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Redox control of skeletal muscle atrophy

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Abstract

Skeletal muscles comprise the largest organ system in the body and play an essential role in body movement, breathing, and glucose homeostasis. Skeletal muscle is also an important endocrine organ that contributes to the health of numerous body organs. Therefore, maintaining healthy skeletal muscles is important to support overall health of the body. Prolonged periods of muscle inactivity (e.g., bed rest or limb immobilization) or chronic inflammatory diseases (i.e., cancer, kidney failure, etc.) result in skeletal muscle atrophy. An excessive loss of muscle mass is associated with a poor prognosis in several diseases and significant muscle weakness impairs the quality of life. The skeletal muscle atrophy that occurs in response to inflammatory diseases or prolonged inactivity is often associated with both oxidative and nitrosative stress. In this report, we critically review the experimental evidence that provides support for a causative link between oxidants and muscle atrophy. More specifically, this review will debate the sources of oxidant production in skeletal muscle undergoing atrophy as well as provide a detailed discussion on how reactive oxygen species and reactive nitrogen species modulate the signaling pathways that regulate both protein synthesis and protein breakdown.

Keywords

oxidative stress; reactive nitrogen species; reactive oxygen species; oxidants; antioxidants; proteolysis; muscle protein synthesis

Introduction

Skeletal muscle fiber size is regulated by the balance of protein synthesis and protein breakdown. For example, when the rate of muscle protein breakdown exceeds the pace of protein synthesis, muscle fibers lose protein and atrophy occurs. The loss of muscle contractile protein results in a decreased capacity to generate force (i.e., muscle weakness). Numerous conditions promote skeletal muscle atrophy including chronic inflammatory diseases and prolonged periods of muscle inactivity. Diseases that are often associated with skeletal muscle atrophy include cancer, kidney disease, heart failure, and chronic obstructive

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pulmonary disease (COPD). Independent of disease, prolonged muscle inactivity (e.g., prolonged bedrest or limb immobilization) also results in muscle atrophy. Regardless of the cause, skeletal muscle atrophy results in muscular weakness and a diminished quality of life [1]. Importantly, excessive loss of muscle mass during disease is also associated with increased morbidity and mortality [2].

The development of a therapeutic intervention to prevent muscle wasting requires an understanding of the cellular signaling pathways that regulate both protein synthesis and proteolysis in muscle. During the past two decades, numerous studies have advanced our knowledge of the cell signaling pathways that regulate muscle size. In this regard, abundant evidence indicates that oxidative stress and/or nitrosative stress, due to increased production of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), plays an important role in modulating the signaling pathways that regulate both protein synthesis and proteolysis in skeletal muscle. Indeed, it is widely accepted that oxidative stress contributes to skeletal muscle atrophy due to both disease and prolonged muscle inactivity [3-6]. Further, evidence also links nitrosative stress to skeletal muscle atrophy resulting from chronic disease or prolonged muscle disuse [7, 8].

This review summarizes our current understanding of the nexus between ROS/RNS and the cell signaling processes leading to skeletal muscle atrophy. We begin with a brief overview of the sources of ROS and RNS production in skeletal muscle. This will be followed by a discussion of the signaling pathways connecting ROS and RNS to increased proteolysis and regulation of protein synthesis. We conclude with a brief discussion of the role that protein oxidation plays in promoting muscle protein breakdown during conditions that produce muscle atrophy.

Sources of ROS and RNS in Atrophying Skeletal Muscle

Both oxidative and nitrosative stress often occur in skeletal muscles undergoing atrophy. In this segment we introduce the pathways responsible for ROS and RNS production in skeletal muscle in response to both chronic inflammatory diseases and prolonged muscle inactivity.

Sources of ROS and RNS production in skeletal muscle during chronic illness

The loss of skeletal muscle mass due to illness is termed cachexia. Severe inflammatory diseases including cancer, kidney failure, COPD, and heart failure are common causes of cachexia. Inflammation in a remote organ (e.g., lungs of COPD patient) often results in systemic inflammation which is characterized by high levels of circulating cytokines; this is important because systemic inflammation serves as a trigger for both oxidative and nitrosative stress in skeletal muscle [4-6].

Inflammatory disease-induced ROS production in skeletal muscles—Several circulating pro-inflammatory mediators including interleukin-6, tumor necrosis factor-α (TNF), C-reactive protein, and sphingomyelinase are elevated in patients with chronic inflammatory disease [6]. Among these factors, TNF likely plays a key role in increased ROS production in skeletal muscle fibers [6]. The link between circulating TNF and ROS production in skeletal muscle fibers has been investigated extensively. Briefly, evidence

indicates that TNF initiates intracellular ROS production by activating the TNF-1 receptor on the sarcolemma; this triggers a cascade of signaling events leading to an increase in superoxide production in the mitochondria (reviewed in [6]). Early events in this biochemical cascade likely involve signaling by one or more sphingomyelinase isoforms leading to the activation of phospholipase A2 which cleaves membrane lipids to release arachidonic acid; this is significant because arachidonic acid is a substrate for ROS-generating enzymes such as lipoxygenases [6, 9]. Moreover, activation of phospholipase A2 has been shown to activate NAD(P)H oxidase to generate superoxide and phospholipase A2 can also stimulate superoxide production in the mitochondria [9].

Another potential link between some diseases that promote cachexia and skeletal muscle ROS production stems from increased plasma levels of angiotensin II (Ang II) which occurs in some diseases[10, 11]. For example, several chronic diseases (e.g., congestive heart failure and chronic kidney disease) are often accompanied by elevated circulating levels of Ang II. This is significant because infusion of Ang II in rodents results in increased circulating levels of several cytokines including TNF [11]. As discussed previously, TNF can promote ROS production in skeletal muscle fibers. Moreover, Ang II binding to angiotensin I receptors on the sarcolemma can activate NAD(P)H oxidase which also generates superoxide radicals [10-12]. Therefore, it is likely that chronic inflammatory diseases promote both mitochondrial and cytosolic ROS production in skeletal muscles via increases in circulating TNF and/or Ang II.

Finally, emerging evidence indicates that myostatin is a pro-oxidant that can promote increased ROS production skeletal muscle fibers [13, 14]. The mechanisms responsible for this myostatin-mediated ROS production remain a topic of debate but evidence suggests that myostatin induces oxidative stress through a TNF-mediated mechanism [13]. Moreover, it appears that both TNF and ROS are potent inducers of myostatin and require NFκB signaling for myostatin expression [13]. Together, these results suggest myostatin and TNF are components of a feed forward system by which myostatin triggers the generation of the second messenger ROS, mediated by TNF, which in turn, stimulates myostatin expression.

RNS production in skeletal muscles during disease-induced muscle wasting— In addition to the production of ROS, many chronic diseases also promote an increase in the production of nitric oxide (NO). NO is produced in muscle by nitric oxide synthases and the expression of inducible nitric oxide synthase (iNOS) is often increased in skeletal muscle during inflammatory disease-induced muscle wasting [15-17]. Specifically, studies in heart failure, cancer, and COPD have demonstrated that increased circulating cytokines (e.g., TNF) stimulate iNOS expression in skeletal muscles [15-18]. This augmented expression of iNOS leads to increased production of nitric oxide (NO) and consequently protein nitration.

Sources of ROS and RNS production in skeletal muscles exposed to prolonged inactivity

Similar to chronic diseases, prolonged periods of muscle disuse results in increased ROS and RNS production in skeletal muscles. Research during the past two decades has provided insight into the sources of both ROS and RNS production in skeletal muscles undergoing disuse-induced atrophy. A discussion of the potential sites of ROS production in inactive skeletal muscles follows.

Prolonged skeletal muscle inactivity promotes increased ROS production— The first evidence that prolonged periods of muscle disuse results in increased oxidative stress in limb skeletal muscles was reported over 20 years ago [19] and these original findings have been confirmed by many investigations (reviewed in [3]). However, unlike inflammatory disease-induced muscle wasting, the disuse-induced ROS production in muscles is not associated with systemic inflammation. Although the causes of disuse-induced oxidative stress in skeletal muscle continues to be investigated, evidence suggests that prolonged skeletal muscle inactivity results in increased superoxide production at multiple locations in the cell including NAD(P)H oxidase, xanthine oxidase, and the mitochondria [20-24]. In regard to the relative contributions of each of these sources of ROS production, recent evidence suggests that mitochondria are a major source of ROS production in inactive skeletal muscles [20, 21, 25]. For example, compared to mitochondria obtained from skeletal muscles of active rodents, mitochondria from muscle exposed to prolonged periods of disuse release significantly more ROS [26] [20, 24]. Importantly, treatment of animals with a mitochondrial-targeted antioxidant prevents inactivity-induced oxidative stress in skeletal muscles [20, 21] [24]. Collectively, these observations suggest that mitochondria are a key source of ROS production in inactive skeletal muscles.

The impact of prolonged muscle inactivity on muscle RNS production remains controversial—At present, the question of whether prolonged muscle inactivity results in increased production of NO remains controversial. On one hand, Suzuki et al. reported that prolonged inactivity in limb skeletal muscles (i.e., 14 days of hindlimb suspension) is accompanied with increased NO levels in the inactive muscle [7]. However, unlike cachectic skeletal muscles, iNOS levels do not increase in limb muscles exposed to prolonged inactivity; therefore, increased NO production via iNOS does not occur in skeletal muscles exposed to prolonged inactivity [7]. Rather, Suzuki and colleagues report that muscle inactivity is associated with a dissociation of neuronal NO synthase (nNOS) from the dystroglycan complex and propose that the release of nNOS from the membrane results in nNOS activation and increased production of NO [7]. The finding that muscle disuse results in increased cytosolic nNOS has been confirmed by others [8, 27, 28]. Nonetheless, it is important to note that there are no published reports documenting higher NO production by non-membrane bound nNOS [29].

