a greater mean rise in CK in men vs. women after endurance cycle ergometry. However, the latter study was confounded by a small sample size, high intersubject variability, and the use of both trained and untrained subjects.

In contrast, two studies[23,139] found no sex differences in muscle damage markers after downhill running, an exercise shown to produce some muscle damage. Sorichter et al.[139] reported higher baseline plasma levels of CK and myoglobin and similar baseline plasma levels of myosin heavy chain fragments and skeletal troponin I in men vs. women. All four of these markers increased after 20 min of downhill running, with men having significantly greater absolute levels after exercise than women. However, when expressed relative to baseline levels, the increase of these four muscle proteins after exercise was not different between men and women. Eston et al.[23] also demonstrated that men and women showed similar CK increases, soreness, and strength loss after a bout of downhill running.

Only a few studies examined damage markers after moderate or high-force eccentric exercise protocols that create damage comparable with that observed in animal models. Miles et al.[140] examined the CK response of non–weight-trained women and men who performed 50 maximal eccentric actions of the elbow flexor muscles. The women had a lower baseline CK (81 units/liter) compared with men (139 units/liter), but they had a similar peak (4295 vs. 4163 units/liter) and relative (4214 vs. 4020 units/liter) increase in serum CK compared with the men in response to the high-force eccentric exercise. In another study,[141] although no significant difference was found between men and women in plasma CK levels after an eccentric protocol involving leg press and leg extension exercises, there was a trend for women to have lower CK values, but the sample size was too small to have sufficient power to test for a difference.[141]

Studies of muscle have also examined histologic and inflammatory damage markers between sexes. Stupka et al.[141] found that there was no difference between men and women in ultrastructural disruption (Z-line streaming) in muscle tissue biopsied at 48 hr after eccentric exercise of the quadriceps. Using neutrophil radio labeling, MacIntyre et al.[94] found that women had an increased presence of labeled neutrophils in muscle 2 and 4 hr after eccentric knee extensions on an isokinetic dynamometer, despite the fact that the men performed significantly more absolute work than the women.

Measures assessing losses in muscle function also seem either not significantly different between sexes or exacerbated in women after moderate or high-force damaging exercise. Borsa and Sauers[142] reported no differences in force loss after concentric/eccentric exercise of the elbow flexors, whereas Rinard et al.[143] found no sex differences in muscle soreness, strength loss after exercise, or in strength recovery during the 7 days after 70 maximal eccentric contractions of the elbow flexor muscles. However, the Rinard et al.[143] study found a greater loss in range of motion for women starting at 72 hr after exercise and persisting through 168 hr after exercise. In a further study of force loss and recovery after eccentric exercise, Sayers and Clarkson[144] found no difference between men and women when examining strength loss after 50 maximal eccentric contractions of the elbow flexors, but they did report that a larger number of women incurred profound strength loss than did men. Of an initial population of 192 subjects, 24 of the 32 subjects that experienced more than 70% strength loss immediately after exercise were women. This profound strength loss is often associated with extended periods of strength impairment such that the subjects in this study had not recovered fully 26 days after exercise.

There are no clear answers on

whether sex differences exist in muscle after either submaximal or maximal exercise protocols. However, the weight of the data suggests that there is either no difference between men and women in their response to damage-inducing exercise or that the differences are small and it is women who show the more pronounced response. The results for humans do not follow those obtained from animal models, in which females show an attenuated response.

SUMMARY

Eccentric contractions produce damage to muscle fibers as documented by morphologic analysis of muscle biopsy samples, although some questions have been raised over the accuracy of this technique to assess muscle damage. Changes in MRI signal intensity have been found after eccentric exercise and are taken to indicate muscle edema, yet these changes, which can last up to 1 mo, are not fully understood. Prolonged force loss seems to be a reliable indicator of damage and may be explained by damage to contractile elements, impairment in excitation-contraction coupling, and inflammation. Analysis of muscle proteins in the blood provides only a qualitative indicator of damage, and the changes observed demonstrate a large intersubject variability. The level of any protein in the blood is a function of both what is being released from the damaged tissues and what is being cleared from the blood. Muscle soreness accompanies muscle damage and may be caused by a combination of factors, including swelling, increases in noxious chemicals, and byproducts of inflammation. Muscle damage is initially caused by mechanical strain on the muscle, and this damage is exacerbated by the inflammatory response in the days after the exercise.

Performance of one bout of eccentric exercise induces an adaptation such that the muscle is less vulnerable to damage from a subsequent bout of eccentric exercise. Although the exact mechanism to explain this repeated bout effect has not been established, there are data to suggest that altered motor unit recruitment, an increase in sarcomeres in series, a blunted inflammatory response, and reduction in stress-susceptible fibers may be involved. Results from animal data clearly show that female animals have a reduced damage response to exercise due to the presence of estrogen. However, this does not seem to be the case in humans. The weight of the data suggests that there is either no difference between men and women in their response to eccentric exercise or even that women may incur greater damage.

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