Oxidative system in aged skeletal muscle

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Summary

Aging is an inevitable biological process that is characterized by a general decline in the physiological and biochemical functions of the major systems. In the case of the neuromuscular system, reductions in strength and mobility cause a deterioration in motor performance, impaired mobility and disability. At the cellular level, aging is caused by a progressive decline in mitochondrial function that results in the accumulation of reactive oxygen species (ROS). As the level of oxidative stress in skeletal muscle increases with age, the age-process is characterized by an imbalance between an increase in ROS production in the organism, and antioxidant defences as a whole. We have reviewed the literature on oxidative stress in aging human skeletal muscles, and to assesss the impact of differences in physiological factors (sex, fiber composition, muscle type and function).

Key words: aged skeletal muscle, markers of skeletal muscle aging, oxidative system, ROS, sarcopenia

Introduction

Aging is a complex process that is characterized by a time-dependent decline in maximal functionality of the tissues and organs of the whole body. Skeletal muscle can be considered the largest organ in the body (1), and age-related loss of skeletal muscle mass and function has a major impact on the life quality of the elderly. After about age 50, muscle mass decreases at an annual rate of 1–2% (2), the cross-sectional area of skeletal muscle is reduced by 25–30% at 70 years and muscle strength declines by about 1.5% per year between ages 50 and 60 and by 3% per year thereafter (2). The original hypothesis of the Theory of Aging was modified in 1972 by the modern version of oxidative stress (OS) theory (3,4), according to which macromolecular damage accumulation is due to the redox imbalance that could be the major trigger of the imbalance between protein synthesis and degradation that leads to muscle atrophy (5). Today a causal relationship between such elements as oxidative macromolecule modifications, mutations of mtDNA, mitochondrial dysfunction and aging are indisputable, but the mechanism by which these molecular and biochemical events occur remains to be established (6).

ROS-induced biochemical alterations in muscle

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are continually produced in the human body as a consequence of normal aerobic metabolism, and are additionally absorbed from the external environment. In muscle fibers, ROS, in particular the superoxide anion, can be produced in varying cellular sites (e.g. plasma membrane, mitochondria, sarcoplasmic reticulum (SR), T tubules, sarcolemma and cytosol) and are generally released in the cytosol of the cell muscle (7). Moreover, muscle cells are able to release the superoxide anion in extracellular space (8). Although this mechanism is still poorly understood, it seems to involve membranal NAD(P)H oxidase. The involvement of other enzymes, such as Phospholipase A2 (PLA2) and Xanthine oxidase, has been suggested. The activation of PLA2 may stimulate NAD(P)H oxidase, while it has been shown that increases in PLA2 activity stimulate ROS production in mitochondria and in cytosol, as well as subsequent release in extracellular space (7). Xanthine dehydrogenase (XDH) is a cytoplasmic enzyme that is converted to Xanthine oxidase, which in turn is able to produce the superoxide anion and, accordingly, hydrogen peroxide. Skeletal muscle generates superoxide and nitric oxide at rest, and this generation is increased by contractile activity. In young and adult animals and man, an increase in activities, and hence in the secondary products that derive from such activities, stimulates redox-sensitive signaling pathways to modify the cellular content of cytoprotective regulators, such as Superoxide dismutases (SOD), Catalase (CAT) and Heat Shock Proteins (HSP), which in turn prevent oxidative damage to tissues. The mechanisms underlying these adaptive responses to contraction include activation of redox-sensitive transcription factors, such as Nu-

clear Factor- KappaB (NF-kB), Activator Protein-1 (AP-1) and Heat Shock Factor 1 (HSF1). All aging tissues, including skeletal muscle, demonstrate an accumulation of oxidative damage that may contribute to the loss of tissue homeostasis (9). The increase in HSP content and in antioxidant defense enzyme activity is evident in the muscles of adult rodents subsequent to isometric contractions; in contrast, this adaptation is not seen in old rodents, due to the lack of appropriate transcription factor activation. It has been demonstrated that this age-related inability to produce HSPs plays a critical role in the development of the functional deficits that occur with aging in skeletal muscle (10).

The literature reports that post-contraction attenuation of the pathways involved in mitochondrial biogenesis in fast muscle fibers (type II) is far more severe in old rats than in young rats (11). ROS affect mitochondrial biogenesis via up regulation of transcriptional regulators, such as peroxisome proliferator-activated receptor-gamma coactivator-1 protein-alpha (PGC-1alpha), and excessively high ROS generation subsequent to contractions may be responsible for diminished mitochondrial biogenesis in the muscles of old rats. The inability of skeletal muscle from old rats to activate redox-sensitive transcription factors such as NF-kB or AP-1 in response to stress is characterized by chronic activation of transcription factors at rest and by an inability to further activate these factors subsequent to muscle contraction (12).

