

# Human Skeletal Muscle Aging and the Oxidative System: Cellular Events

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**Abstract:** As we age, the aerobic and functional capacities of our major physiological systems progressively decline. In the case of the neuromuscular system, reductions in strength and mobility cause a deterioration in motor performance and in turn a greater tendency to fall (with increased risk of fractures), impaired mobility, disability and loss of independence in the elderly. Given the increase in our life expectancy and the consequent growth in the elderly population, these conditions will have an increasing impact on modern healthcare systems, and their prevention and attenuation needs to be addressed. Several intervention strategies have been used to improve motor performance among the aging. (HANGS)At the cellular level, aging is caused by a progressive decline in mitochondrial function that results in the accumulation of reactive oxygen species (ROS) generated by the addition of a single electron to the oxygen molecule As the level of oxidative stress in skeletal muscle increases with age, the production of some antioxidant enzymes increases adaptively to compensate in part. The aging process is characterized by an imbalance between an increase in the production of reactive oxygen species in the organism and the antioxidant defences as a whole.

The goal of this review is to examine the results of existing studies on oxidative stress in aging human skeletal muscles, taking into account different physiological factors (sex, fiber composition, muscle type and function).

**Keywords:** Aging, skeletal muscle, free radicals, oxidative stress, antioxidant enzymes, lipoperoxidation, protein carbonilation, sarcopenia.

## INTRODUCTION

The generation of reactive oxygen species is a normal process in the life of aerobic organisms: up to 5% of oxygen reacting with the respiratory chain is incompletely reduced to ROS [1, 2]. These species are maintained at a level that allows normal muscle contraction by the cellular antioxidant systems, primarily by the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), to [3-5]. The accumulation during aging of ROS results in oxidative stress that can damage cellular components such as lipids, proteins and DNA; and, in turn, the tissues themselves.

There is a lot of evidence to suggest that ROS are directly involved in aging processes. The variation in life span among different species correlates inversely with rates of mitochondrial generation of the oxidant species  $O_2^-$  and  $H_2O_2$ ; experimentally increased life span has been found to be associated with a reduction in the rate of oxidative damage [6]; still more convincingly, over-expression of the free radical scavenging enzymes superoxide dismutase and catalase increases average life span by one-third in drosophila, and preserves physiological function and oxygen consumption [7]. More recently, Schriener and others reported extension of the life span in mice bred for over expression of catalase targeted to the mitochondria [8].

The redox situation within the tissues is a dynamic balance (homeostasis) between the production of reactive oxygen species and antioxidant system capacity, and this determines the level of oxidative damage.

The skeletal muscle is the largest consumer of oxygen in the body, and there is a debate in the literature whether aging in this tissue is associated with change in cellular antioxidant defence mechanisms and/or an progressive increase of ROS described during aging [9]. In fact activities of the antioxidant enzymes in muscles are reported to go up or down or remain unchanged during aging [10]. This lack of consensus in the literature could be related to the variety of mechanisms that can lead to free radical production and that can be modulated by aging, sex, fiber composition, muscle type and the physiological state of the animals.

## Skeletal Muscle Aging

The nature of the mechanisms underlying impaired motor performance in old age involves multi-factor events and is complex and not well understood. The loss of strength in old age is predominantly accounted for by reduced muscle mass, a process known as senile sarcopenia [11]. Sarcopenia is a highly prevalent condition: it has been estimated that 25% of people under the age of 70 and 40% of people aged 80 or older are sarcopenic [12]. As people age the strength in the skeletal muscles gradually decreases at a rate of 1-2% per year after the age of 50 [13] and by 30-40% at the age of 70 [14]. The prevalence of this syndrome is expected to dramatically rise due to the progressive aging of Western populations. Sarcopenia represents a powerful risk factor in older subjects regarding frailty, the loss of independence, and physical disability [15]. In addition, impaired muscle strength has been reported to be highly predictive of incident disability and all-cause mortality in advanced age [16]. Advancing age is associated with a functional decline in muscle mass and strength which is greater in men than in women [17]. Furthermore, sex hormones seem to be an important factor in maintaining muscle mass and strength in men but not in women [12]. The extent of sarcopenia, and thus age-related atrophy, are higher in glycolytic muscles compared to

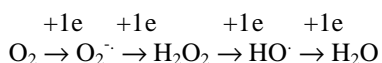
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oxidative muscles [18]. Mammalian skeletal muscle fibers are classified in four major forms according to their myosin heavy chain (MHC) expression. Type I fibers are slow contracting, mainly oxidative, while type II fibers are fast contracting, mainly glycolytic, with a lower number of mitochondria. In humans the structural changes responsible for age-related muscle atrophy and decline in muscle strength are related to the decline in the cross-sectional fiber area (up to 25-30% in subjects aged 70) [19], and to fiber denervation and fiber number loss, with type II fibers being the most affected by aging [20-22]. The remaining type II fibers seem to maintain their efficiency, probably by adjusting their capability to produce energy, as suggested by the absence of age-related changes in the enzyme activities of the anaerobic enzyme machinery of energy production. Type I fibers are little or not at all affected by the aging process [22-24].