In contrast to the Suzuki et al. findings [7], a recent study concludes that 14 days of hindlimb suspension results in a decrease in both muscle nNOS and NO levels [30]. Similarly, nitosative stress is not present in the rat diaphragm following 18 hours of inactivity due to prolonged mechanical ventilation [31]. Therefore, it remains unclear as to whether prolonged muscle inactivity results in increased NO production and clearly additional studies are warranted to resolve this issue.

Redox Regulation of Muscle Atrophy

The hypothesis that redox disturbances contribute to muscle atrophy was first introduced in 1991 [19]. Using a rodent model of skeletal muscle inactivity (i.e., limb immobilization), Kondo and colleagues reported that prolonged muscle inactivity is associated with high levels of lipid peroxidation in the inactive fibers[19]. Moreover, this work revealed that

inactivity-induced muscle atrophy can be attenuated by the antioxidant vitamin E. Although these investigators published several reports that further established a link between ROS and disuse muscle atrophy [32-35], the role that ROS plays did not receive significant experimental attention until the last decade. The next segments will discuss the signaling pathways that form the link between oxidative and nitrosative stress and the regulation of both muscle protein synthesis and degradation.

Oxidative stress inhibits anabolic signaling and muscle protein synthesis

The major signaling pathway that regulates muscle protein synthesis is the insulin-like growth factor-1 (IGF1)-protein kinase b (Akt) signaling pathway. Factors that accelerate signaling through this pathway (e.g., muscular exercise) can promote protein synthesis whereas factors that inhibit one or more signaling steps of the pathway (e.g., oxidative stress) depress protein synthesis. This section highlights the evidence demonstrating that oxidative stress depresses anabolic signaling and protein synthesis in skeletal muscle fibers.

Overview of protein synthesis in skeletal muscles—The role of the IGF1-Akt pathway in controlling protein synthesis is well-established by both gain and loss-of-function experiments. Indeed, numerous studies reveal that inactivation of the IGF1 receptor impairs muscle growth whereas activation of the IGF1 receptor promotes hypertrophy (reviewed in [36]). The activation of the IGF1-Akt pathway promotes protein synthesis by increasing mRNA translation of specific proteins [37].

Signaling via IGF1 begins with the IGF1 ligand binding to its receptor (i.e., tyrosine kinase IGF1 receptor (IGF1R)); this docking results in receptor phosphorylation and recruitment of insulin receptor substrate 1 (IRS1) [38] (Figure 1). Phosphorylation of IRS1 activates the phosphoinositide-3-kinase (PI3K) pathway. Active PI3K triggers a series of steps that activate Akt; active Akt promotes protein synthesis via activation of the mammalian target of rapamycin (mTOR) and its downstream effectors. The Akt-mediated steps leading to mTOR activation involves the Akt-induced phosphorylation and subsequent inhibition of both tuberous sclerosis complex protein 2 (TSC2) and the proline-rich Akt substrate 40 kDa (PRAS40). Inhibition of TSC2 and PRAS40 allows the small GTPase Ras homolog enriched in brain (Rheb) to bind to mTOR and increase mTOR kinase activity (Figure 1).

Note that the kinase mTOR interacts with numerous proteins and exists in two protein complexes: 1) mTORC1 containing the regulatory-associated protein of mTOR (raptor); and 2) mTORC2 which contains the protein rictor (i.e., rapamycin-insensitive companion of mTOR) [36-38]. Abundant evidence indicates that mTORC1 is the primary mediator of protein synthesis in skeletal muscle [36]. Upon activation, the two main effectors phosphorylated by mTORC1 that promote protein synthesis are ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E (4E-BP1) [36, 37].

The importance of phosphorylated (active) S6K1 in promoting protein synthesis is illustrated by the finding that deletion of S6K1 results in muscle atrophy [39]. Although the precise role that S6K1 plays in protein synthesis remains under investigation, it appears that S6K1-mediated phosphorylation of several residues of the S6 ribosomal protein leads to increased translation and therefore, increased protein synthesis [40].

Furthermore, phosphorylated (active) 4E-BP1 leads to the dissociation of 4E-BP1 from the translation initiator eIF4E [38]. This is significant because 4E-BP1 inhibits the positive impact that eIF4E plays in translation. Specifically, following dissociation from 4E-BP1, eIF4E binds with other proteins (i.e., eIF4G and eIF4A) to form the eIF4F complex which is a required step for translation [36].

Elevated ROS production inhibits Akt/mTORC1 signaling and decreases muscle protein synthesis—The impact of ROS on Akt signaling and protein synthesis has been investigated using numerous cell types. Unfortunately, contradictory results exist with some studies reporting that oxidative stress can both promote and inhibit Akt signaling [41]. These divergent results likely arise from the differences in cell types, species of ROS, and the levels of ROS used in the experiments. Because this review focuses on redox control of muscle fiber size, we will focus our discussion on studies involving myocytes or myotubes derived from skeletal or cardiac muscles.

A recent study exposed C2C12 myotubes to a variety of different ROS challenges (e.g., H_2O_2, diamide, and glucose oxidase) and concluded that key components of the Akt signaling pathway differ in their sensitivity to oxidative stress [41]. These investigators reasoned that differences in thiol oxidation sensitivity across various signaling proteins involved in the Akt pathway provides an explanation for many of the contradictory findings in the literature regarding the impact of oxidants on the components of Akt signaling [41]. Importantly, this work revealed that chronic exposure of myotubes to low levels of H_2O_2 leads to oxidative stress and decreased Akt phosphorylation. This decrease in Akt phosphorylation is predicted to result in decreased protein synthesis, increased proteolysis, and muscle wasting [41] (Figure 1).

Although many studies have investigated the impact of ROS on activation of signaling molecules involved in protein synthesis, few studies have directly measured the influence of ROS on cellular protein synthesis. However, a detailed study using ventricular myocytes isolated from the rat heart concluded that exposure of myocytes to H_2O_2 depresses global protein synthesis by ~90% [42]. Moreover, this report revealed that exposure of cardiac myocytes to H_2O_2 resulted in the dephosphorylation of 4E-BP1 which occurred due to increased protein phosphatase activity. This dephosphorylation of 4E-BP1 increases the inhibitory association of 4E-BP1 with eIF4E which results in a decrease in protein synthesis. Similarly, oxidative stress has been shown to decrease the level of 4E-BP1 phosphorylation in other cell types [43]. Additional work suggests that oxidants may also impede protein synthesis in human adenocarcinoma cells by impairing mTOR assembly and therefore, preventing mTOR-mediated phosphorylation of 4E-BP1 [44]. Hence, several lines of evidence suggest that oxidative stress can impair translation, in part, by reducing phosphorylation of the eIF4E repressor protein, 4E-BP1.

A recent report investigated the role that oxidative stress plays in the inactivity-induced decrease in skeletal muscle protein synthesis *in vivo* [45]. Using a mitochondrial-targeted antioxidant to prevent inactivity-induced oxidative stress in diaphragm muscle, this study revealed that oxidative stress plays a key role in inactivity-induced depression of muscle protein synthesis. Moreover, this study demonstrated that prevention of inactivity-induced

oxidative stress in the diaphragm results in higher levels of phosphorylated Atk and mTORC1. Prevention of oxidative stress also resulted in greater phosphorylation of both 4E-BP1 and PRAS40. Together, these findings are consistent with the concept that oxidative stress depresses muscle protein synthesis by decreasing Atk/mTORC1 signaling and subsequently, reducing translation.

In summary, the available evidence indicates that oxidative stress can decrease global protein synthesis in skeletal muscles. This ROS-mediated depression in protein synthesis is likely due to decreases in Akt/mTORC1 signaling resulting in a slower rate of translation (Figure 1).

RNS impact on Akt/mTORC1 signaling and protein synthesis—At present, no studies have investigated the impact of RNS on protein synthesis in skeletal muscle fibers. Indeed, compared to ROS, research on the impact of nitrosative stress on anabolic signaling is limited. Nonetheless, investigations of the impact of RNS on cell signaling in other cell types suggests that nitrosative stress decreases Akt/mTORC1 signaling which would likely decrease translation and consequently, promote a decline in protein synthesis [46, 47]. The apparent mechanism to explain these observations is that exposure of cells to NO results in the activation of ataxia telangiectasia mutated kinase (ATM) and the resultant activation of liver kinase B (LKB1); activation of LKB1 activates AMP-activated protein kinase (AMPK) resulting in a repression of mTORC1 activation [46] (Figure 1). The precise biochemical mechanism responsible for the activation of ATM by RNS remains unclear. However, it is noteworthy that ROS can also activate ATM by direct oxidation of cysteine residues [48, 49].

To summarize, direct evidence that nitrosative stress results in a decrease in muscle protein synthesis does not currently exist. However, RNS-induced nitrosative stress can depress mTORC1 activation in non-muscle cells and this observation is consistent with the notion that nitrosative stress can depress protein synthesis. Future studies are required to confirm that high levels of RNS depress protein synthesis in skeletal muscle fibers.

Oxidative stress promotes proteolysis in skeletal muscles

Four major proteolytic systems exist in skeletal muscle fibers: 1) autophagy; 2) the proteasome system; 3) calpains; and 4) caspase-3. It is also established that skeletal muscle mitochondria also contain three known proteases (e.g., Lon protease) but little is known about the role that these proteases play in muscle atrophy [50]. Abundant evidence reveals that oxidative stress can promote proteolysis of skeletal muscle protein in three different ways. First, oxidative stress promotes gene expression of important proteins involved in several proteolytic systems. Second, oxidative stress in skeletal muscle results in an increase in cytosolic free calcium which can lead to the activation of both calpain and caspase-3. Finally, oxidative stress can also accelerate proteolysis by the oxidative modification of myofibrillar proteins which increases their susceptibility to proteolytic breakdown. In the next segments, we provide a brief discussion of connections between ROS and each of the major proteolytic systems.