Enzymatic antioxidants in aged muscle

Primary antioxidant enzymes include SOD, which hastens the dismutation of superoxide to H_2O_2, Glutathione peroxide (GPx), and CAT, both of which convert H_2O_2 to H_2O. Glutathione (GSH/GSSG) and other antioxidants of low molecular weight play an important role in maintaining sufficient substrate levels for GPx. Mitochondria contain 10–12% of the total GSH content in a cell, but they lack enzymes for GSH biosynthesis; the intramitochondrial pool of GSH is replenished by a rapid net uptake of GSH from cytoplasm (13). GSH is oxidized to GSSG, which cannot be exported to the cytosol and must be reduced back to GSH in the mitochondrial matrix (14). GSH level in human skeletal muscles is quite constant (15,16), a finding that suggests that GSH transport into cells does not change during aging (14). In contrast, GSSG levels increase with age: the GSH/GSSG ratio decreases significantly during aging (17,18), which suggests that aging may cause significant alterations in the glutathione status in male skeletal muscles. Aging effects on antioxidant defense systems in skeletal muscle seem to differ from those observed in the liver, kidney, brain, and heart (19). A marked age-dependent increase in the main antioxidant enzymes (SOD, CAT, GPx) has been shown in rat skeletal muscle (*soleus*) (20). Regarding SOD activities, the literature reports an increase in age-dependent reduction of total SOD and MnSOD in different types of rat skeletal muscle (21), while other studies did not find changes in the activity of total SOD, but reported an increase in MnSOD with age (22) as a result of NF-kB activation (23). In contrast, total SOD activity increases with age in rat's *tibialis* anterior muscle and in the extensor *digitorum longus*, two fast-twitch muscles, whereas MnSOD does not change; this finding is consistent with the hypotheses that the increase in ROS production is greater in oxidative muscles than in glycolitic muscles in the mitochondria, and that an increase in ROS production possibly occurs in glycolytic muscles that are not located in the mitochondria (24). In humans, age-dependent enhancement in MnSOD activity is particularly evident in *rectus abdominis* (RA) (15,18) and in the external intercostal muscle (25), but less so in *vastus lateralis* (VL) (15, 18, 25), a finding which suggests that marked ROS production increases occur mainly in oxidative muscles. Most data on rats report an increase in CAT activity, both in senescent oxidative and in glycolytic muscles (24). This finding is consistent with the possibility that non mitochondrial ROS production increases. In contrast, few and contradictory data are available on human skeletal muscle. Some authors report no change in CAT activity (15), while other studies describe a significant increase (25) or decrease (26). In human skeletal muscles GPx does not change during aging (16) whether in glycolitic or oxidative muscle.

Markers of skeletal muscle aging

ROS accumulation during aging results in oxidative stress that can damage cellular components, such as lipids, proteins and DNA. Levels of oxidative macromolecule damage increased significantly with age, particularly 8-hydroxy-2'-deoxyguanosine (8-OHdG), Malondialdehyde (MDA), as did levels of carbonyl residues such as Carbonyl Proteins (PC) (18,27). This age-dependent increase seems to be common in the brain, heart, kidney (28) and skeletal muscle (27).

Lipid Damage

MDA and 4-hydroxy-2,3-trans-nonenal (4-HNE) are the main products of PUFA peroxidation because they bear numerous double bonds. The main effect on lipid peroxidation of biological membranes is the overall decrease in their fluidity, which makes it extremely easy for the two monolayers to exchange phospholipids, and increases the permeability of the membrane to substances (29). In human skeletal muscle, lipoperoxides (LPO) levels are significantly higher in aged than in young subjects (17,18). Furthermore, LPO levels in young women are significantly lower (by about 50%) than in young men (17,18). This is consistent with the protective effect of female sexual hormones against lipid peroxidation. During the aging process, LPO levels become significantly higher both in women and in men (17,18) which suggests that susceptibility to peroxidation in both sexes increases during aging, as shown in three age groups (17-40; 41-65; 66-91 years old) (17,18). In any case, the level reached in elderly women is lower than that in men (17,18); in particular, MDA values in the VL muscle in aged females (over 70) were comparable to values characteristic of young males (under 40) (26). In human skeletal muscle, the level of lipid peroxidation also depends on muscle fiber composition and on muscle function. Fiber compositions indicate that muscle with more than 40% type II fiber content shows statistically lower LPO levels than does muscle with less than 40% type II fiber content (18). This suggests that increased lipid peroxidation prevails in type I fibers (which are mainly oxidative), where most ROS production probably takes place by mithochondrial alteration. An increase in the LPO level during aging is evident in VL (15,18,25) and in the external intercostal (25), but not in the RA muscle

(15). Fiber I distribution is very similar in RA and VL, which suggests that functional differences in these muscles could be the cause of major lipid peroxidation. On the base of these results it seems that lipid peroxidation level are influenced by a variety of different factors: sex, fiber composition and muscle specificity.