The oxidative capacity of the skeletal muscle reflects the capacity of a working muscle to synthesize adenosine triphosphate (ATP) through aerobic metabolic pathways. Skeletal muscles are unique in their requirement and ability to undertake very rapid changes in energy supply and oxygen flux during contraction. By using different approaches some investigators have reported an age-associated decline in oxidative capacity [25-29]. However, some care has to be taken because different muscles may respond differently to the aging process, as suggested by the contrasting data in the literature. More recently, using a new non-invasive *in vivo* measurement Amara *et al.* describe a reduced mitochondrial efficiency as expressed by a reduction in the ATP molecules produced per O<sub>2</sub> consumed [30] in the human skeletal muscles of healthy early elderly subjects. What is not completely addressed at the moment is the cause of this decline: could it be due to a drop in physical activity that occurs with aging, rather than to aging in and of itself? Habitual physical activity would be expected to play an important role in determining the extent to which oxidative capacity changes with age [31].

### Mitochondria: Source and Target of Reactive Oxygen Species

Reactive oxygen species include oxygen free radicals, the superoxide radical anion (primary product of one-electron dioxygen reduction), the extremely aggressive hydroxyl radical (deriving from subsequent chemical reactions), and the strong non-radical oxidants, singlet oxygen and hydrogen peroxide.



ROS-mediated damage is the result of an increase in electron flux and the corresponding leakage from the mitochondrial respiratory chain. The skeletal muscles (as well as the central nervous system) are particularly vulnerable to "oxidative stress" because they are tissue made up of post mitotic cells that use a large amount of oxygen, thus causing cumulative oxidative damage to the cell structures over time. Furthermore, it has been suggested that the dynamic ability to change the mitochondrial oxidative capacity, typical of this tissue, in response to variations in energy demands makes the tissue particularly prone to ROS-mediated damage. The antioxidant reserve capacity in most tissues is not

marginal, but during aging the production of ROS presents a challenge to the antioxidant systems because it could exceed their capacity.

Mitochondrial structures are very susceptible to oxidative insults given the proximity of mitochondrial DNA (mtDNA) and several functional mitochondrial proteins to the primary ROS source. Consistent with these considerations, markers of oxidative damage to DNA are much higher in the mitochondria as compared to the cytosol [32]. Age-related oxidative stress leads to mitochondrial DNA (mtDNA) damage, which, in turn, results in defective electron transport chain complexes (ETC) [33-35], reduced ETC activity, enhanced production of ROS, and an increase in oxidative damage through a vicious cycle of events ultimately leading to cellular senescence according to the mitochondrial theory of aging [36].

Even a modest, constant ROS production [8, 37] is likely to accumulate oxidative damage affecting DNA, proteins and lipids over time, impairing mitochondrial ATP production and supporting the existence of a "vicious mitochondrial ATP cycle" (see Table 2) [38]. There is a decrease of about 50% in the mitochondrial ATP content and production in the gastrocnemius muscle from aged rats [38], and a similar decline in the human skeletal muscle with advancing age has recently been observed [39].

### OXIDATIVE DAMAGE TO LIPIDS, PROTEINS AND DNA IN HUMAN SKELETAL MUSCLE

Few data is available about oxidative damage during the aging process in human skeletal muscle, and the majority of this data comes from the vastus lateralis muscle. The literature on the human skeletal muscle strongly supports an age-associated increase in damage from oxidative stress to mitochondrial components that includes lipid peroxidation, protein oxidation and mtDNA mutations.

#### Ros-Induced Lipid Damage

One of the principal indicators of free radical damage during aging is lipid peroxides (LPO).

Polyunsaturated fatty acids present on biomembranes are attacked by free radicals in the presence of oxygen. The chain of peroxidative reactions that occurs leads to the formation of hydrocarbon gases (methane, pentane and ethane) and aldehydes (like malonaldehyde, MDA) [40].

