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Muscle Soreness and Damage and the Repeated-Bout Effect

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CHAPTER 5

Muscle Soreness and Damage and the Repeated-Bout Effect

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Unaccustomed exercise consisting of repeated or forced lengthening (eccentric) contractions induces muscle damage. The most noticeable symptom of this damage is the muscle soreness that we experience after performing such exercise, which is often referred to as delayed-onset muscle soreness (DOMS). Eccentric exercise-induced muscle damage is also characterized by morphological changes such as disruption of contractile and recontractile proteins and the plasma membrane, increases in muscle proteins in the blood, prolonged loss of muscle function, swelling, and abnormality detected by ultrasound and magnetic resonance images. These are used as markers of muscle damage, but it is not clear how they are related to each other and are associated with DOMS. A bout of unaccustomed exercise confers a protective effect against DOMS and muscle damage in subsequent bouts of the same or a similar exercise. This effect, referred to as the repeated-bout effect, is a unique feature of eccentric exercise-induced muscle damage. This chapter focuses on the physiology of muscle soreness, the relationship between DOMS and muscle damage, and the repeated-bout effect.

Muscle Soreness

Pain originating from skeletal muscle is caused by an acute or overuse injury or by chronic syndromes such as fibromyalgia and muscular rheumatism. Muscle soreness refers to muscle pain felt after exercise when the muscle is palpated or moved, and is most commonly experienced after the performance of unaccustomed exercise consisting of lengthening (eccentric) contractions. The development of such muscle soreness is generally delayed. Although DOMS is an extremely common symptom, its underlying mechanisms are not clearly understood, nor are the reasons for the delay.

Understanding Pain

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Meurink and Bogduk 1994, pg. 210). We experience various kinds of pain in association with injuries, illnesses, and diseases. We also experience pain in many other non-pathological conditions (e.g., when we hold a heavy object or climb up stairs). Pain is a crucial signal that informs us of an abnormality in the body or a potential risk, but it is difficult to appreciate its value when we are suffering from it. In some cases, pain has no physiological value and yields only suffering. Pain perception is influenced by many factors, such as age, gender, and social or cultural norms about acceptable behavior in relation to pain (Urush et al. 2002). In addition, given the same magnitude of tissue damage, the magnitude of pain perception ranges widely among individuals, since pain is a subjective symptom. These factors add to the complexity of understanding pain.

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Function of Pain
Pain is generally considered a warning signal of actual or perceived tissue damage, and one of its major functions is to protect the organism from injurious stimuli (Millan 1999; Uran et al. 2002). In order for this to occur, (1) pain should be felt before injurious stimuli induce irreversible damage to tissue, (2) the magnitude of the pain should reflect the severity of tissue damage, and (3) the duration of the pain should match the process of tissue damage and recovery. However, pain often occurs without clear evidence of tissue damage; and its onset, magnitude, and duration do not necessarily correspond to tissue damage (Melzack 1982). In cancer, for example, pain does not exist in the developmental phase, but cancer patients often suffer from severe pain in the phase when the disease is no longer treatable.

There are two clinical states of pain: physiological (nociceptive) pain and neuropathic (intractable) pain (Kingsley 2002). The former results from the direct stimulation of pain receptors due to injury of tissue and surrounding organs, and the latter results from injury to the nervous system that causes permanent changes in central nervous system connections (Kingsley 2002). Chronic pain may be associated with changes in the central nervous system (Kingsley 2002). Neuropathic pain does not appear to have a useful physiological function (Millan 1999), and is not necessarily functioning as a warning signal.

Exercise and Muscle Pain
Muscle pain occurs in many situations in sport and exercise, ranging from stretching a stiff muscle to holding a heavy weight to incurring a muscle cramp or muscle tear. The onset of muscle pain varies depending on the cause. Figure 5.1 shows changes in the magnitude of muscle pain relative to its peak in five different situations. All persons experience a muscle tear of the biceps femoris while playing tennis, a muscle cramp in the knee flexors on a different occasion, pain in the knee extensors from running a marathon (42.195 km), pain in the knee extensors from playing soccer for 120 min, and pain from performing maximal eccentric exercise of the elbow flexors. Pain can occur either (a) only during exercise, (b) both during and after exercise, or (c) only after exercise.

Muscle pain is elicited by sustained or rhythmic muscle contractions, with occlusion of blood flow accelerating in onset and increasing the intensity; but it subsides quickly once muscle activity is terminated and normal blood flow is restored (Miles and Clarkson 1994). It seems likely that pain substances produced during muscle contractions, as well as increased intramuscular pressure, are associated with the pain.

Neurophysiology of Muscle Pain
Muscle pain has been less frequently documented than pain originating from cutaneous or visceral tissue. Most of the pain to be expected to have a nociceptor physiologic basis, but it appears that there are some differences between skeletal muscle nociceptors and others.

Pain Receptors (Nociceptors)
Pain receptors, referred to as nociceptors, respond to a noxious (tissue-threating) stimulus. Most of the data on the neurophysiology of pain are from studies of cutaneous nociceptors, less information is available for muscle nociceptors. Muscle pain differs from cutaneous pain in that pain associated with muscle lesions is described as aching and cramping, while cutaneous pain is characterized by sharp, pricking, stabbing, or burning sensations (Mense 1995). A large portion of the afferent nerve terminals lack encapsulated endings; these unencapsulated endings are free nerve endings. The free nerve endings are nociceptors, which are highly branched and have large areas of sensitivity. This is why our idea of the place of origin of a pain sensation is often vague, particularly for muscle pain (Mense 1993). When we feel muscle pain, it is difficult to tell exactly where in the muscle the pain is originating without careful palpation of the muscle. It appears that the pain spreads beyond its actual site of origin.

