Mechanisms and Mediators of the Skeletal Muscle Repeated Bout Effect

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HYLDAHL, R.D., T.C. CHEN, and K. NOSAKA. Mechanisms and mediators of the skeletal muscle repeated bout effect. Exerc. Sport Sci. Rev., Vol. 45, No. 1, pp. 24–33, 2017. Skeletal muscle adapts to exercise-induced damage by orchestrating several but still poorly understood mechanisms that endow protection from subsequent damage. Known widely as the repeated bout effect, we propose that neural adaptations, alterations to muscle mechanical properties, structural remodeling of the extracellular matrix, and biochemical signaling work in concert to coordinate the protective adaptation. Key Words: eccentric exercise, muscle damage, lengthening contractions, delayed-onset muscle soreness, muscle strength, muscle adaptation, muscle soreness

INTRODUCTION

Performing an unaccustomed exercise results in a defined set of symptomatic (e.g., force loss, muscle soreness), systematic (e.g., increased circulating muscle proteins), and histologic (e.g., myofibrillar disruptions) changes that are referred to collectively as muscle damage. Intriguingly, skeletal muscle possesses an intrinsic protective mechanism that reacts to exercise-induced damage by orchestrating an adaptive response that endows resistance to subsequent exercise-induced damage stimuli. This phenomenon—coined the repeated bout effect (RBE)—has been widely observed, yet its underlying mechanisms remain poorly understood. The RBE has been studied primarily in the context of damage-inducing eccentric (lengthening) contractions (ECC), where it has been firmly established that a second bout of ECC, when performed within several weeks of an initial bout, results in a robust and repeatable attenuation of functional declines postexercise (28). The protective characteristics of the RBE also are conferred by electrical stimulation (27), as well as other nondamaging exercises such as low-volume or low-intensity ECC (2,6) and maximal isometric contractions at a long muscle length (4,6). Recent studies also have observed that after unilateral damage, the protective effect is endowed on the contralateral (nonexercised) limb, a peculiarity known as the contralateral RBE (3).

We propose that a better appreciation of the mechanisms that underlie the RBE is fundamental to our evolving understanding of muscle adaptations and has implications for the prevention and management of muscle injury or pathologic conditions. For example, individuals affected with muscular dystrophy are more susceptible to ECC-induced muscle damage, and severe muscle strain injuries are more commonly sustained during forceful ECC. A more detailed understanding of mechanisms underlying skeletal muscle's ability to protect itself from...
contraction-induced injury could be highly relevant to the management of these pathological conditions. Moreover, the temporal understanding of RBE manifestations can impact the optimization of strength and conditioning programs, thereby affecting human performance. From a muscle health perspective, the RBE has shown us how only a small number of muscle contractions can induce significant and widespread physiological changes, indicating the significance of performing muscle contractions, even at very small volumes.

Recent studies have provided insight on how neural adaptations, alterations to muscle mechanical properties, structural remodeling of the extracellular matrix (ECM), inflammation, and biochemical signaling contribute to the emergence of the RBE. We hypothesize that the phenomenon of the RBE is made manifest through a multiplicity of mechanisms that might work independently or in combination to generate muscle protection after exercise. Furthermore, we propose that there are different forms of the RBE, all of which are expressed functionally as reduced muscle damage conferred by a single bout of exercise. In this article, we will explore this hypothesis by first discussing the conditions under which the RBE and contralateral RBE are manifest, along with relevant modifying factors. We will review emerging ideas on the mechanisms that underlie the RBE with special emphasis on alterations at the corticospinal level, muscle-tendon complex behaviors, inflammation, and the role of ECM remodeling. Finally, we will explore the implications of a better mechanistic understanding of the RBE.

FACTORS AFFECTING THE RBE

Muscle damage induced by ECC is evident by histological alterations of muscle fibers (myofilament and/or myofiber level) and their surrounding structures such as ECM (endomysium), perimysium, and epimysium (19), abnormality detected by magnetic resonance imaging (MRI) and/or B-mode ultrasound images, increases in muscle proteins (e.g., creatine kinase, CK, myoglobin, troponin) in the blood, and indirect markers representing symptoms of muscle damage (29). The indirect markers include prolonged decreases in muscle force generating ability, delayed-onset muscle soreness (DOMS), increased muscle stiffness, and muscle swelling (29). The magnitude of histological muscle fiber damage observed by light microscope does not necessarily match with the magnitude of symptoms of muscle damage such as strength loss and DOMS, especially in human studies (19). It has been shown that the changes in the indirect muscle damage markers are not necessarily associated in terms of time course and the magnitude of changes (8). In general, muscle force generating ability shows the greatest decrement immediately after ECC, with a linear restoration of the force during the next 7–14 d. In contrast, muscle soreness develops at 1 d and peaks 2–3 d post-ECC, whereas peak swelling occurs 4–7 d post-ECC, which corresponds to MRI and ultrasound image changes. Blood CK activity increases precipitously at 2 d after ECC, and peaks 4–5 d after ECC. The time course of histological changes at the myofilament, myofiber, and ECM levels are less clear because of the limitations of collecting muscle biopsy samples. However, as subsequently discussed in relation to the ECM, alterations seem to be more pronounced several days postexercise than immediately postexercise (Fig. 1).