By-products of lipid peroxidation are the most frequently studied markers of oxidative tissues: the degree of membrane lipid peroxidation is detected by the MDA level and/or 4-hydroxy-2-nonenal production (4-HNE) [41, 42].

An interesting and possible explanation for age-dependent reduction of membrane fluidity is the oxidation of the membrane lipids [43, 44] induced by ROS and catalyzed by transition metals such as iron and copper [45, 46]. This process is particularly important in mitochondria, which contain cardiolipin as a major component of the inner mitochondrial membrane [47]; cardiolipin is required for the activity of cytochrome oxidase [48] and of other mitochondrial proteins [49]. Cardiolipin is a high-unsaturated molecule, and oxidative stress decreases this lipid to a larger extent than other lipids [50]. The decrease in cardiolipin appears to be

directly related to the reversible decrease in cytochrome oxidase activity [51]. Without the tightly bound cardiolipin, cytochrome C oxidase maintains only 40-50% of its original activity, which indicates that cardiolipins are required for full electron transport activity [48].

In human skeletal muscle, LPO levels are significantly higher in aged subjects than in young subjects [52-56]. The possibility of a sex-dependent difference in the effects of oxidative stress on human skeletal muscle aging has been investigated. The LPO level in young women is significantly lower (about half the value) than in young men [52, 55, 56]. This is consistent with the protective effect of female sexual hormones against lipid peroxidation. Indeed, female estrogens provided significant protection to cultured hippocampal neurons against lipid peroxidation induced by FeSO<sub>4</sub> and amyloid  $\beta$ -peptide, suggesting that these steroids possess antioxidant activities [57].

During the aging process LPO levels become significantly higher both in women and in men [52, 55, 56], which suggests that in the two sexes the susceptibility to peroxidation increases during aging. It should be noted that LPO peroxidation levels increase gradually during aging in both men and women in the three age groups tested (17-40; 41-65; 66-91 years old). Whether the difference in LPO peroxidation between young and aged women could be caused by the loss in the estrogens' protective role after menopause and/or to lifestyle modifications has yet to be determined. In any case, the level reached in elderly women is lower compared to men [52, 55, 56]; in particular, the MDA values in the vastus lateralis muscle in aged females (over 70) were comparable to values characteristic of young males (under 40) [52].

In human skeletal muscle the level of lipid peroxidation also depends on muscle fiber composition and muscle function. Data obtained comparing elderly muscle with different

fiber compositions indicate that muscle with a percentage of type II fiber greater than 40% show a statistically lower LPO level compared to fiber with a percentage of type II fiber less than 40% [56]. This suggests that the increase in lipid peroxidation prevails in type I fibers (which are mainly oxidative), where most ROS production probably takes place.

The increase in the LPO level during aging is evident in the vastus lateralis (VL) [52, 53, 56, 58, 59] and in the external intercostal, as reported by Barreiro [59], but not in the rectus abdominis muscle (RA, see [58]). Fiber I distribution is very similar in RA and VL, thus suggesting that functional differences in these muscles could be the cause of major lipid peroxidation. It is actually difficult to explain why the oxidative lipid levels in RA are different than in VL. For the moment there are two possible explanations: the major contractile function of the VL muscle compared with the RA muscle and/or more peroxidizable substrates present in VL compared to RA.

All data taken together indicate that the LPO peroxidation level is influenced by a number of different variables: sex, fiber composition and muscle specificity.

### Ros-Induced Protein Damage Protein Damage

A great number of studies have shown an increase in oxidized proteins at the intracellular level during senescence. For the most part, oxidative modified proteins are not repaired and must be removed by proteolytic degradation carried out by proteasome, which selectively degrades oxidative modified proteins. The molecular mechanism that causes the accumulation of oxidative modified proteins is not identified. Nevertheless, protein oxidation is likely to have physiological importance because this modification causes a loss of function in the affected proteins and the accumulation will have serious deleterious effects on cellular and organ func-