Skeletal muscle contains four types of afferent fibers: types Aα (slow), Aβ, III (Aδ), and IV (C). The free nerve endings of the latter two respond to nociceptors such as mechanical pressure, heat, cold, and algic substances (e.g., bradykinin, serotonin, potassium ions). Aδ fibers have thin myelinated axons with relatively fast conducting velocities (5-30 m/s); they mediate pain, stretch, and contraction, and innocuous pressure and are sensitive to thermal and chemical stimuli (Millan 1999). In contrast, C fibers are thin and unmyelinated and transmit signals more slowly (0.5-5 m/s). Like Aδ fibers, C fibers respond to thermal stimuli, ischemia, and hypoxia and are sensitive to mechanical stimuli (Millan 1999). Stimulation of C fibers in muscle elicits dull pain, aching pain, and cramping pain. The pain sensation from muscle is thought to be mediated by C fibers and secondarily by Aδ fibers (Hargreaves et al. 1989).

Muscle Nociceptor Locations
In skeletal muscle, the free nerve endings of Aβ and C fibers are located along the walls of arterioles and in the surrounding connective tissue (Mense 1995). It is important to note that there are no pain receptors on the muscle plasmas membrane. Therefore, even if some muscle fibers are damaged and stop functioning, we may not feel muscle pain if nociceptors located far from the damaged area are not affected. Interestingly, devastating muscle cytoptases such as the Duchenne type are not painful (Millan 1993).

It seems that muscle pain is activated by changes in the chemical environment surrounding muscle tissue, or by stimulation of fascia. Pain by primary muscle cell damage (Millan 1993). Someday it may be possible to determine the biochemical process that is responsible for DOMS. It has also been documented that the sympathetic nervous system contributes to the sensation of pain by augmenting or modifying the nociceptors (Hargreaves et al. 1989).

Muscle Nociceptor Stimuli
As already described, muscle nociceptors are polymodal, responding to mechanical, thermal, and chemical stimuli. Effective stimuli for nociceptors in skeletal muscle are strong mechanical forces and endogenous algic substances such as bradykinin, serotonin, and potassium ions (Mense 1993). The nociceptors are sensitized by prostaglandin E2 (PGE2), (Mense 1993) and nitric oxide (O'Connor and Cook 1999). It appears that muscle nociceptors respond to weak mechanical stimuli when they are sensitized.

A reduced sensitivity of nociceptors to a stimulus is termed hyperalgesia, and allodynia refers to the situation in which pain is induced by a stimulus that does not normally provoke pain (Calcutt 2001). In normal conditions, palpating, stretching, or contracting muscles does not induce muscle pain; however, the same stimulus evokes pain when muscle damage has occurred. This indicates that muscle damage changes innocuous stimuli to noxious stimuli; muscle nociceptors become sensitized through the perception experiences allodynia. It seems that in general, endogenous substances produced by muscle damage and inflammation do not stimulate muscle nociceptors directly (though some of them may lead to some other reactions that increase the sensitivity of the nociceptors so that muscle contraction or stretching or palpation pressure becomes painful (figure 5.2)). Swelling may also contribute to hyperalgesia of the nociceptors.

Pain Pathways
Aδ and C fibers bring signals from skeletal muscle to the spinal cord (figure 5.2). Most Aδ and C fibers enter the dorsal root ganglion and synapse primarily in the dorsal horn of the spinal cord (Gray 1994). From the spinal cord, pain signals take the spinothalamic pathways to the thalamus, the midbrain, and the cerebral cortex. There are two different spinothalamic pathways: the spinothalamic (lateral spinothalamic) tract and the lemniscal (anterior spinothalamic) tract (O'Connor and Cook 1999). The lemniscal tract transmits signals primarily from Aβ fibers and appears to be responsible for sharp, fast pain.
In contrast, pain signals from C fibres take the spinothalamic tract and terminate widely in the brainstem and thalamus. It seems that dull, aching pain is conveyed by this tract. The thalamus is the terminal of the spinothalamic pathways and transfers sensory information to the primary somatosensory areas of the cerebral cortex designated Brodmann’s areas 3, 1, and 2 (Guyenet 1994). In addition to these areas, multiple cortical areas such as the secondary somatosensory cortex, the anterior cingulate cortex, the insula, the prefrontal cortex, and the supplementary motor area are activated by pain stimuli (Kingery 2002).

Modulation of Pain and Analgesia

The transmission of pain sensation from the nociceptors to the cerebral cortex is modulated (Millan 1999). Stimulation of nociceptors does not necessarily reach consciousness and elicit pain. For example, it is often reported that athletes do not feel pain during a match even if they have a serious injury. The nervous system can interact with the pain pathways to relieve the perception of pain under some conditions. In this pain control process, often called analgesia, not only the brain but also the spinal cord is involved (Millan 1992). Substrates involved in analgesia include endorphins and enkephalins, and other opioid neuropeptides. Enkephalins are believed to cause presynaptic and postsynaptic inhibition of type Aβ and C fibres in the dorsal horn (Kolton 2000). It is also known that multiple areas of the brain have opioid receptors, and opioids such as endorphins and enkephalins suppress pain signals (O’Connor and Cook 1999). Thus, the magnitude of pain does not directly reflect the magnitude of the stimulus to nociceptors.

It has been documented that exercise increases pain thresholds and pain tolerance; this phenomenon is often referred to as exercise-induced analgesia (Kolton 2000; O’Connor and Cook 1999). Exercise-induced analgesia has been found to occur following running, cycling, and swimming, but little is known about the effect from resistance exercise (Kolton 2000). More than 100 years ago, Hough (1902) reported that performing a second bout of exercise on the day after an activity that induced muscle soreness caused excessive pain for the first 2 to 3 min, which disappeared over the course of 5 to 10 min of exercise. Armstrong (1984) stated that exercising a sore muscle appeared to provide the most effective way of reducing the soreness; however, the nature of exercise induced analgesia for DOMS has not been investigated systematically. A later section of the chapter provides further details on this issue.

