

Mechanisms of disuse muscle atrophy: role of oxidative stress

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Powers, Scott K., Andreas N. Kavazis, and Keith C. DeRuisseau. Mechanisms of disuse muscle atrophy: role of oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 288: R337–R344, 2005; doi:10.1152/ajpregu.00469.2004.—Prolonged periods of skeletal muscle inactivity lead to a loss of muscle protein and strength. Advances in cell biology have progressed our understanding of those factors that contribute to muscle atrophy. To this end, abundant evidence implicates oxidative stress as a potential regulator of proteolytic pathways leading to muscle atrophy during periods of prolonged disuse. This review will address the role of reactive oxygen species and oxidative stress as potential contributors to the process of disuse-mediated muscle atrophy. The first section of this article will discuss our current understanding of muscle proteases, sources of reactive oxygen in muscle fibers, and the evidence linking oxidative stress to disuse muscle atrophy. The second section of this review will highlight gaps in our knowledge relative to the specific role of oxidative stress in the regulation of disuse muscle atrophy. By discussing unresolved issues and suggesting topics for future research, it is hoped that this review will serve as a stimulus for the expansion of knowledge in this exciting field.

redox; proteasome; calpain; caspase-3; reactive oxygen species

PROLONGED PERIODS of muscle disuse due to immobilization, chronic bed rest, physical inactivity, or spaceflight can result in a significant loss of muscle mass and strength (6). Understanding the signaling pathways that contribute to disuse muscle atrophy is important in developing countermeasures to prevent this form of skeletal muscle wasting and preserve physiological function (7). Recent advances in cellular and molecular biology have led to an improved understanding of those factors that contribute to muscle atrophy during both disuse and wasting pathologies. In this regard, growing evidence implicates oxidative stress as an important regulator of pathways leading to muscle atrophy during periods of disuse. For example, redox disturbances (i.e. oxidant stress) in skeletal muscle myotubes increase the expression of key components of the proteasome proteolytic system (42). This important proteolytic system is a prominent contributor to protein breakdown in skeletal muscle during periods of inactivity. Moreover, additional signaling pathways exist between muscle redox imbalance and loss of muscle protein and nuclei (32).

This review will provide a brief synopsis of our current understanding of the role that oxidant stress plays in disuse muscle atrophy. Our approach will be to first present an overview of well-established concepts with reference to important proteolytic pathways that lead to disuse muscle atrophy. We will then discuss potential sources of oxidants in inactive skeletal muscle followed by an overview of the evidence linking oxidative stress to disuse muscle atrophy. A subsequent section will consider unanswered issues related to redox control of muscle proteolysis and loss of myonuclei. By highlighting gaps in our knowledge about oxidant stress and

disuse muscle atrophy, we hope that this review will stimulate future research aimed at improving our understanding in this field.

MODELS OF DISUSE MUSCLE ATROPHY: AN OVERVIEW

Muscle atrophy is present in numerous pathologies such as cancer, sepsis, uremia, and diabetes (19, 26). Moreover, muscle atrophy can also occur in the absence of disease during prolonged periods of reduced muscle activity (6). Indeed, it is well established that prolonged bed rest, limb immobilization, unloading the diaphragm via mechanical ventilation, or spaceflight can produce muscle atrophy in humans. Because it is difficult, if not impossible, to investigate the mechanisms responsible for disuse muscle atrophy in humans, animal models have been developed to mimic the various conditions that produce human disuse muscle atrophy. For example, animal models using hindlimb suspension to unload the hindlimb locomotor muscles have been developed to mimic prolonged bed rest and spaceflight in humans (Table 1). Moreover, animal models of limb immobilization are commonly used in research. Using the rat hindlimb suspension and limb immobilization models, it has been demonstrated that disuse muscle atrophy occurs due to both a decrease in muscle protein synthesis and an increase in the rate of proteolysis (8, 54). In the hindlimb suspension model, the rate of protein synthesis declines rapidly after the onset of muscle unloading (54). This decline in muscle protein synthesis reaches a new steady-state level at ~48 h (54). Additionally, the decrease in protein synthesis is ensued by a large and rapid increase in proteolysis. With limb immobilization, fixation of the limb in a position less than resting length results in rapid atrophy of slow-twitch muscle fibers (6). Collectively, reduced activity of skeletal muscle negatively impacts muscle mass through alterations of the rates of protein synthesis and degradation that ultimately lead to muscle atrophy.