ROS promote autophagy-mediated protein breakdown in skeletal muscles

Autophagy is a highly regulated lysosomal pathway for the degradation of non-myofibril cytosolic proteins and organelles in skeletal muscle [51]. During autophagy organelles (e.g., mitochondria) and cytosolic proteins are separated into double membrane vesicles called autophagosomes; these autophagosomes then fuse with lysosomes to form autolysosomes. After autophagosome formation, the autophagosome constituents are degraded by lysosomal proteases (i.e., cathepsins) [52].

Our understanding of the mechanisms that regulate the major pathway of autophagy (i.e., macroautophagy) has advanced rapidly during the past decade. Specifically, numerous autophagy-related genes exist and are described by the acronym of “Atg” followed by a number to identify the specific gene or protein (e.g., Atg1, Atg2, etc.). The precise role that each of these Atg proteins play in autophagy continues to be investigated but current evidence reveals that more than 30 Atg proteins are involved in autophagy in yeast; many of these same proteins are shared by eukaryotes [53]. Autophagy is often described as a five-step process: 1) induction; 2) expansion; 3) elongation and completion of autophagsome; 4) fusion with lysosome; and 5) degradation of proteins and organelles (Figure 2). The first step (i.e., induction or initiation) of autophagy begins with the formation of the pre-autophagosome structure which occurs by activation of the ULK1 complex (Atg 1 complex in yeast) [54]. Note that this initiation step is negatively regulated by mTORC1; therefore, factors that prevent mTORC1 activation can promote autophagy [54]. Step two involves the assembly of a fractional autophagosome membrane called the phagophore; this stage is often referred to as expansion and requires the recruitment of several Atg proteins including the essential Atg6 (also called beclin-1 in mammals)[54]. Step three involves several Atg genes (e.g., Atg5, Atg7, Atg8 (LC3 in mammals), and Atg12) resulting in elongation and completion of the autophagosome [54]. The next stage (step 4) of autophagy involves the fusion of autophagosome with the lysosome; this step exposes the autophagosome contents (i.e., cytosolic proteins and organelles) to lysosomal proteases (i.e., cathepsins B, D, and L) [54]. The fifth and final step of autophagy involves the cathepsin-mediated degradation of proteins and organelles (i.e., the cargo) contained within the autophagosome.

Compared to the contribution of the other major proteolytic systems to skeletal muscle wasting, autophagy has received relatively limited experimental attention. Nonetheless, it is clear that numerous lysosomal proteases (i.e., cathepsin B, D, & L) are activated in skeletal muscle undergoing disuse atrophy [55]. Moreover, growing evidence suggests that autophagy is accelerated in muscles undergoing atrophy due to fasting, denervation, or inactivity [56-58].

Although numerous reports establish a role for ROS in stimulating autophagy, the mechanisms by which specific ROS promote autophagy remains unclear [54, 59, 60]. Nonetheless, it appears that both superoxide and H_2O_2 can accelerate autophagy in several ways. First, ROS have been shown to activate ATM which activates AMPK; activation of AMPK represses mTORC1 activation [54]. This is significant because active mTORC1 inhibits the activation of ULK1, which is required for the induction of autophagy [61, 62]. Moreover, oxidative stress-induced by exposure of cells to H_2O_2 results in increased expression of key autophagy components such as LC3, beclin 1, and increased formation of

autophagosomes [63]. Further, ROS appears to be essential in the regulation of Atg4 activity and the acceleration of autophagy. Specifically, ROS have been shown to inactivate Atg4 which prevents the premature cleavage of LC3 during autophagosome formation, an essential step in the process of autophagy [64] (Figure 2).

RNS regulation of autophagy

Whether RNS promotes or inhibits autophagy appears to depend upon the cell type investigated along with the levels and duration of RNS exposure [65]. For example, exposure of cells to low levels of RNS appears to increase autophagy [65]. Nonetheless, exposure of non-muscle cells to high levels of nitrosative stress inhibits Akt/mTORC1 signaling [46, 47]. Again, the mechanism to explain this observation is that exposure of cells to high levels of RNS results in the activation of ATM and LKB1. Activation of LKB1 increases the activity of AMPK which represses mTORC1 activation which promotes accelerated autophagy [46]. Therefore, the impact of RNS on autophagy appears to depend upon the level and duration of exposure to RNS. On one hand, low levels of RNS can increase mTORC1 activity which translates into inhibition of autophagy. In contrast, high levels of RNS can inhibit mTORC1 activation and accelerate autophagy.

Both ROS and RNS promote protein breakdown via the proteasome system of proteolysis

The complete ubiquitin-proteasome complex (26S) is comprised of a core proteasome subunit (20S) that provides an enclosed cavity where proteins are degraded. This 20S subunit is coupled with a regulatory complex (19S) connected to each end [66]. The 26S proteasome becomes active when ubiquitin covalently binds to protein substrates and tags them for degradation. The binding of ubiquitin to protein substrates is a multi-stage process that begins with the ubiquitin-activating enzyme (E1). Next, the ubiquitination of specific proteins is achieved by one of several ubiquitin-conjugating enzymes (E2s) followed by specialized protein ligases (E3s) that distinguish specific protein substrates (Figure 3).

Several skeletal muscle specific ubiquitin E3 ligases exist and at least two of these ligases play important roles in skeletal muscle atrophy (e.g., atrogin1 and muscle ring finger-1 (MuRF1)). For example, MuRF1 and atrogin1 knockout mice are resistant to disuse muscle atrophy [36]. The expression of both MuRF1 and atrogin1 are largely under the regulation of the Forkhead box O (FOXO) family of transcription factors. Skeletal muscle expresses three FOXO isoforms: 1) FOXO1; 2) FOXO3; and 3) FOXO4 [36]. When phosphorylated by Akt, all of these transcription factors are excluded from the nucleus. It follows that inactivation of Akt and dephosphorylation of FOXO results in translocation of FOXO to the nucleus and transcriptional activation of atrogin1 and MuRF1 genes. In this regard, evidence indicates that the translocation of FOXO transcription factors into the nucleus is required for the expression of both atrogin1 and MuRF1 [36]. In particular, nuclear translocation of both FOXO1 and FOXO3 is sufficient to increase the expression of these E3 ligases and promote muscle atrophy [51, 58, 67, 68].

Evidence reveals that oxidative stress can increase protein breakdown via the proteasome system in several ways. First, *in vitro* experiments reveal that oxidative stress increases gene expression of several important proteins involved in the proteasome system. For example,

exposure of C2C12 myotubes to ROS (i.e., H_2O_2) increases the expression of muscle specific E3 ligases. Specifically, ROS exposure increases the mRNA levels of both atrogin1 and muscle ring finger-1 (Murf-1) [69, 70]. Also, TNF-α-induced ROS production within murine myotubes promotes increased expression of atrogin1 [70]. Collectively, these findings indicate that exposure of myotubes to ROS increases the expression of muscle specific E3 ligases within the proteasome system (Figure 3). As discussed earlier, exposure of cells to ROS or RNS results in decreased Akt and mTOR1 activation; this relationship between ROS/RNS and Akt signaling likely explains why oxidative or nitrosative stress increases the expression of muscle specific E3 ligases.

Similar to the *in vitro* findings, oxidative stress appears to increase the expression of muscle specific E3 ligases in skeletal muscle *in vivo*. For example, prevention of disuse-induced oxidative stress by treatment of animals with mitochondrial-targeted antioxidants has been shown to protect against inactivity-induced expression of both atrogin1 and MuRF1 [21, 24].

Importantly, oxidative stress has also been shown to allosterically promote proteasome activity. Indeed, *in vivo* experiments reveal that disuse-induced skeletal muscle atrophy results in both oxidative stress and increased activity of the 20S proteasome [71, 72]. Importantly, treatment of animals with the antioxidant trolox can prevent inactivity-induced oxidative stress in skeletal muscle and protect against disuse-induced activation of the 20S proteasome. This finding is consistent with the concept that ROS serve as positive allosteric activators of the 20S proteasome [71, 72]. At present, it is unclear if increased production of RNS have an allosteric influence on 20S proteasome activity.

Finally, another nexus between oxidative stress and augmented proteolysis is the observation that oxidized proteins are more susceptible to proteolytic degradation by the proteasome system. Indeed, detailed evidence that ROS hastens protein breakdown was first provided by Kelvin Davies and colleagues [73-78], who. established that oxidized proteins are rapidly degraded by the ubiquitin-proteasome system. The mechanism to elucidate why oxidation enhances protein breakdown is, in part, due to unfolding of the molecule [74]. Indeed, oxidation of a protein causes a change of the secondary or tertiary structure so that previously screened peptide bonds are exposed to enzymatic hydrolysis [73-75]. Specifically, evidence indicates that several proteases will degrade oxidized proteins more efficiently than normal proteins [66, 79].

To summarize, abundant evidence exists to link increased abundance of ROS and RNS to augment muscle protein breakdown via the proteasome system of proteolysis. The impact of ROS and RNS proteasome activation is largely due to a down-regulation of Akt/mTOR signaling which results in increased FOXO signaling and expression of muscle specific E3 ligases.

ROS promote calpain activation in skeletal muscle

Calpains are Ca^{2+}-activated proteases that engage in selective cleavage of target proteins [80]. Although 15 calpain genes exist in humans, the two primary calpains that contribute to skeletal muscle atrophy are calpain 1 (μ-calpain) and calpain 2 (m-calpain) [81]. Active calpains are known to cleave over 100 celluar proteins including important cytoskeletal

proteins (e.g., titin, nebulin) that anchor contractile elements [81]. Importantly, calpain also cleaves numerous kinases, phosphatases, and can also cleave oxidized contractile proteins such as actin and myosin [79, 81].

