Title

Age-induced oxidative stress: How does it influence skeletal muscle quantity and quality?

Running Head

Aging, oxidative stress and muscular strength

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Abstract

With advancing age, skeletal muscle function declines as a result of strength loss. These strength deficits are largely due to reductions in muscle size (i.e., quantity) and its intrinsic force producing capacity (i.e., quality). Age-induced reductions in skeletal muscle quantity and quality can be the consequence of several factors, including accumulation of reactive oxygen and nitrogen species (ROS/RNS), also known as oxidative stress. Therefore, the purpose of this mini-review is to highlight the published literature that has demonstrated links between aging, oxidative stress, and skeletal muscle quantity or quality. In particular, we focused on how oxidative stress has the potential to reduce muscle quantity by shifting protein balance in a deficit, and muscle quality by impairing activation at the neuromuscular junction, excitation-contraction (EC) coupling at the ryanodine receptor (RyR) and cross-bridge cycling within the myofibrillar apparatus. Of these, muscle weakness due to EC coupling failure mediated by RyR dysfunction via oxidation and/or nitrosylation appears to be the strongest candidate based on the publications reviewed. However, it is clear that age-associated oxidative stress has the ability to alter strength through several mechanisms and at various locations of the muscle fiber.

Keywords: dynapenia, force, reactive oxygen species, sarcopenia, strength
Introduction

Aging results in strength deficits that are associated with frailty, loss of independence and physical disability (40, 51, 64). For instance, adults with low muscle strength have a ~2 to 4 fold greater risk of severe mobility limitations, slow gait speed and mortality compared to older adults with high muscle strength (38). Reductions in strength are primarily due to changes in skeletal muscle size (i.e. quantity) (22) and its intrinsic force producing capacity (i.e., quality) (23, 36, 56). Within the context of aging, loss of muscle quantity (i.e., atrophy) is referred to as sarcopenia (53, 54) while loss of muscle strength is termed dynapenia (11, 12, 37). Although sarcopenia contributes to dynapenia, several reports have demonstrated strength deficits with aging are more rapid than the concomitant loss of muscle size (18, 55), which has led some to suggest muscle quantity plays a relatively minor role in dynapenia when compared to muscle quality (11, 12, 37).

Nonetheless, because skeletal muscle quantity and quality both contribute to dynapenia, it is vital to understand their underlying causes. Sarcopenia is the result of reduced fiber size and number, which translates into fewer myofibrillar proteins to generate force. In general, loss of muscle quantity with age includes any factor that alters protein synthesis and/or degradation (e.g., via inflammation, hormonal content, nutritional status) or influences the number of functional alpha motor neurons (e.g., via apoptosis, neurotoxicity) that innervate the muscle (19, 42, 76). On the other hand, muscle quality is reduced by any factor that lowers force production relative to muscle size or cross-sectional area (CSA), which can be observed by assessing specific force or tension (56). The mechanisms undermining muscle quality are largely linked to neurological and skeletal muscle properties (11, 12, 37, 56). Age-associated neurological properties include changes within the nervous system that impair voluntary activation of the
muscle, such as excitatory drive to the lower motor neurons and/or alpha motor neuron excitability (12, 13, 56). Skeletal muscle properties are alterations inherent to the muscle fibers and include age-related changes in fiber architecture/composition, the excitation-contraction (EC) coupling processes and cross-bridge mechanics (11, 12, 37, 56).

Accumulation of reactive oxygen and nitrogen species (ROS/RNS) is thought to be a common determinant in the loss of both muscle quantity and quality (24). With advancing age, the muscle’s ability to handle increased levels of ROS/RNS is compromised (29), which ultimately impairs cellular homeostasis. Together, this process is commonly referred to as oxidative stress. Accumulation of ROS/RNS can result in redox modifications to nucleic acids, lipids and proteins that result in macromolecular damage and/or dysfunction. However, until recently, the mechanistic relationship between age-induced oxidative stress and loss of muscle strength was relatively unclear. Therefore, the purpose of this mini-review is to highlight the published literature that has demonstrated links between aging, oxidative stress, and skeletal muscle quantity or quality. In particular, we focused specifically on how oxidative stress has the potential to reduce muscle quantity by shifting protein balance in a deficit, and muscle quality by impairing activation at the neuromuscular junction, EC coupling at the ryanodine receptor (RyR) and cross-bridge cycling within the myofibrillar apparatus.