Delayed-Onset Muscle Soreness

Following unaccustomed or severe exercise, we experience the discomfort of a dull, aching pain, combined with stiffness and often stiffness, for several days. The "delay" in DOMS appears to vary among individuals or individuals, but the pain normally increases in intensity in the first 24 h after exercise, peaks from 24 to 72 h, then subsides and disappears by 5 to 7 days postexercise. There is little or no discomfort at rest; the sensation of pain is elicited when mechanical stimuli such as pressure, stretching, or contraction are imposed on the affected muscles.

Muscle soreness develops during exercise such as long-distance running or performance of a marathon, and often gets worse over the days of recovery (figure 5.1). This type of muscle soreness is different from that occurring only after exercise. It may be that the cause of the muscle soreness felt during exercise is not the same as that responsible for the muscle soreness after exercise. However, it seems likely that the cause of the soreness in the days following exercise is similar in the two scenarios. Muscle damage induced by lengthening (eccentric) contractions is associated with the muscle soreness felt over the subsequent days (Armstrong 1984).

Mechanism of DOMS

A number of theories have been proposed to explain DOMS; among these are theories involving lactic acid, muscle spasms, muscle damage, connective tissue damage, and inflammation (Cheung et al. 2003). Theories pertaining to lactic acid and muscle spasm are unlikely to explain DOMS, and there is evidence to rule out these explanations (Cisek and Eaton 1992; Milks and Clarkson 1994).

Delayed-onset muscle soreness was first described by Hough (1902), who concluded that DOMS was "fundamentally the result of ruptures within the muscle." Although "ruptures" of muscle fibres are not associated with DOMS, ultrastructural disruptions of myofilaments, especially at the Z-disc, characterized by broadening, streaming, or rearrangement of the Z-discs as observed under electron microscope, have been reported to accom-
been documented that morphological changes at the muscle fiber level (e.g., non-necrotic cell infiltration) do not correspond with muscle pain (Jones et al. 1986; Newham 1988). Moreover, Yu and colleagues (2002) did not find muscle fiber degeneration or an inflammatory response in human skeletal muscle with DOMS. Thus, the exact relationships between damage and inflammation in muscle or connective tissue and DOMS are still not clearly understood.

**Measurement of DOMS**

There is no generally accepted single best measure of pain (O'Connor and Cook 1999). However, there are several methods for evaluating pain, including the pressure pain threshold and tenderness, and several ways to quantify the soreness level using a questionnaire (McLarty et al. 1995; O'Connor and Cook 1999; Onbach and Gale 1989). Types of scales for assessing muscle soreness include visual analog scales (VAS), numerical rating scales, visual rating scales, descriptor differential scales, and the McGill pain questionnaire. Ortmans and Adler (1975) reported that a VAS consisting of a line (50-200 mm) with "no pain" at the left end and "unbearable pain" at the right end reflected a subject's muscle-associated pain more precisely than a verbal rating scale.

A number of difficulties exist with the use of the VAS to quantify soreness, although this type of scale has been used in many studies. The difficulties include questions regarding the reliability of the instrument. For example, to mark "50," or "unbearably painful," for the peak DOMS level cannot indicate a greater pain level even if they experience greater soreness in subsequent days. This is a disadvantage of using a common scale for quantifying pain. It may be to use an open-ended scale in which subjects can choose any number to represent the pain associated with some intermediate level of stimulation and then scale all subsequent tests in relation to the initial reference stimulus (Jones and Round 1990).

**Defining Muscle Damage**

Muscle is damaged when it receives a harmful physical, chemical, or biological stimulus. In relation to exercise and sport, muscle damage occurs as a result of physical trauma, which is also referred to as muscle injury. In relation to exercise, Safran and colleagues (1989) suggested that muscle injury can be divided into three major types based on clinical presentation. A Type I injury is characterized by muscle soreness that occurs 24 to 48 hours post-exercise (DOMS). A Type II injury is characterized by an acute disabling pain from a muscle tear, ranging from a tear of a few fibers, with fascia remaining intact, to a complete tear of the muscle and fascia. A Type III injury is associated with muscle soreness or cramping that occurs during or immediately after exercise. It may not be accurate to include the Type III injury, because actual injury to muscle does not occur in the case of muscle cramping, although muscle damage may be a part of the process of treating a muscle cramp (e.g., stretching the cramping muscle).

Again, the Type I injury is peculiar to eccentric exercise. Eccentric exercise-induced muscle damage is evident by morphological changes in the intracellular structure and extracellular matrix (Armstrong et al. 1991; Lieber and Friden 2002; Stauber and Smith 1998). The earliest events associated with muscle damage are mechanical, and late events indicate muscle remodeling (Friden and Lieber 2001). Damage to muscle and connective tissue is followed by an inflammatory response that is necessary for regeneration (Kuipers 1994). During this process, neutrophils and macrophages infiltrate damaged muscle fibers and degrade damaged proteins (McLarty et al. 1995). However, it seems possible that this degradation process does not necessarily take place if the damage is not severe (Proctor and Morgan 2001). The relationship between ultrastructural changes and the inflammatory process has not yet been clarified.

**DOMS and Muscle Damage**

As already discussed, DOMS is a symptom particular to eccentric exercise-induced muscle damage. Other symptoms of muscle damage include muscle weakness, stiffness, and swelling. Muscle damage is detected by histological changes, increases in muscle protein in the blood, and abnormality shown by ultrasound and magnetic resonance images. It is important to note that DOMS does not necessarily represent muscle damage and that the level of DOMS and changes in muscle detected by the different methods are not necessarily correlated.

