# Role of Oxidative Stress in Skeletal Muscle Weakness

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Introduction

Our laboratory has a longstanding interest in the weakness of respiratory and limb skeletal muscle caused by reactive oxygen species (ROS). Our current experimental model (Fig 1) proposes that ROS production by muscle fibers is increased in a variety of physiological and pathophysiological conditions. These include chronic inflammation, aging, mechanical unloading, and strenuous exercise.
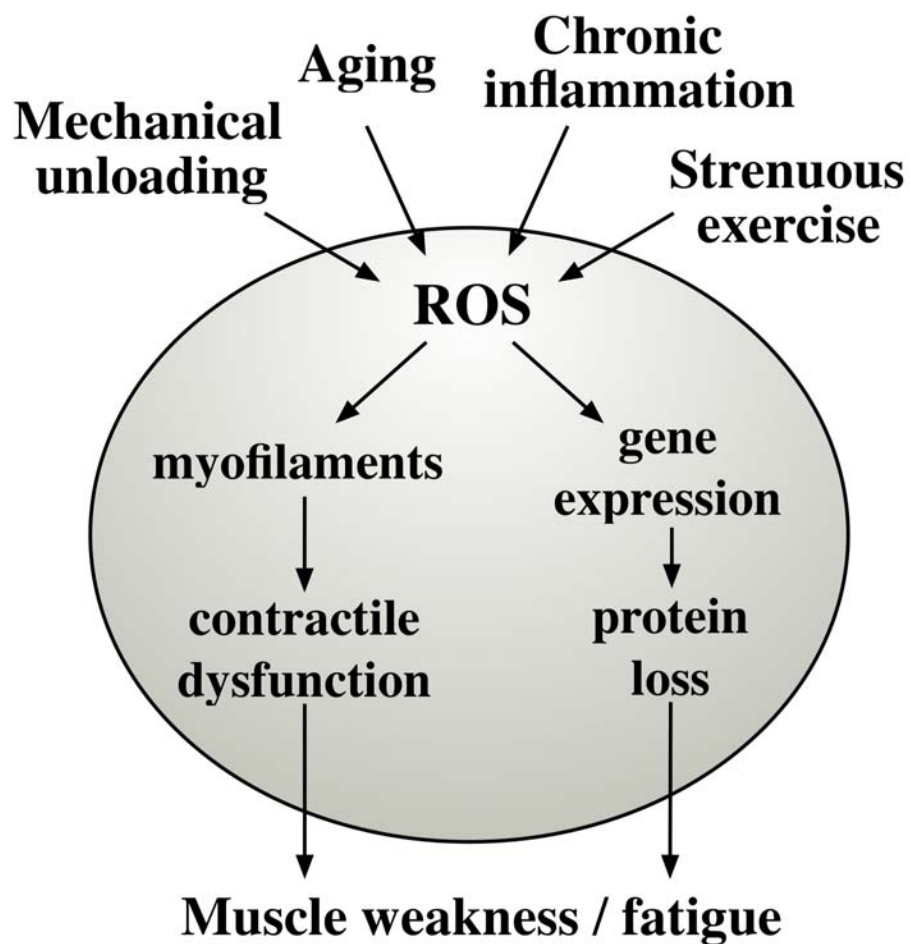
Fig 1. General model of ROS-induced weakness. ROS=reactive oxygen species

Increases in intracellular ROS activity can promote muscle weakness and fatigue via two parallel pathways. First, long-term elevation of ROS activity can act via redox signaling mechanisms to alter muscle gene expression, causing protein loss that diminishes muscle mass ("atrophy"). Second, ROS also can act via post-translational mechanisms to modify constitutively expressed proteins, causing contractile dysfunction that decreases force per cross-sectional area ("specific force"). These two pathways appear to be regulated independently and can have separate or additive effects on mechanical function.

Atrophy-Induced Weakness

As reviewed elsewhere,[1] chronic inflammatory diseases increase ROS activity, causing oxidative stress. This is documented clinically in diseases that range from Duchenne's dystrophy to chronic obstructive pulmonary disease, and from rheumatoid arthritis to cancer. Oxidative stress is commonly associated with changes in antioxidant capacity of the affected tissues and is linked to elevated levels of cytokines, chemokines, and other circulating markers of inflammation. Patients experience muscle wasting in almost all chronic inflammatory conditions. The prevalence approaches 100% in many diseases and inevitably causes weakness.

Proinflammatory cytokines are likely to function as endocrine mediators in chronic disease, stimulating ROS production and muscle atrophy via redox-activated mechanisms. Cytokines in this category include interferon-γ (IFN), interleukin-1 (IL-1), and tumor necrosis factor (TNF). Among these, TNF is most likely to trigger ROS-mediated atrophy.

