HIGHLIGHTED TOPIC | Free Radical Biology in Skeletal Muscle

Oxidative stress and disuse muscle atrophy

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Powers SK, Kavazis AN, McClung JM. Oxidative stress and disuse muscle atrophy. J Appl Physiol 102: 2389–2397, 2007; doi:10.1152/japplphysiol.01202.2006.—Skeletal muscle inactivity is associated with a loss of muscle protein and reduced force-generating capacity. This disuse-induced muscle atrophy results from both increased proteolysis and decreased protein synthesis. Investigations of the cell signaling pathways that regulate disuse muscle atrophy have increased our understanding of this complex process. Emerging evidence implicates oxidative stress as a key regulator of cell signaling pathways, leading to increased proteolysis and muscle atrophy during periods of prolonged disuse. This review will discuss the role of reactive oxygen species in the regulation of inactivity-induced skeletal muscle atrophy. The specific objectives of this article are to provide an overview of muscle proteases, outline intracellular sources of reactive oxygen species, and summarize the evidence that connects oxidative stress to signaling pathways contributing to disuse muscle atrophy. Moreover, this review will also discuss the specific role that oxidative stress plays in signaling pathways responsible for muscle proteolysis and myonuclear apoptosis and highlight gaps in our knowledge of disuse muscle atrophy. By presenting 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.

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

EXTENDED PERIODS of skeletal muscle inactivity (e.g., limb immobilization, chronic bed rest, or spaceflight) result in a loss of muscle mass and strength (1, 11, 83). Knowledge of the cell signaling pathways that regulate disuse muscle atrophy is important in developing an anti-catabolic strategy to prevent or retard protein loss and maintain physiological function (12). Ongoing research in cell signaling has led to an improved understanding of those factors that contribute to muscle atrophy during both disuse and pathologies that promote muscle wasting. In particular, a large volume of research indicates that oxidative stress is an important contributor to numerous cellular signaling pathways that modulate muscle atrophy during prolonged inactivity. For example, exposure of skeletal muscle myotubes to oxidative stress (i.e., hydrogen peroxide) increases the expression of important components of the proteasome proteolytic system (58). Moreover, other signaling pathways exist to connect oxidative stress with cellular processes leading to a loss of cellular protein (44).

The objective of this review is to provide a synopsis of our present knowledge regarding the link between reactive oxygen species (ROS) and proteolytic components of disuse skeletal muscle atrophy. The first segment of this article will introduce experimental models to study muscle atrophy. We will then present an overview of the biochemical events leading to the loss of myonuclei in atrophying muscle followed by a discussion of the principal proteolytic pathways that contribute to disuse muscle atrophy. Next, we will outline the major pathways involved in skeletal muscle radical production followed by a discussion of the signaling pathways linking oxidative stress to increased proteolysis and loss of myonuclei. The final segment of this treatise will identify voids in our knowledge about oxidative stress and disuse muscle atrophy in hopes of stimulating future research in this field.

EXPERIMENTAL MODELS TO INVESTIGATE DISUSE MUSCLE ATROPHY

Skeletal muscle atrophy can occur due to pathology (e.g., cancer, sepsis, or diabetes) and in the absence of disease during extended durations of inactivity (33, 39). For example, long periods of bed rest, limb immobilization, spaceflight, or unloading the diaphragm via mechanical ventilation is associated with skeletal muscle atrophy in humans and other animals. Due to the complexities involved in investigating the mechanisms responsible for disuse muscle atrophy in humans, numerous experimental animal models have evolved to simulate unloading conditions that lead to disuse muscle atrophy (Fig. 1). For example, animal models of limb immobilization (i.e., casting) have been used to investigate the impact of muscle inactivity on muscle size and function (11). Moreover, rodent animal models using a tail suspension technique to unload the hindlimb locomotor muscles have been used to simulate prolonged spaceflight or bed rest in humans (1, 83).

An interesting and clinically relevant model of inactivity-induced skeletal muscle atrophy is controlled mechanical ventilation (i.e., ventilator delivers all of the breaths; Fig. 1). In human medicine, controlled mechanical ventilation is used to
maintain alveolar ventilation in patients incapable of sustaining adequate ventilation. Common applications for mechanical ventilation in medicine include respiratory failure, sepsis, drug overdose, spinal cord injury, and surgery (34). Numerous human and animal studies have reported that prolonged mechanical ventilation results in a rapid onset of diaphragmatic fiber atrophy (25, 54, 56, 75).