Compared with the initial eccentric exercise bout, the subsequent bout of the same exercise performed within several weeks results in less histological changes and abnormality in MRI and B-mode ultrasound images, reduced DOMS and muscle swelling, faster recovery of maximal voluntary muscle contraction (MVC) strength, and smaller increases in CK activity and myoglobin concentration in the blood after the second bout (28). The magnitude of RBE varies among the indicators such that its effect on CK activity is the strongest (no increases in CK activity after the second eccentric exercise bout) when maximal eccentric exercise is repeated within 4 wk. However, the MVC strength loss immediately after eccentric exercise occurs similarly between bouts, although the recovery rate is enhanced by a large extent (e.g., more than 80% faster recovery of MVC strength from immediately to 1 d postexercise) after the second bout. When maximal eccentric exercise of the elbow flexors is repeated 4 wk later, DOMS develops to a smaller extent, but the magnitude of the RBE on DOMS seems less than that of CK activity (Fig. 2). These variations may suggest that there are several aspects of the RBE, and there are several different mechanisms underpinning the RBE demonstrated in different parameters.

The magnitude of muscle damage induced by ECC is affected by many factors: intensity, velocity, number of contractions, muscle length, muscle group (e.g., arm vs leg), exposure to ECC in daily activities, age, and sex. Generally speaking,
greater muscle damage is induced by higher intensity, faster velocity, larger number, longer muscle length ECC (29). Arm muscles are more susceptible to muscle damage than leg muscles (29). Preadolescent children are less susceptible to muscle damage than adolescent children and young adults (5), but elderly adults are not necessarily more susceptible to muscle damage when compared with young and middle-aged adults (25). Controversy exists regarding the difference in muscle damage between men and women, but sex difference does not seem very large in humans (39). A typical RBE is observed when the same exercise is repeated. However, the protective effect against muscle damage can also be conferred by a different eccentric exercise. We (7) compared maximal and three submaximal (40%, 60%, 80% of MVC) intensities of elbow flexor ECC for their effects on the extent of muscle damage (MVC, ROM, upper arm circumference, muscle soreness, CK, myoglobin) induced by maximal ECC of the same arm performed 2–3 wk later. Changes in indirect markers of muscle damage after the first bout were greater in the order of the intensity (maximal > 80% > 60% > 40%). After the second bout, changes in the markers were significantly smaller when compared with the maximal ECC bout performed in the first bout, suggesting that muscle damage was attenuated, but the higher the intensity of the initial bout, the greater the protective effect conferred. We have shown that lower intensity elbow flexor ECC, such as 20% MVC (6), still confers a protective effect against muscle damage induced by maximal ECC performed 3 wk later. However, the magnitude of the protective effect seems to be smaller than that conferred by the 40% (Fig. 2). We (4) reported that when the 40% ECC was repeated every 2 wk for four times, it conferred the same magnitude of protective effect as one bout of maximal ECC. We also found that maximal voluntary isometric contractions \( n = 30 \) at 20° elbow flexion (at a long muscle length) could confer protection against maximal ECC performed 3 wk later, but maximal isometric contractions at 90° elbow flexion (at a short muscle length) did not (6). Importantly, only minor changes in muscle damage markers were observed after the 10% and 20% ECC and the isometric contractions, but they still conferred the protective effect. It does not seem that severe muscle damage is a prerequisite for the RBE. Interestingly, only two maximal voluntary isometric contractions at a long muscle length (20°) still attenuated the magnitude of muscle damage after maximal ECC of the elbow flexors performed 2 or 4 d later, but not immediately after or 7 d later (2). It seems that the smaller the number of the isometric contractions, the shorter the RBE. Importantly, no RBE is induced when the isometric contractions were performed immediately before the maximal ECC.