**ROS/RNS Production and Protein Modification**

Under conditions of oxidative stress, the formation of superoxide (O$_2^-$) and nitric oxide (NO) exceed their removal. These free radicals are commonly referred to as primary ROS/RNS, which can also convert to secondary ROS/RNS such as hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (ONOO$^-$) (28, 47). There are many sources of ROS/RNS, including the mitochondria, cytosol...
and membranes. In skeletal muscle, O$_2^-$ is generated from complexes I and III of the electron transport chain in the mitochondria, in addition to nicotinamide adenine dinucleotide phosphate (NADPH) oxidase located in the sarcoplasmic reticulum (SR) and transverse-tubules (T-tubules), and xanthine oxidase (XO) located in the endothelium (47). Nitric oxide (NO) is synthesized from various isoforms of nitric oxide synthase (NOS), including endothelial (eNOS), neuronal (nNOS) and “inducible” (iNOS) found throughout the cell (63).

Exposure to ROS/RNS can result in post-translational modification to the side chains of various proteins, ultimately affecting their structure and function. These ROS/RNS-induced modifications include nitration, nitrosylation, carbonylation and glycation. Nitration and nitrosylation are both NO-dependent modifications. Specifically, when tyrosine residues are modified 3-nitrotyrosine (3-NT) is formed, while nitrosylation occurs when cysteine residues are modified (39). Carbonylation is the most common protein modification that takes place in the presence of oxidative stress. In this process, carbonyl (C=O) groups are introduced into proteins by direct metal-catalyzed oxidation of certain amino acids or indirectly by reaction with reactive carbonyl species derived from the oxidation of lipids (i.e., lipid peroxidation), which can form 4-hydroxynonenal (HNE) (15). Lastly, protein glycation is involved in the formation of advanced glycation end products (AGEs), which is the addition of a sugar to a protein or lipid. Protein glycation is an emerging marker of oxidative stress due to the fact AGE formation significantly increases in the presence of ROS (45, 46).

Oxidative stress can be prevented by neutralizing ROS/RNS via the antioxidant system. The antioxidant system includes both endogenous and exogenous molecules. Some endogenous antioxidants include enzymes like glutathione peroxidase, superoxide dismutase and
catalase, while exogenous antioxidants include vitamins (e.g., vitamin E, vitamin C) and minerals (zinc, copper, iron) (47). Importantly, these antioxidants assist in maintaining the redox environment of the muscle and attenuate ROS/RNS-induced post-translational modifications.

With aging, oxidative stress is thought to occur due to an overproduction of ROS/RNS and an impaired ability to neutralize them (29, 66). ROS/RNS production increases with aging due to mitochondrial dysfunction caused by age-related mitochondrial DNA mutations, deletions (10, 21) and damage (25, 59). Along with the increase in ROS/RNS production, many of the muscle’s antioxidants are also increased, although this is not a universal response (31). Thus, the burden of defending against ROS/RNS production may be greater than the compensatory change in the antioxidant system (66). Under these conditions, ROS/RNS accumulate causing oxidative stress.

**Skeletal Muscle Contraction**

In order to generate a muscle contraction (Figure 1), the fiber first needs to be activated. In general, this encompasses the release of acetylcholine (i.e., a neurotransmitter) from the presynaptic terminal of the alpha motor neuron at the neuromuscular junction. The acetylcholine transverses the synaptic cleft where it binds to nicotinic acetylcholine receptors located on the postsynaptic motor endplate of the muscle fiber, which in turn generates an action potential on the surface of the sarcolemma.