Abundant evidence indicates that oxidative stress increases the expression of calpains in liver cells, C2C12 myotubes, and human myoblasts. For example, drug-induced oxidative stress increases the abundance of calpain 1 and 2 levels in the liver [82]. Further, exposure of C2C12 myotubes to H_2O_2 increases calpain 1 mRNA levels [69]. Importantly, exposure of human myoblasts to ROS has been shown to increase the expression of both calpain 1 and calpain 2 [83]. Collectively, these studies establish that oxidative stress promotes calpain expression in a variety of cell types including skeletal muscle.

Calpain activity is under allosteric control and studies reveal that oxidative stress increases calpain activity in muscle cells in culture. For example, exposure of C2C12 myotubes to ROS (i.e., 25 μM H_2O_2) increases calpain 1 activation [69]. Similarly, exposure of human myoblasts to H_2O_2 elevates both calpain 1 and calpain 2 activity [83]. Importantly, prevention of inactivity-induced oxidative stress by treatment with an antioxidant prevents calpain 1 activation in inactive skeletal muscle *in vivo* [84]. Collectively, these investigations confirm that oxidative stress in skeletal muscle can activate calpain.

The mechanism(s) responsible for ROS-mediated calpain activation is likely linked to oxidative stress-induced increases in cytosolic levels of free calcium [83, 85]. Calpain activity in cells is regulated by several factors including cytosolic calcium levels and the concentration of the endogenous calpain inhibitor, calpastatin [81, 86]. Indeed, it is well established that a sustained elevation of cytosolic free calcium promotes calpain activation in cells [81]. The direct link between ROS and elevated cytosolic calcium remains under investigation but oxidative stress has been shown to increase in intracellular free calcium [87]. At least two possible mechanisms can explain this observation. First, a potential connection between ROS and increased intracellular calcium is that ROS-mediated formation of reactive aldehydes (i.e., 4-hydroxy-2,3-trans-nonenal) can inhibit membrane calcium ATPase activity [88]. This is significant because inhibition of membrane calcium ATPase activity impairs calcium removal from the cell resulting in increased intracellular calcium levels. Second, ROS-mediated oxidation of the ryanodine receptor in skeletal muscle results in an intracellular calcium leak leading to increased cytosolic levels of free calcium [89]. Together, or independently, each of these mechanisms can promote an increase in cytosolic free calcium (Figure 4).

Impact of RNS on calpain activity in skeletal muscles

As discussed previously, inflammatory diseases promote an increase in both iNOS and NO production in skeletal muscles. In contrast, it is unclear if NO production increases in muscle during prolonged inactivity. Nonetheless, numerous studies have investigated the impact of NO on calpain activity in skeletal muscle fibers and numerous other cell types. In this regard, it is currently unknown if NO impacts calpain expression and it's abundance in the cell. However, several reports show that increased NO production can inhibit calpain activity by promoting s-nitrosylation of this protease [90-94]. Further, recent evidence suggests that an age-related decrease in neuronal NO synthase (nNOS) in skeletal muscle

results in decreased NO production, less s-nitrosylation of calpain, and increased calpain activity [94]. In this context, these investigators postulate that low NO production promotes higher calpain activity and accelerates fiber atrophy leading to sarcopenia [94]. To support this prediction, this investigation revealed that increased NO production in muscle causes S-nitrosylation of calpain and a delayed rate of sarcopenia. This finding seems paradoxical given that high levels of NO production increases transcripts involved in muscle atrophy such as MuRF1 and atrogin1. A potential explanation for these disparate findings is the possibility that an optimal level of NO exists in muscle fibers whereby an increase in NO above optimal levels results in fiber atrophy via activation of the proteasome and autophagy proteolytic pathways. Conversely, low levels of NO (i.e., below optimal) promotes calpain activation and fiber atrophy.

In regard to the link between low levels of NO in muscle and age-related sarcopenia, it is also feasible that a reduction in nNOS levels during aging could contribute to a loss of muscle mass via several mechanisms that are independent from calpain activation. For example, NO appears to be required for muscle growth during periods of increased muscle loading [95]. Further, a loss of nNOS in skeletal muscle is associated with structural defects at the neuromuscular junction [96]. Thus, a deficit in nNOS in muscle due to aging could contribute to sarcopenia through multiple mechanisms, including the failure to inhibit calpain activation.

In summary, the existing evidence indicates that both high and low levels of NO in skeletal muscle impact protease activity. Low levels of NO favors calpain activation whereas high NO levels promote activation of the proteasome and autophagy proteolytic pathways. Additional research is required to confirm or deny the prediction that an optimal level of NO in skeletal muscle is required to maintain protein balance in fibers.

Impact of ROS on activation of Caspase-3 in skeletal muscle

Growing evidence reveals that active caspase-3 contributes to muscle protein degradation and fiber atrophy [97] [98, 99]. 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 decreases the rate of disuse muscle atrophy [98, 99].

Abundant evidence exists to confirm that oxidative stress activates caspase-3 in skeletal muscle. For example, exposure of C2C12 myotubes to ROS (i.e., H_2O_2) activates capase-3 [69, 100]. Further, evidence exists that oxidative stress increases caspase-3 activation in skeletal muscle during disuse muscle atrophy [20, 21, 84]. Collectively, these findings indicate that increased production of ROS activates caspase-3 in muscle fibers.

Regulation of caspase-3 activity in cells involves numerous interrelated signaling pathways. In reference to caspase-3 activity during disuse muscle atrophy, it is possible that caspase-3 is activated by caspase-12 via a calcium release pathway and/or activated by the activation of caspase-9 (via a mitochondrial pathway) [86, 101]. Importantly, both of these pathways can be activated by ROS [86, 102] (Figure 5). However, it remains unclear which of these pathways dominate the ROS-mediated activation of caspase-3 in skeletal muscle during

conditions that promote muscle wasting. Moreover, the impact of increased NO on the activation of caspase-3 in muscle remains unknown.

Summary

To summarize, skeletal muscle atrophy occurs in response to numerous chronic diseases (i.e., COPD, heart failure, etc.) and as a result of prolonged periods of muscle inactivity. Both disease and inactivity-induced muscle atrophy occur due to accelerated proteolysis and a decrease in the rate of protein synthesis. Several lines of evidence directly connect both ROS and RNS to muscle atrophy via redox-mediated control of protein synthesis and proteolysis. Precisely, disease or inactivity-induced production of ROS or RNS in skeletal muscles depresses protein synthesis by inhibition of Akt or mTORC1 activation which reduces the rate of translation. Further, oxidative stress can accelerate proteolysis by: 1) stimulating gene expression and increasing the abundance of key proteins involved in proteolytic systems (i.e., autophagy, calpain, and proteasome); 2) increasing the activity of the 20S proteasome and activation of both calpain and caspase-3; and 3) oxidizing proteins and increasing their susceptibility to proteolytic breakdown.

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References

1. Kress JP, Hall JB. ICU-acquired weakness and recovery from critical illness. N Engl J Med. 2014; 370:1626–1635. [PubMed: 24758618]
2. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. Dis Model Mech. 2013; 6:25–39. [PubMed: 23268536]
3. Powers SK, Smuder AJ, Criswell DS. Mechanistic links between oxidative stress and disuse muscle atrophy. Antioxid Redox Signal. 2011; 15:2519–2528. [PubMed: 21457104]
4. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare R, Dekhuijzen PN, Franssen F, Gayan-Ramirez G, Gea J, Gosker HR, Gosselink R, Hayot M, Hussain SN, Janssens W, Polkey MI, Roca J, Saey D, Schols AM, Spruit MA, Steiner M, Taivassalo T, Troosters T, Vogiatzis I, Wagner PD. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2014; 189:e15–62. [PubMed: 24787074]
5. Tisdale MJ. Mechanisms of cancer cachexia. Physiol Rev. 2009; 89:381–410. [PubMed: 19342610]
6. Reid MB, Moylan JS. Beyond atrophy: redox mechanisms of muscle dysfunction in chronic inflammatory disease. J Physiol. 2011; 589:2171–2179. [PubMed: 21320886]
7. Suzuki N, Motohashi N, Uezumi A, Fukada S, Yoshimura T, Itoyama Y, Aoki M, Miyagoe-Suzuki Y, Takeda S. NO production results in suspension-induced muscle atrophy through dislocation of neuronal NOS. J Clin Invest. 2007; 117:2468–2476. [PubMed: 17786240]
8. Lawler JM, Kunst M, Hord JM, Lee Y, Joshi K, Botchlett RE, Ramirez A, Martinez DA. EUK-134 ameliorates nNOSmu translocation and skeletal muscle fiber atrophy during short-term mechanical unloading. Am J Physiol Regul Integr Comp Physiol. 2014; 306:R470–482. [PubMed: 24477538]
9. Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. Compr Physiol. 2011; 1:941–969. [PubMed: 23737208]
10. Cabello-Verrugio C, Cordova G, Salas JD. Angiotensin II: role in skeletal muscle atrophy. Curr Protein Pept Sci. 2012; 13:560–569. [PubMed: 22974090]