We (1) have shown that slow-velocity elbow flexor ECC (30°/s) conferred protection against fast velocity (210°/s) ECC performed 2 wk later. The magnitude of the protective effect seems similar to that observed when repeating the same velocity ECC. It has been reported that performing an initial exercise bout with a relatively small number of ECC produced the RBE. We (33) reported that two or six maximal ECC of the elbow flexors reduced the magnitude of muscle damage after 24 maximal ECC performed 2 wk later. Interestingly, six maximal ECC that induced less damage than 24 maximal ECC provided a similar magnitude of protection against muscle damage induced by 24 maximal ECC to the condition that 24 maximal ECC were repeated. We (31) also compared 24 maximal ECC of the elbow flexors at a long starting length (50° flexion) and at a short starting length (130° flexion) for their effect on the subsequent 24 maximal ECC starting at the long muscle length performed 4 wk later. We found that the short muscle length ECC produced a partial protective effect (approximately 50%) against muscle damage induced by the long muscle length ECC. These results suggest that severe muscle damage is not a prerequisite for the RBE.
It seems that the RBE occurs to children, young adults, and older adults, although the magnitude of the effect is not necessarily the same. Because children are less susceptible to eccentric exercise-induced muscle damage than young adults, the RBE of children is not expressed as strongly as that of young adults (5). When comparing between young and older adults, the magnitude of muscle damage after the initial eccentric exercise bout is less for older than young adults, but the magnitude of the RBE seems to be similar between them (25). When comparing between resistance-trained and untrained individuals, resistance-trained individuals seem to show less RBE than untrained individuals (29). This is probably because of less initial muscle damage in the trained individuals. It also is important to note that the magnitude of the RBE is less for the knee extensors when compared with the elbow flexors (Fig. 3A). This may be associated with the less muscle damage induced for the knee extensors than elbow flexors because leg muscles are more exposed to ECC in daily activities (26,29). In general, it seems that the greater the potential for muscle damage in the initial exercise bout, the greater the magnitude of the RBE.

As shown in Figures 2 and 3, the magnitude of the protective effect is different among MVC strength, DOMS, and CK. The protective effect on CK is the largest, followed by DOMS and MVC. If a large increase in plasma CK activity indicates plasma membrane damage leading to focal muscle fiber necrosis, it seems likely that little plasma membrane damage is induced after the second bout because no increases in plasma CK activity are seen after the second bout of maximal eccentric exercise. A large protective effect for the plasma membrane seems to be induced even by nondamaging stimulus, as shown in the large protective effect for CK activity after the low-intensity (10%, 20%) eccentric exercise in which increases in CK activity are very small. The protective effect on DOMS is smaller than that of CK but is greater than that of MVC. If DOMS is more associated with muscle fascia and/or endomysium and perimysium damage and inflammation (23), the initial bout of ECC seems to produce some protection on these structures. It has been reported that the decreases in MVC are due to a combination of E-C coupling failure and muscle fiber degeneration, and the prolonged decreases beyond 5 d postexercise were more associated with focal muscle fiber necrosis (40). If this is the case, the protective effect on E-C coupling failure seems minimal. Collectively, these data suggest that at least three different adaptations play a role in the RBE, but other mechanisms such as neural adaptations and altered inflammatory responses may play a role as subsequently discussed. It should also be noted that if the RBE is expressed as "reduction in ECC-induced muscle damage conferred after a single bout exercise," there are at least four kinds of the RBE: 1) the RBE by repeating maximal or the same ECC between bouts, 2) the protective effect against maximal ECC conferred by submaximal ECC that induces some muscle damage, 3) the protective effect conferred by nondamaging or minor-damaging low-intensity ECC or maximal isometric contractions at a long muscle length, and 4) the RBE transferred to the contralateral muscle, which will be explained in the next section.

**THE CONTRALATERAL RBE**

For more than a century, studies have observed the curious existence of a cross-education effect in skeletal muscle, defined by enhanced performance of an uninvolved muscle group consequent to the training of a remote muscle group. Hortobagyi et al. (15) demonstrated that the cross-education effect is substantially greater in the contralateral limb when the ipsilateral limb is trained with ECC compared with concentric contractions (CON). This suggests that, in addition to strength enhancements, the contralateral muscle also may be the beneficiary of the protective adaptation of the RBE after ECC of one limb. Howatson and van Someren (17) showed that the contralateral limb was indeed the recipient of the protective adaptation after unaccustomed ECC (43 maximal ECC of the elbow flexors) of the ipsilateral limb, establishing the...
foundation of our understanding of the contralateral RBE (CL-RBE). Other studies have since clearly established the presence of a CL-RBE in both the elbow flexor and knee extensor muscle groups (3,41). The existence of adaptation in the form of contralateral muscle protection is curious, and could have significant clinical application, particularly for unilaterally immobilized individuals. Thus, identification of the intricacies that might govern the CL-RBE remains a compelling undertaking for future research.

