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Age-dependent ultrastructural alterations and biochemical response of rat skeletal muscle after hypoxic or hyperoxic treatments

F. Amicarelli [a,b,*], A.M. Ragnelli [a], P. Aimola [a], A. Bonfigli [a], S. Colafarina [a,b], C. Di Ilio [c], M. Miranda [a]

[a] *Dipartimento di Biologia di Base ed Applicata, Università di L'Aquila, Via Vetoio-Coppito, 67100 L'Aquila, Italy*
[b] *Chair of General Biology, Istituto Superiore per l'Educazione Fisica, 67100 L'Aquila, Italy*
[c] *Dipartimento di Scienze Biochimiche, Università G. D'Annunzio, 66100 Chieti, Italy*

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Abstract

This work deals with the antioxidant enzymatic response and the ultrastructural aspects of the skeletal muscle of young and aged rats kept under hypoxic or hyperoxic normobaric conditions. It is in fact well known that the supply of oxygen at concentrations higher or lower than those occurring under normal conditions can promote oxidative processes that can cause tissue damage. The enzymes investigated were both those directly involved in reactive oxygen species (ROS) scavenging (superoxide dismutase, catalase and selenium-dependent glutathione peroxidase), and those challenged with the detoxication of cytotoxic compounds produced by the action of ROS on biological molecules (glutathione transferase, glyoxalase I, glutathione reductase), in order to obtain a comparative view of the defence strategies used with respect to aging. Our results support the hypothesis that one of the major contributors to the aging process is the oxidative damage produced at least in part by an impairment of the antioxidant enzymatic system. This makes the aged organism particularly susceptible to oxidative stress injury and to the related degenerative diseases, especially in those tissues with high demand for oxidative metabolism. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Skeletal muscle; Aging; Hypoxia; Hyperoxia; Antioxidant enzyme; Ultrastructure

1. Introduction

Reactive oxygen species (ROS) are produced either by normal physiological processes, or because of the influence of exogenous species that can occur naturally in the biosphere or xenobiotics that are produced by man's activities. These metabolites are cytotoxic, being able to produce deleterious effects on biological macromolecules [1]. All organisms have mechanisms to scavenge the oxidants or to repair the damage caused by ROS, including superoxide dismutases, catalases, peroxidases, glutathione, thioredoxin, heat shock proteins and DNA repair which are quite conserved from prokaryotes to eukaryotes. The expression of the genes coding for these proteins (oxidative stress genes) is induced by changes in the concentration of ROS, suggesting that cells have developed a mechanism to sense the ROS [2,3]. However, after an increase in the production of free radicals or a decrease in the defense against toxic species or both, oxidative stress can occur. Oxidative stress reflects the consequences of a mismatch between the

* Corresponding author, at address a. Fax: +39 (862) 433273; E-mail: cellular@univaq.it

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rate of formation of free radicals and the ability of the cell to transform them to less toxic species. It is a metabolic feature of many physiological conditions such as strenuous physical exercise, stimulation of intracellular oxidases, hypoxia, hyperoxia, degenerative diseases, and drug induced toxicity [4–8]. Moreover, the free radical theory of aging assumes that the increase in peroxidative damage associated with aging is one of the major causes of the age-related cellular and molecular damage and is mainly due to a widespread decline in physiological functions and to a defective adaptive regulation of a number of enzymes [9–11].

One of the main consequence of this reduced age-dependent homeostatic response is a progressive impairment in the ability to adapt to environmental changes and a greater susceptibility to oxidative stress injury.

In previous works we studied the response of antioxidant and detoxifying enzymes in the liver and lung of young and aged rats after hypoxic or hyperoxic normobaric treatments, as models of oxidative stress [12,13]. The results showed an age-dependent biochemical adaptive behaviour to both hypoxic and hyperoxic conditions.

We have now extended our study to rat skeletal muscle, by investigating both the ultrastructural aspects and the antioxidant enzymatic response to hypoxic or hyperoxic treatments during the aging process.

The enzymes investigated were both those directly involved in active oxygen species scavenging (superoxide dismutase, catalase and selenium-dependent glutathione peroxidase), and those challenged with the detoxication of cytotoxic compounds produced by the action of ROS on biological molecules (glutathione transferase, glyoxalase I, glutathione reductase), in order to obtain a wide comparative view of the defence strategies used with respect to aging.

2. Materials and methods

2.1. Hypoxic treatment

Experiments were performed on two groups of male Wistar rats (250–400 g) of eight animals each. One group (hypoxic group) was exposed to 10% oxygen (76 Torr of oxygen) for 12 days in a large plexiglas chamber, as previously reported [12,13]. The chamber was recirculated with a pump; CO_2 was removed from the chamber air with baralyme and continuously monitored by a capnograph. Boric acid was mixed with the litter to minimize the emission of urinary ammonia. The room and the chamber temperature was maintained around 25°C. The other group (control group) was maintained on breathing air in the same room (21%, 156 Torr of oxygen). Each of these groups was composed of four aged rats (25 months) and four young rats (2 months).