Cell culture studies show that TNF acts directly on differentiated muscle cells to stimulate cytosolic ROS activity.[2] In contrast, oxidant activity is unaffected by exposure to IFN, IL-1, interleukin-6, or c-reactive protein.[3,4] TNF administration also stimulates loss of muscle protein, both in cultured muscle cells[2] and in experimental animals.[5] In patients with chronic disease, elevated serum TNF levels are strongly correlated with muscle atrophy and peripheral weakness. Muscle atrophy appears to be caused by the rise in oxidant activity since administration of exogenous antioxidants can blunt muscle loss in TNF-treated animals.[5]

Studies of muscle wasting during chronic inflammation have focused largely on regulation of proteolysis. Muscle proteins are disassembled and degraded via the coordinated actions of multiple parallel mechanisms. Dissociation and release of proteins from the myofibrillar lattice likely are caused by the actions of selective proteolytic enzymes, ie, calpains and caspases. Degradation of muscle protein occurs through autophagy and, to a greater extent, the ubiquitin-proteasome pathway. The latter pathway (Fig 2) includes three classes of regulatory E-proteins that interact to sequentially activate and conjugate ubiquitin to specific substrate proteins. Ubiquitin accumulation targets the doomed substrate for proteolysis via the 26S-proteasome.
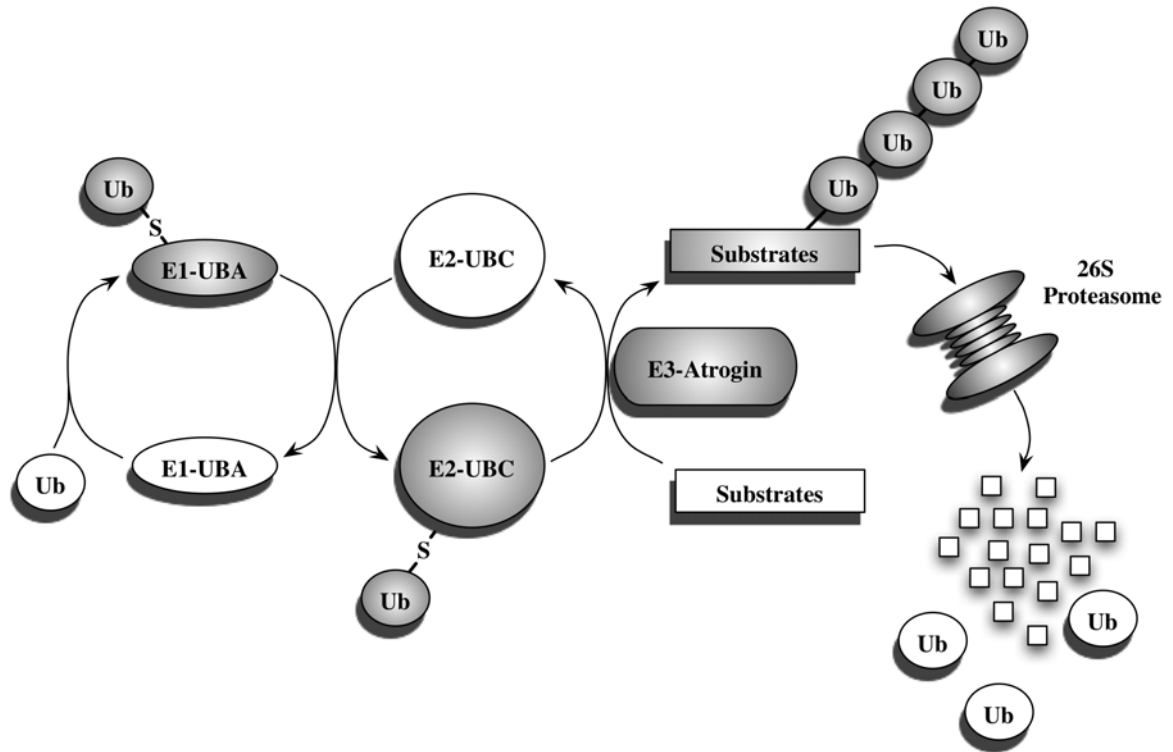
Fig 2. The ubiquitin proteasome pathway.[6] Ubiquitin monomers (Ub) are activated by the E1 or ubiquitin-activating protein (E1-UBA), transferred to the E2 or ubiquitin-conjugating protein (E2-UBC), and attached to substrate proteins in cooperation with E3 proteins such as atrogin1/MAFbx (E3-atrogin); repetition of this process creates ubiquitin polymers that trigger substrate degradation by the 26S proteasome.

From "Response of the ubiquitin-proteasome pathway to changes in muscle activity" by Reid MB. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 2005;288:R1423. © 2005 by American Physiological Society. Reprinted with permission.