BIOCHEMICAL ALTERATIONS IN SKELETAL MUSCLE DURING PROLONGED INACTIVITY

Well-controlled studies, using both the tail suspension and limb immobilization models of muscle inactivity, indicate that disuse muscle atrophy occurs due to both an increase in proteolysis and a decrease in muscle protein synthesis (13, 82). For example, a study using the hindlimb suspension model of muscle atrophy reported that the rate of protein synthesis declines quickly after the onset of muscle unloading (82). This decrease in muscle protein synthesis reaches a new “lower” steady-state rate of protein synthesis at ~48 h (82). Furthermore, this disuse-induced decrease in muscle protein synthesis is followed by a large increase in muscle protein degradation. Hence, reduced activity of skeletal muscle results in a net loss of total muscle protein due to both a decrease in protein synthesis and an increase in proteolysis.

Similar to studies using locomotor skeletal muscle, recent studies indicate that mechanical ventilation-induced diaphragmatic atrophy is a result of both elevated proteolysis and decreased protein synthesis (19, 73, 74). However, during prolonged mechanical ventilation, the time course of disuse-induced proteolysis in the diaphragm is more rapid than the rate of protein breakdown observed in limb muscles exposed to periods of unloading. In fact, it would take at least 96 h to achieve the same level of atrophy in locomotor muscle unloaded by hindlimb suspension as observed in the diaphragm following 12 h of mechanical ventilation (61, 82). In addition, the rate of mechanical ventilation-induced atrophy also exceeds that reported in the diaphragm with denervation (26), spinal cord hemisection (91), and corticosteroid administration (87). These facts suggest that the signaling associated with myofiber atrophy during mechanical ventilation may involve different or accelerated mechanisms compared with other models of muscle atrophy.

Moreover, it is now clear that muscular dystrophy (72), denervation (14)-, aging (20, 78)-, hindlimb unloading (4, 5, 22)-, and mechanical ventilation (61)-induced disuse muscle atrophy are associated with a loss of myonuclei, and growing evidence indicates that the loss is due to a form of apoptosis called “myonuclear apoptosis” (3, 4, 71). Apoptosis is a highly regulated form of programmed cell death that is characterized by specific morphological and biochemical events (55, 66). Recently it has been reported that a loss of myonuclei via apoptotic pathways can occur without cell death in multinucleated muscle fibers during disuse muscle atrophy (4, 66, 71). The loss of nuclei within atrophying muscle fibers appears to be a strategy to maintain a constant ratio of nuclei per fiber area (4, 71). It has been postulated that this constant ratio of nuclei to fiber area (i.e., myonuclear domain) within skeletal muscle is intended to maintain an optimal nuclear-to-cytoplasm ratio such that nuclei are added to muscle fibers during growth and nuclei are lost during atrophy (Fig. 2; Refs. 4, 71).

Identical to locomotor skeletal muscle, our laboratory recently discovered that unloading the diaphragm during prolonged mechanical ventilation results in caspase-3 activation and myonuclear apoptosis (61). The pathways responsible for this disuse-induced myonuclear apoptosis are a hot topic of debate and potential candidate pathways will be outlined in the next segment.

PATHWAYS RESPONSIBLE FOR MYONUCLEAR APOPTOSIS

Myonuclear apoptosis can be induced by three overlapping pathways: J) sarcoplasmic (endoplasmic) reticulum (SR), 2) receptor mediated, or 3) mitochondrial. Each of these apoptotic pathways can trigger the activation of a unique group of proteases called “caspases.” To date, over 14 different caspases have been identified in mammalian cells (66). Collectively, caspases are endoproteases responsible for the final execution of cell death or nuclear destruction. In the cell, caspases are expressed as inactive precursors (i.e., procaspases), which activate on cleavage, resulting in a series of events leading to

Fig. 1. Human conditions that promote skeletal muscle atrophy and the corresponding animal models that are used to investigate them.

Fig. 2. Alterations in myonuclear number during skeletal muscle hypertrophy and atrophy. Note that during hypertrophy or disuse atrophy, myonuclear number is altered in conjunction with changes in myofibrillar protein content and myofiber cross-sectional area (CSA), resulting in a constant ratio of cytoplasmic area to nuclear number (myonuclear domain).
apoptosis. An illustration of the three primary apoptotic pathways along with selected caspases and pro/anti-apoptotic proteins is presented in Fig. 3.