2.2. Hyperoxic treatment

Experiments were performed on two groups of male Wistar rats. One group of eight rats (250–400 g) was exposed to 98–100% oxygen (760 Torr) for 60 h in a large plexiglas chamber, as previously reported [12,13]. All the other experimental conditions were the same as for the hypoxic treatment. Each of these groups was divided into two groups: one of young rats (2 months) and the other of aged rats (25 months).

2.3. Cytosol preparation

After hypoxic or hyperoxic treatment, rats were anaesthetized with Nembutal (30 mg/kg i.p.) and portions of anterior tibial muscle were immediately removed. Excised muscles were quickly dissected free of fat and tendon and maintained at −80°C until analysis. Immediately prior to biochemical analysis, tissues were thawed, minced and homogenized. The homogenization was performed in 10 mM potassium phosphate buffer pH 7.0 (1:10 w/v) made 1 mM with respect to Triton X-100 (for catalase and superoxide dismutase assays) or made 1 mM with respect to dithiothreitol (for glutathione peroxidase, glutathione transferase, glutathione reductase and glyoxalase I assays) with a Potter homogenizer. The homogenate was centrifuged for 60 min at $105\,000\times g$ with a Spinco L-50 centrifuge. The supernatants were recovered and used for enzymatic activity measurements.

2.4. Enzymatic assays

2.4.1. Catalase

Catalase (CAT; EC 1.11.1.6) activity was measured according to a spectrophotometric method [14]. The reduction of H_2O_2 at 240 nm was followed on a Perkin-Elmer spectrophotometer at 25°C. One unit was defined as 1 μmole H_2O_2 reduced/min.

2.4.2. Superoxide dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the epinephrine method as described by Sun and Zigman [15]. The inhibitory effect of superoxide dismutase on the autoxidation of epinephrine (0.1 mM) in 50 mM sodium carbonate buffer pH 10.0 was assayed spectrophotometrically at 480 nm and 25°C. Percent inhibition values were converted into activities using a purified Cu-Zn bovine superoxide dismutase as a standard. One unit of superoxide dismutase was defined as the amount of the enzyme required to halve the rate of substrate autoxidation.

2.4.3. Glutathione peroxidase

Quantification of glutathione peroxidase (GSH-Px; EC 1.11.1.9) activity was done by the method of Paglia and Valentine [16] as modified by Di Ilio et al. [17]. The activity of the Se-dependent GSH-Px was measured with H_2O_2 (0.25 mM) as substrate. The oxidation of NADPH was followed at 25°C on a Perkin-Elmer spectrophotometer at 340 nm. One unit was defined as 1 μmole of glutathione (GSH) oxidized/min.

2.4.4. Glutathione reductase

Glutathione reductase (GSSG-Rx; EC 1.6.4.27) activity was measured as described previously [18]. The assay mixture contained 50 mM potassium phosphate buffer pH 7.4, 1 mM EDTA, 1 mM GSSG and 0.16 mM NADPH. The blank did not contain GSSG. The oxidation of NADPH was followed at 25°C on a Perkin-Elmer spectrophotometer at 340 nm. One unit was defined as 1 μmole of NADPH oxidized/min.

2.4.5. Glutathione transferase

Glutathione transferase (GST; EC 2.5.1.18) activity was recorded at 340 nm and 25°C by the method described by Habig and Jakoby [19]. The standard assay mixture contained 0.1 M potassium phosphate buffer pH 6.5, 1 mM EDTA, 2 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene. The conjugation reaction was monitored at 25°C on a Perkin-Elmer spectrophotometer following the increase in absorbance at 340 nm. One unit was defined as 1 μmole of GSH conjugated/min.

2.4.6. Glyoxalase I

Glyoxalase I (GLXI; EC 4.4.1.5) activity was assayed as described by Mannervik et al. [20]. The assay solution contained 0.1 M sodium phosphate buffer pH 7.2, 2 mM methylglyoxal and 1 mM GSH. The reaction was monitored at 25°C, on a Perkin-Elmer spectrophotometer, following the increase in absorbance at 240 nm. One unit was defined as 1 μmole of *S*-lactoylglutathione produced/min.

2.5. Protein assay

The protein concentration was determined by the biuret method, using seroalbumin as standard.

2.6. Reagents and enzymes

Reagents and enzymes were purchased from Sigma and were of the purest grade.

2.7. Statistical analysis

Statistical analysis was carried out using Student's *t*-test. $P < 0.05$ was taken as the level of significance.

2.8. Electron microscopy

Tibialis anterior muscles were removed from animals of each of the six groups described above and small samples were obtained. The pieces of muscle tissue were immediately fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 h at 0–4°C and postfixed in 1% buffered osmium tetroxide for 2 h at 0–4°C; then they were dehydrated in an ethanol series and embedded in Durcupan ACM resin. Ultrathin sections were cut with a Sorvall Porter-Blum MT2-B ultramicrotome, stained with 5% uranyl acetate in 70% ethanol and lead citrate and observed with a JEOL JEM 100C electron microscope.

