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Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats

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Abstract

The aim of this study was to investigate the effect of aerobic exercise training on activities and mRNA levels of catalase (CAT), glutathione peroxidase (GPX), Cu,Zn- and Mn-superoxide dismutases (SOD), TBARS content, and xanthine oxidase (XO) activity, in soleus muscle from young and aged rats. The antioxidant enzyme activities and mRNA levels were markedly increased in soleus muscle with aging. TBARS content of soleus muscle from the aged group was 8.3-fold higher as compared with that of young rats. In young rats, exercise training induced an increase of all antioxidant enzyme activities, except for Cu,Zn-SOD. XO also did not change. The TBARS content was also increased (2.9-fold) due to exercise training in soleus muscle from young rats. In aged rats, the activities of CAT, GPX and Cu,Zn-SOD in the soleus muscle did not change with the exercise training, whereas the activities of Mn-SOD (40%) and XO (27%) were decreased. The mRNA levels of Mn-SOD and CAT were decreased by 42% and 24%, respectively, in the trained group. Exercise training induced a significant decrease of TBARS content (81%) in the soleus muscle from aged rats. These findings support the proposition that exercise training presents an antioxidant stress effect on skeletal muscle from both young and aged rats.

Keywords: Aging; Antioxidant enzymes; Exercise training; ROS; Skeletal muscle

1. Introduction

Morphological and physiological changes occur in cardiovascular, nervous, digestive, urinary, endocrine, reproductive, bone and muscular systems during the aging process. The changes that occur in skeletal muscle markedly affect the quality of life of aged people. The loss of muscle mass (sarcopenia) causes difficulties to perform daily tasks and increases the risk of falls, being the main cause of accidents of elderly subjects (Morley et al., 2001; McArdle et al., 2002; Fitts, 2003).

The association of reactive oxygen species (ROS) with the aging process was firstly proposed by Harman (1956) (free radicals theory of aging), and later by Linnane et al. (1989), who postulated the mitochondria participation in this process (mitochondrial theory of aging). ROS play a very important role in the changes induced by aging in various tissues (Fulle et al., 2004). In fact, ROS accumulation and the subsequent oxidative damage to cells are prevented by antioxidant enzymes such as Cu,Zn- and Mn-superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) (Halliwell and Gutteridge, 1999; Wei and Lee, 2002; Rodriguez et al., 2004). The aging process causes an unbalance between ROS production and antioxidant capacity of the tissues leading to cell damage (Wei and Lee, 2002).

Physical activity promotes various metabolic and functional adaptations in the skeletal muscle. Resistance exercise is recommended for the treatment and prevention of sarcopenia (Trappe et al., 2000), providing significant improvement in the function of skeletal muscle in aged patients such as the speed of walking and the ability to perform daily tasks (Frontera et al., 2000). Despite the improvements obtained in skeletal muscle with the practice of resistance exercise, some researchers

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believe that aerobic exercise is an ideal exertion for aged subjects due to the benefits for cardiovascular function.

The sites of ROS production in muscles are: xanthine oxidase system in the cytosol, NADPH oxidase complex in plasma membrane (Lagranha et al., 2005), and the electron transport chain in the mitochondria (Bejma and Ji, 1999; Banerjee et al., 2003). During aerobic contractions, skeletal muscle produces significant amounts of superoxide anion ($O_2^{\bullet -}$) due to its increase in oxygen uptake that reaches up to 90 times the values obtained at rest (Ji, 1995; Ji et al., 1998; Sen, 2001; Khassaf et al., 2003; Mastaloudis et al., 2004). The skeletal muscle of aged subjects becomes more susceptible to oxidative damage, mainly during exercise, due to physiological, morphological and biochemical changes such as mitochondria dysfunction that leads to a still higher ROS production rate (Bejma and Ji, 1999; Fulle et al., 2004).

Xanthine oxidase (XO) activity is low in skeletal muscles (Borges et al., 2002). However, the increase in oxidative stress and ADP accumulation observed in aging, due to the decrease in skeletal muscle oxidative capacity (Baker et al., 2006), lead to an increase of XO activity, contributing for the high anion superoxide ($O_2^{\bullet -}$) production. To our knowledge this enzyme activity has not been measured after exercise training in aged rats.