**Indirect Markers of Muscle Damage**

Two typical symptoma of eccentric exercise-induced muscle damage are muscle soreness and loss of muscle function, and these effects have been used as markers of muscle damage (Faulkner et al. 1997). Muscle damage is also assessed via increases in muscle-specific proteins in the blood such as creatine kinase (CK) or myoglobin (Clarkson et al. 1995). Swelling of muscles, detected by increases in circumference or magnetic resonance or ultrasound images, is often included among the markers of muscle damage (Howell et al. 1996; Nomura and Clarkson 1996). Among indirect markers, muscle function measures such as muscle strength and range of motion (ROM) are considered the best tools for quantifying muscle damage (Warran et al. 1999).
Connections Between DOMS and Muscle Damage

As already discussed, although DOMS is one of the symptoms associated with muscle damage, it may not be a direct reflection of muscle damage. It is possible for severe DOMS to develop with little or no indication of muscle damage, and severe muscle damage does not necessarily result in severe DOMS. The dissociation between DOMS and other indicators of muscle damage has been documented (Newham 1988, Rockenburg et al. 1993, Nosaka et al. 2002a).

**Time Course**

Although nocturnal eccentric exercise results in DOMS and a number of functional, structural, and biochemical changes, it is important to note that the time courses of these changes differ (figure 5.4). After maximal eccentric exercise of the elbow flexors, muscle soreness does not develop immediately whereas muscle strength shows its largest decrease at this point. Range of motion is more affected a couple of days after exercise but recovers more quickly than muscle strength. When muscle soreness subsides, swelling of the upper arm peaks, and abnormality in magnetic resonance or ultrason images is greatest around this time period. Plasma CK activity peaks around five days after exercise and returns to baseline two to three weeks later. Not only do the time courses of changes in the markers of muscle damage differ from one another, but also none of them match the time course of muscle soreness (Newham 1988). All this clearly shows that muscle soreness is not a cause of loss of muscle function, swelling is not a direct stimulus for muscle soreness, and muscle soreness does not peak when plasma CK peaks.

**Correlations Between DOMS and Other Markers Among Subjects**

It has been reported that the level of DOMS correlates poorly with the magnitude of changes in other indicators of muscle damage (Nosaka et al. 2002a; Rockenburg et al. 1993). As shown in figure 5.5, the peak muscle soreness score does not correlate strongly with other markers. It is generally thought that the larger the decrease in muscle strength following eccentric exercise, the greater the magnitude of muscle damage. However, subjects who show large decreases in maximal isometric strength do not necessarily have severe soreness.

It is also interesting that resistance-trained subjects and untrained subjects reported a similar magnitude of peak muscle soreness after performing maximal eccentric exercise of the elbow flexors, although changes in other markers of muscle damage such as maximal isometric strength and plasma CK activity were significantly larger for untrained compared to trained subjects (figure 5.6). This suggests that the magnitude of muscle damage is less for resistance-trained than for untrained individuals after eccentric exercise, but that resistance-trained individuals may feel similar magnitude of DOMS with significantly less muscle damage.

**The Magnitude of DOMS and Muscle Damage in the Same Subject**

The examples shown so far are based on groups of subjects. It may be that findings of little or no relationship between DOMS and muscle damage are due to differing pain perception among subjects. However, there is evidence to support the idea that DOMS does not reflect the magnitude of muscle damage even within a given individual, depending on the type and intensity of exercise as well as on which muscles are being used.

- **Type and intensity of exercise.** When the same subject performed different intensities of eccentric exercise using the same muscle group, all of the indirect markers of muscle damage showed larger changes for maximal intensity than for 50% intensity. However, no significant difference in muscle soreness was observed between the exercises (Nosaka and Newton 2002c). Moreover, two different types of exercise of the elbow flexors (maximal eccentric exercise in which 24 forced eccentric muscle actions were performed under maximal force generation; endurance exercise in which elbow flexion and extension movements were repeated for 30 min) performed by the same subject induced a similar magnitude of muscle soreness, but the magnitudes of changes in other markers of muscle damage such as muscle strength and plasma CK activity were significantly different (figure 5.7). These findings also support the notion that the magnitude of DOMS does not necessarily reflect the magnitude of muscle damage.

- **Difference between arm and leg muscles.** The response to eccentric exercise differs between the leg and arm muscles. Having subjects perform both arm and leg exercises, Jamurtas and colleagues (2005) compared effects of an eccentric exercise of the elbow flexors with those of an eccentric exercise of the knee extensors, matching the number of eccentric actions (six sets of 12 reps) and the relative intensity using 75% of pre-determined maximal eccentric torque. The arm eccentric exercise induced larger decreases and slower recovery of strength, as well as larger increases in blood markers of muscle damage (CK, myoglobin), than the leg exercise. However, DOMS did not differ between the two exercises (figure 5.8). These findings again support the idea that the magnitude of DOMS does not represent the magnitude of muscle damage.

The **Message of DOMS**

Delayed-onset muscle soreness may play a protective role by acting as a warning to reduce muscle activity and prevent further injury. However, we may find that further activity, although causing more pain initially, in fact alleviates muscle soreness. Hough (1902) reported that performing a second bout of exercise with a sore muscle caused excessive pain for the first 2 to 3 min but that the pain disappeared over the course of 5 to 10 min. Saxon and Donnelly (1995) investigated the effect of submaximal (50%) concentric exercise performed one to
sereness and damage (Figure 5.9a). This suggests that the light concentric exercise was effective for attenuating DOMS. However, the pullulative effect of the light exercise was temporary and did not influence changes in overall muscle soreness (figure 5.9b). No adverse effects of the light concentric exercise on recovery of muscle function were evident, either. Thus, it seems unlikely that DOMS is a warning signal not to use the affected muscle.