Table 1
Antioxidant and detoxifying enzyme activities in young and aged rat skeletal muscle subjected to hypoxia and hyperoxia

Enzyme	Control		Hypoxia		Hyperoxia	
	Young	Aged	Young	Aged	Young	Aged
Catalase	2210 ± 143 (n = 8)	1780 ± 110* (n = 8)	2100 ± 67 (n = 8)	1360 ± 65*** (n = 8)	2445 ± 140 (n = 8)	2700 ± 74 (n = 8)
Superoxide dismutase	670 ± 37 (n = 8)	2157 ± 53*** (n = 8)	540 ± 40 (n = 8)	1700 ± 17*** (n = 8)	610 ± 18 (n = 8)	2440 ± 74*** (n = 8)
Glutathione peroxidase	32 ± 7 (n = 8)	135 ± 13*** (n = 8)	24 ± 3 (n = 8)	95 ± 3** (n = 8)	71 ± 5.6 (n = 8)	123 ± 38 (n = 8)
Glutathione transferase	16 ± 0.33 (n = 8)	19 ± 4.0 (n = 8)	5 ± 0.3 (n = 8)	15 ± 0.5** (n = 8)	7.55 ± 1.1 (n = 8)	12 ± 0.6* (n = 8)
Glutathione reductase	16 ± 1.00 (n = 8)	17 ± 1.0 (n = 8)	14 ± 1.00 (n = 8)	17 ± 0.0* (n = 8)	15.1 ± 1.6 (n = 8)	2.55 ± 0.15** (n = 8)
Glyoxalase I	836 ± 86 (n = 8)	463 ± 28** (n = 8)	751 ± 59 (n = 8)	588 ± 27 (n = 8)	35.8 ± 3.7 (n = 8)	41.4 ± 3.9 (n = 8)

Specific activities expressed as International mUnits/mg protein. T = 25°C. n = sample dimension. Values are means ± S.E.M.
*$P < 0.05$ vs. young, using paired Student's t-test. **$P < 0.005$ vs. young, using paired Student's t-test. ***$P < 0.0005$ vs. young, using paired Student's t-test.

3. Results

3.1. Biochemical analysis

Aging induces a variation of some antioxidant and detoxifying enzymatic activities. In particular a significant increase in GSH-Px and SOD activities and a significant decrease in GLX I activity can be observed (Table 1).

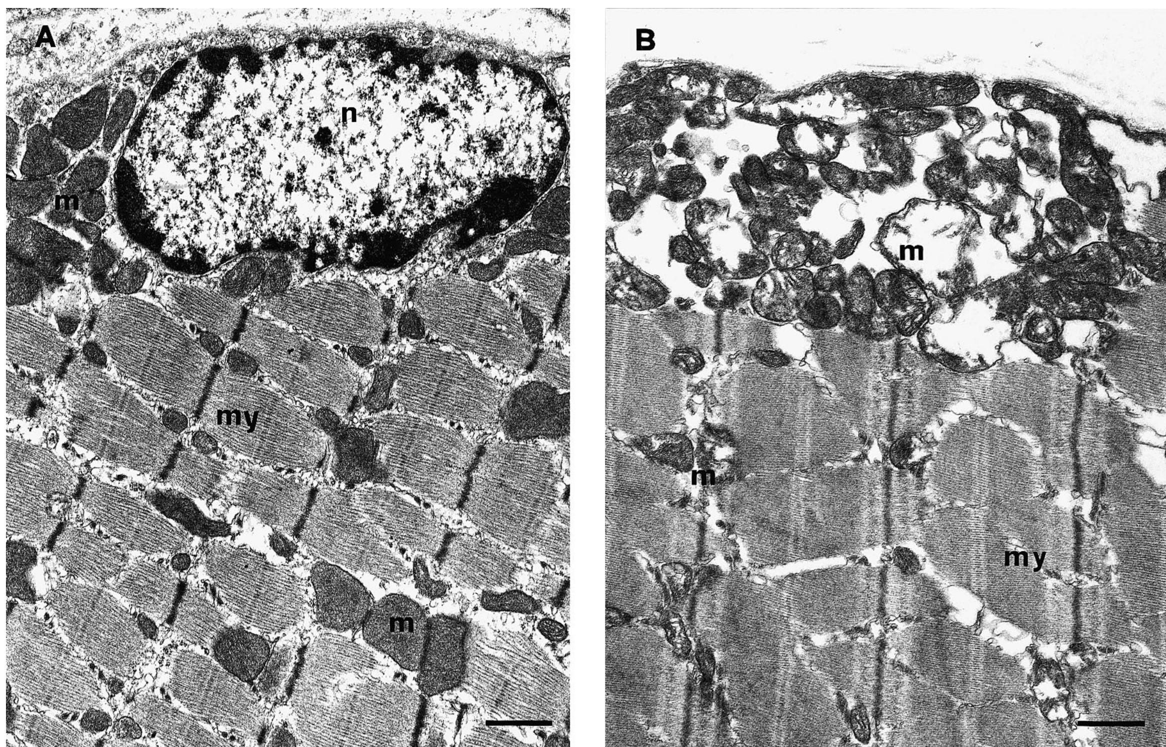

Fig. 1. Ultrastructural appearance of skeletal muscle in young (A) and old (B) untreated rats. m, mitochondria; my, myofibrillar elements; n, nucleus. Bar: 1 μm.