In spite of the information above, the effect of exercise and its association with ROS in the aging process of skeletal muscle still remains to be fully established. Some studies have shown increases in SOD, GPX and CAT activities after aerobic exercise training in young rats (Meydani et al., 1992; Jenkins, 1993; Powers et al., 1994; Leeuwenburgh et al., 1997; Hollander et al., 2000). However, similar experiment was rarely performed in aged rats (Parise et al., 2005).

Some studies examined the effect of exercise training on the heart (Kakarla et al., 2005; Quindry et al., 2005; Gul et al., 2006; Rinaldi et al., 2006) and testis (Aksoy et al., 2006) of aged rats. Concerning the skeletal muscles, Lawler et al. (1994), determined the activities of GPX and SOD in diaphragm of female rats after an acute exercise, Lawler et al. (1993), examined the activity of GPX in leg muscles of female rats after a single session of exercise, Gunduz et al. (2004), measured the activities of CAT, GPX and SOD after swimming and Navarro-Arevalo and Sanchez-del-Pino (1998), evaluated the activity of SOD in soleus muscle of male rats. In these studies, the authors investigated the activities of the antioxidant enzymes only. There is only one study that examined both activity and mRNA levels of the antioxidant enzymes in the lung of aged rats after an acute exercise (Hatao et al., 2006). Therefore, a systematic study on the effect of an aerobic exercise training on activity and expression of the antioxidant enzymes in skeletal muscle from aged rats remains to be carried out.

In addition to the loss of muscle mass, aging leads to a shift of type II to type I fibers (Balagopal et al., 2001). Muscles presenting high percentage of type I fibers are less affected by aging, avoiding large differences between aged and young animals. According to Adams et al. (1994), rat soleus muscle presents about 97% of myosin heavy chain I (MHC I) and only 3% of MHC IIa. Therefore, soleus muscle has a very homogenous fiber composition and so it is an ideal model to investigate physiological changes induced by exercise training in aged and young rats.

The information above led us to investigate the effect of an aerobic exercise training on activities and mRNA levels of CAT, GPX, Cu,Zn- and Mn-SOD, and XO, and thiobarbituric acid reactive substances (TBARS) content, as a qualitative index of lipid peroxidation and oxidative stress, in soleus muscle from young and aged rats.

2. Methods

2.1. Animals

Male 2 (young) and 21 (aged) months of age Wistar-Kyoto (WKY) rats, weighing 220–280 g and 320–370 g, respectively, at the beginning of the experiment, were obtained from the Institute of Biomedical Sciences (University of São Paulo, São Paulo, Brazil). The lifespan of the Wistar-Kyoto rats is of approximately 32 months (Linz et al., 1997). The rats were maintained at 23 °C under a light/dark cycle of 12 h/12 h. The Animal Care Committee of the Institute of Biomedical Sciences approved the experimental procedure of this study. The rats had free access to food composed of 63.4% carbohydrate, 25.6% protein, and 11% lipid (NUVILAB CR1, NUVITAL Nutrientes LTDA, Curitiba, PR) and received water *ad libitum*. The rats were divided into four groups: young untrained, young trained, aged untrained, and aged trained.

2.2. Exercise training protocol

Young and aged rats were pre-selected by their ability to run on a treadmill (Inbramed, KT-300); four to five sessions at 0.3 km/h up to 0.5 km/h, 0% grade, 10 min/day. The pre-selected animals were then randomly assigned to trained or untrained groups. Low-intensity exercise training protocol was performed 5 days per week, 1 h per day, over 13 weeks, as previously described (Dufloth et al., 1997). The exercise training intensity was progressively increased by a combination of time and speed, attaining 1 h per day by week 3 and maximal velocity on week 7. These values were maintained thereon. The training protocol corresponded to 50–60% of maximal exercise capacity, as measured in both young and aged rats by means of a maximal exercise test. This test consisted of graded exercise on a treadmill, with increments of 0.3 km/h for young rats, and 0.2 km/h for aged rats, every 3 min, starting with 0.3 km/h up to the maximal intensity attained for each rat. Tests were performed for each rat at the beginning of the training protocol and by week 6 to establish the training intensity. The test was repeated at week 12 to compare the efficacy of the training protocol. Untrained rats were handled every day and submitted once a week to a short period of mild exercise (5–10 min, 0.4–0.8 km/h, 0% grade). This procedure allowed untrained rats to adapt themselves to the experimental conditions. The animals were killed 2 days after the last session of exercise. Untrained rats were killed under similar conditions.