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Table 1. *Nonpathological conditions that result in human skeletal muscle atrophy and the corresponding animal model*

Human Condition Resulting in Muscle Atrophy	Animal Model
Limb immobilization	Limb immobilization
Spaceflight	Hindlimb suspension
Bed rest	Hindlimb suspension; limb immobilization
Diaphragm unloading via mechanical ventilation	Mechanical ventilation

Another interesting animal model used to investigate disuse muscle atrophy is controlled mechanical ventilation (MV) that unloads the diaphragm. Controlled MV is used in human medicine to maintain alveolar ventilation in patients incapable of maintaining adequate ventilation on their own. In adult patients, controlled MV is primarily used in numerous clinical situations [e.g., drug overdose, spinal cord injury, surgery (22)]. During controlled MV, all breaths are delivered by the ventilator and the diaphragm is completely inactive (45). Numerous animal studies have reported that prolonged MV results in a rapid onset of diaphragmatic fiber atrophy (13, 40, 51). Moreover, recent investigations reveal that MV-induced atrophy occurs as a result of both elevated proteolysis and decreased protein synthesis (50, 51). MV-induced diaphragm atrophy is clinically significant because ventilator-induced diaphragmatic weakness contributes to difficult weaning from MV. Indeed, the most frequent cause of difficult weaning is respiratory muscle failure due to inspiratory muscle weakness and/or a decline in inspiratory muscle endurance (57).

PROTEOLYTIC PATHWAYS IN SKELETAL MUSCLE

Several proteolytic systems contribute to the degradation of muscle proteins. The most investigated proteases in skeletal muscle are lysosomal proteases, Ca²⁺-activated proteases (i.e. calpain), and the proteasome system. Although lysosomal proteases are activated in skeletal muscle undergoing disuse

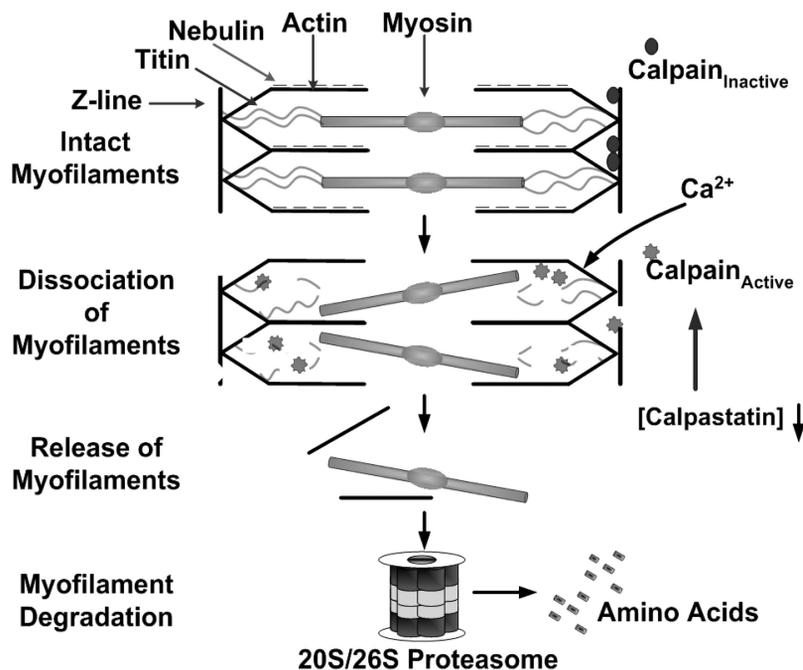
atrophy, the importance of these proteases appears limited (12, 20, 47). In contrast, strong evidence indicates that both calpain and the proteasome system play important roles in muscle protein breakdown during muscle atrophy (12, 24, 47). Moreover, new evidence reveals that another protease, caspase-3, may also contribute to select forms of muscle atrophy (11).

The bulk of muscle proteins (50–70%) exist in actomyosin complexes (55). While the proteasome system can degrade monomeric contractile proteins (i.e. actin and myosin), this protease does not degrade intact actomyosin complexes (14). Hence, myofilaments must be released from the sarcomere as monomeric proteins before degradation by the proteasome system (55, 59) (Fig. 1). This observation suggests that the release of myofilaments is the rate-limiting step in muscle protein degradation. Evidence indicates that both calpain and caspase-3 are capable of producing actomyosin disassociation (11, 14, 55). Therefore, activation of one or both of these proteases is required to achieve proteolytic degradation of myofilaments during muscle disuse.