Although the magnitude of the protective effect has varied between studies and muscle groups, most of the data indicate that the protective effect on the contralateral limb is approximately 40%-60% less than that of the ipsilateral (exercised) limb. In a very recent study, we (3) compared the magnitude of the CL-RBE for different time intervals between two bouts of eccentric exercise of the elbow flexors; 0.5, 6, 12, and 24 h, 7, 28, or 56 d in comparison to the RBE by the same arm separated by 2 wk. We found that changes in indirect muscle damage markers were smaller after the second bout than those after the first bout, when the opposite arm performed the second exercise bout at 24 h, 7 d, and 28 d. However, the changes were not significantly different between bouts for the interval between bouts of 0.5, 6, and 12 h, as well as 56 d. The difference in the changes in all variables between the first and second bouts was smaller for the CL-RBE observed at 24 h (70%), 7 d (55%), and 56 d (36%) than the RBE of the same arm (91%), showing that the magnitude of the CL-RBE was reduced with increasing time between bouts from 1 d to 4 wk. The magnitude of the protective effect was smaller for CL-RBE at 7 d when compared with that of the ipsilateral RBE at 14 d (Fig. 3B) confirming that the magnitude of the CL-RBE is approximately 50% of that of the ipsilateral RBE. These results suggest that the CL-RBE is less persistent than the ipsilateral RBE (>8 wk), and requires a day to be conferred. Although there is limited research to date on the mechanisms that mediate the CL-RBE, it seems that some of the same mechanisms that are involved in the traditional ipsilateral RBE are at play in the CL-RBE. Some of those mechanisms, including potential neural and inflammatory adaptations, are discussed in the subsequent sections.

POTENTIAL MECHANISMS UNDERLYING THE PROTECTIVE ADAPTATION

Neural Adaptations

Previous studies showed that neural adaptations were associated with the RBE. For instance, a shift in motor unit recruitment toward low-threshold motor units during the second bout of ECC has been suggested to be associated with the RBE (28). Using intramuscular EMG recordings, it was reported that motor unit synchronization increased up to 7 d after an eccentric exercise (9). Such enhancement of motor unit synchronization was described as a strategy of the central nervous system to promote coordination between synergist muscles (9). Thus, it is assumed that the central nervous system adjusts the motoneuron pool to protect the muscle from further damage by distributing mechanical constraint over a greater motor unit sample, when submaximal eccentric exercise is repeated. In volitional maximal eccentric contractions, motor unit synchronization would increase force production, which potentially makes muscles more susceptible to damage, if no other protective mechanisms are involved.

A contribution of neural function in muscle damage has been pointed out, but no previous study has systematically investigated supraspinal and spinal components in the adaptation process. Some studies examined ECC-induced neural responses a short time after exercise when muscle damage symptoms (e.g., decrease in MVC strength) still existed (9). Less is known about long-term adaptations of the central nervous system after a bout of ECC. To the best of our knowledge, only two studies investigated long-term changes in the central nervous system after ECC training of plantar flexor muscles (e.g., 12). These studies suggested that both volitional drive from the supraspinal centers, assessed by V wave measurements, and transmission efficiency in Ia afferent synapses, estimated by H-reflex measurements, increased after training (12). The enhancement of descending tracts shown in these studies could increase the α-motoneuron excitability and/or decrease the presynaptic inhibition of Ia terminals. However, it is not known whether long-term neural modulations are induced by one ECC bout and would persist for more than 4 wk when the RBE has been shown to strongly exist.

It has been shown that spinal pathway modulations from mechanoreceptor afferents (e.g., muscle spindles, Golgi tendon organs) increase the inhibitory feedback and reduction in the neural descending drive in return (11). Some studies showed a unique activation strategy in eccentric contractions in comparison with concentric contractions such that voluntary activation was lower and/or motor unit recruitment did not follow the size principle in eccentric contractions (11). If this is the case, it is possible that the central nervous system is unable to maximize the motor unit recruitment and discharge rate during ECC, at least for subjects who are unaccustomed to ECC, and this might be associated with the RBE.

