

HIGHLIGHTED TOPIC | Aging and Exercise

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

Cory W. Baumann, Dongmin Kwak, Haiming M. Liu, and LaDora V. Thompson

Department of Physical Medicine and Rehabilitation, University of Minnesota Medical School, Minneapolis, Minnesota

Submitted 11 April 2016; accepted in final form 12 May 2016

Baumann CW, Kwak D, Liu HM, Thompson LV. Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality? *J Appl Physiol* 121: 1047–1052, 2016. First published May 19, 2016; doi:10.1152/jappphysiol.00321.2016.—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.

dynapenia; force; reactive oxygen species; sarcopenia; strength

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 an ~2- to 4-fold greater risk of severe mobility limitations, slow gait speed, and mortality compared with 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 compared with 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 degra-

dation (e.g., via inflammation, hormonal content, and 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 α -motor neuron excitability (12, 13, 56). Skeletal muscle property alterations are 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. How-

Address for reprint requests and other correspondence: L. V. Thompson, Dept. of Physical Medicine and Rehabilitation, Univ. of Minnesota, Medical School, Minneapolis, Minnesota (e-mail: thomp067@umn.edu).

ever, 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) exceeds 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_2O_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 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 posttranslational 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 that AGE formation significantly increases in the presence of ROS (45, 46).

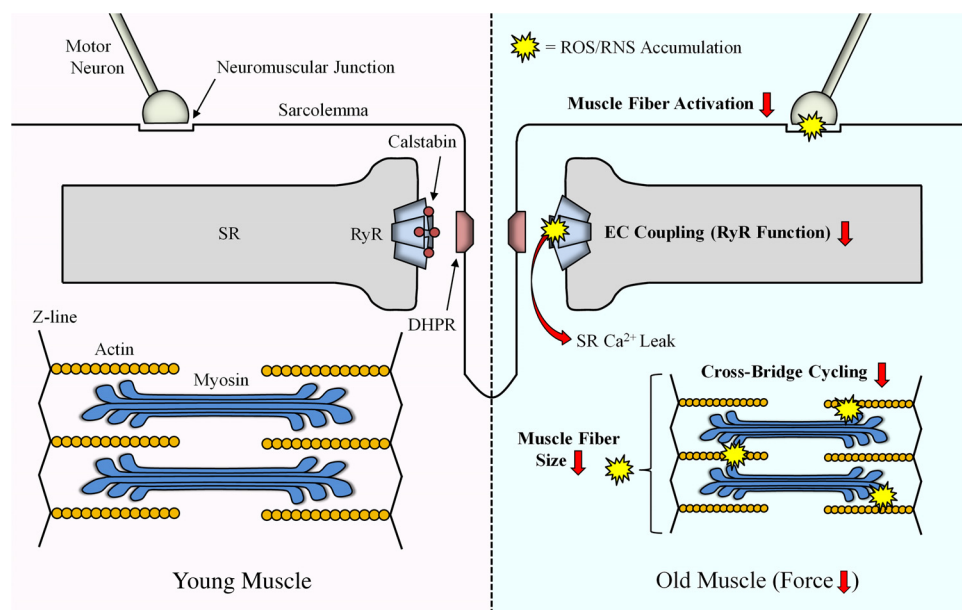
Oxidative stress can be prevented by neutralizing ROS/RNS via the antioxidant system. The antioxidant system includes both endogenous and exogenous molecules. Some endogenous antioxidants include enzymes like glutathione peroxidase, superoxide dismutase, and catalase, while exogenous antioxidants include vitamins (e.g., vitamin E and 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 posttranslational 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

To generate a muscle contraction (Fig. 1), the fiber first needs to be activated. In general, this encompasses the release of acetylcholine (ACh) (i.e., a neurotransmitter) from the presynaptic terminal of the α -motor neuron at the neuromuscular junction. The ACh transverse the synaptic cleft where it

Fig. 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 deficit, 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.



binds to nicotinic ACh 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 posttranslational 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

Because of increased levels of oxidative stress in aged muscle, ROS/RNS accumulation has been suggested to play a key role in sarcopenia (24, 41) (Fig. 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_2O_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 in 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 upregulating 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 are needed to validate whether 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 (Fig. 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 with 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 whether 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 α -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 subconductance 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 (20, 43, 44, 62, 75). 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 to 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 posttranslationally modified actin and myosin proteins and/or site-specific modi-

fications of key amino acid residues important to the actin-myosin interaction (66). However, redox modifications to actin and myosin are quite variable on the basis of 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 (Fig. 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.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Jeffrey S. Otis (Georgia State University) and Choung-Hun Kang (University of Minnesota) for their critical review during the preparation of this article.