Muscle exposure to either TNF or ROS increases activity of the ubiquitin-proteasome pathway. This rise in pathway activity is regulated primarily at the transcriptional level. Genes that code for ubiquitin, E-proteins, and proteasome subunits are upregulated in response to TNF or ROS exposure. These include genes for muscle-specific E2 proteins (E2-14k and UbcH2/E2-20k) and E3 proteins (E3α, atrogin1/MAFbx, and muscle-specific RING finger-1 [MuRF-1]) that are strongly associated with muscle catabolism. E2 and E3 proteins determine specificity of the ubiquitin-proteasome pathway. They are

rate-limiting elements of the pathway, determining the maximal rate of ubiquitin conjugation to substrates. Increased expression of E2 and E3 proteins can influence the composition of proteolyzed substrates and can accelerate the overall rate of proteasomal degradation, favoring net loss of muscle protein.

The signal transduction mechanisms that mediate this response are best understood for two muscle-specific E3s that strongly influence muscle atrophy. Atrogin/MAFbx expression is regulated positively by the Forkhead-O (FoxO) family of transcription factors. FoxO is tonically inhibited by Akt in muscle and other cell types. FoxO signaling is stimulated by TNF or ROS exposure and by other metabolism-associated stimuli. Another positive regulator of atrogin1/MAFbx is p38 mitogen-activated protein kinase (p38 MAPK), an essential mediator of gene expression in response to TNF, which also is activated by ROS exposure.[7] MuRF-1 is a second atrophy-associated E3 in muscle. Like atrogin1/MAFbx, MuRF-1 expression is positively regulated by FoxO. MuRF-1 is also sensitive to nuclear factor-κB (NF-κB), a transcription factor that is activated by inflammatory mediators, including TNF and ROS.[8]

Contractile Dysfunction

The weakness experienced by individuals with chronic inflammatory disease is not a simple function of muscle atrophy. Loss of force can exceed loss of muscle. This reflects contractile dysfunction of the remaining muscle, which generates less specific force than healthy muscle. This is observed in pathophysiological states that include chronic heart failure, cancer, COPD, and the sarcopenia of aging.

Serum TNF levels are elevated in these same diseases, a common denominator that may be responsible for contractile dysfunction. TNF depresses specific force in a variety of experimental preparations. These include animals injected with TNF,[9] transgenic mice engineered for cardiac overexpression of TNF,[10] and wild-type muscle preparations incubated with TNF in vitro.[10] This is associated with a rise in cytosolic oxidant activity[10] that appears to be essential for the fall in specific force. Pretreatment with antioxidants preserves specific force of muscle exposed to TNF either in vitro[10] or in vivo.[9] Interestingly, post-hoc incubation with an antioxidant can partially reverse dysfunction after prolonged TNF exposure.[10]

The intracellular target of TNF-stimulated oxidants appears to be myofibrillar proteins. In intact muscle fibers, TNF depresses specific force of tetanic contractions without altering cytosolic calcium transients or resting calcium concentration.[11] More directly, specific force is depressed in skinned fibers isolated from muscles of TNF-treated animals.[9] Activating calcium levels are controlled under these experimental conditions, clearly demonstrating dysfunction of the myofibrillar lattice.