**Table 1. LPO, PC and DNA Damage in Aged vs. Young**

AGED vs. YOUNG	LPO	PC	DNA
Pooled data	↑Mecocci <sup>99</sup> ↑Pansarasa <sup>99</sup> ↑Pansarasa <sup>00</sup> ↑Fanò <sup>01</sup> ↑Marzani <sup>04</sup>	↑Mecocci <sup>99</sup> (NS) Pansarasa <sup>99</sup>	↑Mecocci <sup>99</sup> ↑Fanò <sup>01</sup> ↑Parise <sup>04</sup>
Aged Women vs Aged Men	↓ Pansarasa <sup>00</sup> ↓Fanò <sup>01</sup> ↓ Marzani <sup>04</sup>	↓ Pansarasa <sup>00</sup> (NS) Fanò <sup>01</sup>	↓ Fanò <sup>01</sup>
Fiber II vs Fiber I	↓ Marzani <sup>04</sup>		
Muscle Type Vastus lateralis	↑Mecocci <sup>99</sup> ↑Fanò <sup>01</sup> ↑Marzani <sup>04</sup> ↑Marzani <sup>05</sup> ↑Barreiro <sup>06</sup>	↑Mecocci <sup>99</sup> (NS) Fanò <sup>01</sup> ↑Parise <sup>04</sup> (NS) Marzani <sup>05</sup> (NS)Barreiro <sup>06</sup>	↑Mecocci <sup>99</sup> ↑Fanò <sup>01</sup> ↑Parise <sup>04</sup>
Rectus abdominis	(NS) Marzani <sup>05</sup>	(NS) Marzani <sup>05</sup>	
External intercostal	↑Barreiro <sup>06</sup>	↑Barreiro <sup>06</sup>	

(NS) not significant; (upper case number) year paper published.

tion. Recently, Moskovitz and others have shown that transgenic mice knockouts of methionine sulfoxide reductase, which repairs oxidized methionine, have a reduced life span and show an increase in carbonilated proteins (CP/PC) [60].

The protein carbonylation is a marker of protein oxidation caused by oxidation of the side chains of the amino acid residues lysine, arginine, proline and threonin; this may occur either directly or indirectly as a consequence of lipid peroxidation [61]. Oxidative damage targets specific proteins during aging. Proteins that are reported to be affected by oxidative damage are respiratory chain enzymes [62], adenosin-triphosphatase (ATPase) [61-64], the adenine nucleotide translocator, and transhydrogenase [62]. In particular, among the respiratory chain enzymes, complex I is particularly susceptible to oxidative damage, and the oxidative modifications of proteins by reactive species is closely related to the aetiology and/or progression of different disorders and diseases (for a recent review see [65]), such as Parkinson's disease [66]. The carbonyl protein content increases drastically in the last third of the life span (see Table 1, [65]), reaching a level such that on average 1 out of every 3 protein molecules carry the modification [61]. A trend towards increasing amounts of protein carbonyl content has been observed during aging in male skeletal muscles, but only when subjects younger than age 40 and older than age 70 were compared [50] [55]. The protein carbonyl content basal levels in young women and men are comparable [58]. Both in men and women the protein carbonyl content tends to increase during aging, but in women the changes are not statistically significant and have a lower value [52, 55]. A comparison has been carried out between muscles with different functions in humans [58] and rats [67, 68]. In human muscle some authors reported an increase in the PC level during aging in both the vastus lateralis [53, 69] and the external intercostal [59]; in other cases the increase is not statistically significant in the vastus lateralis [52, 58, 59] and the rectus abdominis [58].

The difficulty in showing significant changes in PC in the chronic physiological process could be due to the dynamic features of the process: during aging the myofibrillar carbonyl content has been shown to increase significantly 3 hours after acute oxidative stress, only to return to its basic level within a few hours [70]. This rapid removal of oxidized myofibrillar proteins is unexpected in a tissue like skeletal muscle, which is characterized by a relatively slow protein turnover.

### **Mitochondrial DNA Damage**

Mitochondrial DNA (mtDNA) is a double stranded ring (about 16.5 Kb) encoding 13 subunits of the ETC as well as 2 rRNA and 22 tRNA proteins necessary for their translocation. Mitochondrial DNA lacks introns and is devoid of histones and other DNA-associated proteins; it is located in the mitochondrial matrix, close to the major source of ROS. For these reasons the probability of the oxidative modification of a coding region of mtDNA is very high, and mtDNA is thus extremely susceptible to oxidative damage (for a recent review see [71]). The repair devices of mtDNA, even if present, are largely insufficient to overcome extensive DNA damage, as demonstrated after an oxidative stress to cultured cells: the damage to mtDNA is higher and persists longer

than that to nuclear DNA [72]. Similarly, a ten times higher age-dependent accumulation of oxidative damage in mitochondrial DNA compared to nuclear DNA was shown in the human cerebral cortex [73]. Isolated single muscle fibers with ETC dysfunction accumulate mtDNA point mutations [74, 75] and deleted mtDNA [35, 75].