Regarding the CL-RBE, it seems reasonable to assume that neural adaptations play a main role. For example, Howatson et al. (16) showed that corticospinal excitability in the relaxed right flexor carpi radialis (FCR) increased more during ECC than CON of the left FCR, intracortical facilitation decreased during CON but increased during ECC, and interhemispheric inhibition to the nonactive motor cortex diminished during CON but became nearly abolished during ECC. They did not find any differences in the decreased amplitude of the H-reflex in the relaxed right FCR between ECC and CON of the left wrist flexors. These data suggest that ipsilateral motor cortex output and a shift to lower interhemispheric and intracortical inhibition are greater during ECC than CON. Kidgell et al. (22) also showed that eccentric training modulated corticospinal excitability and inhibition of the untrained limb to a greater extent than concentric training. It is interesting to investigate whether these neural changes peculiar to ECC occur after a single bout of ECC. We did not find the CL-RBE when the second bout was performed at 0.5, 6, or 12 h after the first bout, and 8 wk after the first bout (3). Thus, it seems that more than 12 h are required for neural adaptations to be induced, and the adaptations disappear between 4 and 8 wk. It may be that neural adaptations are not limited to the neural system, and other adaptations such as changes in muscle-tendon complex behaviors and modifications of immune responses.
shown in the following sections can perhaps be triggered by neural changes (41).

**Muscle-Tendon Complex Behaviors**

Previous *in vitro* and animal studies have suggested that some sarcomeres are overstretched during ECC, which is thought to be a key mechanism of muscle damage (36). It has been speculated that the RBE is due to an addition of sarcomeres in series to reduce mechanical strain to muscle fibers (36). Some studies suggested that an optimal angle to produce peak force shifted to a long muscle length after eccentric exercise, which was regarded as an evidence of increased sarcomere numbers in series (36). However, it does not seem that a shift of optimal angle to a longer muscle length can explain the RBE because the shift does not last for 4 wk when the RBE is still evident (7). This suggests that the optimal angle shift is not a sensitive marker of sarcomere remodeling, or sarcomere remodeling cannot fully explain the RBE.

Instead of changes at the sarcomere level, some studies have shown changes in muscle fascicle behavior between the first and second bouts of eccentric exercise (24,34). It should be noted that the fascicle length changes are considerably affected by tendon compliance and fascicle orientation represented by pennation angle (13). Guilhem et al. (13) reported a significant correlation \( r = 0.68 \) between the maximal gastrocnemius medialis fascicle length change during maximal eccentric plantar flexions and the magnitude of strength loss at 2 d after exercise. We (34) have shown that a reduced vastus lateralis fascicle elongation during the second eccentric cycling bout was associated with less DOMS. Similarly, we (24) also found that the RBE (i.e., a reduction in muscle damage markers) was associated with smaller displacement of biceps brachii myotendinous junction during the second bout of eccentric exercise when compared with the initial bout. These studies suggested a possibility that an increase in tendon compliance reduces mechanical strain transmitted to muscle fascicles; however, direct evidence to support a tendon adaptation after a bout of ECC has not been presented.

An intriguing question is whether muscle-tendon behavior change is produced by performing low-intensity ECC or maximal isometric contractions at a long muscle length. In a preliminary study, we found that the magnitude of increase in biceps brachii myotendinous junction displacement through five sets of six maximal ECC of the elbow flexors was reduced by 30 low-intensity (10% MVC) ECC or two maximal voluntary isometric contractions at 20° elbow flexion performed 2 d before the maximal ECC. It is interesting that muscle-tendon behavior is changed by the contractions that do not seem to affect the muscle-tendon complex significantly. Although it may be less likely, an important question is whether muscle-tendon behavior is affected by eccentric exercise of a contralateral limb. Further research is required to investigate how the muscle-tendon behavior changes are endowed and how it is associated with neural adaptations and/or peripheral adaptations.

**Extracellular Matrix Structural Remodeling**

Skeletal muscle ECM is a complex network of collagens, proteoglycans and glycoproteins that envelopes single muscle fibers and ensheathes whole muscle. It supports the cellular structure, is a source of muscle passive stiffness, and interfaces through focal adhesion complexes at the sarcolemma to facilitate the transfer of mechanical force from within the myofiber to the tendon. A primary function of ECM is to buffer myofibers from mechanical strain by increasing passive tension, thereby protecting the muscle from injury. Therefore, we and others have questioned whether remodeling of the ECM might also contribute to the RBE.