11. Yoshida T, Tabony AM, Galvez S, Mitch WE, Higashi Y, Sukhanov S, Delafontaine P. Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: potential therapeutic targets for cardiac cachexia. Int J Biochem Cell Biol. 2013; 45:2322–2332. [PubMed: 23769949]
12. Kwon OS, Smuder A, Wiggs MP, Hall SE, Sollanek K, Morton A, Talbert E, Toklu HZ, Tumer N, Powers SK. AT1 receptor blocker losartan protects against mechanical ventilation-induced diaphragmatic dysfunction. J Appl Physiol (1985). 2015 jap 00237 02015.
13. Sriram S, Subramanian S, Sathiakumar D, Venkatesh R, Salerno MS, McFarlane CD, Kambadur R, Sharma M. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-kappaB. Aging Cell. 2011; 10:931–948. [PubMed: 21771249]
14. Ziaaldini MM, Koltai E, Csende Z, Goto S, Boldogh I, Taylor AW, Radak Z. Exercise training increases anabolic and attenuates catabolic and apoptotic processes in aged skeletal muscle of male rats. Exp Gerontol. 2015; 67:9–14. [PubMed: 25910622]
15. Nin N, Cassina A, Boggia J, Alfonso E, Botti H, Peluffo G, Trostchansky A, Batthyany C, Radi R, Rubbo H, Hurtado FJ. Septic diaphragmatic dysfunction is prevented by Mn(III)porphyrin therapy and inducible nitric oxide synthase inhibition. Intensive Care Med. 2004; 30:2271–2278. [PubMed: 15349724]
16. Adams V, Yu J, Mobius-Winkler S, Linke A, Weigl C, Hilbrich L, Schuler G, Hambrecht R. Increased inducible nitric oxide synthase in skeletal muscle biopsies from patients with chronic heart failure. Biochem Mol Med. 1997; 61:152–160. [PubMed: 9259980]
17. Boveris A, Alvarez S, Navarro A. The role of mitochondrial nitric oxide synthase in inflammation and septic shock. Free Radic Biol Med. 2002; 33:1186–1193. [PubMed: 12398926]
18. Agusti A, Morla M, Sauleda J, Saus C, Busquets X. NF-kappaB activation and iNOS upregulation in skeletal muscle of patients with COPD and low body weight. Thorax. 2004; 59:483–487. [PubMed: 15170030]
19. Kondo H, Miura M, Itokawa Y. Oxidative stress in skeletal muscle atrophied by immobilization. Acta Physiol Scand. 1991; 142:527–528. [PubMed: 1950601]
20. Min K, Smuder AJ, Kwon OS, Kavazis AN, Szeto HH, Powers SK. Mitochondrial-targeted antioxidants protect skeletal muscle against immobilization-induced muscle atrophy. J Appl Physiol (1985). 2011; 111:1459–1466. [PubMed: 21817113]
21. Powers SK, Hudson MB, Nelson WB, Talbert EE, Min K, Szeto HH, Kavazis AN, Smuder AJ. Mitochondria-targeted antioxidants protect against mechanical ventilation-induced diaphragm weakness. Crit Care Med. 2011; 39:1749–1759. [PubMed: 21460706]
22. Whidden MA, McClung JM, Falk DJ, Hudson MB, Smuder AJ, Nelson WB, Powers SK. Xanthine oxidase contributes to mechanical ventilation-induced diaphragmatic oxidative stress and contractile dysfunction. J Appl Physiol (1985). 2009; 106:385–394. [PubMed: 18974366]
23. McClung JM, Van Gammeren D, Whidden MA, Falk DJ, Kavazis AN, Hudson MB, Gayan-Ramirez G, Decramer M, DeRuisseau KC, Powers SK. Apocynin attenuates diaphragm oxidative stress and protease activation during prolonged mechanical ventilation. Crit Care Med. 2009; 37:1373–1379. [PubMed: 19242334]
24. Talbert EE, Smuder AJ, Min K, Kwon OS, Szeto HH, Powers SK. Immobilization-induced activation of key proteolytic systems in skeletal muscles is prevented by a mitochondria-targeted antioxidant. J Appl Physiol (1985). 2013; 115:529–538. [PubMed: 23766499]
25. Talbert EE, Smuder AJ, Min K, Kwon OS, Powers SK. Calpain and caspase-3 play required roles in immobilization-induced limb muscle atrophy. J Appl Physiol (1985). 2013; 114:1482–1489. [PubMed: 23471945]
26. Kavazis AN, Talbert EE, Smuder AJ, Hudson MB, Nelson WB, Powers SK. Mechanical ventilation induces diaphragmatic mitochondrial dysfunction and increased oxidant production. Free Radic Biol Med. 2009; 46:842–850. [PubMed: 19185055]
27. Vitadello M, Germinario E, Ravara B, Libera LD, Danieli-Betto D, Gorza L. Curcumin counteracts loss of force and atrophy of hindlimb unloaded rat soleus by hampering neuronal nitric oxide synthase untethering from sarcolemma. J Physiol. 2014; 592:2637–2652. [PubMed: 24710058]
28. Sandona D, Desaphy JF, Camerino GM, Bianchini E, Ciciliot S, Danieli-Betto D, Dobrowolny G, Furlan S, Germinario E, Goto K, Gutsmann M, Kawano F, Nakai N, Ohira T, Ohno Y, Picard A,

Salanova M, Schiffl G, Blottner D, Musaro A, Ohira Y, Betto R, Conte D, Schiaffino S. Adaptation of mouse skeletal muscle to long-term microgravity in the MDS mission. PLoS One. 2012; 7:e33232. [PubMed: 22470446]

29. Shenkman BS, Nemirovskaya TL, Lomonosova YN. No-dependent signaling pathways in unloaded skeletal muscle. Front Physiol. 2015; 6:298. [PubMed: 26582991]
30. Lomonosova YN, Kalamkarov GR, Bugrova AE, Shevchenko TF, Kartashkina NL, Lysenko EA, Shvets VI, Nemirovskaya TL. Protective effect of L-Arginine administration on proteins of unloaded m. soleus. Biochemistry (Mosc). 2011; 76:571–580. [PubMed: 21639837]
31. Van Gammeren D, Falk DJ, Deering MA, Deruisseau KC, Powers SK. Diaphragmatic nitric oxide synthase is not induced during mechanical ventilation. J Appl Physiol (1985). 2007; 102:157–162. [PubMed: 16931563]
32. Kondo H, Miura M, Itokawa Y. Antioxidant enzyme systems in skeletal muscle atrophied by immobilization. Pflugers Arch. 1993; 422:404–406. [PubMed: 8437891]
33. Kondo H, Miura M, Kodama J, Ahmed SM, Itokawa Y. Role of iron in oxidative stress in skeletal muscle atrophied by immobilization. Pflugers Arch. 1992; 421:295–297. [PubMed: 1528723]
34. Kondo H, Miura M, Nakagaki I, Sasaki S, Itokawa Y. Trace element movement and oxidative stress in skeletal muscle atrophied by immobilization. Am J Physiol. 1992; 262:E583–590. [PubMed: 1590370]
35. Kondo H, Nakagaki I, Sasaki S, Hori S, Itokawa Y. Mechanism of oxidative stress in skeletal muscle atrophied by immobilization. Am J Physiol. 1993; 265:E839–844. [PubMed: 8279538]
36. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. FEBS J. 2013; 280:4294–4314. [PubMed: 23517348]
37. Ham DJ, Caldow MK, Lynch GS, Koopman R. Leucine as a treatment for muscle wasting: a critical review. Clin Nutr. 2014; 33:937–945. [PubMed: 25444557]
38. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. Crit Rev Biochem Mol Biol. 2014; 49:59–68. [PubMed: 24237131]
39. Mounier R, Lantier L, Leclerc J, Sotiropoulos A, Foretz M, Viollet B. Antagonistic control of muscle cell size by AMPK and mTORC1. Cell Cycle. 2011; 10:2640–2646. [PubMed: 21799304]
40. Magnuson B, Ekim B, Fingar DC. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem J. 2012; 441:1–21. [PubMed: 22168436]
41. Tan PL, Shavlakadze T, Grounds MD, Arthur PG. Differential thiol oxidation of the signaling proteins Akt, PTEN or PP2A determines whether Akt phosphorylation is enhanced or inhibited by oxidative stress in C2C12 myotubes derived from skeletal muscle. Int J Biochem Cell Biol. 2015; 62:72–79. [PubMed: 25737250]
42. Pham FH, Sugden PH, Clerk A. Regulation of protein kinase B and 4E-BP1 by oxidative stress in cardiac myocytes. Circ Res. 2000; 86:1252–1258. [PubMed: 10864916]
43. O'Loghlen A, Perez-Morgado MI, Salinas M, Martin ME. N-acetyl-cysteine abolishes hydrogen peroxide-induced modification of eukaryotic initiation factor 4F activity via distinct signalling pathways. Cell Signal. 2006; 18:21–31. [PubMed: 15907373]
44. Zhang L, Kimball SR, Jefferson LS, Shenberger JS. Hydrogen peroxide impairs insulin-stimulated assembly of mTORC1. Free Radic Biol Med. 2009; 46:1500–1509. [PubMed: 19281842]
45. Hudson MB, Smuder AJ, Nelson WB, Wiggs MP, Shimkus KL, Fluckey JD, Szeto HH, Powers SK. Partial Support Ventilation and Mitochondrial-Targeted Antioxidants Protect against Ventilator-Induced Decreases in Diaphragm Muscle Protein Synthesis. PLoS One. 2015; 10:e0137693. [PubMed: 26361212]
46. Tripathi DN, Chowdhury R, Trudel LJ, Tee AR, Slack RS, Walker CL, Wogan GN. Reactive nitrogen species regulate autophagy through ATM-AMPK-TSC2-mediated suppression of mTORC1. Proc Natl Acad Sci U S A. 2013; 110:E2950–2957. [PubMed: 23878245]
47. Song P, Wu Y, Xu J, Xie Z, Dong Y, Zhang M, Zou MH. Reactive nitrogen species induced by hyperglycemia suppresses Akt signaling and triggers apoptosis by upregulating phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) in an LKB1-dependent manner. Circulation. 2007; 116:1585–1595. [PubMed: 17875968]
48. Guo Z, Deshpande R, Paull TT. ATM activation in the presence of oxidative stress. Cell Cycle. 2010; 9:4805–4811. [PubMed: 21150274]