Protein carbonylation

Proteins are easy targets of oxidative modifications as induced by ROS and lipid peroxidation products (MDA and HNE). Protein oxidative damage involves both the loss of thiol groups, and modifications to amino acids that constitute the polypeptide chain, in particular histidine. Since oxidized protein is harmful to the maintenance of cellular homeostasis, it requires rapid removal by proteolytic digestion. PC content increases drastically in the last third of the life span, and reaches a level such that on average 1 out of every 3 protein molecules carries modification (30). Both in men and in women, PC content tends to increase during aging, but in women these changes are not statistically significant (17). Increasing amounts of PC content were observed and compared during aging in male skeletal muscles, but only in subjects younger than age 40 and older than age 70 (17). Muscles with different functions in humans have been compared (15), and some authors reported an increase in PC level during aging in both VL (31) and external intercostal (25); in other cases, the increase was not statistically significant either in the VL (15,25) or in the RA (15).

Mitochondrial DNA damage: 8-OHdG

Investigations of human skeletal muscle have established a correlation between age and the accumulation of mtDNA deletions and mutations (32); it has also been suggested that mtDNA mutations start to occur after the fourth decade of life, and that they accumulate with age in post-mitotic tissues (33). Data obtained in male skeletal muscles indicate an increase in DNA damage during aging (31); analogous damage occurs in women, but at lower levels than in men (26). This difference may be due to females' high estrogen levels, since these hormones could contribute to enhancing antioxidant defences in female muscles (34). An increase in MDA content is significantly correlated with an increase in 8-OHdG, a finding which suggests a direct correlation between lipid peroxidation and DNA damage in human skeletal muscles (27). It has been demonstrated that deletion patterns of mtDNA over a lifetime are tissue-specific and that they are more pronounced in the skeletal muscle (35).

The role of oxidative stress in age-related Sarcopenia

As we age, significant changes in muscle mass and quality take place. Irwin Rosenberg first coined the term *sarcopenia* in 1989 (36). An age-dependent decrease in total muscle mass and cross-sectional area (CSA) and an infiltration of muscle by fat and connective tissue (myosteatosis) co-occur (37) with a macrophage infiltration release of pro-inflammatory cytokines (such as TNF-a, IL-6, IL-1) (38). Furthermore, satellite cell activation/proliferation, proliferation of SR and of the T-tubular system, and excitation–contraction coupling appear to decrease as the result of an accumulation of oxidative products (39). Intrinsic factors play a key role in the development of *sarcopenia*:

1) reduction in anabolic hormones, such as testosterone, estrogens, growth hormone and insulin-like growth factor-1; 2) increased inflammatory activity as measured by the cytokines IL-6 or TNF-alpha; 3) some changes in mitochondrial function of muscle cells; 4) increased apoptotic activity in myofibers (40); 5) accumulation of free radicals (41).

A plausible theory for age-related impairments in muscle tissue considers oxidative damage to satellite cells. These latter are adult stem cells involved in normal muscle growth, as well as in regeneration subsequent to injury or disease. Satellite cell antioxidant capacity decreases during the later years of life. Some authors recently reported that a single bout of oxidative stress in satellite cells induced a loss of viability, a shorter lifespan and a substantial decrease in the cells' proliferative capacity (42). This finding supports the hypothesis that CAT and Gluthatione transferase antioxidant activity reduces in satellite cells (43).

Influence of gender, type and function in age–related oxidative alteration in skeletal muscle

The cross-sectional muscle area decreases by about 40% between 20 and 60 years of age (44), and this drop seems to be about two times higher in men than in women (45), as may be predicted by evidence that fat-free mass decreases faster in men than in women (46). Studies on oxidative stress confirm that men are more susceptible than women to age-dependent changes in enzymatic and non-enzymatic antioxidant mechanisms in skeletal muscle**.** GSSG levels were significantly higher in 66-91 year-old men than in analogous women; moreover, men have higher lipid peroxidation levels, and MnSOD, which increases significantly during aging, is particularly evident in females (17). Furthermore, twofold higher levels of LPO have been found, and lower SOD activity, in subjects with less than 40% of type II fibers as compared with subjects with more than 40% of type II fibers (16). Muscle function seems to play a specific role in age-related changes in different muscles (15). The respective effects of aging were compared in VL, a typical movement muscle, and on RA, a typical posture muscle; fiber I/II distribution proved to be very similar in RA and VL, which suggests that the differences in use of these muscles is the origin of their differing age-related responses (15). MnSOD increase is more evident in RA than in VL. LPO, in contrast, increases more during aging in VL than it does in RA (15). The GSH/GSSG *ratio* decreased exclusively in RA, while in VL it did not change (15). These findings showed that muscle function could be significant for biochemical adaptations.