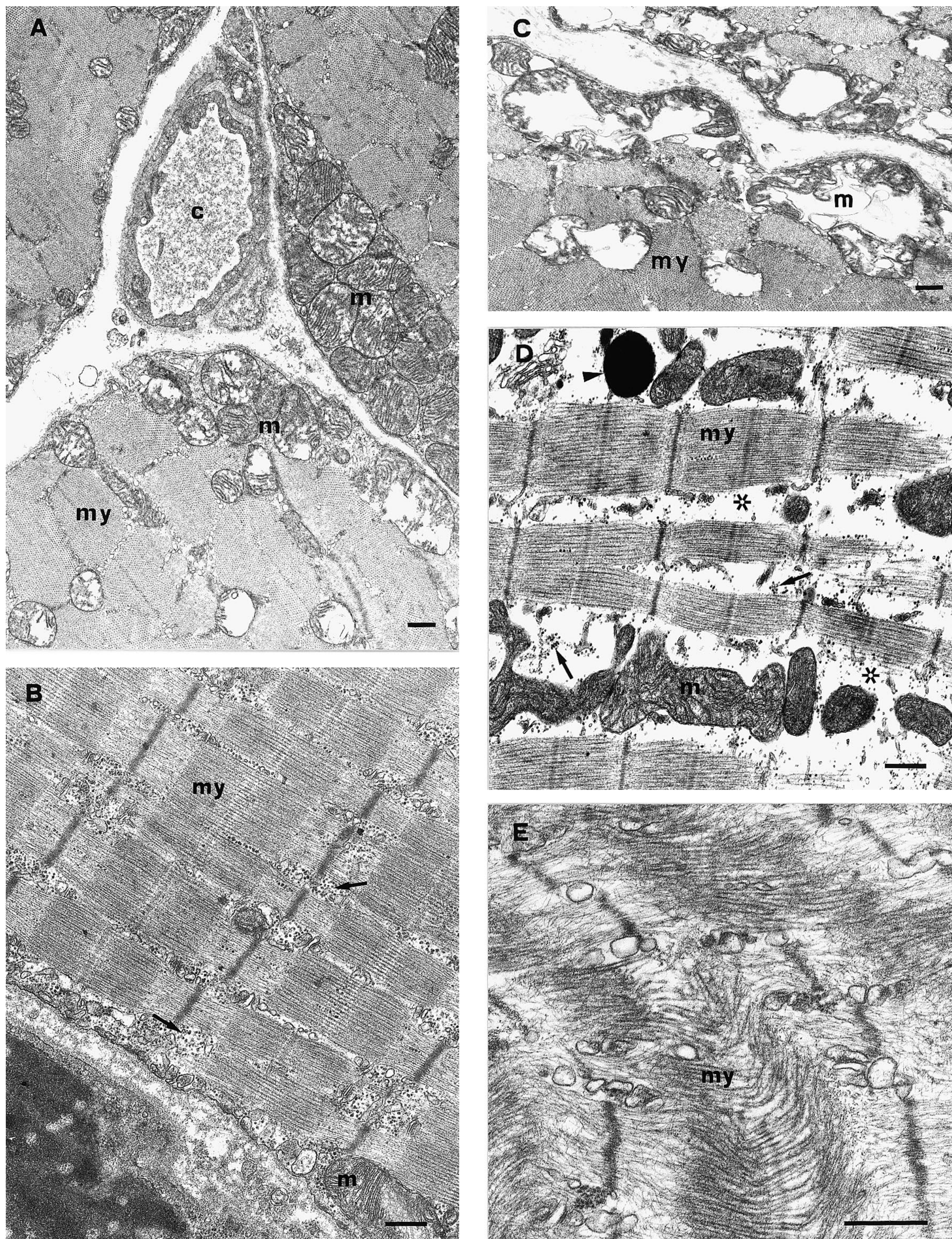

Fig. 2. Hypoxic treatment. Longitudinal (B,D,E) and transverse (A,C) sections of young (A,B) and old (C,D,E) rat skeletal muscle. c, capillary; m, mitochondria; my, myofibrillar elements; ↑, glycogen; ▲, lipofuscin granule; *, intermyofibrillar space. Bar: 0.5 μm.

3.2. Age-dependent antioxidant and detoxifying enzymatic activities after hypoxic treatment

Hypoxic treatment does not induce any peculiar variations of the enzymatic activities considered, either in the young or in the old individuals, with the exception of the GST activity that decreases significantly in the young (Table 1).