Several years ago, we performed a genome-wide transcriptomic analysis on muscle biopsy samples after ECC to better understand the molecular changes driving adaptation to unaccustomed ECC. The screen identified a total of 463 modified genes that were significantly changed from 1.5- to 85-fold pre-to postexercise (21). We systematically categorized the 463 differentially expressed genes into physiological networks that have been defined for skeletal muscle in previous studies. The highest rated network corresponded to ECM structure and function. Interestingly, many of the highest responding genes in the ECM group were those of the matricular protein family including tenasin C (TNC; increased 11.6-fold), cysteine-rich angiogenic inducer 61 (CYR61; increased 33.7-fold), and thrombospondin-1 (TSP-1; 8.2-fold increase). To put those data into context, of the 463 differentially expressed genes, less than 1% of the genes reached a fold change greater than fivefold. The screen clearly indicated that the ECM, particularly the matricular proteins, was a significant point of adaptation after unaccustomed ECC.

To follow up on the transcriptomic finding, we investigated the response of TNC in human muscle after repeated bouts of ECC. TNC markedly increased after the first bout of ECC. More interesting, TNC expression was attenuated after the second bout relative to the first bout of ECC (20). Mackey et al. (27) likewise reported a similar finding for TNC after two bouts of damaging electrically stimulated muscle contractions. Matricular proteins such as TNC are thought to contribute to deadhesive activity in the ECM, creating a transient intermediate adhesive state between the ECM and its cellular constituents. The deadhesive state is thought to be a necessary transition after muscle injury because it facilitates both proliferation and cytokinesis of satellite and inflammatory cells, which are essential for effective muscle regeneration (27). The collective findings suggest a transient, intermediate adhesive state that persists for at least 2 d after a muscle-damaging stimulus that is absent on a repeated bout. Given the correspondence of TNC expression with force loss after repeated bouts of ECC, we asked whether there might be a relationship between these two variables. Indeed, we showed significant positive correlation between strength loss and TNC expression 2 d postexercise. Although a causative relationship between these two variables is far from certain, the data provide compelling hypotheses for how ECM remodeling might affect strength loss after ECC and influence the RBE.

Relative to the deadhesive activity after muscle damage, analysis of collagen (type I, III, and IV) gene expression indicates that restructuring of the ECM collagen matrix occurs on a delayed time course. Data from both our laboratory and Mackey et al. (27) show that increases in collagen transcriptional activity are not evident 2 d after muscle damage, but rather are increased 4 wk after either ECC or electrical stimulation (Fig. 1). The biopsy snapshots from these studies give little
ample, we showed that transcription of the α7 integrin is markedly increased after an initial bout of ECC yet is not changed in response to a repeated bout. Increased expression of integrins may act to strengthen the ECM/myofiber interface after an initial bout of muscle damage to protect against subsequent damage. An interesting question to consider is whether other membrane-supporting (i.e., dystrophin or other dystroglycan complex proteins) or cytoskeletal proteins react in a likewise manner to support the membrane and strengthen interactions between the myofiber and ECM scaffold.

Figure 1 provides a schematic illustration of ECM remodeling after muscle damage and how it might impact the manifestation of the RBE. Indeed, ECC clearly results in widespread adaptation to both ECM structural and myofiber/ECM interfacing elements. However, compared with studies on myofibrillar adaptation, studies investigating how ECM changes impact overall muscle adaptation and the RBE are relatively few and represent a wide-open area for future investigation.

Modified Inflammatory Response

The infiltration of successive waves of immune cells into skeletal muscle is a highly orchestrated, well-characterized, and necessary occurrence for successful regeneration and adaptation after damage. The animal literature has provided the most detailed description of the immune response after muscle injury. In these more severe animal models of muscle injury, it is clear that regeneration of muscle is dependent on the transient and sequential appearance of both phagocytic, proinflammatory cells, followed by successive waves of differentially polarized macrophages. In human skeletal muscle, the inflammatory response after unaccustomed ECC also has been investigated, although not to the same extent as animals. Generally, the majority of studies using voluntary ECC as a damaging stimulus report the endomysial infiltration of myeloid-derived leukocytes, and increases in intramuscular proinflammatory cytokine expression in the hours and days after exercise. In the more severe damaging protocols, leukocytes, including macrophages and neutrophils, can be found invading individual, presumably damaged, myofibers. Recently, evidence has emerged that some of the same immune-mediated mechanisms that drive rodent muscle regeneration are present in humans — namely alterations in macrophage polarity and their association with muscle progenitor cells (37).