Calpain-mediated proteolysis. Calpains (calpain I and II) are Ca²⁺-dependent cysteine proteases that are activated in skeletal muscle during periods of inactivity (14). Although calpains do not directly degrade the contractile proteins actin and myosin, calpain releases sarcomeric proteins by cleaving cytoskeletal proteins (e.g., titin, nebulin) that anchor the contractile elements (31, 47) (Fig. 1).

Calpain activity is regulated by several factors, including cytosolic calcium levels and the concentration of the endogenous calpain inhibitor calpastatin (14). Hence, calpain activity is increased by any factor that elevates cytosolic calcium concentrations and/or decreases calpastatin levels (14). In this regard, it is known that muscle inactivity is associated with calcium overload and calpain activation (38). Although the mechanism responsible for this inactivity-mediated calcium overload is unknown, it has been argued that oxidative stress could play an important role in ionic disturbances in cells (37).

Fig. 1. Illustration of the calpain-mediated release of myofilaments during mechanical ventilation (MV)-induced diaphragmatic atrophy. Because the proteasome cannot degrade intact myofibrillar proteins, it is possible that calpain activation is a required first step to disassemble the sarcomere. This calpain-mediated release of myofibrils would permit the degradation of these sarcomeric proteins by the proteasome system.



A biological explanation for this thesis is that oxidant-mediated formation of reactive aldehydes (i.e., 4-hydroxy-2,3-trans-non-enal) reduces plasma membrane Ca^{2+} ATPase activity (52). It follows that an oxidative stress-induced decrease in membrane Ca^{2+} ATPase activity would retard Ca^{2+} removal from the cell and promote intracellular Ca^{2+} accumulation. Nonetheless, whether, this mechanism is solely responsible for inactivity-mediated calcium overload in muscle remains unknown.

Caspase-3 and muscle atrophy. Numerous signaling pathways can trigger the activation of a unique group of proteases termed "caspases" (46). Collectively, caspases are endoproteases that degrade proteins and, in some cases, cause programmed cell death (apoptosis). In the cell, caspases are expressed as inactive precursors (i.e. procaspases), and activation of caspases can result in events leading to protein breakdown and apoptosis.

New evidence suggests that caspase-3 may play an important role in muscle protein degradation during diabetes-induced muscle atrophy (11). Specifically, caspase-3 activation promotes degradation of actomyosin complexes, and, inhibition of caspase-3 activity suppresses the overall rate of proteolysis in diabetes-mediated cachexia (11).

Control of caspase-3 activity is complex and involves several interconnected signaling pathways. In the case of diabetes-induced muscle atrophy, it seems possible that caspase-3 is activated by activation of caspase-12 (via a calcium release pathway) and/or activation of caspase-9 (via a mitochondrial pathway). A key interaction between these caspase-3 activation pathways is that both of these corridors can be activated by reactive oxygen species (ROS) (46) (Fig. 2). The calcium release pathway activates caspase-3 activity via a signaling path that culminates in a caspase-12-derived activation of caspase-3 (46). Notice that calpain activation can also contribute to caspase-3 activation via this calcium-mediated pathway (9) (Fig. 2). The mitochondrial pathway of caspase-3 activation

is complex and can be initiated by numerous interacting signals including ROS and a high pro- to anti-apoptotic protein ratio in the mitochondria (Fig. 2) (41). ROS can lead to mitochondrial release of cytochrome *c*, resulting in the activation of caspase-9 and the subsequent activation of caspase-3 (41).

Finally, it is noteworthy that calpastatin is a substrate for both caspase-3 and calpain. Therefore, increases in caspase-3 or calpain activity lower calpastatin levels in cells and promote calpain activation (11, 14, 58). Furthermore, increased calpain activity can lead to the activation of caspase-3 (9). Hence, cross-talk between the calpain and caspase-3 proteolytic systems could play an important role in the regulation of myofilament release in skeletal muscle during periods of disuse.