Several studies have shown that performing eccentric exercise in the early recovery days after the initial bout (two to three days) with sore muscles does not exacerbate muscle damage or retard the recovery process (Chen 2003; Nosaka and Newton 2002; 2005). These results suggest that muscle soreness is not necessarily a warning signal not to use the muscle. Such muscle pain may be telling us that we should not use the muscle, but we find that the pain is reduced when we use the sore muscle anyway. Thus it is still unclear how we should treat this type of muscle pain. We often face a situation in which we need to consider whether to ignore and overcome pain or accept and try to remove it.

Repealed-Bout Effect

Once an individual has experienced severe DOMS after performing an uncustomed exercise, the exercise is no longer uncustomed. After the person performs a similar exercise a couple of weeks later, severe DOMS no longer develops. It is as if muscles adapt to exercise rapidly to protect themselves from muscle damage. This phenomenon of a protective effect against muscle damage has been termed the repeated-bout effect (McHugh 2005). Along with attenuated DOMS after the exercise, the repeated-bout effect is characterized by faster recovery of muscle strength and range of motion, reduced swelling and muscle soreness, and smaller increases in muscle proteins (e.g., CK, myoglobin) in the blood following the second bout of a given eccentric exercise compared to the first (Figure 5.10). It has also been demonstrated that plasma responses are attenuated in the second bout (Poula et al. 1999). Fewer abnormalities in ultrasound or magnetic resonance images are evident after the subsequent bout as well (Foley et al. 1999).

It is important to note that the protective effect conferred by the initial bout of eccentric exercise does not necessarily "prevent" muscle damage, but attenuates the magnitude of changes in markers of muscle damage or enhances the recovery process. It appears that the adaptation is more specific to the muscles involved in the exercise, as evidenced by a study in which subjects performed the second eccentric exercise bout with a different arm from the initial bout. No significant difference in the changes in isometric strength is evident between the right and left arm bouts separated by two weeks, but the first and second bouts performed by the same arm showed a distinct difference in strength recovery (figure 5.11).

Hutton and van Someren (2007) reported that changes in maximal isometric strength, serum CK activity, and muscle soreness were attenuated when a second bout of eccentric exercise of the elbow flexors was performed by the contralateral arm two weeks later; however, the magnitude of protection in the contralateral arm was much less than that shown in the ipsilateral limb. Newton et al. (2007) compared changes in the markers of muscle damage between arms after maximal eccentric exercise of the elbow flexors separated by four weeks by counterbalancing the use of dominant and nondominant arms for the first bout among subjects. Changes in maximal isometric strength, range of motion, upper arm circumference, plasma CK activity, and muscle soreness measurements were not significantly different between arms, but a significant difference between the bouts was evident for maximal isometric torque, circumference, and plasma CK activity, such that the changes were significantly smaller after the second bout compared with the first bout. These suggest that some effect is transferred from one arm to the other, but the effect is weak, and the repeated-bout effect appears more strongly for the muscle that previously performed the same eccentric exercise.

Characteristics of Repealed-Bout Effect

The repeated-bout effect occurs whenever uncustomed exercise is repeated within a certain period of time. However, many factors influence the magnitude of the effect, such as time between bouts, the number of eccentric contractions, muscle length, and exercise mode. Additionally, it seems that intersubject variability exists in relation to
this effect, and the magnitude of the effect differs among the markers of muscle damage.

**Effect of Time Between Bouts**

If the time period between the uncustomized and subsequent exercise bout is too long, the repeated-bout effect does not occur. However, it appears that the repeated-bout effect lasts for at least several weeks and that its length is dependent on markers of muscle damage (Nosaka et al. 2005a). It was reported that changes in indirect markers of muscle damage following exercise were suppressed more when the interbout interval was 6 weeks compared to 10 weeks (Nosaka et al. 1991). As shown in figure 5.12, faster recovery of strength and range of motion, reduced swelling, less development of muscle soreness, and smaller increases in muscle protein in the blood following a second eccentric exercise bout compared with the initial bout persisted for more than six months for eccentric exercise of the elbow flexors. However, the magnitude of the protective effect appears to decrease gradually as the time between bouts increases, and the time course of attenuation of the protective effect varies among the measures. No protective effects seem to last more than 4 years.

Several studies showed that when the second eccentric exercise was performed two weeks after the first bout, prior to full recovery of muscle function, prolonged decreases in muscle function and development of muscle soreness, but no increases in CK activity, occurred (Clarkson and Tremblay 1988; Newham et al. 1987; Nosaka et al. 2005b). When the second bout was performed within a week of the initial exercise, in the early recovery phase, no adverse effects on markers of damage were observed, although acute decreases in muscle function occurred immediately after exercise (Chen 2003; Echols and Clarkson 1989; Nosaka and Newton 2002b, 2002d).

**Effect of Number of Eccentric Contractions**

Muscles do not appear to require the same exercise stimuli in the two bouts in order to show the repeated-bout effect. It has been reported that performing an initial eccentric bout with a relatively small number of eccentric actions produced the repeated-bout effect. An initial bout of 24 maximal eccentric repetitions reduced plasma CK activity and the magnitude of the strength loss and, by 24 hours when a 70-repetition bout was performed two weeks later (Clarkson and Tremblay 1988). It has also been demonstrated that 10, 30, or 50 eccentric actions provided equal protection for a bout of 50 eccentric actions three weeks later, in which increases in plasma CK activity were attenuated and the magnitude of isometric force loss was reduced (Brown et al. 1997).