The continuous exposure to free radical attack leads to stochastic errors in the mtDNA-encoded polypeptide chains that accumulate with age. ROS were shown to induce extensive fragmentation and deletions in mtDNA in a transformed human fibroblast cell line [77]. The consequence of these alterations in mtDNA affect the four mitochondrial enzymatic complexes involved in energy conservation, causing defective electron transfer and oxidative phosphorylation, thereby establishing a vicious circle of mtDNA mutations and "oxidative stress" (see Table 2) [78]. A correlation between mitochondrial DNA deletion and respiratory chain enzyme activities in aging rats [35] and human skeletal muscles is reported [79].

The relationship between mtDNA mutations and the aging process could be investigated by generating mice that display elevated mtDNA mutation frequencies throughout their tissues (3-11 times higher than in wild mice) due to error-prone replication by an exonuclease derivative of the nuclear-encoded mtDNA polymerase  $\gamma$ . These "mutator mice" (called D257A) display a large array of phenotypes resembling aspects of accelerated aging; these include reduced life span, hair loss, muscle loss, reduced fertility and osteoporosis. This transgenic mouse provides a model by which we can examine the mechanistic features of the aging process [80, 81].

Investigations of the human skeletal muscle have established a correlation between age and the accumulation of mtDNA deletions [82] and mutations [83-85]. The degree of DNA oxidative damage could be measured by means of 8-hydroxy-2-deoxyguanosine (8-OHdG). 8-OHdG is the most frequent base formed after exposing mammalian chromatin to an ionizing radiation-generated free radical. It can mispair with adenine one percent of the time, thereby leading to point mutations, and can cause the misreading of adjacent residues [86, 87].

Data obtained in male skeletal muscles indicate an increase in DNA damage during aging [52, 69] for pooled male and female data [53]. As in men, DNA damage in women increases with age but to a lesser extent [52]. Mecocci and others have shown that the increase in malondialdehyde content is significantly correlated with an increase in 8-OHdG, suggesting a direct correlation between lipid peroxidation and DNA damage in human skeletal muscles [53].

It has recently been demonstrated that deletion patterns of mtDNA over a lifetime is tissue-specific and more pronounced in the skeletal muscle and heart [88].

Taken together, these data suggest that during normal aging molecular oxidative damage to macromolecules, and particularly to lipid and DNA, accumulates in the mitochondria. It should be noted that in human skeletal muscle oxidative damage is probably lower in aged women compared with aged men. In particular, it seems that women are more

**Table 2. Activities of CuZnSOD, MnSOD and CAT in Aged vs. Young**

AGED vs. YOUNG	CuZnSOD	MnSOD	CAT
Pooled data	(NS) Parise <sup>04</sup>	↑Pansarasa <sup>99</sup> (NS) Pansarasa <sup>00</sup> ↑Marzani <sup>04</sup> ↑Parise <sup>04</sup>	(NS) Pansarasa <sup>99</sup> Marzani <sup>05</sup>
Aged Women vs. Young Women		↑Pansarasa <sup>00</sup>	
Aged Women vs. Aged Men		(NS) Pansarasa <sup>00</sup>	(NS) Pansarasa <sup>00</sup> (NS) Fanò <sup>01</sup>
Fiber II vs Fiber I		(NS) Pansarasa <sup>02</sup>	(NS) Pansarasa <sup>02</sup>
Muscle Type Vastus lateralis	(NS) Parise <sup>04</sup> (NS) Marzani <sup>05</sup>	(NS) Marzani <sup>04</sup> ↑Parise <sup>04</sup> (NS) Marzani <sup>05</sup> (NS) Barreiro <sup>06</sup>	↓Fanò <sup>01</sup> ↑Parise <sup>04</sup> (NS) Marzani <sup>05</sup> ↑Barreiro <sup>06</sup>
Rectus Abdominis	(NS) Marzani <sup>05</sup>	↑Marzani <sup>04</sup> ↑Marzani <sup>05</sup>	(NS) Marzani <sup>05</sup>
Exsternal Intercostal		↑Barreiro <sup>06</sup>	↑Barreiro <sup>06</sup>

(NS) not significant; (upper case number) year paper published.

protected against molecular oxidative stress during premenopausal life period, suggesting a significant antioxidant role of oestrogen.

The increase in DNA damage is more evident in the male skeletal muscle compared to female samples (up to fourfold) [50]. This difference may be due to high estrogen levels, since these hormones could contribute to enhancing antioxidant defences in female muscles [89, 90]. During the fertile age these hormones probably produce less oxidative damage accumulation with respect to males, determining a more favourable condition in the aging process. Other causes, such as different sex-related muscle activity, could also play a role.