**SR stress-induced apoptosis.** The SR-mediated pathway of apoptosis is activated via SR stress, resulting in calcium release leading to activation of caspase-12 (due to either caspase-7 or calpain activation). Activation of caspase-12 can then lead to activation of caspase-3 (Fig. 3). Activated caspase-3 then translocates to the nucleus where it cleaves and deactivates “inhibitor of caspase-activated DNase” (66). This liberates caspase-activated DNase, an endonuclease, to fracture genomic DNA, leading to one of the most important morphological events of apoptosis (i.e., DNA fragmentation; Ref. 66). This apoptotic DNA fragmentation can be identified via histological techniques, including fluorescent labeling of damaged DNA via probes for single-stranded DNA or terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) or electrophoretic procedures to reveal DNA fragmentation (i.e., DNA laddering).

Note that increases in cytosolic calcium due to SR stress can also promote apoptosis via activation of calpain. Calcium-mediated activation of calpain has been postulated to contribute to apoptosis in several ways. For example, calpain can cleave the Bcl2 (B-cell CLL/lymphoma-2) family member Bid (BH3 interacting domain death agonist), to an active pro-apoptotic molecule, tBid (truncated Bid). tBid promotes the formation of the mitochondrial permeability transition pore and cytochrome c release from the mitochondria, leading to nuclear apoptosis (15). Moreover, it has been reported that calpain cleaves a variety of other proteins to promote apoptosis (15). Hence it is possible that calpain activation is an important stimulus to promote myonuclear apoptosis in skeletal muscle during prolonged periods of unloading.

**Receptor-mediated apoptosis.** A widely studied pathway leading to apoptosis is the corridor induced by the binding of a ligand to one or more extracellular receptors. These ligand-receptor complexes transmit their signals via a series of protein-protein interactions (66). The tumor necrosis factor receptor super family is a critical trans-membrane receptor class that binds both the cytokine tumor necrosis factor-α (TNF-α) and Fas Ligand (a transmembrane protein on cytotoxic T lymphocytes and other immune cells; Ref. 52). Receptor-mediated apoptotic pathways can be initiated by ligand-induced (e.g., TNF-α) binding, leading to the activation of caspase-8 and -10, promoting the activation of caspase-3 and subsequent damage to genomic DNA (66).

**Mitochondrial-mediated apoptosis.** The mitochondrial pathway of apoptosis can be initiated via various signals, including ROS (i.e., oxidative stress) and high levels of cellular calcium. These factors can induce opening of the mitochondrial permeability transition pore and promote the release of cytochrome c from the mitochondria into the cytosol (Fig. 3; Ref. 66). In the cytosol, cytochrome c triggers a signaling pathway leading to the activation of caspase-9 and subsequently promoting caspase-3 mediated nuclear DNA fragmentation. Independent of caspase activation, mitochondrial release of apoptosis-inducing factor (AIF) or endonuclease G (Endo G) can also induce nuclear DNA fragmentation in atrophying skeletal muscle (22, 66).

In general, the release of mitochondrial apoptotic factors into the cytoplasm is regulated by the Bcl-2 family of apoptotic regulators (17, 24, 66). Within this family, several pro-apoptotic (e.g., Bax, Bid, PUMA, etc.) and anti-apoptotic (e.g., Bcl-2, Bcl-XL, etc.) members exist (66). For example, Bcl-2 protects the mitochondria against the release of pro-apoptotic molecules, whereas Bax (Bcl2-associated X-protein) and Bid facilitate apoptosis by contributing to the opening of the mitochondrial permeability transition pore, resulting in the release of cytochrome c and AIF (24, 66). Interestingly, Bcl-2 and Bax influence each other’s function such that the relative
concentration of these two proteins may determine whether the cell undergoes apoptosis when faced with stimuli such as oxidative stress or calcium overload (24, 66).

PROTEOLYTIC PATHWAYS IN SKELETAL MUSCLE

Numerous proteolytic systems contribute to the degradation of muscle proteins. The principal proteases in skeletal muscle can be classified into three major categories: 1) lysosomal proteases, 2) Ca\(^{2+}\)-activated proteases (i.e., calpain), and 3) the proteasome system. Furthermore, recent evidence reveals that another protease, caspase-3, may also contribute to select forms of muscle atrophy (21, 61). A brief overview of each of these major proteolytic systems follows.