Nosaka and colleagues (2001b) investigated whether a small volume of an initial eccentric exercise bout could still confer the repeated-bout effect on a second bout of a larger volume eccentric exercise that was performed two weeks later. The volume for the initial exercise bout was less than 1% (two maximal eccentric actions: 2ECC), of the number of contractions to be performed in the second bout (24 maximal eccentric actions: 24ECC). All variables changed significantly after 2ECC, but the amount of change in isometric strength and muscle soreness after the 2ECC was significantly smaller than that for 24ECC (figure 5.13). After the second bout, the group that performed 2ECC performed a repeated-bout effect that was indicated by a faster recovery of isometric strength and less development of muscle soreness. The group that initially performed 2ECC (2-24ECC) also demonstrated the repeated-bout effect, although the magnitude of the effect was not as strong as that of the 24ECC. These results suggest that the repeated-bout effect can be produced by two maximal eccentric actions, and it is not necessary to perform a high number of eccentric actions in the first bout to elicit a repeated-bout effect.

**Effect of Muscle Length**

It is known that changes in markers of muscle damage are significantly smaller following eccentric exercise of the elbow flexors in which the elbow joint is not fully extended compared to when it is fully extended (Nosaka and Sakamoto 2001). An interesting question is whether eccentric exercise without full extension movements can protect against muscle damage induced by eccentric exercise with full extension.

![Figure 5.12](image1.png)  
**Figure 5.12** Magnitude of repeated-bout effect for maximal isometric strength (MVC), range of motion (ROM), upper arm circumference (UB), plasma creatine kinase activity (CK), and muscle soreness (SQR) when the interval between bouts was 2, 4, 6, 12, 24, 36, or 52 weeks. For MVC, ROM, and CK, the values at four days postexercise in relation to the preexercise values were compared between bouts. For CK and SQR, peak values after exercise were compared between bouts.

![Figure 5.13](image2.png)  
**Figure 5.13** Changes in (a) maximal isometric strength and (b) muscle soreness following two (2ECC: filled circles) or 24 (24ECC: filled squares) maximal eccentric actions of the elbow flexors and in the subsequent bout of exercise (in which 24 maximal eccentric actions of the same muscle group were performed by the arm that previously performed 2ECC [2-24ECC: open circles] or 24ECC [24-24ECC: open squares]). Modified from Nosaka et al. (2000).
eccentric exercise of the same muscle. They suggested that to prevent or reduce eccentric exercise-induced muscle damage, it seems necessary to stimulate the muscles using the same muscle actions and intensity in the damaging exercise.

Eliot and colleagues (1996) showed that muscle soreness, strength loss, and increases in plasma CK activity after a downhill run were reduced when 100 maximal isokinetic eccentric actions had been performed two weeks earlier. This suggests that a different mode of exercise of the same muscle group confers the repeated-bout effect. It is not clear yet how much of a protective effect is conferred by a different mode of exercise of the same muscle.

One unpublished study addressed the extent of protection conferred by a submaximal elbow flexor endurance task against the effects of maximal eccentric exercise of the same muscle group. A group of subjects flexed (1 s) and extended (1 s) their elbow joint rhythmically for 30 min (180 actions) with a wristband load set at 10% of their maximal isometric strength and then, four to six weeks later, performed maximal eccentric exercise of the elbow flexors consisting of 24 forcible extensions of the elbow joint from a flexed (90°) to an extended position (180°). Another group performed the two bouts of maximal eccentric exercise with the same arm.

Changes in indicators of muscle damage were significantly smaller following the endurance exercise compared with the maximal exercise (Figure 5.15). After the maximal eccentric exercise, the subjects who had performed the endurance exercise initially showed smaller changes in the indicators of muscle damage compared with those observed after the first bout of maximal eccentric exercise performed by the other group of subjects. However, the magnitude of the protective effect against the effects of maximal eccentric exercise in the subjects who had initially performed the endurance exercise was not as strong as that shown by the subjects who repeated the maximal eccentric exercise. This suggests that protection against the effects of maximal eccentric exercise can be partially conferred by the endurance exercises using submaximal endurance exercise that results in minor damage.

Protective Effect Induced by Isometric or Nondamaging Exercise

It seems that severe muscle damage is not necessary in order to confer a protective effect on subsequent exercise.

As already mentioned, low-intensity (40%) eccentric exercise could confer protection of 20% to 60% on the indices of muscle damage following a subsequent 100% exercise bout performed two to three weeks later (Chen et al. 2007). Lavender and Nosaka (2007) have shown recently that light eccentric exercise (with a dumbbell set at 10% of maximal isometric strength) induces some protection against the effects of a subsequent bout of eccentric exercise with a heavier weight (a dumbbell set at 40% of maximal isometric strength) carried out two days later. However, whether the protective effect conferred by 10% eccentric exercise lasts longer, say for two weeks, has not been confirmed. Some animal studies have shown that isometric exercise can produce protective effects against the effects of eccentric exercise. Koh and Brooks (2001) reported that maximal isometric contractions or passive stretches of the extensor digitorum longus (EDL) muscles in mice did not cause degeneration of muscle fibers but induced protection against muscle damage from maximal eccentric actions performed three days later. McArdle and colleagues (2004) also reported that nondamaging isometric contractions of the soleus and EDL muscles via electrical stimulation, conducted 4 or 12 h prior to a damaging protocol, reduced CK release from the muscles of mice. However, no human studies have yet shown the extent of the protective effect conferred by isometric exercise against the effects of eccentric exercise.