Proteasome-mediated proteolysis. In the proteasome system of proteolysis, proteins can be degraded by either the 20S core proteasome or the 26S proteasome (16, 17, 20). The 26S proteasome is composed of the 20S core proteasome with a regulatory 19S complex (also called PA700) connected to each end (10). The 19S regulatory complex possesses ATPase activity and plays an important role in ATP-dependent degradation of ubiquitinated proteins (10). In the 26S proteasome pathway, ubiquitin covalently binds to protein substrates and marks them for degradation. The ubiquitinated protein is recognized and bound by the 19S regulators of the 26S proteasome. Energy from ATP hydrolysis removes the polyubiquitin chain and unfolds the substrate protein; this unfolded protein is then fed into the 20S core proteasome where it is degraded in a process that does not require ATP (17). Furthermore, new evidence reveals that the 20S core proteasome can selectively degrade oxidatively modified proteins without ubiquitination (16, 17). Thus it seems possible that oxidant stress can accelerate muscle protein breakdown via 20S core proteasome alone.

The binding of ubiquitin to protein substrates requires the ubiquitin-activating enzyme (E1), specific ubiquitin-conjugat-

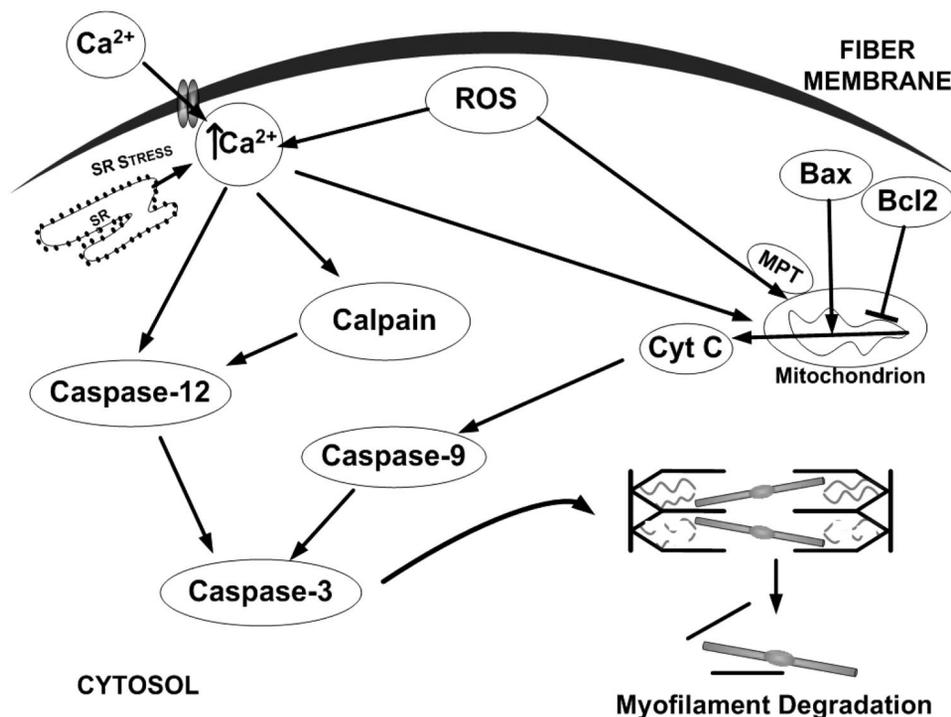


Fig. 2. Simplified overview of signaling pathways leading to activation of caspase-3. Caspase-3 can be activated by oxidative stress, increased cellular calcium, and increased calpain activity. Also, note the multiple lines of cross talk between pathways. ROS, reactive oxygen species; MPT, mitochondrial permeability transition; Cyt C, cytochrome *c*; SR, sarcoplasmic reticulum.

ing enzymes (E2), and in many cases specific ubiquitin protein ligase enzymes (E3). The ubiquitination of specific proteins is provided by one of a variety of E2s and by specific E3s. For example, studies reveal that the specific ubiquitin-conjugating enzyme E2_{14k} is a critical regulator of skeletal muscle ubiquitin-protein conjugation (42). Furthermore, E2_{14k} interacts with a specific E3 ligase (E3 α) to promote muscle wasting in a variety of catabolic states. Additionally, two unique ubiquitin E3 ligases, atrogin1 (also called muscle atrophy F-box) and muscle ring finger-1, have been discovered in skeletal muscle (5, 15). Growing evidence indicates that these ligases play important roles in skeletal muscle atrophy (5, 15). Importantly, ROS has been shown to upregulate gene expression of these key proteasome components (42).