The cellular mechanism by which TNF stimulates contractile dysfunction is beginning to emerge. We know the response is mediated via the TNF receptor subtype 1 (TNFR1) because TNF administration does not cause dysfunction in TNFR1-deficient mice.[9] Fig 3 illustrates the major post-receptor events that remain undefined.
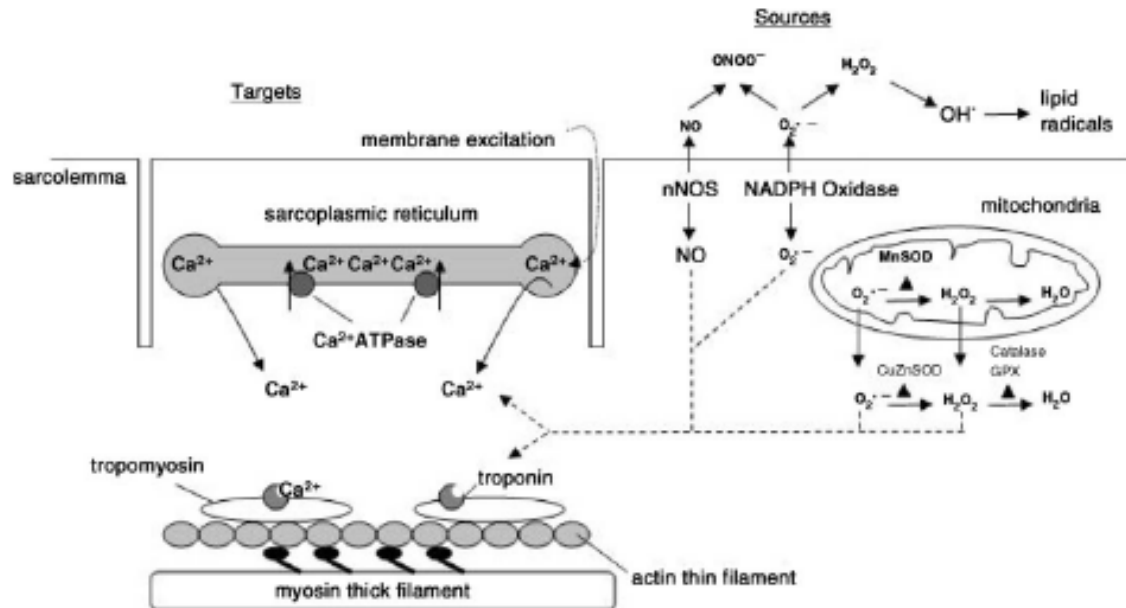
Fig 3. Oxidant sources and targets in muscle fibers.[12] $ONOO^-$ =peroxynitrite, H_2O_2=hydrogen peroxide, NO=nitric oxide, H_2O=water, $OH^•$=hydroxyl radical, $O_2•^-$ =superoxide anion, NADPH=nicotinamide adenine dinucleotide phosphate, Ca^{2+}=calcium, MnSOD=manganese superoxide dismutase, CuZnSOD=copper-zinc superoxide dismutase, ATP= adenosine triphosphatase, GPX=glutathione peroxidase.

One issue is the composition and source of TNF-stimulated oxidants. Skeletal muscle fibers contain multiple ROS sources, including the mitochondrial electron transport chain and a sarcolemmal NADPH oxidase complex. The source of oxidants stimulated via TNFR1 activation has not been tested in skeletal muscle. Further, fast-type muscle fibers constitutively express both the neuronal and endothelial isoforms of nitric oxide (NO) synthase, ie, nNOS and eNOS. NO derivatives are detectable in the sarcoplasm of rodent muscle fibers and have not been ruled out as possible co-mediators of TNF-stimulated dysfunction.

A second issue is the protein chemistry of myofibrillar dysfunction. What proteins are affected and what modifications compromise function? Regulatory proteins of the myofibrillar lattice that influence force production include actin, myosin heavy chain, myosin light chain, tropomyosin, and the troponin complex. Each is subject to post-translational modifications that diminish force and is a potential target of TNF-stimulated oxidants. Oxidants may act directly on proteins to stimulate carbonylation, nitration, sulfhydryl oxidation, and formation of nitrotyrosine or 4-hydroxy-2-nonenol adducts. Oxidants also may act indirectly via redox-sensitive regulatory proteins. For example, oxidizing conditions might alter the activity of a specific kinase or phosphatase and thereby disrupt the phosphorylation state of myofibrillar proteins. These issues remain unresolved, emphasizing the potential value of future research into the cellular mechanism of inflammation-induced dysfunction.

Summary

The weakness that commonly occurs in chronic inflammatory disease appears to be mediated by circulating proinflammatory mediators. The best recognized is TNF, which acts via the TNFR1 complex to increase cytosolic ROS activity in muscle fibers and cause weakness via two processes. Muscle atrophy is linked to increases in procatabolic signaling, E3 upregulation, ubiquitin conjugation, and protein loss. Contractile dysfunction is caused by ROS-mediated modifications to myofibrillar proteins that decrease specific force. Atrophy and dysfunction are parallel processes that appear to be

largely independent. Both are attractive targets for nutritional or pharmacologic interventions to preserve muscle function in patients with chronic inflammatory disease.

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Q&A

Q: Interestingly, you shared the results of Akt activation by TNF. Two years ago, we showed that PIF had the same effect. However, we had problems getting the referees to accept that we could get activation of Akt. I think they forgot that this is up to about 2 hours: Within the first 2 hours of adding the catabolic stimulus to muscle, a whole new set of proteins was synthesized. That is, we showed that if we added cycloheximide, we did not get protein degradation because a lot of protein is synthesized. So it is not surprising that anabolic signals are increased at the same time as catabolic signals. Can you comment?

Dr Reid: I was fascinated by your data because with PIF the responses are hours out. The signaling events we are looking at occur within minutes. We see an increase in Akt phosphorylation within 15 minutes or so of our TNF stimulus. In our hands, the MAPKs, Akt, Fox0 responses are a little bit later, but NF-kB activation happens within the first 5 to 30 minutes.

Q: We see most of those, including ROS production, within 15 minutes, but Akt lasted longer.