**Lysosomal proteases.** Lysosomes are a component of the degradative machinery in many mammalian cells. Lysosomes are membrane-bound vesicles containing acid hydrolases that include proteases, glycosidases, lipases, and phosphatases (8). Therefore, lysosomes are intracellular compartments dedicated to the degradation of macromolecules.

The four major lysosomal proteases are cathepsins L, B, D, and H (8, 79). Although these cathepsins are ubiquitously expressed in all tissues, the concentrations of cathepsins in cells vary, with the greatest levels found in tissues containing inherently high rates of protein turnover (i.e., kidney, liver, and spleen), whereas tissues with slow protein turnover (i.e., skeletal muscle) possess much lower levels (8). Moreover, cathepsin levels differ between skeletal muscle fiber types. Type I (slow, oxidative) fibers exhibit higher levels of cathepsins compared with type IIb (fast, glycolytic) fibers (8).

Although some debate continues, it is generally agreed that lysosomal protease activity increases in disuse muscle atrophy (8, 27, 79, 85). For instance, work by Taillardier et al. (79) indicates that 9 days of hindlimb suspension promoted a 2.5-fold increase in lysosomal protease activity in rat soleus muscle. In an effort to determine the quantitative contribution of cathepsins to muscle atrophy, these investigators inhibited cathepsin activity during periods of muscle disuse and evaluated the rate of muscle atrophy. Their results revealed that inhibition of cathepsin activity resulted in only a small decrease (<18%) of the loss of muscle protein associated with disuse muscle atrophy. Furthermore, because lysosomal proteases are not involved in the degradation of myofibrillar proteins, it seems unlikely that cathepsins play a dominant role in the proteolysis associated with inactivity in skeletal muscles (79).

**Calpain-mediated proteolysis.** Calpains are Ca\(^{2+}\)-dependent cysteine proteases that are found in all vertebrate cells (28). Although numerous members of the calpain family of proteases exist, the two best-characterized calpains found in skeletal muscle are termed \(\mu\)-calpain and \(m\)-calpain and refer to micromolar and millimolar amounts of calcium required to activate each respective calpain isoform (28). Although calpains are not at present known to directly degrade the contractile proteins actin and myosin, they participate in sarcomeric protein release by cleaving cytoskeletal proteins (e.g., titin, nebulin) that anchor contractile elements (43, 67). Moreover, calpain is known to degrade several kinases and phosphatases, including calcium/calmodulin-dependent kinase (CaM kinase II), protein kinase C (PKC-\(\alpha\), PKC-\(\beta\), PKC-\(\beta\)II, and PKC-\(\gamma\)), and calcineurin (28, 32, 81).

In vivo calpain activity is regulated by several factors including cytosolic calcium levels and the concentration of the endogenous calpain inhibitor, calpastatin (28). More specifically, calpain activity is increased by a sustained elevation in free calcium in the cytosol and/or a decrease in cytosolic calpastatin levels (28). In regard to calpain’s contribution to disuse muscle atrophy, it is clear that skeletal muscle inactivity is associated with an increase in both cytosolic calcium levels and calpain activity (51). Although the mechanism responsible for this inactivity-mediated calcium overload is unknown, it is possible that intracellular production of ROS could play a key role in disturbances in calcium homeostasis (42). A potential biochemical mechanism to link oxidative stress with calcium overload is that ROS-mediated formation of reactive aldehydes (i.e., 4-hydroxy-2,3-trans-nonenal) can inhibit plasma membrane Ca\(^{2+}\)-ATPase activity (77). Hence, an oxidative stress-induced decrease in membrane Ca\(^{2+}\)-ATPase activity would impede Ca\(^{2+}\) removal from the cell and promote intracellular Ca\(^{2+}\) accumulation. Nonetheless, it is currently unknown as to whether this mechanism is the single explanation for inactivity-mediated calcium overload in muscle.

**Caspase-3 and muscle atrophy.** Recent evidence suggests that the protease caspase-3 contributes to protein degradation and muscle atrophy (21, 61). In this regard, caspase-3 activation promotes degradation of actomyosin complexes, and inhibition of caspase-3 activity suppresses the overall rate of proteolysis in diabetes-mediated cachexia and myofiber atrophy in mechanical ventilation disuse (21, 61).