Mechanisms of Repeated-Bout Effect

The mechanisms underlying the repeated-bout effect have yet to be fully elucidated, although several potential mechanisms have been addressed. McHugh (2003) reviewed studies associated with the repeated-bout effect and categorized the potential mechanisms as neural, mechanical, and cellular adaptations (Table 5.1). He concluded that "there may be several mechanisms for the repeated-bout effect and those mechanisms may complement each other or operate independently of each other (McHugh 2003, p. 56)."

The neural adaptations include more efficient recruitment of motor units, increased synchrony of motor unit firing, better distribution of the workload among fibers, improved usage of synergist muscles, and increased slow-twitch (fat) fiber recruitment. To investigate any neural adaptations associated with the repeated-bout effect,
Table 5.1 Potential Mechanisms for the Repeated-Bout Effect

<table>
<thead>
<tr>
<th>Neural adaptations (spinal cord, CNS)</th>
<th>Mechanical adaptations (noncontractile elements)</th>
<th>Cellular adaptations (contractile machinery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased recruitment of slow-twitch motor units</td>
<td>Increased passive or dynamic muscle stiffness</td>
<td>Longitudinal addition of sarcomeres (shift in the length-tension relationship)</td>
</tr>
<tr>
<td>Activation of larger motor unit pool</td>
<td>Removal of intermediate filament system to promote mechanical rearrangement (desmin, titin, etc.)</td>
<td>Adaptation in inflammatory response</td>
</tr>
<tr>
<td>Increased motor unit synchronization</td>
<td>Increased intracellular oxidative tissue</td>
<td>Adaptation to maintain excitation–contraction coupling</td>
</tr>
<tr>
<td>Learning effect</td>
<td></td>
<td>Strengthened plasma membrane</td>
</tr>
</tbody>
</table>

Nosaka and colleagues (2002b) compared two bouts of stretching of muscles receiving percutaneous electrical stimulation. Since the electrical stimulation bypasses the involvement of central motor drive, the expectation was to examine whether the central nervous system is involved in the repeated-bout effect. The results showed that the repeated bout of exercise resulted in less damage than the first bout, with all of the indirect markers of muscle damage showing smaller changes, and that recovery was significantly faster following the second bout (Figure 5.16). If neural adaptations were primarily responsible for the repeated-bout effect, similar effects on the criterion measures would have been observed following the two eccentric exercise bouts. The findings suggest that the repeated-bout effect did not result from changes in the motor output of the central nervous system. Some evidence exists to support the neural adaptation theory. Howatson and van Someren (2007) reported a significant attenuation of muscle damage in the second bout of eccentric exercise performed by the contralateral arm two weeks later. Newton et al. (2007) also showed that changes in maximal isometric torque, upper arm circumference, and plasma CK activity were significantly smaller after the second bout performed by the opposite arm than the first bout. A possible explanation for how the attenuation of the changes in some of the criterion measures was confounded by the first bout performed by the contralateral arm may lie in the phenomenon of cross education effect or some learning effect (Howatson and van Someren 2007; Newton et al. 2007).

According to another theory, suggested by Praske and Morgan (2001), increases in sarcomere number in series are associated with the protective effect. The increases in sarcomere number in series are indirectly assessed by a shift in optimum angle toward a longer muscle length (Praske and Morgan 2001). Philippou and colleagues (2004) recently reported a shift in the optimum angle of the elbow joint for producing maximal force by approximately 1°. If the shift could last for more than several weeks, this theory is attractive; however, the duration of this adaptation has yet to be determined. Chen and colleagues (2007) compared the effect of four different intensities (100%, 80%, 60%, and 40%) of eccentric exercise on optimum angle shift and the extent of muscle damage induced by subsequent maximal eccentric exercise (ECC2) performed two to three weeks later with a 10% load. A rightward shift of the optimum angle following ECC1 was significantly greater for the 100% and 80% than for the 60% and 40% exercises, and decreased significantly from immediately to five days postexercise. By the time ECC2 was performed, only 100% exercise retained a significant shift (4°). Although the magnitude of the repeated-bout effect following ECC2 was significantly smaller for the 40% and 60% groups, all groups showed significantly reduced changes in criterion measures following ECC2 in comparison to the ECC1 100% bout. This suggests that the repeated-bout effect is not dependent on the shift in optimum angle. Thus, it seems unlikely that the protective effect can be explained solely by increases in the number of sarcomeres in series.

Inglis and colleagues (2004) recently showed that the enhanced strength recovery of muscle foot dorsiflexor muscles with repeated lengthening exercise could be attributed to elevated rates of protein synthesis. This could explain the faster recovery of muscle function after a second bout compared with an initial bout as seen in human studies. Newham and colleagues (1987) have postulated that muscle fibers become more resilient and are able to withstand a given eccentric exercise after stress-susceptible fibers are removed and replaced by regenerated fibers. This theory appears to explain the repeated-bout effect very well if the newly regenerated fibers become susceptible again to eccentric exercise in 8 to 12 weeks. Figure 5.17 presents a graphic image of the stress-susceptible fiber hypothesis, which proposes six stages in the repeated-bout effect:

- **Stage 1:** Before performing the first eccentric exercise bout (ECC1), some of the muscle fibers are stress-susceptible fibers.
- **Stage 2:** These fibers are likely to be damaged by ECC1 and to degenerate, and severe muscle damage is induced, but other fibers may survive.
- **Stage 3:** After ECC1, the damaged fibers are regenerated and may be remodeled and become “strong” fibers.
- **Stage 4:** When the second bout of eccentric exercise (ECC2) occurs at this stage, the number of stress-susceptible fibers is small, and less muscle damage is produced.
- **Stage 5:** No stress-susceptible fibers exist, and little muscle damage is induced when eccentric exercise is performed at this stage. When eccentric exercise is performed regularly, it may be that muscles are in the Stage 5 condition.
- **Stage 6:** Because of protein turnover, some muscle fibers become stress-susceptible fibers again.