Role of nutrition against muscle oxidative damage and sarcopenia

The beneficial effects of nutrition on the prevention of oxidative stress have been attributed to the presence of antioxidants contained mainly in vegetables: carotenoids, plant polyphenols, tocopherols, ascorbate, and selenium. Among the antioxidants contained in foods, carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene) are considered to be the best biological markers for fruit and vegetable intake (47). In this regard, population-based studies on the association between carotenoid blood levels and oxidative

damage attempt to understand the impact of healthy diet on the aging process. Some studies show that low serum/plasma carotenoid levels are independently associated with poor muscle strength and a decline in walking speed, as well as being related to *sarcopenia* and related muscle dysfunctions (48,49). In the InCHIANTI study, low β-carotene intake was associated with low physical performance (50) and disability (51).

Possible use of dietary supplementation

The literature provides little evidence of the possible effects of antioxidant supplementation on skeletal muscle aging (52,53). It has been showed in an animal aging model that vitamin E and C supplementation as well as a blend of polyphenols and carotenoids for 10 months resulted in significantly increased activity of the GSH system in muscle (54); another study indicated that mixed supplementation with rutin, vitamin E, vitamin A, Zinc and selenium restores the ability to stimulate protein synthesis subsequent to leucine administration (52). Recently, Resveratrol, a natural polyphenol found in grapes, peanuts and berries, has shown a protective effect against oxidative stress in skeletal muscle through the expression of antioxidant enzymes in young and old rats (55).

Some authors showed that vitamin C and E supplementation attenuated the increase in markers of oxidative stress in response to chronic repetitive muscle loading, but supplementation did not increase muscle mass and maximal force production (53). Furthermore, possible improvements in premature aging signs (e.g. muscle waste) were investigated in BMAL1 knockout mice by supplementation with N-acetyl-L-cysteine (NAC); the results suggested an extended lifespan, but excluded significant effects on *sarcopenia* (56).

The literature also reports negative results for supplementation; antioxidant supplements (vitamins E and C) determine increased levels of antioxidant biomarkers in men undergoing surgical immobility, while clinically substantial and beneficial effects of antioxidant supplementation are elusive (57).

Altogether, the above studies suggest that a diet supplemented with a combination of antioxidants may possibly increase antioxidant defenses, lower muscle oxidative damage and improve muscle protein balance during senescence. We must consider that an adequate number of human clinical trials on this topic is not yet available; most of the positive results are obtained in animal models and still await confirmation in humans.

Conclusions

It is now clear that such phenomena as oxidative macromolecule modifications, mtDNA mutations, mitochondrial dysfunction are causally related to aging, but the mechanisms by which these molecular and biochemical events occur remain to be established. During aging all tissues, including skeletal muscle, demonstrate an accumulation of oxidative damage that may contribute to loss of tissue homeostasis (9). From a molecular point of view, one explanation could be that HSP is implied in antioxidant defense enzyme systems; while this role is evident in the muscles of adult rodents, it is not seen in old rodents, due to the lack of appropriate transcription factor activation. It has been demonstrated that this age-related inability to produce HSPs plays a critical role in the development of the functional deficits that occur with aging in skeletal muscle (10). From an antioxidant system perspective, GSH levels are quite constant, while GSSG levels increase with age, so that the GSH/GSSG *ratio* decreases, which in turn suggests that aging may cause significant alterations to the redox system in skeletal muscles (17,18). Consistently with the documented increase in mitochondrial ROS production, MnSOD increases in oxidative muscle during aging, which suggests that the origin of oxidant stress in aging may be compartmentalized. Accordingly, altered membrane mitochondria could increase free radical production and cell damage, and it could induce apoptosis, both of which are key mechanisms behind *sarcopenia*. Other antioxidant enzymes show contradictory data in aging human skeletal muscle (15, 25, 26). In conclusion, antioxidant supplementation, which is receiving growing attention, could benefit muscle protein metabolism during aging, but a large number of humans clinical trials is required to establish the correct relationship between antioxidant supplements and *sarcopenia*.

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