GRANTS

This study was partially supported by National Institute on Aging/National Institutes of Health Training Grant T32-AG029796.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.W.B., D.K., and L.V.T. conception and design of research; C.W.B. and D.K. prepared figures; C.W.B., D.K., H.L., and L.V.T. drafted manuscript; C.W.B., D.K., H.L., and L.V.T. edited and revised manuscript; C.W.B., D.K., H.L., and L.V.T. approved final version of manuscript.

REFERENCES

- Ahern GP, Junankar PR, Dulhunty AF. Subconductance states in single-channel activity of skeletal muscle ryanodine receptors after removal of FKBP12. *Biophys J* 72: 146–162, 1997.
- Altun M, Besche HC, Overkleeft HS, Piccirillo R, Edelmann MJ, Kessler BM, Goldberg AL, Ulfhake B. Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. *J Biol Chem* 285: 39,597–39,608, 2010.
- 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* 14: 196–207, 2011.
- Baumann CW, Rogers RG, Gahlot N, Ingalls CP. Eccentric contractions disrupt FKBP12 content in mouse skeletal muscle. *Physiol Rep* 2: pii: e12081, 2014.
- Bellinger AM, Mongillo M, Marks AR. Stressed out: the skeletal muscle ryanodine receptor as a target of stress. *J Clin Invest* 118: 445–453, 2008.
- Bellinger AM, Reiken S, Carlson C, Mongillo M, Liu X, Rothman L, Matecki S, Lacampagne A, Marks AR. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nat Med* 15: 325–330, 2009.
- Bellinger AM, Reiken S, Dura M, Murphy PW, Deng SX, Landry DW, Nieman D, Lehnart SE, Samaru M, LaCampagne A, Marks AR. Remodeling of ryanodine receptor complex causes “leaky” channels: a molecular mechanism for decreased exercise capacity. *Proc Natl Acad Sci USA* 105: 2198–2202, 2008.
- Braga M, Hikim APS, Datta S, Ferrini MG, Brown D, Kovacheva EL, Gonzalez-Cadavid NF, Sinha-Hikim I. Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. *Apoptosis* 13: 822–832, 2008.
- Brillantes AMB, Ondrias K, Scott A, Kobrinsky E, Ondriašová E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE, Marks AR. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 77: 513–523, 1994.
- Bua E, Johnson J, Herbst A, Delong B, McKenzie D, Salamat S, Aiken JM. Mitochondrial DNA-deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. *Am J Hum Genet* 79: 469–480, 2006.
- Clark BC, Manini TM. Sarcopenia ≠ dynapenia. *J Gerontol A Biol Sci Med Sci* 63: 829–834, 2008.
- Clark BC, Manini TM. What is dynapenia? *Nutrition* 28: 495–503, 2012.
- Clark BC, Taylor JL. Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. *Curr Aging Sci* 4: 192–199, 2011.
- D’Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canevari M, Saltin B, Bottinelli R. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *J Physiol* 552: 499–511, 2003.
- Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta* 329: 23–38, 2003.
- Dargelos E, Brulé C, Combaret L, Hadj-Sassi A, Dulong S, Poussard S, Cottin P. Involvement of the calcium-dependent proteolytic system in skeletal muscle aging. *Exp Gerontol* 42: 1088–1098, 2007.
- Delbono O, O’Rourke K, Ettinger W. Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. *J Membr Biol* 148: 211–222, 1995.
- Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieryer P, Boudreau R, Manini TM, Nevitt M, Newman AB. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* 90: 1579–1585, 2009.
- Doherty TJ. Aging and sarcopenia. *J Appl Physiol* 95: 1717–1727, 2003.
- dos Santos SL, Baraibar MA, Lundberg S, Eeg-Olofsson O, Larsson L, Friguet B. Oxidative proteome alterations during skeletal muscle ageing. *Redox Biol* 5: 267–274, 2015.
- Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, Barja G, Leeuwenburgh C. Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol* 284: R474–R480, 2003.
- Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol* 88: 1321–1326, 2000.
- Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R. Skeletal muscle fiber quality in older men and women. *Am J Physiol Cell Physiol* 279: C611–C618, 2000.
- Fulle S, Protasi F, Di Tano G, Pietrangelo T, Beltramin A, Boncompagni S, Vecchiet L, Fanò G. The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Exp Gerontol* 39: 17–24, 2004.
- Gianni P, Jan KJ, Douglas MJ, Stuart PM, Tarnopolsky MA. Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Exp Gerontol* 39: 1391–1400, 2004.
- Ivannikov MV, Van Remmen H. Sod1 gene ablation in adult mice leads to physiological changes at the neuromuscular junction similar to changes that occur in old wild-type mice. *Free Radic Biol Med* 84: 254–262, 2015.
- Jackson JR, Ryan MJ, Alway SE. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. *J Gerontol A Biol Sci Med Sci* 66: 751–764, 2011.
- Jackson MJ. Redox regulation of muscle adaptations to contractile activity and aging. *J Appl Physiol* 119: 163–171, 2015.
- Jackson MJ, McArdle A. Age-related changes in skeletal muscle reactive oxygen species generation and adaptive responses to reactive oxygen species. *J Physiol* 589: 2139–2145, 2011.
- Jang YC, Van Remmen H. Age-associated alterations of the neuromuscular junction. *Exp Gerontol* 46: 193–198, 2011.
- Ji LL. Exercise-induced modulation of antioxidant defense. *Annals NY Acad Sci* 959: 82–92, 2002.
- Jiménez-Moreno R, Wang ZM, Gerring RC, Delbono O. Sarcoplasmic reticulum Ca^{2+} release declines in muscle fibers from aging mice. *Biophys J* 94: 3178–3188, 2008.
- Lambole C, Wyckelsma V, McKenna M, Murphy R, Lamb G. Ca^{2+} leakage out of the sarcoplasmic reticulum is increased in type I skeletal muscle fibres in aged humans. *J Physiol* 594: 469–481, 2016.
- Lowe DA, Surek JT, Thomas DD, Thompson LV. Electron paramagnetic resonance reveals age-related myosin structural changes in rat skeletal muscle fibers. *Am J Physiol Cell Physiol* 280: C540–C547, 2001.
- Lowe DA, Warren GL, Snow LM, Thompson LV, Thomas DD. Muscle activity and aging affect myosin structural distribution and force generation in rat fibers. *J Appl Physiol* 96: 498–506, 2004.
- Lynch N, Metter E, Lindle R, Fozard J, Tobin J, Roy T, Fleg J, Hurley B. I. Muscle quality age-associated differences between arm and leg muscle groups. *J Appl Physiol* 86: 188–194, 1999.
- Manini TM, Clark BC. Dynapenia and aging: an update. *J Gerontol A Biol Sci Med Sci* 67: 28–40, 2012.
- Manini TM, Visser M, Won-Park S, Patel KV, Strotmeyer ES, Chen H, Goodpaster B, De Rekeneire N, Newman AB, Simonsick EM. Knee extension strength cutpoints for maintaining mobility. *J Am Geriatr Soc* 55: 451–457, 2007.
- Martínez-Ruiz A, Cadenas S, Lamas S. Nitric oxide signaling: classical, less classical, and nonclassical mechanisms. *Free Radic Biol Med* 51: 17–29, 2011.
- Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. *Exp Gerontol* 41: 1234–1238, 2006.
- Meng SJ, Yu LJ. Oxidative stress, molecular inflammation and sarcopenia. *Int J Mol Sci* 11: 1509–1526, 2010.
- Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. Sarcopenia. *J Lab Clin Med* 137: 231–243, 2001.
- Murakami H, Guillet C, Tardif N, Salles J, Migné C, Boirie Y, Walrand S. Cumulative 3-nitrotyrosine in specific muscle proteins is associated with muscle loss during aging. *Exp Gerontol* 47: 129–135, 2012.
- Oh-Ishi M, Ueno T, Maeda T. Proteomic method detects oxidatively induced protein carbonyls in muscles of a diabetes model Otsuka Long-Evans Tokushima Fatty (OLETF) rat. *Free Radic Biol Med* 34: 11–22, 2003.