As discussed previously, control of caspase-3 activity in the cell is complex and involves numerous interconnected signaling pathways. In the case of diabetes-induced muscle atrophy, it seems possible that caspase-3 is activated by caspase-12 (via a calcium release pathway) and/or activation of caspase-9 (via a mitochondrial pathway). A potential interaction between these caspase-3 activation pathways is that both of these can be activated by ROS (Fig. 3; Ref. 66).

Moreover, note that calpastatin is a substrate for both caspase-3 and calpain. It follows that increases in caspase-3 or calpain activity lower calpastatin levels in cells and promote calpain activation (21, 28, 88). Furthermore, increased calpain activity can lead to the activation of caspase-3 (15). Therefore, cross-talk between the calpain and caspase-3 proteolytic systems could play a role in the regulation of myofilament release in skeletal muscle during periods of disuse.

The proteolytic release of myofilaments during disuse muscle atrophy is important because the bulk of muscle proteins (50–70%) exist in actomyosin complexes (84). Although the proteasome system can degrade monomeric contractile proteins (i.e., actin and myosin), it is unable to break down intact actomyosin complexes (28). Hence, myofilaments must be released from the sarcomere as monomeric proteins prior to degradation by the proteasome system (84, 89). This fact dictates that the release of myofilaments is potentially the rate-limiting step in muscle protein degradation. Again, evidence reveals that both calpain and caspase-3 are capable of producing actomyosin disassociation (21, 28, 84). Hence, activation of one or both of these proteases is a requirement for proteolytic degradation of myofilaments during conditions that result in muscle atrophy.

**Proteasome-mediated proteolysis.** The total proteasome complex (26S) is comprised of a core proteasome subunit...
(20S) coupled with a regulatory complex (19S) connected to each end of the 20S core (30, 31, 33, 84). Interestingly, proteins can be degraded by either the 26S proteasome or the 20S protease core.

The 26S proteasome degrades ubiquitinated proteins. It follows that the 26S proteasome degradation pathway is only active after ubiquitin covalently binds to protein substrates and marks them for degradation. The binding of ubiquitin to protein substrates is often a three-step process that initially requires the ubiquitin-activating enzyme (E1). Following activation, the ubiquitination of specific proteins is provided by one of a variety of E2s and by specialized E3s that recognize specific protein substrates. In this regard, numerous studies reveal that the ubiquitin-conjugating enzyme E214k is an important regulator of skeletal muscle ubiquitin-protein conjugation (58, 70, 80). Furthermore, E214k interacts with a specific E3 ligase (i.e., E3α) to promote muscle protein degradation in catabolic states. In addition, other unique ubiquitin E3 ligases (e.g., atrogin1/muscle atrophy F-box and muscle ring finger-1) exist in skeletal muscle, and these ligases play essential roles in skeletal muscle atrophy (10, 29, 70, 80).

The process of ubiquinated protein degradation requires a coordinated effort of both the 19S regulatory complex and the 20S proteasome core. The 19S regulatory complex possesses inherent ATPase activity and is a required participant in ATP-dependent degradation of ubiquitinated proteins (18). The ubiquitinated protein is recognized and bound by the 19S regulators of the 26S proteasome, and energy from ATP hydrolysis removes the polyubiquitin chain and unfolds the substrate protein. The unfolded protein then enters the 20S core proteasome and is degraded in a process that does not require energy from ATP (31, 70). Also, note that evidence reveals that the 20S core proteasome can degrade oxidized proteins without ubiquitination (30, 31). Therefore, ROS-mediated oxidation of proteins can promote muscle protein breakdown via 20S core proteasome alone.

PRODUCTION OF ROS IN QUIESCENT SKELETAL MUSCLES

It is now clear that ROS are produced in both inactive and contracting skeletal muscles (44, 69). When ROS production in cells exceeds the antioxidant capacity to eliminate these oxidants, oxidative stress occurs. A pro-oxidant state in cells can alter the structure and function of proteins, lipids, and nucleic acids, resulting in cellular injury and, in extreme circumstances, cell death.