Due to differences in the fiber composition of people, we can expect that muscles with a higher percentage of fiber II in the skeletal muscle will be less susceptible to lipid peroxidation during aging.

## ENZYMATIC ANTIOXIDANT SYSTEMS

ROS are produced in mitochondria during respiratory ETC and should be rapidly transformed into more inactive species by the antioxidant enzymes SOD, GPx and CAT. The level of potentially toxic superoxide radical in cells is controlled by SOD, while the level of hydrogen peroxide is controlled by catalase and glutathione peroxidase.

There is great controversy in the literature as to whether or not aging is associated with an increase or decrease of enzymatic antioxidant defences in the cell. There are few works on the antioxidant system during aging in human skeletal muscles that indicate that the antioxidant system undergoes significant alteration, but the results need to be carefully interpreted taking into account sex, muscle fiber composition and specificity.

## Superoxide Dismutase

SOD is one of the primary enzymatic antioxidant defences, and it readily converts superoxide radicals ( $O_2^-$ ) into  $H_2O_2$ . An increase in total SOD activity corresponds with enhanced resistance to oxidative stress [91]. SOD is comprised primarily of cytosolic (CuZnSOD) and mitochondrial (MnSOD) SOD localized in the inner membrane of the mitochondria. Depending on the source of antioxidant stress, these two isoforms are differentially affected.

MnSOD activity in skeletal muscle, both in rats [92] and men [54, 57, 69], is reported to increase significantly with aging and to respond to age-related oxidative stress in the mitochondria by up-regulation.

Interestingly, MnSOD activity [93, 94], like glutathione peroxidase activity, [93, 95, 96] is always found to increase during aging in the oxidative soleus muscle in rats. In the rat's tibialis anterior muscle and in the extensor digitorum longus, two fast-twitch muscles, total SOD activity increases with age, whereas MnSOD did not change, which is consistent with a greater increase in ROS production in oxidative muscles compared to glycolytic muscles in the mitochondria and with a possible increase in ROS production in glycolytic muscles not located in the mitochondria [67, 93].

In humans, as in rats, the age-dependent enhancement in MnSOD activity is particularly evident in the rectus abdominis [56, 58] and in the external intercostal muscle [59] rather than in the vastus lateralis [56, 58, 59, 69], suggesting a marked increase in ROS production mainly in oxidative muscles, where the marked alteration of ETC functionality results in an electron leakage increment.

The MnSOD activity value is comparable between young men and women, doubling during aging in both sexes [55]. The MnSOD level seems to play an active role in both

women and men during aging. This regulatory mechanism partially counteracts the increase in free radicals in the mitochondria in senescent tissues, and in particular provides an induction of the defences against  $O_2^{\cdot-}$  that may be generated at a higher rate in mitochondria during aging.

These results strongly suggest that mitochondrial stress due to increased leakage in ETC during aging is associated with the production of superoxide. This free radical has a short half-life and can freely cross membranes *in vivo* [97]; for this reason mitochondrial stress will occur in the mitochondria. According to this hypothesis, cytoplasmic SOD did not show any change or decrease in activity during aging [52, 55, 69].

The effect of age on total SOD activity in the skeletal muscle is a subject of debate. Some studies of rats show an age-dependent increase in total SOD activity in several types of skeletal muscle, except the soleus [92, 67, 98]. Some researchers report a decreasing trend in total SOD [54, 57] and CuZnSOD activity in human skeletal muscles during aging [58], whereas others report no change in total [52, 69] and cytoplasmic SOD activity [69]. The different results in the literature concerning SOD activity could be related to the variety of mechanisms that can lead to free radical production and that can be modulated not only by age and sex but also by fiber and muscle type.

In human skeletal muscle the relationship between SOD activity, aging and sex was investigated [55] in the following age groups: young (17-40 years of age), adult (41-65) and aged (66-91). Total SOD activity in the human skeletal muscle in young women is about four-to-five times higher than in men of the same age. In elderly women, as in men, SOD activity decreases and values become comparable. It was suggested that total SOD activity could be a limiting factor in muscle defences against oxidative damage in humans. In the vastus lateralis muscle, there was no significant difference in the SOD activity of males and females [52].