Understanding the mechanisms underlying the repeated-bout effect may be the key to understanding eccentric exercise–induced muscle damage.

Figure 5.17 Stress-susceptible fiber hypothesis.

According to Koh (2002), heat shock proteins (HSPs) may be involved in protecting skeletal muscle fibers from eccentric exercise–induced muscle damage. A microwave diathermy treatment that increased muscle temperature to over 40°C, 16 to 20 h prior to exercise, resulted in a significantly faster recovery of muscle strength, a smaller change in RMM, and less muscle soreness; however, the protection afforded by the diathermy treatment was significantly less effective than in the second bout, performed four to six weeks after the initial bout (Nosaka et al. 2007).

McArdle and colleagues (2004) proposed that activation of the home oxygenase-1 (HO-1) gene resulting from increased reactive oxygen and nitrogen species (ROS) production was associated with the protective effect. Mikkelsen and colleagues (2006) have recently shown that stimulation of the Nrf-2/p66Shc complex improved force recovery in fast-twitch fibers by 40% to 90% with a 50 min electrical stimulation protocol. It is possible that these proteins are associated with protective effects. Further studies are needed to advance understanding of the mechanisms underlying the repeated-bout effect.

**Summary**

This chapter has focused on DOMS, muscle damage, and the relationship between DOMS and muscle damage, and the repeated-bout effect. Despite advances in our understanding of eccentric exercise–induced muscle damage, we still do not have a complete picture of the phenomenon. More than a century ago, Hough (1902) carefully observed "muscular soreness" and thought creatively in an attempt to explain the cause of DOMS. He stated, "The abnormal condition of the muscle frequently escapes notice, unless attention is specially directed to it by making it work or by over-extension." We still need to pay more attention to the condition of muscles so as not to miss what they can tell us. The key points of this chapter can be summarized as follows:
• Muscle soreness is sensed in the brain as a signal from muscle; however, the stimuli that prompt nociceptors or other receptors to evoke muscle soreness have not been fully elucidated.
• Delayed-onset muscle soreness is associated with eccentric exercise–induced muscle damage; however, it is still unclear how a sequence of events in the process of muscle damage induces DOMS.
• Physiological changes used as indicators of eccentric exercise–induced muscle damage include decreases in muscle function, swelling, increases in muscle proteins in the blood, and abnormalities shown by ultrasound and magnetic resonance images.
• The magnitude of DOMS does not necessarily reflect the extent of muscle damage, and the time course of DOMS does not represent the time course of changes in indicators of muscle damage.
• Muscle adapts rapidly after eccentric exercise to prevent muscle damage, and this effect (repeated-bout effect) lasts for several weeks to several months.
• The repeated-bout effect is produced even if the initial bout is less demanding than the second bout in terms of the number of eccentric actions, muscle length, and the force generated during eccentric exercise; and conferral of the protective effect does not necessarily require muscle damage.
• Neural, mechanical, and cellular adaptations are likely involved in the mechanisms underlying the repeated-bout effect; however, a unified theory explaining the mechanisms remains elusive.

CHAPTER 6
Satellite Cells and Muscle Repair
Karin Shortreed, MSc; Adam Johnston, MSc; and Thomas J Hawke, PhD

The skeletal muscle of adult mammals displays a remarkable ability for growth and repair throughout the life span. This adaptability is largely the result of a population of stem cells, termed myogenic satellite cells, resident within the skeletal muscle itself. This chapter focuses on the capacity of skeletal muscle for growth and repair and the role of these unique cells in the regenerative process. The regulation of myogenic satellite cells in health and disease, as well as the role of various extrinsic factors in affecting myogenic satellite cell function, is also discussed.

Skeletal Muscle Stem Cell Populations

Adult skeletal muscles contain various cell populations that display stem cell–like characteristics, including the capacity for self-renewal, proliferation, and plasticity (capacity to become multiple fates). In particular, the myogenic satellite cell and the muscle side-population (SP) cell are the most well characterized of the skeletal muscle stem cell populations to date.

Myogenic Satellite Cells

The myogenic satellite cell was named on the basis of its location at the periphery of the adult muscle fiber. Although these undifferentiated stem cells were identified over 40 years ago (Meineri 1964) and are the most thoroughly characterized of the resident muscle stem cell populations, a great deal of attention has recently been refocused on these cells as we begin to further appreciate their stem cell–like capacities.

In unperturbed muscle, the quiescent myogenic satellite cell resides outside of the muscle fiber plasma membrane but underneath the overlying basal lamina (figure 6.1a, a & c). In this resting state, the nuclei of these cells comprises approximately 2% to 5% of all muscle nuclei and display dense heterochromatin (genetically inactive region of chromosomes), reduced organelle content, and high nuclear-to-cytoplasmic volume ratio, consistent with their low transcriptional activity (figure 6.1b). Satellite cells exit their quiescent state and enter a proliferative phase in response to stressors such as trauma (figure 6.1d). Studies of rodent skeletal muscles have demonstrated a relationship between myogenic satellite cell content and muscle fiber types (see Hawke and Garry 2001 for review), with oxidative muscle fibers demonstrating a five to six times greater myogenic satellite cell content than glycolytic muscle fibers (table 6.1). As human skeletal muscle displays a more heterogeneous fiber type composition, this phenomenon is less observed in humans.

In response to cellular and extracellular cues associated with intense exercise or muscle damage, the myogenic satellite cells exit their quiescent state (become "activated"), proliferate, and migrate to the site of injury to repair or replace damaged muscle fibers by fusing together, fusing to existing muscle fibers, or both. Although it has been suggested that other stem cell populations contribute to skeletal muscle regeneration, the evidence to date suggests that myogenic satellite...
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