Until the early 1990s it was widely believed that ROS production was limited in noncontracting skeletal muscle and that oxidative injury does not occur in inactive muscles. However, many studies indicate that oxidative injury occurs during periods of disuse in locomotor skeletal muscles (45, 47–50, 53) and in the unloaded diaphragm during prolonged mechanical ventilation (23, 75, 90). It is currently unknown which ROS producing pathways are dominant contributors to inactivity-induced oxidative injury in skeletal muscles. Indeed, it is possible that oxidative stress in inactive skeletal muscle may be due to the interaction of several major oxidant production pathways, including xanthine oxidase production of superoxide, nitric oxide synthase production of nitric oxide, NADPH oxidase-mediated production of superoxide, and mitochondrial production of superoxide (7, 23, 37, 38, 50, 86; Fig. 4).

**WHAT INTRACELLULAR SIGNALING PATHWAYS LINK OXIDATIVE STRESS TO MUSCLE ATROPHY?**

Recent studies reveal that ROS species can serve as second messengers in cellular signal transduction pathways that regulate both normal physiological signaling and pathological signaling leading to proteolysis and cell death via apoptosis. This seemingly contradictory signaling function of ROS is presumably linked to the level of ROS and the overall redox state in the cell. That is, low levels of ROS promote cell adaptation and survival, whereas high levels of ROS modulate signaling pathways that lead to proteolysis and cell death.

Abundant research demonstrates that oxidative stress contributes to disuse muscle atrophy. The first evidence that oxidants played a key signaling role in the regulation of disuse muscle atrophy was provided by Kondo et al. (46). The pioneering work of Kondo and colleagues revealed that immobilization of skeletal muscles is associated with increased radical production, resulting in oxidative injury in inactive muscle fibers (46, 47, 50). Importantly, this work also demonstrated that disuse muscle atrophy could be delayed by exogenous antioxidants. These early observations have subsequently been confirmed by others (6, 9, 60).

How does inactivity-induced oxidative stress in skeletal muscle contribute to muscle atrophy? At present, a complete answer to this question is not available. Nonetheless, it appears that oxidative stress could contribute to disuse muscle atrophy by influencing one or more of the following cell signaling pathways: 1) regulation of cytosolic calcium levels and subsequent activation of calpain (as previously discussed); 2) control of mitogen activated protein kinase (MAPK) signaling; and 3) activation of the nuclear factor (NF)-κB pathway. A brief discussion of these possibilities follows.

**OXIDATIVE STRESS ACTIVATES MAPK SIGNALING**

The redox regulation of MAPK provides another potential link between oxidative stress and skeletal muscle atrophy. It is well established that MAPKs play a key role in cell signaling because control of numerous cellular signaling pathways is achieved via activation or deactivation of regulatory proteins through phosphorylation (16). Indeed, the highly conserved MAPK family is one of the major kinase families that regulate the conversion of cell signals into cellular responses. These protein kinases contribute to the regulation of life and death decisions made in response to various stress signals (e.g.,
oxidative stress), as the actions of both pro- and antiapoptotic factors are often regulated by the phosphorylation status of key elements in the execution of apoptosis or survival (59).

All eukaryotic cells possess multiple MAPK pathways. The three best characterized MAPK subfamilies are c-Jun NH2-terminal kinase (JNK), p38 MAPK, and extracellular signal-regulated kinase (ERK; Ref. 64). All three of these MAPK pathways are structurally similar, but functionally distinct. Importantly, ERK, JNK, and p38 have all been shown to be activated by oxidative stress and could potentially participate in pathways influencing muscle protein breakdown or myonuclear apoptosis. The classic MAPK activation cascade consists of three sequential intracellular protein kinase activation steps leading to the activation of numerous protein kinases, nuclear proteins, and transcription factors, resulting in downstream signal transduction (16, 64). A brief overview of the functions of ERK, p38, and JNK follows.

**ERK1/2.** The first identified and best studied MAPK cascade is the ERK pathway (16). ERK is composed of two isoforms, ERK1 and ERK2, and are collectively referred to as ERK1/2 (16, 64). ERK1/2 can be activated by a number of mitogens, including epidermal growth factor, platelet-derived growth factor, transforming growth factor, and insulin. In addition, ERK1/2 can be activated by endotoxin and oxidative stress (16). The activation of ERK1/2 by oxidative stress is consistent with the hypothesis that low and adequate levels of ROS are mitogenic (64, 68). Activation of ERK1/2 regulates the transcriptional activity of AP-1, NF-κB, c-Myc, and the cell survival protein Bcl-2 (68). Although it has been reported that ERK1/2 can promote apoptosis, ERK1/2 can also function as an anti-apoptotic factor following oxidative stress (64). To date, however, there is little evidence to link ERK1/2 activation with disuse muscle atrophy.