From this point, the action potential propagates along the sarcolemma down the T-tubules. Depolarization of the membrane stimulates the voltage-sensitive dihydropyridine receptors (DHPRs) located in the T-tubules, which in turn activate the ryanodine receptors
(RyRs). The RyR is a Ca\(^{2+}\) release channel composed of four monomers embedded in the SR membrane. Each RyR monomer interacts with numerous ancillary proteins and is vulnerable to post-translational modification. These RyR modifications and interactions are known to influence channel function, both positively and negatively, and thus, SR Ca\(^{2+}\) release (5, 73). In this review, we refer to the aforementioned sequence of events (i.e., membrane depolarization to the release of Ca\(^{2+}\) from the SR) as EC coupling.

Successful activation of the RyR results in a rise of cytoplasmic Ca\(^{2+}\) that is sufficient to increase Ca\(^{2+}\) binding to troponin C, which exposes specific active sites on the actin filaments. These active sites are specific for actin-myosin interactions. Importantly, actin and myosin transition between two structural states, strong and weak, which are coupled with ATP hydrolysis. Specifically, binding of ATP to myosin is a weak structural state and with no force production. Upon hydrolysis and the release of phosphate (a byproduct of ATP hydrolysis) myosin is in a strong structural state, which generates force. Thus, with continuous ATP binding and hydrolysis, cyclic interactions of actin and myosin occur resulting in the sliding of actin filaments past myosin filaments toward the center of the sarcomere. As a whole, we refer to this process as cross-bridge cycling.

ROS/RNS and Muscle Quantity

Due to increased levels of oxidative stress in aged muscle, ROS/RNS accumulation has been suggested to play a key role in sarcopenia (24, 41) (Figure 1). Evidence for this is largely supported by investigations that have observed a relationship between oxidative stress and muscle mass (43, 61, 70), or have shown loss of muscle mass can be attenuated by antioxidant
supplementation (60). However, within the published literature, there is evidence for and against these claims.

For instance, it was reported that total protein carbonyl levels and sarcoplasmic, myofibrillar and mitochondrial 3-NT content were correlated with muscle mass within aging mice (70) and rats (43), respectively. In contrast, others failed to find correlations between carbonyl levels in the sarcoplasmic, myofibrillar and mitochondrial protein fractions and loss of muscle mass in aging rats (43) and humans (72). Mixed results have also been observed after antioxidant supplementation. For example, long-term antioxidant supplementation with FI (a cysteine-based antioxidant) (60) and resveratrol (27) were both shown to reduce markers of oxidative stress in aging mice muscle. Specifically, FI increased the muscle’s glutathione to glutathione disulfide ratio, resveratrol lowered the concentration of H$_2$O$_2$, while both alleviated lipid peroxidation. Despite these changes, only supplementation of FI proved beneficial in preserving muscle quantity, as assessed by muscle weight and CSA (60). Taken together, it appears that age-related muscle loss may be linked to the accumulation of specific ROS/RNS markers rather than “oxidative stress” overall. Moreover, it must be remembered that proteins are constantly being degraded and synthesized, and thus, if markers of oxidative stress are assessed at a single point time, it would only provide a snapshot of a relatively dynamic process.

If age-induced ROS/RNS accumulation results in sarcopenia, the effects are likely mediated by shifting protein balance in a deficit, specifically increasing proteolysis. Although the precise mechanisms responsible for this process are unclear in old muscle, they may be comparable to those observed following prolonged periods of contractile inactivity. As reviewed
by Powers et al. (48, 49), ROS production can promote protein breakdown by up-regulating components of the ubiquitin-proteasome system and/or through allosteric activation of muscle proteases (i.e., caspases, calpains). Similar to that of prolonged inactivity, aging is also associated with greater proteasome expression/content (2), and caspase (8) and calpain activity (16), which may be linked to ROS/RNS production. It is also feasible that increased ROS/RNS production could contribute to sarcopenia by impeding muscle protein synthesis. Obviously, more data is needed to validate if aged-induced oxidative stress decreases muscle quantity and if so, by what underlying mechanism(s).