After 48 h of the last session of exercise training, soleus muscle was removed for determination of the antioxidant enzyme activities and mRNA levels and TBARS content.

2.3. Enzyme activity assays

The extraction buffer for the measurement of CAT, GPX, Cu,Zn- and Mn-SOD, and XO activities contained 0.1 M sodium phosphate, pH 7.0. CAT activity was determined by measuring the breakdown of hydrogen peroxide at 230 nm (Aebi, 1984). GPX activity was determined as described by Wendel (1981), and Mannervik (1985), following the rate of NADPH oxidation, at 340 nm, and 37 °C, in an assay medium containing 50 mM phosphate buffer (pH 7.0), 0.3 mM NADPH, glutathione reductase (0.25 U/mL) and 5 mM reduced glutathione. The reaction was initiated by the addition of *t*-butyl

hydroperoxide (1.5 mM). Cu,Zn- and Mn-SOD activities were determined according to the method of Flohe and Otting (1984) by measuring, at 25 °C, the decrease in the rate of cytochrome *c* reduction in a xanthine–xanthine oxidase superoxide generating system consisting of 10 μM cytochrome *c*, 100 μM xanthine, 50 mM sodium phosphate buffer (pH 10.0), and the necessary quantity of xanthine oxidase to yield a variation of 0.025 absorbance per min at 550 nm. Mn-SOD activity was determined by the addition of 1 mM KCN to the assay of total SOD activity. This drug suppresses the activity of Cu,Zn-SOD (Higuchi et al., 1985). Citrate synthase activity was measured as previously described by Srere et al. (1963), and Pithon-Curi et al. (2004). XO activity was determined by formation of uric acid from xanthine through the increase in absorbance at 293 nm according to Prajda and Weber (1975).

2.4. Real time PCR

The expression of the antioxidant enzymes was evaluated by real time PCR (Higuchi et al., 1992) using a ROTOR GENE 3000 equipment (Corbett Research, Mortlake, Australia). Total RNA was obtained from 50 to 100 μg of soleus muscle using Trizol reagent™ (Invitrogen Life Technologies, Rockville, MD, USA), as previously described (Chomczynski and Sacchi, 1987). Briefly, soleus muscle was lysed using 1 mL Trizol reagent and then, after 5 min incubation at room temperature, 200 μL chloroform were added to the tubes and centrifuged at 12,000 × *g*. The aqueous phase was transferred to another tube and the RNA was pelleted by centrifugation (12,000 × *g*) with isopropyl alcohol. RNA pellets were washed with 75% ethanol by centrifugation at 7500 × *g* for 5 min and dried in air. RNA pellets were than eluated in RNase-free water and treated with DNase I. Afterwards, RNA was stored at −70 °C until to the reverse transcription reaction being performed. RNA was quantified by measuring absorbance at 260 nm. The purity of the RNA preparations was assessed by the 260/280 nm ratio and on a 1% agarose gel electrophoresis stained with ethidium bromide at 5 μg/mL (Sambrook and Russel, 2001).

cDNA probes were synthesized using 4 μg of total RNA and a mix of the following reagents: 146 ng of ''random primers'', 200 U of reverse transcriptase (Invitrogen Life Technologies, Rockville, MD, USA), reaction buffer 5× (50 mM Tris–HCl, pH 8.0, 75 mM KCl, 3 mM $MgCl_2$), 5 mM DTT, 500 μM dNTP in a final volume of 20 μL. The reaction was incubated for 2 min, at 25 °C, assembling the oligonucleotides and RNA hybridization, followed by heating at 42 °C for 50 min. cDNA was stored at −20 °C prior to the real time PCR assay.