OXIDANT PRODUCTION IN INACTIVE SKELETAL MUSCLES

It is well established that radicals and other ROS are produced in both inactive and contracting skeletal muscles (32, 48). When oxidant production in skeletal muscle exceeds the antioxidant capacity to buffer oxidants, oxidative stress occurs. Oxidation can alter the structure and function of lipids, proteins, and nucleic acids, leading to cellular injury and even cell death.

Historically, it was believed that ROS production is low in noncontracting skeletal muscle and oxidative injury is not present. However, numerous studies have demonstrated that oxidative injury occurs during periods of disuse in locomotor skeletal muscles (33–37, 39) and in the unloaded diaphragm during prolonged MV (51, 60). At present, it is unknown which ROS-producing pathways are responsible for this observed oxidative injury within inactive skeletal muscles. Nonetheless, it seems plausible that oxidative stress in inactive skeletal muscle may be due to the interaction of at least five different oxidant production pathways (32): 1) generation of ROS by the

xanthine oxidase pathway; 2) production of NO via nitric oxide synthase (NOS); 3) formation of ROS (hydroxyl radicals) by increased cellular levels of reactive iron; 4) NADPH oxidase; and 5) mitochondrial production of superoxide radicals (Fig. 3). A brief synopsis of each of these pathways follows.

Xanthine oxidase. Xanthine oxidase (XO) is produced in cells via sulfhydryl oxidation or proteolysis of xanthine dehydrogenase by calcium-activated proteases (i.e., calpain) (21). In the presence of oxygen and purine substrates (i.e., hypoxanthine, xanthine), XO catalyzes the formation of superoxide radicals and uric acid. When compared to the highly reactive hydroxyl radical, superoxide radicals are somewhat innocuous in chemical terms. Nonetheless, superoxide radicals can lead to the formation of other more damaging reactive species. For example, superoxide production by the XO pathway can react with nitric oxide (NO \cdot) to form the highly reactive and biologically damaging peroxynitrite (ONOO $^-$) (18).

Nitric oxide. Endogenous production of nitric oxide (NO \cdot) via nitric oxide synthases (NOS) can result in the formation of several reactive nitrogen species (RNS), including ONOO $^-$. Production of ONOO $^-$ and other RNS are associated with cellular injury due to increased lipid peroxidation and nitrosylation of proteins (28). Three isoforms of NOS exist (53): 1) inducible NOS (iNOS), which is calcium independent; 2) endothelial NOS (eNOS), which is calcium activated; and 3) neuronal NOS (nNOS), which is also calcium activated. Both nNOS and eNOS are expressed in skeletal muscle (29). Furthermore, in addition to calcium activation, NOS activity is also influenced by phosphorylation and heat shock protein 90 (1). Evidence indicates that NOS activity is increased in immobilized skeletal muscle, resulting in increased production of NO \cdot (32).

Reactive iron. Transition metals such as iron and copper can participate in chemical reactions that produce ROS (18). For

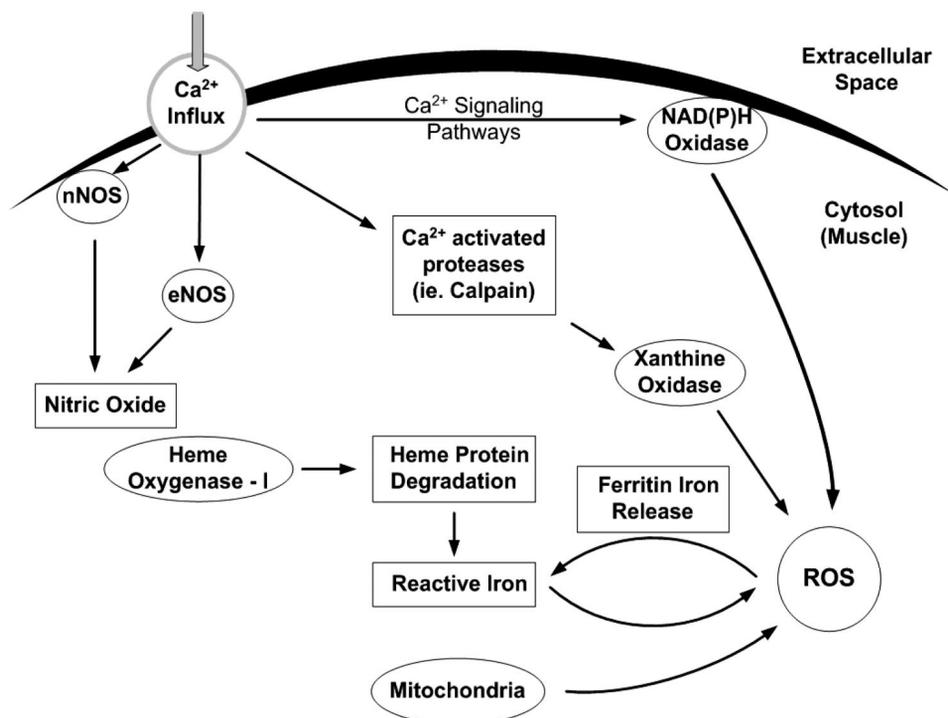
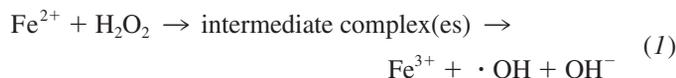
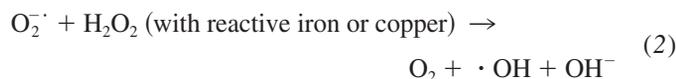