3.3. Age-dependent antioxidant and detoxifying enzymatic activities after hyperoxic treatment

Hyperoxic treatment drastically affects the enzymatic pattern in an age-dependent fashion. In the young GSH-Px doubles its values, while GST decreases significantly. In the old hyperoxic treatment induces a strong increase in CAT activity and a drastic reduction of GSSG Rx activity. Both in the young and in the old rats GLX I strongly decreases under hyperoxic conditions (Table 1).

3.4. Ultrastructural analysis

Ultrastructural investigation of the young control anterior tibial muscle shows the typical organization of the red muscle fibres with several mitochondria, especially localized below the sarcolemma, near the nucleus and endowed with a great number of cristae (Fig. 1A). Only very few degenerating mitochondria are evident and are probably representative of the normal turn-over of the organelles (not shown). In the old control, the peculiar difference observable with respect to the young is a great number of degenerating and swollen mitochondria with altered matrix and cristae (Fig. 1B).

Hypoxic treatment induces muscle fibre alterations both in young and old rat muscles. In the young individuals mitochondria appear swollen, in some cases cristae are in degeneration (Fig. 2A). In the old muscle the mitochondrial derangement is higher, and a wide and spread vacuolization with traces of inner membrane can be observed (Fig. 2C).

In the young rats, myofibrillar elements appear normal: numerous glycogen granules are present in the intermyofibrillar spaces, grouped especially near the Z line and the triads (Fig. 2B).

The longitudinal sections of the old hypoxic muscle show a marked increase in the intermyofibrillar space and loss of myofilaments (Fig. 2D). Moreover, an anomalous myofilament assembly can be observed that gives rise to irregular myofibrillar elements (Fig. 2E).

Hyperoxic stress causes damage to the muscle structure similar to that found after hypoxic stress, but more marked both in young and in old subjects. However, in the latter the damage appears higher. The main alteration visible in the young muscle is a strong mitochondrial degeneration, mainly at the cristae and matrix levels. The sarcomeric and myofibrillar organization maintains its normal arrangement (Fig. 3A). In the old rats, hyperoxia induces, on the contrary, a deep derangement of the myofibrillar elements that, when observed in transversal sections, seem to lose their individuality (Fig. 3B,C). In some cases it is possible to note, like under hypoxic conditions, the absence of myofilaments and enlarged intermyofibrillar spaces, together with triad degeneration (Fig. 3B). Mitochondria appear swollen, with deranged cristae. Some myelinic figures are also present as an index of structural alteration (Fig. 3B).

4. Discussion

The ultrastructural investigation shows that the main damage induced by the aging process to the rat skeletal muscle structure is a mitochondrial derangement. These data are consistent with the findings of an age-dependent decline of the respiratory chain activity in human skeletal muscle, observed by Boffoli et al. [21,22]. In fact, an impairment of the respiratory chain activity may result in the production of partially reduced oxygen species that, in turn, may produce damage to mitochondrial structures. On the basis of this hypothesis, the age-related mitochondrial decay is mainly due to an oxidative damage caused by cellular energy deficits. These defects might be one of the reasons of the decrease in the mass and functional capacity typical of aging, mostly in those tissues, such as skeletal muscle, having a high demand for oxidative phosphorylation [23,24]. Moreover, reactive oxygen species leakage from aged mitochondria is also well documented [25,26]. In this context, the significant increase in SOD and GSH-Px specific activity we find in the aged rats compared to