To perform the real time PCR reaction, 1 μL of cDNA was used in a final volume of 25 μL, containing 100 μM of dNTPs, reaction buffer 10× (10 mM Tris–HCl, 50 mM KCl, 2 mM $MgCl_2$), 1 U of Taq DNA polymerase (Invitrogen Life Technologies, Rockville, MD, USA), 0.1 μM of each primer (sense and antisense), and SYBR GREEN (1000× diluted) (Invitrogen Life Technologies, Rockville, MD, USA) as fluorescent dye. The sequences of the primers were designed using information contained in the public database in the GeneBank of the National Center for Biotechnology Information (NCBI). The sequences of the utilized primers were: 5′ATTGCCGTCCGATTCTCC3′ and 5′CCAGTTACCATCTTCAGTGTAG3′ for sense and antisense primers of the CAT gene, respectively; 5′GTCCACCGTGTATGCCTTC3′ and 5′CTCTTCATTCTTGCCATTCTCC3′ for sense and antisense primers of the GPX gene, respectively; 5′GACCTGCCTTACGACTATG3′ and 5′TACTTCTCCTCGGTGACG3′ for sense and antisense of the Mn-SOD gene, respectively; 5′CCAGCGGATGAAGAGAGG3′ and 5′CCAATCACACCACAAGCC3′ for sense and antisense primers of the Cu,Zn-SOD gene, respectively; and 5′ACCACAGTCCATGCCATCAC3′ and 5′TCCACCACCCTGTTGCTGTA3′ for sense and antisense of the G3PDH gene, respectively.

Quantification of gene expression was carried out by the method described by Liu and Saint (2002), using G3PDH gene as inner control, which is the most stable gene for real time RT-PCR measurements in skeletal muscle (Jemiolo and Trappe, 2004; Mahoney et al., 2004). Amplification efficiency of each sample was calculated as described by Ramakers et al. (2003).

2.5. Determination of TBARS content

TBARS were measured as described by Winterbourn et al. (1985), in the same extraction medium as that for the antioxidant enzyme assays. Muscles were homogenized in saline buffer and the precipitated proteins were removed by centrifugation at 12,000 × *g* for 10 min. The supernatant (500 μL) was mixed with 500 μL thiobarbituric acid (1% in 50 mM NaOH) and 500 μL of HCl at 25%. The samples were then heated in a boiling water bath for 10 min and, after cooling, TBARS were extracted with 1.5 mL of butanol. The mixture was centrifuged at 12,000 × *g* for 10 min and the absorbance of the supernatant was determined at 532 nm. Thiobarbituric acid reacts with products of lipid peroxidation, mainly malondialdehyde, producing a colored compound.

2.6. Protein determination

Tissue protein was measured by the Bradford method (Bradford, 1976), using bovine serum albumin as standard.

2.7. Statistical analysis

Statistical analyses of the results were performed by using the two-way analysis of variance (ANOVA) and Bonferroni's post hoc test (GraphPad Prism 4, Graph Pad Software, Inc., San Diego, CA, USA). Results were considered statistically significant for $P < 0.05$.

3. Results

3.1. Effects of the aging process

As a consequence of aging the maximal running speed reached by the aged rats in the treadmill was approximately 60% lower than that of young animals (Table 1).

The antioxidant enzyme activities were markedly increased in soleus muscle from aged rats as compared to young animals; CAT (43%), total SOD (86%), Mn-SOD (3.4-fold) (Fig. 1A–D, respectively). The activities of GPX and of Cu,Zn-SOD were not markedly different between young and aged rats (Fig. 1B and E, respectively). The activity of XO was increased by 35% due to aging (Fig. 1F).

The mRNA levels of the antioxidant enzymes were also increased in soleus muscle from aged rats. Aging caused a marked increase in mRNA levels of CAT (4.7-fold), GPX (17-fold), Mn-SOD (2.8-fold), and Cu,Zn-SOD (3.9-fold) (Fig. 2A–D, respectively). The TBARS content of soleus muscle from the aged group was 8.3-fold higher as compared with that of young rats (Fig. 3).

Table 1
Maximal running speed reached in the last treadmill test and citrate synthase activity in the soleus muscle from young and aged rats

Trainability indicators	Young rats		Aged rats	
	Untrained group	Trained group	Untrained group	Trained group
Maximal running speed reached (km/h)	1.4 ± 0.08	2.1 ± 0.17**	0.7 ± 0.03+	1.04 ± 0.05**
Citrate synthase activity (nmol/(min mg protein))	427 ± 15.5	584 ± 34.3**	445.2 ± 21.9	564 ± 33.1*

The values are presented as mean ± S.E.M. of young and aged rats. *$P < 0.05$; **$P < 0.01$; +$P < 0.001$ when compared to young untrained group.