Fig. 3. Diagram illustrating pathways capable of producing reactive oxygen species (ROS) and nitric oxide in skeletal muscle during periods of disuse. See text for details. nNOS, neuronal nitric oxide synthase; eNOS, endothelial nitric oxide synthase.

example, in the presence of Fe^{2+} , H_2O_2 is converted to the highly reactive hydroxyl radical ($\cdot\text{OH}$) via the Fenton reaction (*reaction 1*)



Further, metal catalysts can also contribute to the formation of hydroxyl radicals via the Haber-Weiss reaction (*reaction 2*)



In healthy cells, iron is tightly bound to the iron-binding protein ferritin (18). Iron bound to ferritin does not normally participate in either Fenton or Haber-Weiss reactions. However, a release of iron from ferritin or heme-proteins can result in the formation of low-molecular-weight iron compounds (i.e. reactive iron) that are capable of participating in the aforementioned radical-producing reactions. In this regard, both H_2O_2 and superoxide radicals have been shown to promote the release of iron from ferritin, and heme-oxygenase-1 is capable of releasing iron bound to heme-proteins (18). In either case, an increase in cellular levels of reactive iron is a potential contributor to oxidant-mediated cellular injury. In reference to iron-mediated oxidant stress in skeletal muscle, immobilization of the rat soleus muscle has been shown to promote increases in total muscle iron levels (34, 35). This increase in muscle iron was associated with elevated lipid peroxidation in the immobilized muscle, and systemic delivery of an iron chelator decreased the oxidant stress associated with muscle immobilization (34).

NAD(P)H oxidase. New evidence indicates that a nonphagocytic and nonmitochondrial NAD(P)H oxidase is found in both human and rodent skeletal muscle (27). NAD(P)H oxidases are membrane-associated enzymes that catalyze the one-electron reduction of molecular oxygen using either NADH or NADPH as electron donors. Numerous factors can increase NAD(P)H oxidase activity in cells, including the calcium-sensitive protein kinase C-ERK1/2 pathway (27). Because skeletal muscle inactivity results in an increase in intracellular calcium concentration, it seems plausible that NAD(P)H oxidase activity would increase, resulting in elevated superoxide production. Nonetheless, at present it is unclear if skeletal muscle inactivity results in an increase in NAD(P)H oxidase activity.

Mitochondrial production of ROS. It is well established that the transport of electrons along the electron transport chain results in the formation of superoxide radicals. In fact, it has been estimated that at physiological levels of oxygen, 1–3% of the total oxygen reduced in the mitochondria may form superoxide radicals (18). Therefore, in skeletal muscle, it is likely that mitochondrial-mediated superoxide production is greatest during heavy muscular exercise when ATP requirement is high and is lowest during periods of muscle inactivity when the ATP requirement is minimal (25). Thus, during periods of low or non-existent muscular activity (e.g., immobilization, mechanical ventilation, etc.), mitochondrial production of ROS is at a low level. Hence, it appears that mitochondrial contributions to disuse-mediated oxidative injury in skeletal muscle would be minimal.