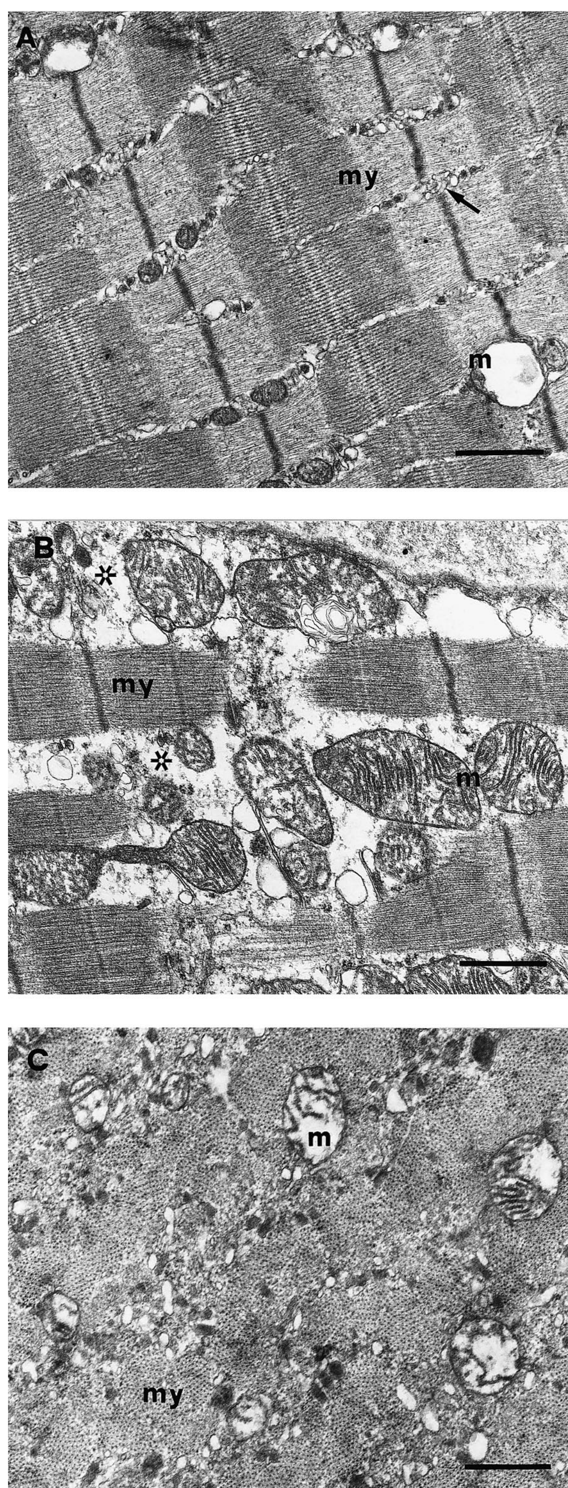

Fig. 3. Hyperoxic treatment. Longitudinal (A,B) and transverse (C) sections of young (A) and old (B,C) rat skeletal muscle. m, mitochondria; my, myofibrillar elements; *, intermyofibrillar space; ↑, sarcoplasmic reticulum. Bar: 1 μm.

the young ones (Table 1) may be indicative of an adaptive mechanism aiming at a better scavenging action towards the overproduction of partially reduced oxygen species. It is worth mentioning that an age-dependent increase in GSH-Px activity was previously found by us also in rat liver and lung [13] and is probably able to counteract the decrease in CAT activity that occurs in the aged rat skeletal muscle, likewise in liver and lung [13]. Also the GLX 1 activity trend with respect to age parallels what we already found in rat liver and lung [13]. In particular, it dramatically decreases (Table 1), causing in the old individuals a reduced efficiency in the detoxication from various 2-oxoaldehydes and, first of all, from the highly cytotoxic methylglyoxal, whose deleterious effects on biological molecules may result quite severe in this tissue with high glycolytic activity, being a natural by-product of this metabolic pathway [27]. The aging process does not affect, on the contrary, GST and GSSG-Rx activity levels.

To better understand how the organism is able to protect itself from oxidative stress injury, we should consider the ratios between the antioxidants enzymes, more than their single specific activities [28]. When a CAT/SOD and GSH-Px/SOD ratio increase occurs, this might indicate an activation of the antioxidant enzyme defence against ROS, while a decrease in one or both ratios would be indicative of a lower scavenging efficiency. In the latter case, oxidative damage may occur. Another important ratio is GSSG-Rx/GSH-Px, that shows how the system is able to recycle reduced GSH. When this ratio increases, the system would be able to keep the GSH levels high and to provide protection to the cells; when this ratio decreases, the cell might not produce enough GSH to get rid of organic and inorganic peroxides or ROS.

Table 2 shows that aging induces in rat skeletal muscle a significant decrease both in CAT/SOD and in GSSG-Rx/GSH-Px ratio, strengthening the hypothesis that the mitochondrial alterations shown by ultrastructural analysis in the aged rat muscle are imputable to oxidative damage. Further supports to these findings are data from the literature speaking about an age-dependent depletion of reduced glutathione in the liver of rat and mice [29].

The biochemical response of the antioxidant enzymes to hypoxic oxidative stress in the young and

Table 2
Values of the CAT/SOD, GSH-Px/SOD and GSSG-Rx/GSH-Px ratios in young and aged rat skeletal muscle subjected to hypoxia and hyperoxia

Ratio	Control		Hypoxia		Hyperoxia	
	Young	Aged	Young	Aged	Young	Aged
CAT/SOD	3.30	0.82	3.90	0.80	4.00	1.11
GSH-Px/SOD	0.05	0.06	0.045	0.06	0.12	0.06
GSSG-Rx/GSH-Px	0.50	0.12	0.58	0.18	0.21	0.02

old muscle is quite similar, since in both cases a decrease in these enzymatic activities occurs, although more marked in the aged rats. However, the old hypoxic muscle shows higher levels of SOD and GSH-Px activities with respect to the young one, as well as in the control groups. After hypoxic treatment, the values of CAT/SOD and GSSG-Rx/GSH-Px ratios in the old rat muscle are significantly lower than in the young muscle, suggesting an age-dependent reduced homeostatic ability towards physiological and environmental stresses. The ultrastructural investigation is consistent with these observations. Hypoxia, in fact, causes profound structural alterations in the muscle organization of the old subjects, concerning not only mitochondria and fibre derangement, as in young muscle, but also a wide loss of myofibrillar elements and a marked increase in the intermyofibrillar spaces (Fig. 2B,D). It is possible that the marked mitochondrial degeneration present in the old hypoxic muscle gives rise to a massive leakage of partially reduced oxygen species that causes a generalized damage to muscle structure (Fig. 2D,E).