49. Guo Z, Kozlov S, Lavin MF, Person MD, Paull TT. ATM activation by oxidative stress. Science. 2010; 330:517–521. [PubMed: 20966255]
50. Ngo JK, Pomatto LC, Davies KJ. Upregulation of the mitochondrial Lon Protease allows adaptation to acute oxidative stress but dysregulation is associated with chronic stress, disease, and aging. Redox Biol. 2013; 1:258–264. [PubMed: 24024159]
51. Zhao J, Brault JJ, Schild A, Cao P, Sandri M, Schiaffino S, Lecker SH, Goldberg AL. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab. 2007; 6:472–483. [PubMed: 18054316]
52. Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, Ballabio A, Bamber BA, Bassham DC, Bergamini E, Bi X, Biard-Piechaczyk M, Blum JS, Bredesen DE, Brodsky JL, Brumell JH, Brunk UT, Bursch W, Camougrand N, Cebollero E, Cecconi F, Chen Y, Chin LS, Choi A, Chu CT, Chung J, Clarke PG, Clark RS, Clarke SG, Clave C, Cleveland JL, Codogno P, Colombo MI, Coto-Montes A, Cregg JM, Cuervo AM, Debnath J, Demarchi F, Dennis PB, Dennis PA, Deretic V, Devenish RJ, Di Sano F, Dice JF, Difiglia M, Dinesh-Kumar S, Distelhorst CW, Djavaheri-Mergny M, Dorsey FC, Droge W, Dron M, Dunn WA Jr, Duszenko M, Eissa NT, Elazar Z, Esclatine A, Eskelinen EL, Fesus L, Finley KD, Fuentes JM, Fueyo J, Fujisaki K, Galliot B, Gao FB, Gewirtz DA, Gibson SB, Gohla A, Goldberg AL, Gonzalez R, Gonzalez-Estevez C, Gorski S, Gottlieb RA, Haussinger D, He YW, Heidenreich K, Hill JA, Hoyer-Hansen M, Hu X, Huang WP, Iwasaki A, Jaattela M, Jackson WT, Jiang X, Jin S, Johansen T, Jung JU, Kadowaki M, Kang C, Kelekar A, Kessel DH, Kiel JA, Kim HP, Kimchi A, Kinsella TJ, Kiselyov K, Kitamoto K, Knecht E, Komatsu M, Kominami E, Kondo S, Kovacs AL, Kroemer G, Kuan CY, Kumar R, Kundu M, Landry J, Laporte M, Le W, Lei HY, Lenardo MJ, Levine B, Lieberman A, Lim KL, Lin FC, Liou W, Liu LF, Lopez-Berestein G, Lopez-Otin C, Lu B, Macleod KF, Malorni W, Martinet W, Matsuoka K, Mautner J, Meijer AJ, Melendez A, Michels P, Miotto G, Mistiaen WP, Mizushima N, Mograbi B, Monastyrska I, Moore MN, Moreira PI, Moriyasu Y, Motyl T, Munz C, Murphy LO, Naqvi NI, Neufeld TP, Nishino I, Nixon RA, Noda T, Nurnberg B, Ogawa M, Oleinick NL, Olsen LJ, Ozpolat B, Paglin S, Palmer GE, Papassideri I, Parkes M, Perlmutter DH, Perry G, Piacentini M, Pinkas-Kramarski R, Prescott M, Proikas-Cezanne T, Raben N, Rami A, Reggiori F, Rohrer B, Rubinsztein DC, Ryan KM, Sadoshima J, Sakagami H, Sakai Y, Sandri M, Sasakawa C, Sass M, Schneider C, Seglen PO, Seleverstov O, Settleman J, Shacka JJ, Shapiro IM, Sibirny A, Silva-Zacarin EC, Simon HU, Simone C, Simonsen A, Smith MA, Spanel-Borowski K, Srinivas V, Steeves M, Stenmark H, Stromhaug PE, Subauste CS, Sugimoto S, Sulzer D, Suzuki T, Swanson MS, Tabas I, Takeshita F, Talbot NJ, Talloczy Z, Tanaka K, Tanida I, Taylor GS, Taylor JP, Terman A, Tettamanti G, Thompson CB, Thumm M, Tolkovsky AM, Tooze SA, Truant R, Tumanovska LV, Uchiyama Y, Ueno T, Uzcategui NL, van der Klei I, Vaquero EC, Vellai T, Vogel MW, Wang HG, Webster P, Wiley JW, Xi Z, Xiao G, Yahalom J, Yang JM, Yap G, Yin XM, Yoshimori T, Yu L, Yue Z, Yuzaki M, Zabirnyk O, Zheng X, Zhu X, Deter RL. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy. 2008; 4:151–175. [PubMed: 18188003]
53. Feng Y, Yao Z, Klionsky DJ. How to control self-digestion: transcriptional, post-transcriptional, and post-translational regulation of autophagy. Trends Cell Biol. 2015; 25:354–363. [PubMed: 25759175]
54. Navarro-Yepes J, Burns M, Anandhan A, Khalimonchuk O, del Razo LM, Quintanilla-Vega B, Pappa A, Panayiotidis MI, Franco R. Oxidative stress, redox signaling, and autophagy: cell death versus survival. Antioxid Redox Signal. 2014; 21:66–85. [PubMed: 24483238]
55. Bechet D, Tassa A, Taillandier D, Combaret L, Attaix D. Lysosomal proteolysis in skeletal muscle. Int J Biochem Cell Biol. 2005; 37:2098–2114. [PubMed: 16125113]
56. Sandri M. New findings of lysosomal proteolysis in skeletal muscle. Curr Opin Clin Nutr Metab Care. 2011; 14:223–229. [PubMed: 21415731]
57. Hussain SN, Mofarrahi M, Sigala I, Kim HC, Vassilakopoulos T, Maltais F, Bellenis I, Chaturvedi R, Gottfried SB, Metrakos P, Danialou G, Matecki S, Jaber S, Petrof BJ, Goldberg P. Mechanical ventilation-induced diaphragm disuse in humans triggers autophagy. Am J Respir Crit Care Med. 2010; 182:1377–1386. [PubMed: 20639440]
58. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, Burden SJ, Di Lisi R, Sandri C, Zhao J, Goldberg AL, Schiaffino S, Sandri M. FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab. 2007; 6:458–471. [PubMed: 18054315]

59. Scherz-Shouval R, Elazar Z. Regulation of autophagy by ROS: physiology and pathology. Trends Biochem Sci. 2011; 36:30–38. [PubMed: 20728362]
60. Pajares M, Jimenez-Moreno N, Dias IH, Debelec B, Vucetic M, Fladmark KE, Basaga H, Ribaric S, Milisav I, Cuadrado A. Redox control of protein degradation. Redox Biol. 2015; 6:409–420. [PubMed: 26381917]
61. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. Curr Opin Cell Biol. 2010; 22:124–131. [PubMed: 20034776]
62. Kundu M, Thompson CB. Autophagy: basic principles and relevance to disease. Annu Rev Pathol. 2008; 3:427–455. [PubMed: 18039129]
63. Kim RJ, Hah YS, Sung CM, Kang JR, Park HB. Do antioxidants inhibit oxidative-stress-induced autophagy of tenofibroblasts? J Orthop Res. 2014; 32:937–943. [PubMed: 24644146]
64. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J. 2007; 26:1749–1760. [PubMed: 17347651]
65. Lee J, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J. 2012; 441:523–540. [PubMed: 22187934]
66. Grune T, Merker K, Sandig G, Davies KJ. Selective degradation of oxidatively modified protein substrates by the proteasome. Biochem Biophys Res Commun. 2003; 305:709–718. [PubMed: 12763051]
67. McLoughlin TJ, Smith SM, DeLong AD, Wang H, Unterman TG, Esser KA. FoxO1 induces apoptosis in skeletal myotubes in a DNA-binding-dependent manner. Am J Physiol Cell Physiol. 2009; 297:C548–555. [PubMed: 19553561]
68. Southgate RJ, Neill B, Prelovsek O, El-Osta A, Kamei Y, Miura S, Ezaki O, McLoughlin TJ, Zhang W, Unterman TG, Febbraio MA. FOXO1 regulates the expression of 4E-BP1 and inhibits mTOR signaling in mammalian skeletal muscle. J Biol Chem. 2007; 282:21176–21186. [PubMed: 17510058]
69. McClung JM, Judge AR, Talbert EE, Powers SK. Calpain-1 is required for hydrogen peroxide-induced myotube atrophy. Am J Physiol Cell Physiol. 2009; 296:C363–371. [PubMed: 19109522]
70. Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, Reid MB. TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. FASEB J. 2005; 19:362–370. [PubMed: 15746179]
71. Betters JL, Criswell DS, Shanely RA, Van Gammeren D, Falk D, Deruisseau KC, Deering M, Yimlamai T, Powers SK. Trolox attenuates mechanical ventilation-induced diaphragmatic dysfunction and proteolysis. Am J Respir Crit Care Med. 2004; 170:1179–1184. [PubMed: 15374845]
72. McClung JM, Kavazis AN, Whidden MA, DeRuisseau KC, Falk DJ, Criswell DS, Powers SK. Antioxidant administration attenuates mechanical ventilation-induced rat diaphragm muscle atrophy independent of protein kinase B (PKB Akt) signalling. J Physiol. 2007; 585:203–215. [PubMed: 17916612]
73. Davies KJ, Lin SW, Pacifici RE. Protein damage and degradation by oxygen radicals. IV. Degradation of denatured protein. J Biol Chem. 1987; 262:9914–9920. [PubMed: 3036878]
74. Davies KJ, Delsignore ME. Protein damage and degradation by oxygen radicals. III. Modification of secondary and tertiary structure. J Biol Chem. 1987; 262:9908–9913. [PubMed: 3036877]
75. Davies KJ, Delsignore ME, Lin SW. Protein damage and degradation by oxygen radicals. II. Modification of amino acids. J Biol Chem. 1987; 262:9902–9907. [PubMed: 3036876]
76. Davies KJ. Protein damage and degradation by oxygen radicals. I. general aspects. J Biol Chem. 1987; 262:9895–9901. [PubMed: 3036875]
77. Davies KJ, Goldberg AL. Proteins damaged by oxygen radicals are rapidly degraded in extracts of red blood cells. J Biol Chem. 1987; 262:8227–8234. [PubMed: 3597373]
78. Davies KJ, Goldberg AL. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. J Biol Chem. 1987; 262:8220–8226. [PubMed: 3597372]