45. Ott C, Jacobs K, Haucke E, Santos AN, Grune T, Simm A. Role of advanced glycation end products in cellular signaling. *Redox Biol* 2: 411–429, 2014.
46. Pietropaoli D, Ortu E, Severino M, Ciarrocchi I, Gatto R, Monaco A. Glycation and oxidative stress in the failure of dental implants: a case series. *BMC Res Notes* 6: 296, 2013.
47. Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. *Compr Physiol* 1: 941–969, 2011.
48. Powers SK, Smuder A, Judge A. Oxidative stress and disuse muscle atrophy: cause or consequence? *Curr Opin Clin Nutr Metab Care* 15: 240–245, 2012.
49. Powers SK, Smuder AJ, Criswell DS. Mechanistic links between oxidative stress and disuse muscle atrophy. *Antioxid Redox Signal* 15: 2519–2528, 2011.
50. Prochniewicz E, Thomas DD, Thompson LV. Age-related decline in actomyosin function. *J Gerontol A Biol Sci Med Sci* 60: 425–431, 2005.
51. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, White L. Midlife hand grip strength as a predictor of old age disability. *JAMA* 281: 558–560, 1999.
52. Renganathan M, Messi MaL, Delbono O. Overexpression of IGF-1 exclusively in skeletal muscle prevents age-related decline in the number of dihydropyridine receptors. *J Biol Chem* 273: 28,845–28,851, 1998.
53. Rosenberg IH. Sarcopenia: origins and clinical relevance. *Clin Ger Med* 27: 337–339, 2011.
54. Rosenberg IH. Summary comments. *Am J Clin Nutr* 50: 1231–1233, 1989.
55. Russ D, Grandy J, Toma K, Ward C. Ageing, but not yet senescent, rats exhibit reduced muscle quality and sarcoplasmic reticulum function. *Acta Physiol* 201: 391–403, 2011.
56. Russ DW, Gregg-Cornell K, Conaway MJ, Clark BC. Evolving concepts on the age-related changes in “muscle quality”. *J Cachexia Sarcopenia Muscle* 3: 95–109, 2012.
57. Ryan M, Carlson BM, Ohlendieck K. Oligomeric status of the dihydropyridine receptor in aged skeletal muscle. *Mol Cell Biol Res Commun* 4: 224–229, 2000.
58. Shan J, Betzenhauser MJ, Kushnir A, Reiken S, Meli AC, Wronska A, Dura M, Chen BX, Marks AR. Role of chronic ryanodine receptor phosphorylation in heart failure and β -adrenergic receptor blockade in mice. *J Clin Invest* 120: 4375–4387, 2010.
59. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci USA* 102: 5618–5623, 2005.
60. Sinha-Hikim I, Sinha-Hikim AP, Parveen M, Shen R, Goswami R, Tran P, Crum A, Norris KC. Long-term supplementation with a cystine-based antioxidant delays loss of muscle mass in aging. *J Gerontol A Biol Sci Med Sci* 68: 749–759, 2013.
61. Siu PM, Pistilli EE, Alway SE. Age-dependent increase in oxidative stress in gastrocnemius muscle with unloading. *J Appl Physiol* 105: 1695–1705, 2008.
62. Snow LM, Fugere NA, Thompson LV. Advanced glycation end-product accumulation and associated protein modification in type II skeletal muscle with aging. *J Gerontol A Biol Sci Med Sci* 62: 1204–1210, 2007.
63. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiol Rev* 81: 209–237, 2001.
64. Taekema DG, Gussekloo J, Maier AB, Westendorp RG, de Craen AJ. Handgrip strength as a predictor of functional, psychological and social health. A prospective population-based study among the oldest old. *Age Ageing* 39: 331–337, 2010.
65. Thompson LV. Contractile properties and protein isoforms of single skeletal muscle fibers from 12- and 30-month-old Fischer 344 brown Norway F1 hybrid rats. *Aging (Milano)* 11: 109–118, 1999.
66. Thompson LV. Age-related muscle dysfunction. *Exp Gerontol* 44: 106–111, 2009.
67. Thompson LV, Brown M. Age-related changes in contractile properties of single skeletal fibers from the soleus muscle. *J Appl Physiol* 86: 881–886, 1999.
68. Thompson LV, Durand D, Fugere NA, Ferrington DA. Myosin and actin expression and oxidation in aging muscle. *J Appl Physiol* 101: 1581–1587, 2006.
69. Thompson LV, Lowe DA, Ferrington DA, Thomas DD. Electron paramagnetic resonance: a high-resolution tool for muscle physiology. *Exerc Sport Sci Rev* 29: 3–6, 2001.
70. Tohma H, El-Shafey AF, Croft K, Shavlakadze T, Grounds MD, Arthur PG. Protein thiol oxidation does not change in skeletal muscles of aging female mice. *Biogerontology* 15: 87–98, 2014.
71. Umanskaya A, Santulli G, Xie W, Andersson DC, Reiken SR, Marks AR. Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging. *Proc Natl Acad Sci USA* 111: 15,250–15,255, 2014.
72. Valls MRB, Wilkinson DJ, Narici MV, Smith K, Phillips BE, Caporossi D, Atherton PJ. Protein carbonylation and heat shock proteins in human skeletal muscle: relationships to age and sarcopenia. *J Gerontol A Biol Sci Med Sci* 70: 174–181, 2015.
73. Van Petegem F. Ryanodine receptors: structure and function. *J Biol Chem* 287: 31,624–31,632, 2012.
74. Wang ZM, Messi ML, Delbono O. L-type Ca^{2+} channel charge movement and intracellular Ca^{2+} in skeletal muscle fibers from aging mice. *Biophys J* 78: 1947–1954, 2000.
75. Watanabe H, Ogasawara M, Suzuki N, Nishizawa N, Ambo K. Glycation of myofibrillar protein in aged rats and mice. *Biosci Biotechnol Biochem* 56: 1109–1112, 1992.
76. Zembron-Lacny A, Dziubek W, Rogowski L, Skorupka E, Dabrowska G. Sarcopenia: monitoring, molecular mechanisms, and physical intervention. *Physiol Res* 63: 683–691, 2014.
77. Zhong S, Lowe DA, Thompson LV. Effects of hindlimb unweighting and aging on rat semimembranosus muscle and myosin. *J Appl Physiol* 101: 873–880, 2006.