An age related antioxidant and detoxifying enzyme variation occurs under hyperoxic oxidative stress, showing that a good level of inducibility is maintained also in the old individuals. Nevertheless, if we consider the CAT/SOD and GSH-Px/SOD ratios as an index of the status and adaptation level of the antioxidant enzymes, it might be argued that the aged muscles are much more vulnerable to oxidative stress (Table 2). Moreover, after hyperoxic treatment, the strong decrease in GSSG-Rx activity found in the old group would result in a much less efficient recycling of GSH, although the GSSG-Rx/GSH-Px ratio drastically diminishes also in the young muscle (Table 2). The structural damage caused by hyperoxic oxidative stress is higher than that observed after hypoxic oxidative stress both in young and in old rat skeletal muscle, where, in any case, the damage is more severe. The spread and wide injury inflicted by hyperoxic stress both to mitochondria and to myofibrillar and sarcomeric structures might probably also be due to the drastic reduction in GSH turn-over, that might contribute to the extent of oxidative damage, especially in the old skeletal muscle where the GSSG-Rx/GSH-Px ratio becomes very low.

The high oxygen tension produces a drastic lowering of GLX I activity, not related to age. It is well known that inhibition of glycolysis by respiration and as a consequence a lowering of the glycolytic intermediates should occur. The possible reduced methylglyoxal concentration might negatively affect GLX I activity.

Both under hypoxic and hyperoxic oxidative stress the rat skeletal muscle GST activity of the young undergoes a significant lowering. It has been reported that GST subunit 1 gene contains an antioxidant responsive element (ARE), that allows eukaryotic cells to respond to oxidative stress [30]. It is thus possible that the observed variation in GST specific activity might reflect a change in the expression pattern of the GST subunits. Our previous findings of an age-related alteration of GST subunit composition in rat liver after hypoxic and hyperoxic oxidative stress [12], as well as those of Veera Reddy et al. about the induction of specific rat hepatic GST subunits after exercise-induced oxidative stress [31] are coherent with this explanation.

In conclusion, our data further support the hypothesis that one of the major contributors to the aging process is oxidative damage produced at least in part by an impairment of the antioxidant enzymatic system. This makes the aged organism particularly susceptible to oxidative stress injury and to the

related degenerative diseases, especially in those organs and tissues with high demand for oxidative metabolism. The possible occurrence of exercise induced oxidative stress [32,33], as well as the protective effect of antioxidant dietary supplementation [4,34,35] should also be taken into account in designing physical exercise schedules suitable for aged individuals.