**p38.** p38 is an important MAPK member that is activated in response to various physiological stresses such as osmotic stress, endotoxins, and oxidative stress (64). The link between oxidative stress and p38 activation occurs via apoptosis stimulating kinase 1 (ASK1; Ref. 59). ASK1 is a ubiquitously expressed member of the MAPK family that activates both p38 and JNK by phosphorylating and activating respective MAPK kinases (Fig. 5). Five isoforms of p38 have been identified: p38α, p38β, p38β2, p38γ, and p38δ and expression of these isoforms varies among tissues (16). For example, p38α is highly expressed in bone marrow and leukocytes, p38β is expressed in heart and brain, and p38γ is predominantly expressed in skeletal muscle (16).

Among the phosphorylation targets of p38 are several important transcription factors, including p53, NF-κB, and ATF2. Of particular importance to apoptosis is the fact that activation of the tumor suppressor protein p53 results in the expression of the pro-apoptosis protein Bax. Moreover, p38 signaling has been shown to promote the expression of atrogin 1 in myotubes (57). Collectively, these data suggest a potential role for oxidative stress-induced activation of p38 in disuse muscle atrophy.

**JNK.** JNK (also known as stress-induced kinase) has three isoforms (JNK1, JNK2, JNK3) that are encoded by three different genes. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 is only expressed in brain, heart, and testis (16). JNK can be activated in response to many of the same stimuli that activate p38, such as osmotic stress, endotoxins, and oxidative stress. Moreover, similar to p38, oxidative stress-induced activation of JNK occurs via the ASK1 pathway (Fig. 5; Ref. 76).

The specific molecular targets of JNK include the transcriptional factors AP-1, p53, and c-Myc and many other nontranscriptional factors such as Bcl-2 family members. In this regard, there is growing evidence that JNK plays an important role in oxidative stress-mediated apoptosis. Indeed, because ROS themselves are not able to activate caspases, ROS-mediated apoptosis requires another death-signaling pathway.

**Fig. 5.** Simplified overview of ROS signaling pathways leading to activation of mitogen-activated protein kinases in skeletal muscle. Skeletal muscle cytosolic or mitochondrial ROS activates apoptosis-stimulating kinase 1 (ASK1) stimulating phosphorylation (p) of jun NH2-terminal kinase (JNK) and/or p38 regulation of myonuclear apoptosis-associated transcription factors (AP1, p53, c-Myc, NFkB). JNK/p38 activation by ASK1 can lead to cytochrome c release and caspase-3 activation. In contrast, low levels of ROS (dashed lines) can result in the phosphorylation of the extracellular-regulated kinases 1 and 2 (ERK 1/2), which functions in cellular survival, mtPTP, mitochondrial permeability transition pore.
such as JNK (59, 76). Preliminary evidence implicating JNK as a mediator of ROS-induced apoptosis can be summarized in three observations. First, JNK is readily activated in cells by exposure to physiological levels of ROS (59, 76). Secondly, in ASK1−/− cells, JNK is not activated in response to ROS and ASK−/− cells are resistant to ROS-mediated apoptosis, although the role of p38 could not be eliminated (76). Finally, overexpression of a dominant negative mutant of JNK provides some resistance to ROS-induced apoptosis (76). Collectively, these findings support a link between ROS, JNK, and apoptosis. However, it is currently unknown if JNK activation is responsible for the myonuclear apoptosis that occurs during disuse muscle atrophy.

**MAPK cross talk.** While each member of the MAPK super family is considered unique, each member forms only a part of the complex and interactive network of phosphorylation signaling pathways (16). Indeed, each member of the MAPK cascade shares communication with a number of upstream and downstream kinases along with transcription factors that interact and integrate these different pathways. Therefore, this redundancy results in a complex cross-talk and signal convergence among MAPK family members. Moreover, each MAPK family member has feedback loops that impact their activation and regulate the activation of other MAPK family members (16).