SIGNALING LINKS BETWEEN OXIDATIVE STRESS AND PROTEOLYSIS

Several lines of evidence suggest that oxidative stress in inactive skeletal muscle contributes to disuse muscle atrophy. The first evidence that oxidants contributed to disuse muscle atrophy was provided by Kondo et al. (32). This work revealed that immobilization of skeletal muscles was associated with oxidative injury in the muscle. Further, these investigators reported that disuse muscle atrophy could be retarded by the delivery of exogenous antioxidants. Specifically, these investigators treated rats with the lipid-soluble antioxidant vitamin E and reduced immobilization-induced muscle atrophy by ~20%. The ability of vitamin E to diminish disuse muscle atrophy has been confirmed by Appell et al. (4). Furthermore, prevention of oxidant stress through the administration of the antioxidant cysteine effectively suppressed protein ubiquitination and myosin heavy chain fragmentation in the gastrocnemius muscle after hindlimb suspension in rats. Importantly, these experiments demonstrated that maintenance of the muscle redox status attenuated disuse muscle atrophy (23). Moreover, recent work from our laboratory has shown that prevention of oxidative stress in the diaphragm during mechanical ventilation results in a reduced rate of muscle proteolysis (56). Collectively, these experiments suggest that oxidative stress contributes to disuse muscle atrophy via regulation of proteolysis. Nonetheless, it should be noted that not all antioxidant interventions are capable of retarding disuse muscle atrophy (30).

How does prevention of disuse-related oxidative stress in skeletal muscle diminish the rate of muscle proteolysis and atrophy? Several possibilities exist. First, it is possible that disuse-induced oxidative stress leads to calcium overload and activation of calcium-activated proteases (e.g., calpain) in skeletal muscles. This postulate is supported by evidence that oxidative stress can promote calcium overload in cells (38). A potential mechanism to explain oxidant-mediated calcium overload in cells is as follows. Oxidant-generated formation of reactive aldehydes (i.e. 4-hydroxy-2,3-trans-nonenal) has been shown to reduce plasma membrane Ca^{2+} ATPase activity (52). Hence, oxidative stress-induced decrease in membrane Ca^{2+} ATPase activity would retard Ca^{2+} removal from the cell and therefore contribute to cellular Ca^{2+} accumulation. It follows that increased intracellular Ca^{2+} levels would activate calpain and other calcium-activated proteases resulting in augmented proteolysis of diaphragmatic cytoskeletal proteins and the release of myofilaments for subsequent degradation by the proteasome system (14, 55).

A second potential link between oxidative stress and skeletal muscle atrophy is the control of caspase-3 activity. Regulation of caspase-3 activity is complex and involves several interconnected signaling pathways. In the case of disuse-induced muscle atrophy, it is plausible that caspase-3 is activated by one of the following activation pathways: 1) activation of caspase-12 (via a calcium release pathway) or 2) activation of caspase-9 (via a mitochondrial pathway). A key interaction between these caspase-3 activation pathways is that both of these corridors can be triggered by ROS (46) (Fig. 2).

The calcium release pathway can promote caspase-3 activity via a signaling path that culminates in a caspase-12-derived activation of caspase-3 (46). Note that this pathway can be

accelerated by increased calpain activity and other signaling molecules (46) (Fig. 2).

The mitochondrial pathway of caspase-3 activation is complex and can be initiated by numerous interacting signals, including ROS and a high pro- to anti-apoptotic protein ratio in the mitochondria (Fig. 2). ROS can lead to mitochondrial release of cytochrome *c*, resulting in the activation of caspase-9 and the subsequent activation of caspase-3. Further, numerous pro-apoptotic (e.g., Bax) and anti-apoptotic (e.g., Bcl-2) proteins exist in the cell. A high Bcl-2-to-Bax ratio in the cell promotes mitochondria integrity, whereas a high Bax-to-Bcl-2 ratio favors mitochondrial release of cytochrome *c*, leading to the activation of caspase-9 and subsequently activation of caspase-3 (46) (Fig 2).