79. Smuder AJ, Kavazis AN, Hudson MB, Nelson WB, Powers SK. Oxidation enhances myofibrillar protein degradation via calpain and caspase-3. Free Radic Biol Med. 2010; 49:1152–1160. [PubMed: 20600829]
80. Campbell RL, Davies PL. Structure-function relationships in calpains. Biochem J. 2012; 447:335–351. [PubMed: 23035980]
81. Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev. 2003; 83:731–801. [PubMed: 12843408]
82. Pal S, Ghosh M, Ghosh S, Bhattacharyya S, Sil PC. Atorvastatin induced hepatic oxidative stress and apoptotic damage via MAPKs, mitochondria, calpain and caspase12 dependent pathways. Food Chem Toxicol. 2015; 83:36–47. [PubMed: 26051349]
83. Dargelos E, Brule C, Stuelsatz P, Mouly V, Veschambre P, Cottin P, Poussard S. Up-regulation of calcium-dependent proteolysis in human myoblasts under acute oxidative stress. Exp Cell Res. 2010; 316:115–125. [PubMed: 19651121]
84. Whidden MA, Smuder AJ, Wu M, Hudson MB, Nelson WB, Powers SK. Oxidative stress is required for mechanical ventilation-induced protease activation in the diaphragm. J Appl Physiol (1985). 2010; 108:1376–1382. [PubMed: 20203072]
85. Dargelos E, Poussard S, Brule C, Daury L, Cottin P. Calcium-dependent proteolytic system and muscle dysfunctions: a possible role of calpains in sarcopenia. Biochimie. 2008; 90:359–368. [PubMed: 17881114]
86. Powers SK, Kavazis AN, McClung JM. Oxidative stress and disuse muscle atrophy. J Appl Physiol (1985). 2007; 102:2389–2397. [PubMed: 17289908]
87. Kourie JI. Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol. 1998; 275:C1–24. [PubMed: 9688830]
88. Siems W, Capuozzo E, Lucano A, Salerno C, Crifo C. High sensitivity of plasma membrane ion transport ATPases from human neutrophils towards 4-hydroxy-2,3-trans-nonenal. Life Sci. 2003; 73:2583–2590. [PubMed: 12967682]
89. Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, Shiomi T, Zalk R, Lacampagne A, Marks AR. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. Cell Metab. 2011; 14:196–207. [PubMed: 21803290]
90. Koh TJ, Tidball JG. Nitric oxide inhibits calpain-mediated proteolysis of talin in skeletal muscle cells. Am J Physiol Cell Physiol. 2000; 279:C806–812. [PubMed: 10942731]
91. Xu PT, Li Q, Sheng JJ, Chang H, Song Z, Yu ZB. Passive stretch reduces calpain activity through nitric oxide pathway in unloaded soleus muscles. Mol Cell Biochem. 2012; 367:113–124. [PubMed: 22547201]
92. Li YP, Liu R, Zhang WG, Fu QQ, Liu N, Zhou GH. Effect of nitric oxide on mu-calpain activation, protein proteolysis, and protein oxidation of pork during post-mortem aging. J Agric Food Chem. 2014; 62:5972–5977. [PubMed: 24910006]
93. Michetti M, Salamino F, Melloni E, Pontremoli S. Reversible inactivation of calpain isoforms by nitric oxide. Biochem Biophys Res Commun. 1995; 207:1009–1014. [PubMed: 7864886]
94. Samengo G, Avik A, Fedor B, Whittaker D, Myung KH, Wehling-Henricks M, Tidball JG. Age-related loss of nitric oxide synthase in skeletal muscle causes reductions in calpain S-nitrosylation that increase myofibril degradation and sarcopenia. Aging Cell. 2012; 11:1036–1045. [PubMed: 22950758]
95. Sellman JE, DeRuisseau KC, Betters JL, Lira VA, Soltow QA, Selsby JT, Criswell DS. In vivo inhibition of nitric oxide synthase impairs upregulation of contractile protein mRNA in overloaded plantaris muscle. J Appl Physiol (1985). 2006; 100:258–265. [PubMed: 16166235]
96. Shiao T, Fond A, Deng B, Wehling-Henricks M, Adams ME, Froehner SC, Tidball JG. Defects in neuromuscular junction structure in dystrophic muscle are corrected by expression of a NOS transgene in dystrophin-deficient muscles, but not in muscles lacking alpha-and beta1-syntrophins. Hum Mol Genet. 2004; 13:1873–1884. [PubMed: 15238508]
97. Du J, Wang X, Miereles C, Bailey JL, Debigare R, Zheng B, Price SR, Mitch WE. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. J Clin Invest. 2004; 113:115–123. [PubMed: 14702115]

98. McClung JM, Kavazis AN, DeRuisseau KC, Falk DJ, Deering MA, Lee Y, Sugiura T, Powers SK. Caspase-3 regulation of diaphragm myonuclear domain during mechanical ventilation-induced atrophy. Am J Respir Crit Care Med. 2007; 175:150–159. [PubMed: 17082496]

99. Nelson WB, Smuder AJ, Hudson MB, Talbert EE, Powers SK. Cross-talk between the calpain and caspase-3 proteolytic systems in the diaphragm during prolonged mechanical ventilation. Crit Care Med. 2012; 40:1857–1863. [PubMed: 22487998]

100. Siu PM, Wang Y, Alway SE. Apoptotic signaling induced by H2O2-mediated oxidative stress in differentiated C2C12 myotubes. Life Sci. 2009; 84:468–481. [PubMed: 19302811]

101. Powers SK, Kavazis AN, DeRuisseau KC. Mechanisms of disuse muscle atrophy: role of oxidative stress. Am J Physiol Regul Integr Comp Physiol. 2005; 288:R337–344. [PubMed: 15637170]

102. Primeau AJ, Adhihetty PJ, Hood DA. Apoptosis in heart and skeletal muscle. Can J Appl Physiol. 2002; 27:349–395. [PubMed: 12442352]

Highlights

- Skeletal muscle atrophy occurs in response to numerous chronic diseases (i.e., COPD, heart failure, etc.) and as a result of prolonged periods of muscle inactivity.
- Both disease and inactivity-induced muscle atrophy occur due to accelerated proteolysis and a decrease in the rate of protein synthesis.
- Several lines of evidence directly connect increased production of both reactive oxygen species and reactive nitrogen species to muscle atrophy via redox-mediated control of protein synthesis and proteolysis.

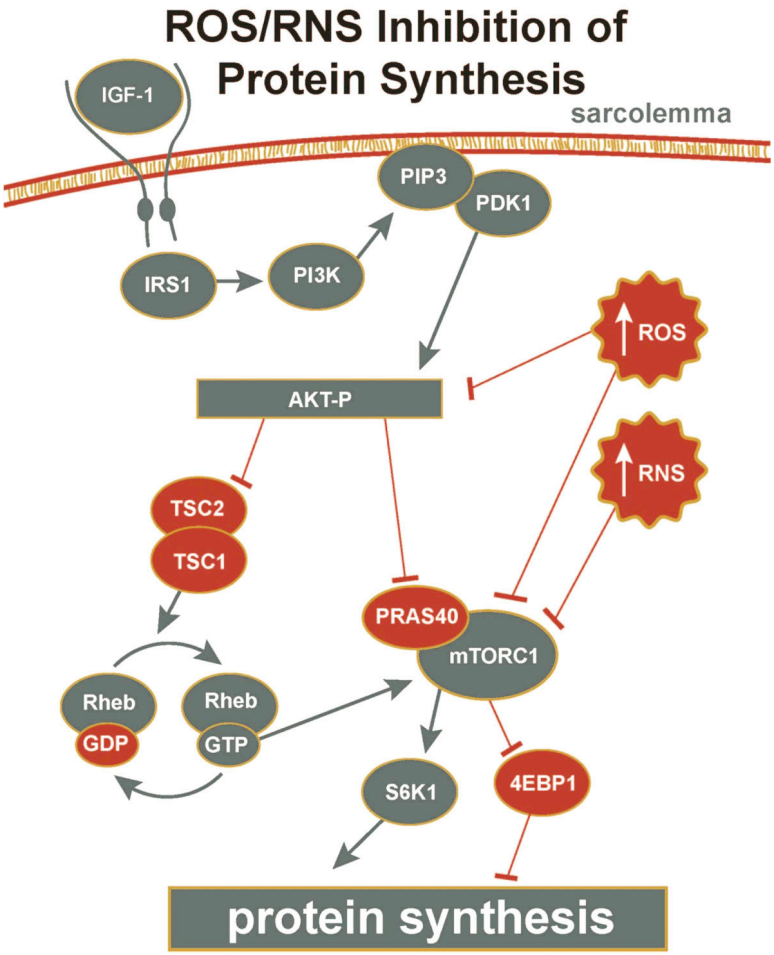

Figure 1.

Schematic of the Akt/mTORC1 signaling pathway leading to accelerated translation and increased protein synthesis. Note that both ROS and RNS have been shown to impede one or more steps leading to a decrease in muscle protein synthesis. See text for details.