Pansarasa *et al.* compare total SOD and MnSOD activity in muscles with different fiber composition in elderly people (65-90 years of age). Total SOD activity appears to be significantly higher in enriched type II fiber muscles [99]. Strength training, which results in muscle hypertrophy and improved muscular strength and power [100], could be an important factor in delaying the progressive loss of type II fibers in elderly subjects as well [101, 102]. This translates into improved functionality, including walking mechanics, speed, endurance, and stair-climbing power [103, 104]. Despite its demonstrated benefits, strength training continues to receive only brief mention in published exercise guidelines. Health care providers normally recommend only aerobic exercise to their aged patients [105], forgetting that strength training together with aerobic exercise is more likely to be useful for the management of sarcopenia. In type I fiber-enriched muscles there is an increase in oxidative damage as measured by lipid peroxidation and a decline in total superoxide scavenger capability.

In short, during aging the increased ROS production is differently located and gives rise to different compensatory effects of the antioxidant system according to the different functional and metabolic muscle properties: in glycolytic

skeletal muscles total SOD activity increases without changes in MnSOD, suggesting that the increase in ROS and the subsequent increase in the antioxidant system is outside the mitochondria; in oxidative muscle the increase in ROS as well as the compensatory effect concerns the mitochondrial environment.

### Catalase

The decomposition of hydrogen peroxide to form water and oxygen is carried out in the cell by glutathione peroxidase and CAT.  $H_2O_2$  has the ability to freely cross membranes [106] and has a relatively long half-life. Like SOD, GPx and catalase are located in both the mitochondria and the cytosol, where they provide important cellular protection against free radical damage to membrane lipids, proteins and nucleic acids. Catalase enzyme is widely distributed in the cell, with the majority of activity occurring in the mitochondria and peroxisomes [107].

Studies performed on catalase activities have dealt with rat skeletal muscles, but there is a small amount of data from human skeletal muscles. Concerning CAT activity, most data on rats report an increase in catalase activity both in senescent oxidative muscles and glycolytic muscles [67, 93, 96, 108], which is consistent with a possible increase in non-mitochondrial ROS production.

The small amount of data on  $H_2O_2$  detoxifying enzymes from the human skeletal muscle is contradictory: some authors report no change in catalase activity in men during aging [51]. No differences are observed in catalase activity with aging under all conditions tested: between men and women, between muscles with different fiber compositions, and between oxidative and glycolytic muscles [58]. Other authors describe a significant increase [59, 69] or decrease in CAT activity in the vastus lateralis [52].

Nevertheless, the contradictory data in the literature could be due to the dynamics of catalase activity during aging: in men, as in rats, a two-phase trend was described: an initial decrease in adult animals followed by a significant enhancement in aged animals [55, 92].

### Glutathione Status

Of the two  $H_2O_2$  detoxifying enzymes, glutathione peroxidase has a much greater affinity to hydrogen peroxide than to catalase; but when the increased production of hydrogen peroxide exceeds the capabilities of glutathione peroxidase, catalase activity could compensate for the inability of GPx to scavenge hydrogen peroxide.

Glutathione (L- $\gamma$ -glutamyl-L-cystenyl-glycine), an essential tripeptide found in virtually all animal cells, plays a protective role as an  $H_2O_2$  detoxifying enzyme. Reduced glutathione (GSH) is used as a substrate for GPx to detoxify hydrogen peroxide and form glutathione disulfide. Glutathione disulfide (GSSG) must be recycled into GSH through glutathione reductase in order to maintain protection against  $H_2O_2$ . As a major thiol source in the body, GSH is also a scavenger of singlet oxygen and hydroxyl radicals.

Therefore, an index of the cell's ability to cope with stressful conditions is the redox status, the ratio of GSH /

GSSG disulfide [40]. As with other enzymatic antioxidant activity, the ratio of GSH / GSSG can undergo dynamic changes in different physiological and pathological situations.

Skeletal muscle has one of the lowest GSH amounts (about 1 mM) compared to other tissue such as the eye lens and liver, which have the highest GSH concentration (about 10 mM). Furthermore, the muscle takes up GSH mainly from extracellular sources [109] because of its scarce ability to synthesize GSH *de novo*.

The GSH level in human skeletal muscles is quite constant [58, 99], suggesting that GSH transport into the cell did not change during aging [96]. On the contrary, GSSG levels increase with age: the GSH/GSSG ratio decreases significantly during aging [55, 56], suggesting that aging may cause significant alterations in the glutathione status in male skeletal muscles. In human skeletal muscles GPx does not change during aging [52, 55, 58, 99] in either glycolytic or oxidative muscle.