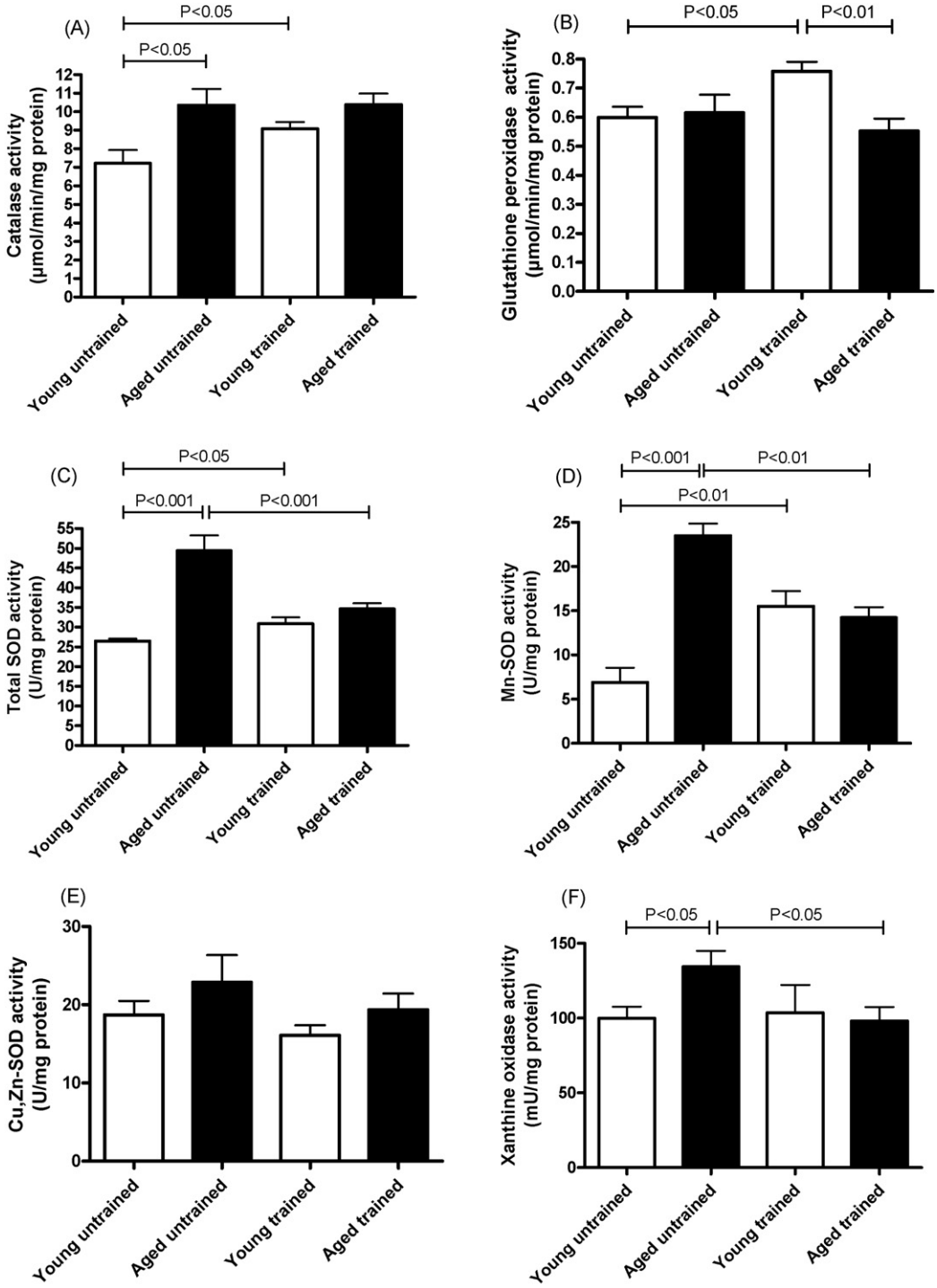

Fig. 1. Effects of exercise training on catalase, glutathione peroxidase, total SOD, Mn-SOD, Cu,Zn-SOD and xanthine oxidase activities of soleus muscle from young and aged rats. The values are presented as mean ± S.E.M. Two-way ANOVA with Bonferroni's post hoc test was used for comparison between groups.