A third potential link between oxidative stress and muscle disuse atrophy involves the redox regulation of the proteasome proteolytic system. For example, we have demonstrated that oxidative stress accelerates muscle protein degradation in the unloaded diaphragm via the proteasome system (56). This observation may have several origins. For instance, oxidative stress has been shown to upregulate the expression of E2_{14k}, muscle atrophy F-box/atrogin1, and muscle ring finger-1 in myotubes (42). Theoretically, increased expression of E3 ubiquitin ligases (i.e., atrogin1, and muscle ring finger-1) in skeletal muscle would lead to accelerated proteasome proteolysis and muscle atrophy (5). Indeed, Li et al. (42) have postulated that oxidant stress accelerates muscle protein breakdown by augmenting the 26S proteasome system of proteolysis. Furthermore, new evidence also indicates that the 20S core proteasome can degrade oxidatively modified proteins without ubiquitination (16, 17). Therefore, it seems plausible that oxidant stress can accelerate muscle protein breakdown via both the 26S and 20S core proteasome.

OXIDATIVE STRESS AND MUSCLE ATROPHY: UNANSWERED ISSUES

A fundamental unsettled question is “Which ROS pathways are responsible for oxidant production in unloaded skeletal muscle?” Additionally, if more than one oxidant production pathway is involved, what is the relative contribution of each pathway to the overall level of oxidant stress? Unfortunately, our current understanding of the factors that regulate ROS production in skeletal muscles during various states (i.e. during contractions or periods of inactivity) is limited. Heightened awareness of these issues will provide the necessary knowledge required to develop therapeutic strategies to prevent oxidant production or scavenge ROS to prevent oxidative injury in the cell during prolonged periods of inactivity.

Although evidence exists that specific antioxidants can retard disuse muscle atrophy, it is unclear if oxidant production is an absolute requirement for muscle atrophy or simply contributes to the rate of muscle atrophy. In a connected question, do ROS alone act as second messengers to regulate muscle atrophy or is ROS-mediated oxidative injury a requirement for oxidant regulation of muscle atrophy? A related and more specific question is “Which protease systems are controlled by ROS?” Furthermore, does the redox control of protease activity occur by virtue of allosteric regulation (e.g., control of cytosolic calcium levels) and/or via increased gene expression of

proteases? Clearly, each of these issues is an important topic for future research.

Another fundamental but unanswered question is “Does oxidative stress negatively impact protein synthesis in unloaded skeletal muscle?” It is well known that disuse muscle atrophy occurs due to both an increase in proteolysis and a decrease in protein synthesis. Furthermore, growing evidence indicates that oxidative stress can profoundly inhibit protein synthesis in a variety of cell types (2, 43, 44). Nonetheless, to date, all research related to ROS and muscle atrophy has focused on control of proteolysis. Given that numerous redox-sensitive transcriptional activating factors exist, it is plausible that ROS play an important role in the control of protein synthesis (2, 43, 44).

An ongoing constraint in redox biology research is the problem of quantifying different ROS in living tissues. Indeed, this technical limitation has hindered advancements in this field during the past decades. The development of sensitive and reliable techniques to quantify the production of reactive species in cells would permit rapid advancement in many areas of oxidative stress research.

Finally, recent evidence indicates that disuse muscle atrophy is associated with a loss of myonuclei from muscle fibers (3). It has been postulated that this disuse-related loss of myonuclei occurs from a special form of apoptosis termed “nuclear apoptosis” (3, 49). This form of apoptosis does not result in fiber death but appears to be a biological mechanism to eliminate nuclei from fibers during periods of atrophy in order to maintain a constant cytosol-to-nuclei ratio (i.e., constant myonuclear domain). At present, the signaling pathways responsible for nuclear apoptosis are unknown. Nonetheless, it is well known that ROS can contribute to signaling pathways that lead to apoptosis (41, 46). Therefore, it is conceivable that oxidative stress in muscle fibers is a “trigger” for the loss of myonuclei during disuse-induced muscle atrophy. However, such a pathway remains theoretical as experiments to support this postulate remain unpublished.

CONCLUSIONS

Disuse muscle atrophy is an important clinical problem. Several lines of evidence link ROS to disuse muscle atrophy via redox control of proteolysis. Importantly, a growing number of studies suggest that antioxidants can serve as therapeutic agents in delaying the rate of disuse muscle atrophy. Nonetheless, numerous unanswered questions remain. Hopefully, questions outlined in this review stimulate muscle biologists to pursue research in the area of ROS and skeletal muscle atrophy. Technical advances in cell and molecular biology will provide powerful tools to address these important questions that may ultimately lead to therapeutic countermeasures to retard disuse muscle atrophy. Clearly, the field of skeletal muscle atrophy is at an exciting stage.

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