Young females have higher GSH levels and lower GSSG levels than young males, along with comparable GPx activity: the GSH/GSSG ratio is significantly higher in young women compared to young men [49, 96]. During aging the increase in the GSSG level in men is three times higher than in women; consequently the GSH/GSSG ratio between the sexes becomes statistically significant.

In young rats, skeletal muscle fibers with greater oxidative capacity (like the soleus muscle) have higher levels of GSH and total glutathione content than those with lower oxidative potential (like the deep or superficial vastus lateralis muscle [110]). In addition, GPx activity was higher in oxidative muscles, causing less vulnerability and more resistance to an exercise-induced oxidative stress, whereas the modest levels of GSH and GPx in the deep or superficial vastus lateralis muscle were associated with greater lipid peroxidation under exercise stress. For these reasons, GSH and GSSG muscle levels, as well as the GSH/GSSG ratio, are expected to reflect the different muscle functions.

Comparing the human skeletal muscle in elderly people with different fiber composition or glycolytic and oxidative muscles, GSH, GSSG and GSH/GSSG show the same value [58, 99]. Despite an increase in type I fiber in both muscles with aging, the data [58] showed that the GSH and GSSG levels remained unchanged, thus suggesting that during aging there are no alterations of membrane glutathione transport and that there is a fiber-specific adaptation of the GSH system in the skeletal muscle [96].

### Dietary Antioxidants and Sarcopenia

The six major dietary carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin and lycopene) comprise an important component of the antioxidant defense system in humans. Recent epidemiological studies suggest that carotenoids or carotenoid-rich foods are protective against a decline in muscle strength and walking disability among older community-dwelling adults. The carotenoids protect against oxidative stress by quenching singlet oxygen, scavenging free radicals, and inhibiting lipid peroxidation [111]. Low serum/plasma carotenoids are independently

associated with poor skeletal muscle strength and impaired physical performance. Among 669 women aged 70–79 years in the Women's Health and Aging Studies (WHAS) I and II, low serum carotenoid levels were associated with poor muscle strength [112]. In multivariate models adjusting for age, race, smoking, cardiovascular disease, arthritis and serum IL-6, low total carotenoid levels were associated with low grip, hip and knee strength. In the InCHIANTI study, a population-based study of older adults in the Chianti region of Tuscany, Italy, low  $\beta$ -carotene intake was associated with low physical performance [113].

Further work is needed to determine the relationship between sarcopenia and other dietary antioxidants in older adults, such as  $\alpha$ -tocopherol, ascorbate, selenium and dietary polyphenols, in terms of predicting changes in balance performance and nerve conduction velocity, such as walking disability. If these observations are widely corroborated, this could provide a strong rationale and justification for dietary intervention studies aimed at reducing or preventing sarcopenia among older adults.

### CONCLUSIONS

During organism aging the production of reactive oxygen species is increased as a result of the functional deterioration of mitochondria. There is strong evidence that increased free radical generation may be the underlying reason for several age-related pathogenesises.

However, few studies have actually measured aging-induced free radicals in human skeletal muscles. It has been suggested that age-related oxidative stress may be a function of a reduction in enzymatic antioxidant capacity, but this has not been demonstrated in human skeletal muscle. In fact, all investigations of aging in human skeletal muscle have reported significantly higher levels of at least one antioxidant enzyme. The increased production of free radicals with aging plays a major role in targeting adaptive responses in human skeletal muscle. The adaptive mechanisms involve the antioxidant scavenger system and appear to be sex-, fiber- and muscle-specific. Furthermore, the amount of dietary antioxidant introduced could play a specific and important role.

MnSOD increases in oxidative muscle during aging, consistent with an increase in mitochondrial ROS production, suggesting that there may be a compartmentalization regarding the origin of oxidant stress in aging. In fact, during the aging process abnormal mitochondria are seen to accumulate with enhanced ROS production (9). The accumulation of altered membrane [115] mitochondria [116, 117] might enhance free radical production and cell injury. It is well established that following oxidative stress stimuli and an accumulation of mtDNA damage, mitochondria can induce apoptosis. The accelerated apoptosis of muscle fibers may represent a key mechanism underlying sarcopenia. Sarcopenia of aging is associated with considerable disability and mortality and represents a major modifiable risk factor for frailty. Future studies are necessary to investigate the relationship between specific signalling pathways of programmed cell death and the progression of sarcopenia in geriatric populations. This would allow us to identify specific markers to evaluate the effectiveness of targeted inter-

vention strategies designed to counteract the overwhelming and ever-growing prevalence of physical disability in older populations.

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