3.2. *Effects of exercise training on young rats*

The effectiveness of the exercise training protocol was determined by the maximal speed reached in the treadmill and the results of citrate synthase activity measured in the soleus muscle. The maximal speed reached was 56% higher after 13 weeks of training when compared to the speed observed in the first test (Table 1). Citrate synthase activity of the soleus muscle was increased by 37% due to the exercise protocol (Table 1). The maximal oxygen uptake (VO_2, mL/(kg min)) (data not shown) also confirmed the effectiveness of the exercise training protocol used presenting an increase of 40% (43.16 ± 1.17 to 60.59 ± 1.33) ($P < 0.0001$).

Exercise training induced an increase of all antioxidant enzyme activities except for Cu,Zn-SOD that did not change significantly (Fig. 1E). The activities of CAT (Fig. 1A) and

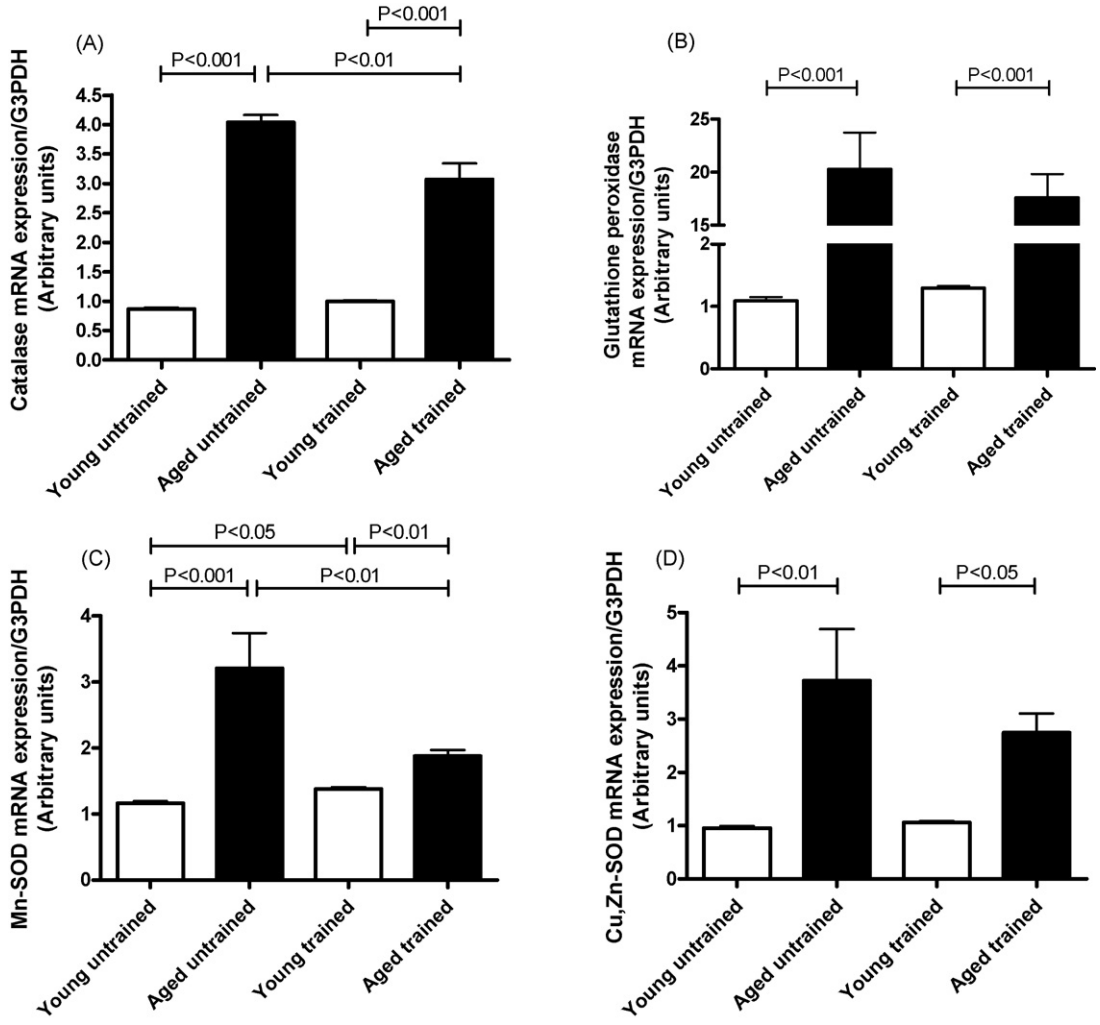

Fig. 2. Effects of exercise training on catalase, glutathione peroxidase, Mn- and Cu,Zn-SOD mRNA levels of soleus muscle from young and aged rats. The values are presented as mean ± S.E.M. Two-way ANOVA with Bonferroni's post hoc test was used for comparison between groups.