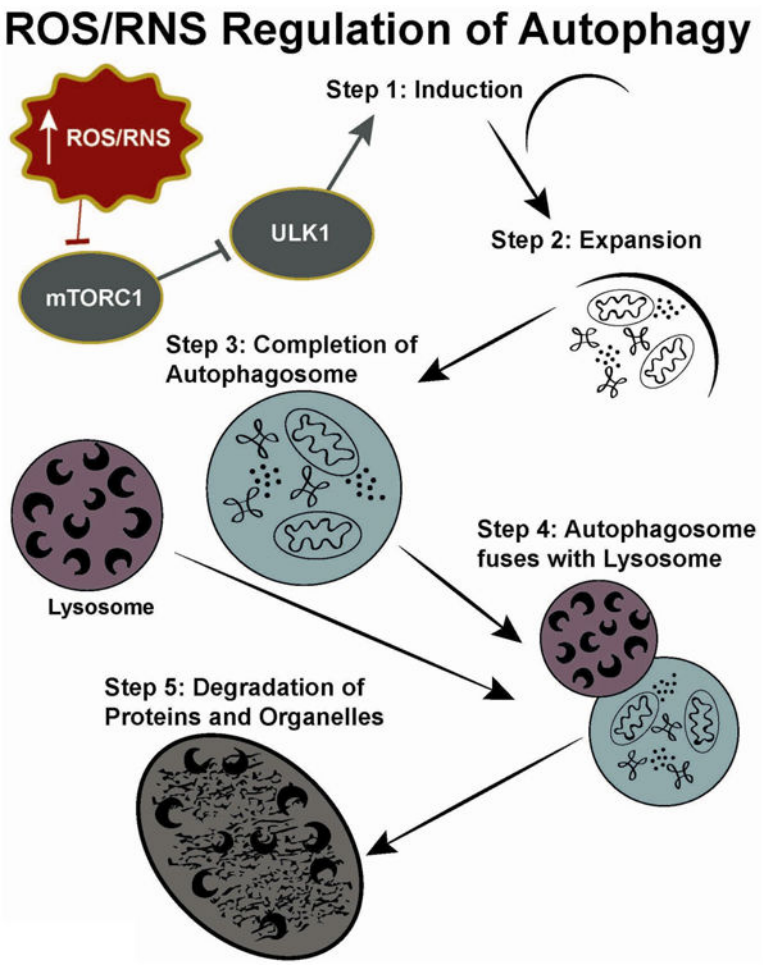

Figure 2.

Illustration of the steps leading to autophagy. Autophagy progresses during a five step process: 1) Induction; 2) Expansion; 3) Completion of autophagosome; 4) Autophagosome fuses with lysosome; and 5) Degradation of proteins and organelles. As discussed in the text, both ROS and RNS have the potential to accelerate autophagy flux. See text for details.

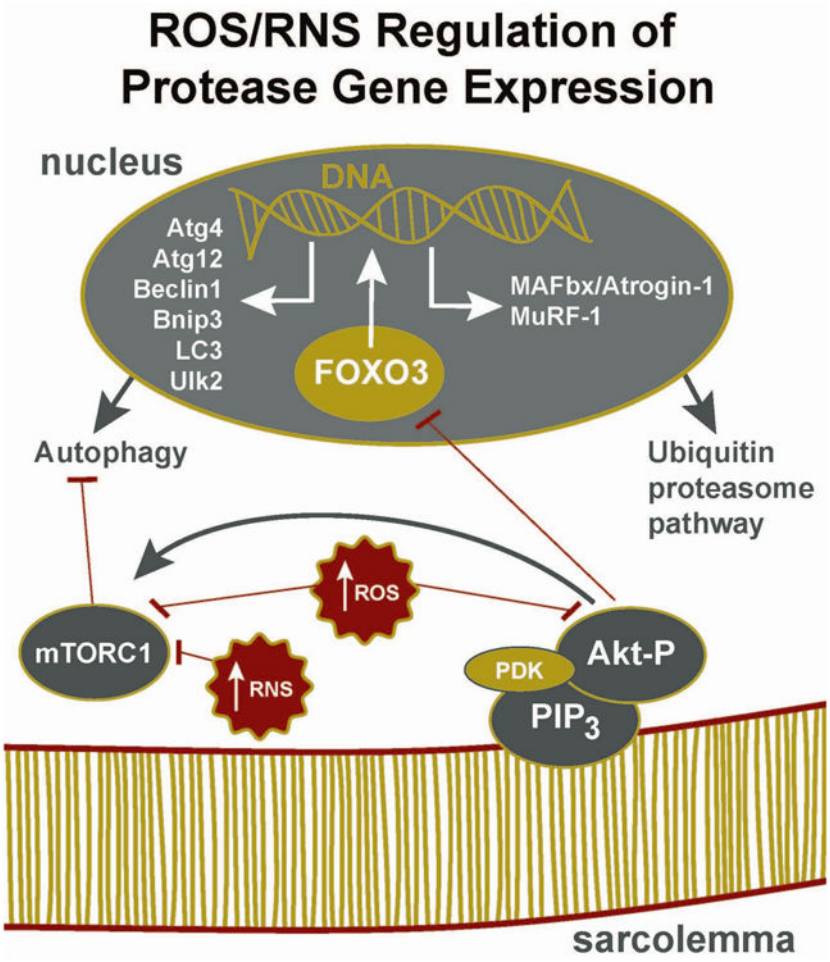

Figure 3.

Simplified diagram illustrating the role of FOXO3 in promoting gene expression of both muscle specific E3 ligases (i.e., atrogin 1 and MuRF1) and autophagy genes. Note that both ROS and RNS have the potential to accelerate protein breakdown via the proteasome and autophagy pathway. See text for more details.

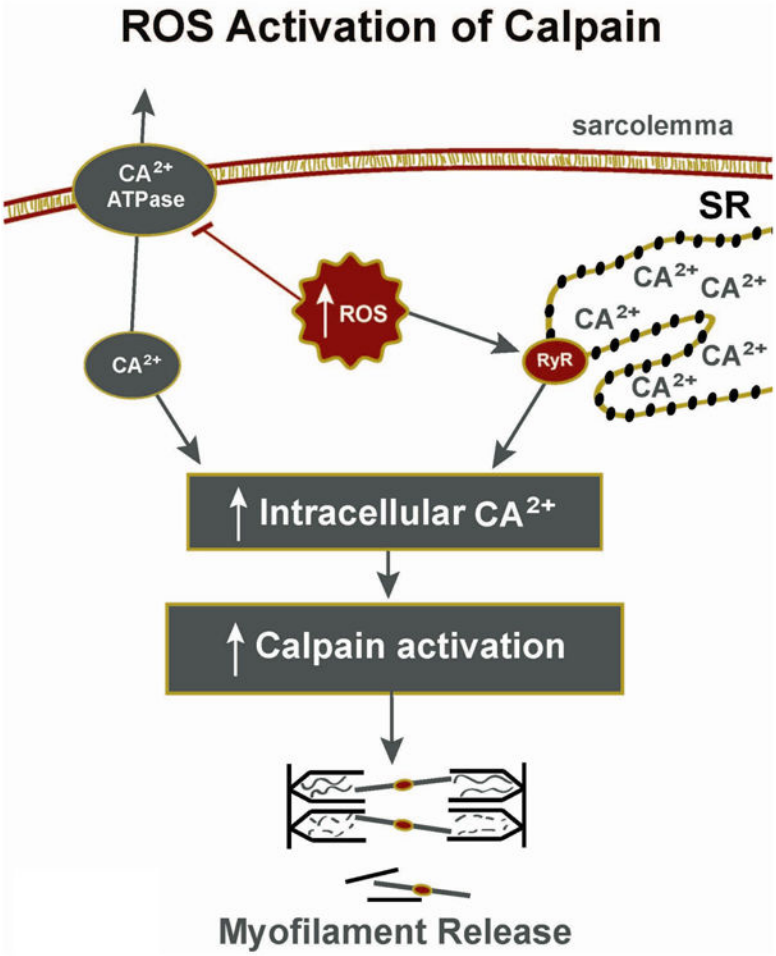

Figure 4.

Simplistic overview of the role that ROS plays in promoting an increase in intracellular calcium and calpain activation in skeletal muscle. See text for more details.

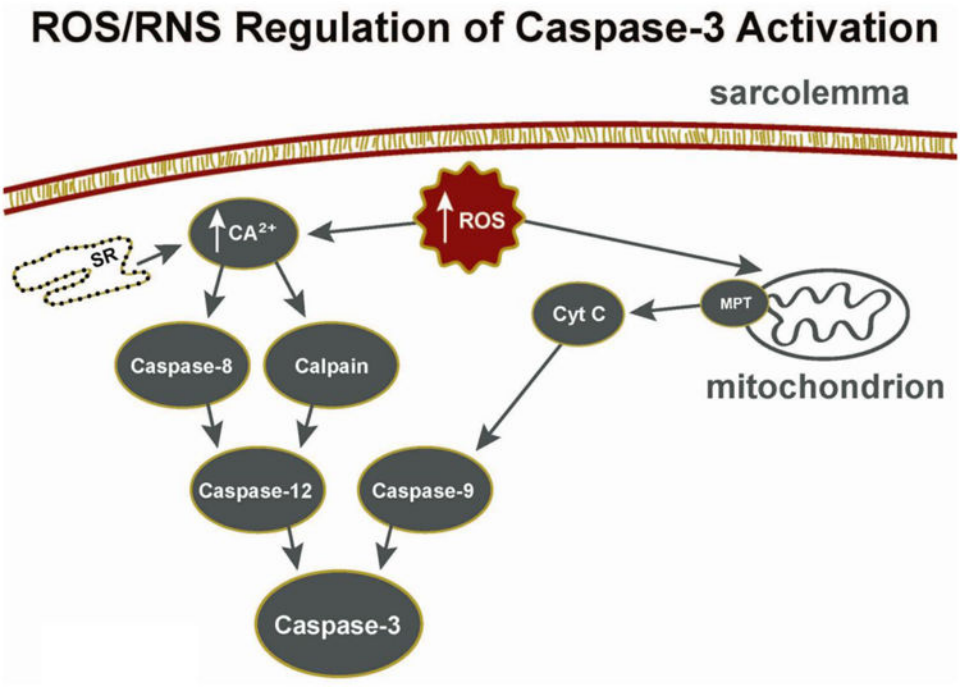

Figure 5.

Summary of the role that ROS/RNS play in activation of caspase-3 in skeletal muscle. Note that ROS can promote caspase-3 activation via both an intrinsic pathway (mitochondrial) or extrinsic pathway involving the activation of caspase-12. See text for more details.