Whereas the inflammatory response to a single bout of muscle damage has been investigated more extensively, how the inflammatory response responds to a repeated bout of exercise and the extent to which it is modified after a repeated bout has been less studied. Studies in mice have shown a blunted inflammatory response when a bout of ECC is preceded by a bout of passive stretches or a prior bout of ECC (35), indicating that the RBE might involve a blunted inflammatory response after the second bout. The blunted inflammatory response was thought to result in a reduced secondary damage response and, therefore, attenuation of muscle damage markers. We recently hypothesized that a repeated bout of ECC in humans would likewise be associated with a diminished inflammatory response, concurrent with reductions in soreness and force losses. Unexpectedly, we found quite the opposite (10). Whereas an initial bout of ECC resulted in little change to intramuscular markers of inflammation, a second bout, completed 4 wk later, resulted in marked increases in multiple proinflammatory cytokines and significant macrophage and T-cell infiltration. Our observation that CD8+ T-cells appear in the muscle after the second bout of ECC was particularly interesting because this was the first evidence of lymphoid-derived cell infiltration in human muscle after ECC. Moreover, T-cells often were seen infiltrating damaged myofibers, suggesting that they might contribute to the regenerative response of the muscle and the manifestation of the RBE. However, at this point, the role of T-cells in the regeneration and/or adaptation of human muscle after ECC is unclear and awaits future inquiry.

Given these observations, we suspect that an enhanced and more targeted acute inflammatory response may actually help speed recovery of muscle after a repeated bout of exercise. This might be possible via a memory mechanism, through which an initial bout of ECC primes the muscle to more effectively recruit inflammatory cells. To explore this idea, we determined whether the increase in T-cell infiltration observed after a repeated bout of ECC might have been related to increased muscle expression of major histocompatibility complex-1 (MHC-1). MHC-1 proteins are found on the cell surface and display proteasome-derived peptides for CD8+ T-cell-mediated surveillance. MHC-1 is not highly expressed in healthy skeletal muscle, but can be significantly upregulated in muscle of inflammatory myopathy patients. Immunohistochemical analysis of the biopsy samples revealed no significant differences in MHC-1 staining (percentage of positive area) at any time point pre- or post- either bout of exercise (10). Nevertheless, many subjects displayed an altered staining pattern, with MHC-1 appearing at the sarcolemma and in the cytoplasm 27 d after the initial bout of ECC (the time point right before the second bout of exercise). Although it is still unclear whether inflammatory alteration is simply a product of reduced muscle damage at the second bout, or if it might be driving the RBE, the data collected thus far present several intriguing questions for future research.

A modified inflammatory response also might play a role in the CL-RBE as well. In what is the only CL-RBE study to date to directly assess changes within the muscle, we recently analyzed biopsy samples from both the ipsilateral and contralateral knee extensor muscles before and after two bouts of ECC that were separated by 4 wk (41). Indirect measures of muscle damage (force loss) confirmed the presence of a CL-RBE. More intriguingly, analysis of the muscle tissue showed that activation of the inflammatory-related transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) increased after the first bout of ECC in the ipsilateral leg and was attenuated at the second bout when the contralateral leg was exercised. These data suggest that inflammatory signaling measured after ECC is attenuated on the second bout when the contralateral muscle is exposed to ECC. Because the contralateral leg was not exposed to the initial bout of ECC, there was no direct mechanical stimulus for molecular or cellular adaptation. Thus, a plausible explanation is that NF-κB is an effector of an upstream mechanistic pathway that is transferred to the nonexercised leg, possibly through neural adaptation.
Alternatively, the data leave open the possibility that a myokine secreted from the exercised muscle promotes cellular adaptation in the homologous contralateral muscle. However, if a circulating factor was involved, one would expect that all muscles, not just the homologous contralateral muscle would be conferred the protective effect. Although no RBE studies have addressed whether muscles other than the homologous muscle are protected, the cross-education literature does present some evidence that muscles other than the homologous muscle may be affected (38). However, a strong conclusion is precluded by a paucity of studies addressing this specific question.

Clearly, there remains a host of unanswered questions related to how an altered inflammatory environment might affect the manifestation of the RBE and CL-RBE. First off, whether the enhanced inflammatory response after a repeated bout of ECC actually speeds recovery and contributes to the protective adaptation needs to be established. Other interesting directions would be whether the RBE is associated with the infiltration of immune cells of a specific polarity (anti- vs proinflammatory) or T-cell lineage (cytotoxic, helper, or regulatory) and to more fully characterize the role of lymphoid-derived cells in the adaptive response. The existence of possible circulating factor(s) that induces an RBE in remote muscles is intriguing and has clear exercise mimetic implications. The continued study of this area using muscle damage models in humans is important because it might have direct application to inflammatory-related muscle pathologies.