GPX (Fig. 1B) were increased by 26%, and of total SOD (Fig. 1C) by 16% due to exercise training, whereas the activity of Mn-SOD was raised by 2.3-fold (Fig. 1D), and of XO remained unchanged (Fig. 1F).

Concerning mRNA levels of the enzymes, the exercise training induced a slight increase of all enzymes being significant for Mn-SOD only. The TBARS content was also increased (2.9-fold) due to exercise training in young rats (Fig. 3).

3.3. Effects of exercise training on aged rats

The effectiveness of the exercise training protocol for aged rats was also determined by the maximal running speed in the treadmill and citrate synthase activity in the soleus muscle. The maximal speed reached was 38% higher after 13 weeks of training when compared to the speed observed in the first test (Table 1). Citrate synthase activity of the soleus muscle was increased by 27% due to exercise training (Table 1). The maximal distance reached and maximal period of time in the test was measured (data not shown) and showed an increase of 73% and 38%, respectively, also confirming the effectiveness of the exercise training protocol used for aged rats.

The activities of CAT, GPX and Cu,Zn-SOD in the soleus muscle of aged rats were not increased by the exercise training (Fig. 1A, B and E, respectively). Activities of total SOD (Fig. 1C), Mn-SOD (Fig. 1D) and XO (Fig. 1F) were decreased by 30%, 40%, and 27%, respectively, due to exercise training. The mRNA levels of GPX (Fig. 2B) and Cu,Zn-SOD (Fig. 2D) were not altered by the exercise training. Mn-SOD (Fig. 2C) and CAT (Fig. 2A) mRNA levels were decreased by 42% and 24%, respectively, in the trained group when compared to untrained rats. Exercise training caused significant decrease of TBARS content (81%) (Fig. 3).

4. Discussion

Sarcopenia is a process that often occurs with aging, being characterized by loss of skeletal muscle mass and function (Greenlund and Nair, 2003). This loss is more pronounced in muscles that contain a high percentage of type II fibers, as shown in several studies that found a significant decrease of

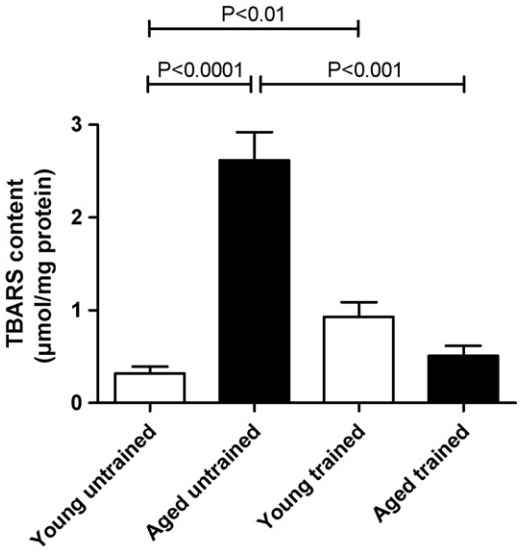

Fig. 3. Effects of exercise training on TBARS content in soleus muscle from young and aged rats. The values are presented as mean ± S.E.M. Two-way ANOVA with Bonferroni's post hoc test was used for comparison between groups.

MHC II mRNA, and a maintenance or even an increase of MHC I mRNA with aging (Locke et al., 1994; Lexell, 1995; Balagopal et al., 2001). The aim of this study was to investigate the changes in the enzymatic antioxidant system in soleus muscle during aging and due to exercise training. As previously mentioned, soleus muscle was chosen because it is one of the most recruited muscles during the exercise training protocol used presenting a large proportion of type I fibers (Adams et al., 1994; Delp and Duan, 1996) that is less affected by aging and also has high activities of CAT, GPX and SOD compared to muscle with a large percentage of type II fibers (Powers et al., 1994).