ROS/RNS and Muscle Quality

As mentioned earlier, strength deficits with aging are more rapid than the concomitant loss of muscle quantity, which suggests dynapenia is largely due to changes in muscle quality. Age-induced ROS/RNS accumulation has been proposed to impair muscle quality at various locations. These include muscle fiber activation at the neuromuscular junction, EC coupling at the RyR and cross-bridge cycling within the myofibrillar apparatus (Figure 1).

-Muscle Fiber Activation

At the neuromuscular junction, ROS production was recently demonstrated to be greater in the levator auris longus muscle in old mice compared to young mice (26). Interestingly, these changes were associated with less neurotransmitter being released at the synaptic cleft, which could cause failure in the generation of an action potential by the sarcolemma. However, in that study, the old muscle appeared to compensate or offset these changes by becoming more excitable (26). Clearly, this is an understudied area of research and future investigations will be needed to determine if age-induced oxidative stress at the neuromuscular junction alters muscle...
quality; in particular, before the muscle begins to compensate for the age-related reductions seen
in neurotransmitter content. With that said, it is also worth noting that persistent oxidative stress
may alter morphology of the alpha motor neuron and the neuromuscular junction, ultimately
resulting in the loss of innervation and fiber number (quantity). For a detailed review of this
topic, see Jang and Van Remmen (30).

-EC Coupling

Theoretically, if any step in the EC coupling pathway is disrupted (i.e., EC coupling failure),
voltage-induced SR Ca\(^{2+}\) release will be impaired and less cytoplasmic Ca\(^{2+}\) will be available to
bind with troponin C. Impaired SR Ca\(^{2+}\) release is associated with reductions in specific force in
both rodent (3, 32, 55) and human models of aging (33). Potential reasons for EC coupling
failure include an uncoupling between DHPR and RyR (17, 32, 74), modifications to the RyR (3,
33, 55) and/or a decrease in DHPR density and thus the DHPR-RyR ratio (52, 57), although the
latter was not observed in recent investigations (26, 33).

Accumulation of ROS/RNS is known to modify or remodel the RyR. With advancing
age, the RyR becomes increasingly oxidized and nitrosylated, which has been shown to promote
RyR dysfunction in both mice (3, 71) and human muscle (33). Interestingly, these redox
modifications do not appear to directly alter RyR function per se, but rather through their effects
on the ancillary proteins that are associated with the channel’s activity. One in particular is
calstabin (FK506 binding protein, FKBP12), a 12 kDa protein that normally binds to each RyR
monomer, which is thought to stabilize the channel preventing it from opening to sub-
conductance states (1, 9). In fast-twitch muscle from aged mice, oxidation and nitrosylation of
RyR depleted the channel of calstabin, diminishing the calstabin-RyR interaction (3, 71). These changes resulted in “leaky” RyR channels that manifested as increases in single-channel open probability and Ca\(^{2+}\) spark frequency (3, 71). Under these conditions, SR lumen Ca\(^{2+}\) content decreases reducing SR Ca\(^{2+}\) release, and thus specific force upon voltage-induced activation. Importantly, RyR dysfunction mediated by depletion of calstabin is not only observed with aging (3, 55), but also heart failure (58), muscular dystrophy (6), chronic muscle fatigue (7) and contraction-induced injury (4).

Accordingly, methods used to reduce oxidation and/or nitrosylation of RyR or attenuate the loss of calstabin have proven beneficial in restoring muscle quality. For instance, in vitro antioxidant treatment of dithiothreitol (DTT) was able to reverse age-related SR Ca\(^{2+}\) leak and increase SR Ca\(^{2+}\) stores from intact fast-twitch and slow-twitch fibers from old mice (71) and human muscle (33), respectively. Furthermore, treatment of S107 (i.e., a drug that preserves calstabin-RyR binding) (3) and overexpression of mitochondrial catalase (71) were both able to stabilize calstabin to RyR, prevent SR Ca\(^{2+}\) leak and increase specific force in EDL muscle from old mice. Taken together, these results demonstrate that age-related reductions in specific force (i.e., muscle quality) are largely due to EC coupling failure stemming from redox-induced RyR dysfunction.