**IMPLICATIONS OF A BETTER UNDERSTANDING OF THE RBE**

It is our opinion that the continued study of exercise-induced muscle damage and the RBE will reveal important fundamental insights into the regenerative and adaptive program of human muscle that may be applied to both pathological (i.e., diseases such as the inflammatory myopathies and muscular dystrophy) and nonpathological (i.e., muscle building and fitness) situations. For example, adaptive responses to repetitive exercise stimuli are consolidated. However, as seen in the RBE, only one bout of exercise can trigger a significant and meaningful adaptation. Furthermore, adaptations induced by resistance training are not limited to the skeletal muscles used in the training, but affect whole body systems. Without the orchestration of the nervous, skeletal, muscular, cardiovascular, respiratory, endocrine, lymphatic, and digestive systems, exercise is not possible. Instead of monitoring responses of these systems to training, which can take weeks, the RBE can be used as a model to investigate how the systems are coordinated to confer the effect. Thus, the RBE can shed light on the mechanisms of training adaptations, with the clear advantage that they can be studied in a much shorter time frame than performing a training study.

In like manner, we see a wide range of applications for the further study of the protective adaptation conferred by the RBE. For instance, patients with Duchenne muscular dystrophy are exceedingly sensitive to the muscle-damaging effects of eccentric exercise and display greater evidence of failed regeneration and fatty infiltration in muscles that perform ECC (18). In immobilization, muscle is atrophied, muscle function decreases, and recovery can take a long time. As previously discussed, training of the noninjured limb can attenuate the impairments. A better understanding of how muscles in the contralateral limb protect themselves from exercise-induced damage (CL-RBE) has implications in this case.

Study of the RBE also may facilitate a better understanding of pathological phenomena in systems other than muscle. In
extreme cases, exercise-induced muscle damage can lead to a potentially life-threatening condition called exertional rhabdomyolysis. In a subset of patients who experience exertional rhabdomyolysis, the accumulation of renal myoglobin can lead to kidney toxicity, acute kidney injury, and possible renal failure. The amount of myoglobin in the systemic circulation at any given time is dependent on the extent of muscle damage and the ability of the kidneys to successfully clear the myoglobin from the blood. CK is used clinically as a surrogate measure for myoglobin, and CK concentrations of 5- to 10-fold greater than normal (50–300) are generally considered to be clinically relevant. A rather interesting observation made by Hoffman et al. (14) was that finishers of the 2010 Western States Endurance Run experienced a remarkably high level of serum CK activity (mean of 32,956 IU/L and range of 1500–264,300 IU/L) immediately after the run. However, only 2 of the 328 finishers required hospitalization for extensive renal treatment. Moreover, in the subjects who gave follow-up blood samples, CK drastically decreased during the course of 51 h after the ultra-marathon. This is in contrast to what we typically observe in unaccustomed exercisers, where CK activity generally peaks 2–5 d postexercise and returns to normal by 1 wk. These data would suggest that ultraendurance athletes have adapted, through training, to more successfully clear myoglobin from the circulation without incurring renal damage. We note the same effect, albeit to a lesser extent, in an experimentally induced RBE and suspect that renal adaptation may account for the more effective clearance of myoglobin with repeated exposures. Thus, the RBE represents an excellent model to study the mechanisms of renal adaptation in the context of rhabdomyolysis and kidney injury.

CONCLUSIONS

More than 10 yrs ago, McHugh (28) concluded that the neural, mechanical, and cellular adaptations involved in the RBE might complement each other or operate independently of each other, and that a unified theory to explain the RBE remains elusive. A decade on, and a significant number of studies later, many observations have been made to help further clarify the conclusions of McHugh. As previously discussed, potential mechanisms include neural adaptations, alterations to muscle mechanical properties, structural remodeling of the ECM, and biochemical signaling. It seems most likely that these work in concert to coordinate the RBE (Fig. 4). However, they also may work independently. For example, it seems most likely that the early (1–2 d) protective adaptations are driven by neural mechanisms, whereas later adaptations can be attributed more to ECM remodeling. An intriguing question to consider is if there is a trigger to initiate the adaptation process, what is it? If we can perhaps find a condition that does not induce the RBE, this may lead to a better idea of how the RBE is induced. For example, it is very important to note that more than 12 h was necessary for the CL-RBE to be induced (3), and no protective effect was induced when maximal voluntary isometric contractions were performed immediately before maximal ECC (2). It seems that something is happening in the first 24 h after the initial exercise bout. In conclusion, more research is required to further understand the underpinning mechanisms of the RBE. It is our assertion that the continued study of the RBE will have broad application to the understanding of muscle adaptation in both physiological and pathological contexts.

References