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References

[1] B. Halliwell, J.M.C. Gutteridge, Oxygen toxicity, oxygen radicals, transition metals and disease, Biochem. J. 219 (1984) 1–14.
[2] R. Schreck, P. Baeuerle, A role for oxygen radicals as second messenger, Trends Cell Biol. 1 (1991) 39–42.
[3] G. Storz, B.S. Polla, Transcriptional regulators of oxidative stress inducible genes in prokaryotes and eukaryotes, in: V. Feige, R.I. Morimoto, I. Jahara, B. Polla (Eds.), Stress Inducible Cellular Responses, Birkhäuser Verlag, Basel, 1996, pp. 239–254.
[4] K. Veera Reddy, T. Charles Kumar, M. Prasad, P. Reddana, Exercise induced oxidant stress in the lung tissue: role of dietary supplementation of vitamin E and selenium, Biochem. Int. 26 (1992) 863–871.
[5] L.E. Costa, S. Llesuy, A. Boveris, Active oxygen species in the livers of rats submitted to chronic hypobaric hypoxia, Am. J. Physiol. 264 (1993) C1395–C1400.
[6] D. Jamieson, B. Chance, E. Cadenas, A. Boveris, The relation of free radical production to hyperoxia, Annu. Rev. Physiol. 48 (1986) 703–719.
[7] C.A. Rice Evans, A.T. Diplock, Current status of antioxidant therapy, Free Radic. Biol. Med. 15 (1993) 77–96.
[8] P.G. Wells, L.M. Winn, Biochemical toxicology of chemical teratogenesis, Crit. Rev. Biochem. Mol. Biol. 31 (1996) 1–40.
[9] D. Harman, Free radical theory of aging. The free radical diseases, Age 57 (1984) 111–131.
[10] D. Harman, Free radical theory of aging, in: I. Emerit, B. Chance (Eds.), Free Radicals and Aging, Birkhäuser Verlag, Basel, 1992.
[11] L.E. Rikans, K.R. Hornbrook, Lipid peroxidation, antioxidant protection and aging, Biochim. Biophys. Acta 1362 (1997) 116–127.
[12] C. Di Iilio, S. Angelucci, T. Bucciarelli, A. Pennelli, R. Petruzzelli, C. Di Giulio, M. Miranda, F. Amicarelli, P. Sacchetta, Alteration of glutathione transferase subunits composition in the liver of young and aged rats submitted to hypoxic and hyperoxic conditions, Biochim. Biophys. Acta 1312 (1996) 215–226.
[13] F. Amicarelli, C. Di Ilio, L. Masciocco, A. Bonfigli, O. Zarivi, M.R. D'Andrea, C. Di Giulio, M. Miranda, Aging and detoxifying enzymes responses to hypoxic and hyperoxic treatment, Mech. Aging Dev. 37 (1997) 215–226.
[14] H. Luck, Catalase, in: H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Verlag Chemie, Weinheim, 1965, pp. 885–894.
[15] M. Sun, S. Zigman, An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation, Anal. Biochem. 90 (1978) 81–89.
[16] D.E. Paglia, W.N. Valentine, Studies on the qualitative and quantitative characterization of erythrocytes glutathione peroxidase, J. Lab. Clin. Med. 70 (1967) 158–169.
[17] C. Di Ilio, P. Sacchetta, M. Lo Bello, G. Caccuri, G. Federici, Selenium independent glutathione peroxidase activity associated with cationic forms of glutathione transferase in human hearth, J. Mol. Cell. Cardiol. 18 (1986) 983–991.
[18] C. Di Ilio, G. Polidoro, A. Arduini, A. Muccini, G. Federici, Glutathione peroxidase, glutathione reductase, glutathione S-transferase and γ-glutamyl-transpeptidase activities in human early pregnancy placenta, Biochem. Med. 29 (1983) 143–148.
[19] W.H. Habig, W.B. Jacoby, Assay for differentiation of glutathione S-transferase, Methods Enzymol. 77 (1981) 398–405.
[20] B. Mannervik, A.C. Aronsson, E. Marmstal, G. Tibellin, Glyoxalase I (rat liver), Methods Enzymol. 77 (1981) 297–301.
[21] D. Boffoli, S.C. Scacco, R. Vergari, G. Solarino, G. Santacroce, S. Papa, Decline with age of the respiratory chain activity in human skeletal muscle, Biochim. Biophys. Acta 1226 (1994) 73–82.
[22] D. Boffoli, S.C. Scacco, R. Vergari, M.T. Persio, G. Solarino, R. Laforgia, S. Papa, Ageing is associated in females with a decline in the content and activity of the b-c_1 complex in skeletal muscle mitochondria, Biochim. Biophys. Acta 1315 (1996) 66–72.
[23] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. USA 91 (1994) 10771–10778.
[24] B.N. Ames, M.K. Shigenaga, T.M. Hagen, Mitochondrial decay in aging, Biochim. Biophys. Acta 1271 (1995) 165–170.
[25] R.S. Sohal, B.H. Sohal, Hydrogen peroxide release by mitochondria increases during aging, Mech. Aging Dev. 57 (1991) 187–202.
[26] R.S. Sohal, Aging, cytochrome oxidase activity and hydrogen peroxide release by mitochondria, Free Radic. Biol. Med. 14 (1993) 583–588.
[27] P.J. Thornalley, The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life, Biochem. J. 269 (1990) 1–11.
[28] S.M. Somani, K. Husain, E.C. Schlorff, Response of antiox-

idant system to physical and chemical stress, in: I.S. Baskins, H. Salem (Eds.), Oxidant, Antioxidants and Free Radicals, Taylor and Francis, London, 1996, pp. 125–141.

[29] K. Nakata, M. Kawase, S. Ogino, C. Kinoshita, H. Murata, T. Sakaue, K. Ogata, S. Ohmori, Effects of age on levels of cysteine, glutathione and related enzyme activities in livers of mice and rats and an attempt to replenish hepatic glutathione level of mouse with cysteine derivatives, Mech. Ageing Dev. 90 (1996) 195–207.

[30] J.D. Hayes, D.J. Pulford, The glutathione S-transferase supergene family: regulation of GST* and the contribution of the isoenzymes to cancer chemoprotection and drug resistance, Crit. Rev. Biochem. Mol. 30 (1995) 445–600.

[31] K. Veera Reddy, D. Anuradha, T. Charles Kumar, P. Reddanna, Induction of Ya1 subunit of rat hepatic glutathione S-transferases by exercise-induced oxidative stress, Arch. Biochem. Biophys. 323 (1995) 6–10.

[32] S.K. Powers, J. Lawler, D. Criswell, F.K. Lien, D. Martin, Aging and respiratory muscle metabolic plasticity: effects of endurance training, J. Appl. Physiol. 72 (1992) 1068–1073.

[33] J. Hammeren, S. Powers, J. Lawler, D. Criswell, D. Martin, D. Lowenthal, M. Pollock, Exercise training-induced alterations in skeletal muscle oxidative and antioxidant enzyme activity in senescent rats, Int. J. Sports Med. 13 (1992) 412–416.

[34] A.Z. Reznich, E. Witt, M. Matsumoto, L. Packer, Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats, Biochem. Biophys. Res. Commun. 189 (1992) 801–805.

[35] C.T. Kumar, K. Veera Reddy, M. Prasad, K. Thyagaraju, P. Reddanna, Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress, Mol. Cell. Biochem. 111 (1992) 109–115.