-Cross-Bridge Cycling

In addition to less Ca\(^{2+}\) released from the SR, accumulation of ROS/RNS has also been suggested to reduce muscle quality by impairing cross-bridge cycling. This idea is largely supported by age-related reductions in specific force from human and rat permeabilized fibers (14, 23, 65, 67), but also findings that have observed myofibrillar proteins are redox sensitive.
Importantly, permeabilized fibers do not have an intact membrane, which means force generation occurs independently of the EC coupling process and therefore, directly reflects the actin-myosin interactions. Thus, the loss of specific force observed in aged permeabilized fibers would be unrelated to the aforementioned RyR dysfunction.

Age-related reductions in specific force often range from 20-30% in both human and rat permeabilized fibers (14, 23, 50, 65, 67), and are associated with defective actin-myosin interactions. More specifically, there is an overall decline in the fraction of myosin heads in the strong-binding, force-generating structural state (34, 35, 69, 77), which translates into less force for a given amount of actin and myosin. Loss of specific force could be due to an accumulation of post-translationally modified actin and myosin proteins and/or site-specific modifications of key amino acid residues important to the actin-myosin interaction (66). However, redox modifications to actin and myosin are quite variable based on investigations that have assessed their susceptibility to glycation (62, 75), carbonylation (20, 44) or the formation of 3-NT and HNE adducts (68), which suggests accumulation of modified actin and myosin proteins is not the main contributor to age-dependent reductions in permeabilized skeletal muscle fiber quality. Rather, evidence for site-specific, redox modifications appears to be more promising. For instance, oxidative modifications to cysteine residues of myosin (not actin) have been shown to increase with age but more importantly, are thought to reduce efficient cross-bridge cycling by inhibiting actin-activated myosin ATPase activity (50).

Although it is difficult to determine the degree to which these redox modifications influence cross-bridge cycling, it is likely that they are at least partially responsible for the reductions in specific force observed in permeabilized fiber experiments. It is also possible that
other myofibrillar proteins are redox sensitive and contribute to age-induced muscle dysfunction. For example, troponin T and myosin-binding protein C were both found to be increasingly carbonylated in the rectus abdominis of old human subjects (20). However, it is currently unknown how these modifications would influence force production.

Conclusions

Loss of strength is a common consequence of aging, and can be attributed to reductions in both skeletal muscle quantity and quality. Although numerous factors influence these parameters, protein damage and dysfunction are commonly accredited to age-related elevations in oxidative stress. Here, we highlighted several investigations that have focused specifically on aging, oxidative stress, and skeletal muscle quantity or quality. Taken together, accumulation of ROS/RNS has been reported to affect several components involved in force generation, including skeletal muscle size, fiber activation, EC coupling and cross-bridge cycling (Figure 1). Of these, EC coupling failure mediated by RyR dysfunction via oxidation and/or nitrosylation appears to be the strongest candidate based on the publications reviewed. However, it is clearly evident that age-associated oxidative stress has the ability to alter strength at various sites through several different redox modifications.

Upcoming investigations should not only focus on whether ROS/RNS modifications increase with age, but also their structural and functional impact as measured by skeletal muscle quantity and quality. Furthermore, new knowledge and the respective technological advancements will allow the analysis of other potential markers of oxidative stress. In the near future, it is likely we will see a significant increase in the number of specific redox modifications
and how they associate with muscle proteins, ultimately enhancing our understanding of specific aging phenotypes.

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Figure Legend

Figure 1. Possible mechanisms by which age-related oxidative stress reduces skeletal muscle quantity and/or quality. Accumulation of reactive oxygen and nitrogen species (ROS/RNS) in old muscle results in protein modification and/or damage that could reduce quantity (i.e., fiber size) by shifting protein balance in a deficits, and/or quality by impairing muscle fiber activation at the neuromuscular junction, excitation-contraction (EC) coupling at the RyR and cross-bridge cycling within the myofibrillar apparatus. SR; sarcoplasmic reticulum, RyR; ryanodine receptor, DHPR; dihydropyridine receptor.