**REDOX REGULATION OF NF-κB SIGNALING**

Another potential link between oxidative and muscle disuse atrophy involves the redox regulation of the NF-κB family of transcriptional activators. NF-κB comprises a family of five transcription factors (p65, Rel B, c-rel, p52, and p50) and, when activated, can promote a wide range of cellular outcomes depending on the cell type (reviewed in Refs. 36, 41, 42). All five of the NF-κB family members are expressed in skeletal muscle and Hunter et al. (35) first reported that skeletal muscle inactivity leads to increased NF-κB transcriptional activity. Since this discovery in 2002, accumulating evidence indicates that a specific NF-κB pathway is required for disuse muscle atrophy (reviewed in Refs. 36, 41). Nonetheless, although NF-κB regulates the expression of over 100 genes, a detailed understanding of the specific NF-κB targets required for disuse muscle atrophy remains incomplete.

Although it has long been believed that NF-κB activation is under redox control, how ROS regulate NF-κB transcriptional activity remains contentious. For example, ROS are known to enhance the signal transduction pathways (e.g., activation of kinases) that promote both NF-κB activation in the cytoplasm and translocation to the nucleus (40, 62). This observation is consistent with the concept that ROS can promote NF-κB activation and subsequent gene expression. In contrast, the DNA binding activity of oxidized NF-κB is diminished, suggesting that ROS may also inhibit NF-κB transcriptional activity (40). Therefore, although NF-κB was once considered to be a prototypic redox sensitive transcription factor, the fact that ROS can both promote and inhibit NF-κB transcriptional activation has lead to considerable debate regarding the redox control of NF-κB signaling (62). Clearly, future research will be required to unravel the uncertainties about the redox regulation of NF-κB in physiologically relevant settings in skeletal muscle during prolonged periods of inactivity.

**CONCLUSIONS AND UNANSWERED QUESTIONS**

Inactivity-induced skeletal muscle atrophy can occur due to a variety of conditions, including prolonged bed rest, limb immobilization, and spaceflight. Several lines of evidence directly link ROS to disuse muscle atrophy via ROS-mediated regulation of proteolysis. Importantly, several studies suggest that antioxidants can serve as therapeutic agents in delaying the rate of disuse muscle atrophy.

Although it is clear that ROS-mediated signaling contributes to disuse muscle atrophy, numerous unanswered questions remain. For example, does the role that ROS play in muscle atrophy differ between different models of skeletal muscle inactivity (e.g., immobilization vs. microgravity)? Another key question is “which ROS pathways are active in unloaded skeletal muscle”? Moreover, “if multiple oxidant production pathways are active, what is the relative contribution of each pathway to the regulation of cell signaling pathways involved in disuse muscle atrophy”? Resolution of these issues will provide the information needed to develop therapeutic strategies to prevent oxidant production or scavenge ROS to prevent oxidative injury in the muscle fiber during prolonged periods of inactivity.

Is the production of ROS a requirement for disuse muscle atrophy or does oxidative stress merely regulate the rate of muscle atrophy? A related question is, “do ROS simply act as second messengers to control muscle atrophy or is ROS-mediated oxidative injury a requirement for oxidant regulation of muscle atrophy”? Both of these questions are important and unresolved.

Another unresolved question is “does oxidative stress negatively influence the rate of protein synthesis in skeletal muscle during periods of inactivity”? To date, the majority of previous research related to ROS and muscle atrophy has focused on proteolysis, and it is unclear if ROS contribute to the downregulation of protein synthesis that occurs during disuse in skeletal muscle; hence, this is an interesting area for future work. In this regard, emerging evidence reveals that oxidative stress can inhibit protein synthesis in several cell types (2, 63, 65).

A final area for future research relates to the pathways responsible for myonuclear apoptosis in skeletal muscle. As discussed earlier, disuse muscle atrophy is associated with a loss of myonuclei from muscle fibers due to myonuclear apoptosis (3, 71). Although it is well known that ROS can contribute to signaling pathways, leading to apoptosis, it is unknown if ROS represent the primary signal to promote myonuclear apoptosis in atrophying skeletal muscle (55, 66). Hopefully, questions outlined in this review stimulate muscle biologists to pursue research in the area of ROS and skeletal muscle atrophy. Future scientific advances in cell signaling will provide the tools required to answer these important questions that will ultimately result in therapeutic approaches to prevent or diminish disuse muscle atrophy.

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Oxidative Stress and Disuse Muscle Atrophy

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