Some studies have found a decrease in skeletal muscle antioxidant enzyme activities with aging. Pansarasa et al. (1999, 2000), showed a decrease in total SOD activity in skeletal muscle from aged individuals, when compared to young subjects, and Fano et al. (2001), found a decrease of CAT activity in the vastus lateralis muscle with aging. However, Luhtala et al. (1994), Leeuwenburgh et al. (1994) and Gianni et al. (2004), observed an increase of the activities of CAT, total SOD and GPX activities in skeletal muscles from humans and rodents. Ji et al. (1998), observed that the aging process leads to an increase of ROS production and antioxidant enzyme activities in skeletal muscle. These divergences observed in the literature are mainly due to differences in the type of animal, age and/or skeletal muscle used. In the present study, a significant increase of CAT and Mn-SOD activities was observed in soleus muscle with age. The changes in these enzyme activities probably occurred partially due to an increase in mRNA levels. Taking into consideration that Mn-SOD is a mitochondrial enzyme, and this organelle is the main site for ROS production during exercise (Banerjee et al., 2003), and also due to the increase of the mRNA levels in response to exercise training, an increase of this enzyme activity was expected.

ROS may modulate the antioxidant enzyme activities by regulating the mRNA levels through activation of signaling pathways (Fulle et al., 2004). According to Franco et al. (1999), the induction of antioxidant enzyme mRNA levels coincide with increases in oxidative damage of proteins, supporting the postulated relationship between oxidative stress and antioxidant enzyme mRNA expression. In fact, ROS play a very important role to regulate several cell functions, acting as second messengers, and activating specific redox-sensitive transcription factors, such as AP-1 and NF-κB (Dalton et al., 1999; Zhou et al., 2001; Khassaf et al., 2003). AP-1 and NF-κB response elements are present in the promoter regions of genes encoding CAT, GPX, Mn-SOD and Cu,Zn-SOD (Zhou et al., 2001). Combinations of AP-1 and NF-κB with other redox-sensitive transcription factors may determine which antioxidant enzyme is going to be induced and to what extend.

AP-1 is a dimeric transcription factor composed of activating (c-Fos and c-Jun) and inhibitory (Fra-1 and Fra-2) subunits, which can generate different heterodimers and so modulating expression of target genes. NF-κB also acts as a dimeric factor, which is maintained in the cytosol in an inactive status by binding to the I-κB inhibitory subunits (Catani et al., 2004). In muscle cells, ROS can induce degradation of the inhibitory I-κB protein subunits bound to NF-κB subunits (p65, p50 and RelB), leading to a rapid translocation of NF-κB to the nucleus activating the transcription of specific genes (Muller et al., 1997). AP-1 is also activated by ROS. Oxidative stress induces the binding of AP-1 complex proteins (c-Jun and c-Fos) to DNA (Gomez del Arco et al., 1997). Zhou et al. (2001) showed that the use of specific NF-κB inhibitors blocked the oxidant-induced expression of CAT and GPX, confirming the hypothesis that ROS can modulate antioxidant enzyme mRNA levels by activating redox-sensitive transcription factors, such as NF-κB and AP-1. In fact, the modulation of these transcription factors induced by exercise in skeletal muscle was also observed by others (Hollander et al., 2001; Aoi et al., 2004).

In the present work, mRNA levels of CAT and GPX in soleus muscle showed a large increase with aging. This may be due to the high oxidative stress observed in aging. In fact, Franco et al. (1999) reported that muscle exposed to oxidative stress presented a marked increase of CAT and GPX mRNA levels. However, there was no increase of GPX activity in soleus muscle with aging. Cu,Zn-SOD activity also did not change. In fact, others have also observed similar results in response to different stimuli. No change of these enzyme activities was observed in lymphocytes (Joksic et al., 2000; Pajovic et al., 2000; Khassaf et al., 2003), and in diaphragm, soleus and gastrocnemius muscles (Higuchi et al., 1985; Pereira et al., 1994; Caillaud et al., 1999; Vincent et al., 1999; Gregorevic et al., 2001; Gunduz et al., 2004). In addition, these studies pointed out that Mn-SOD is probably the major inducible SOD isoform. An increase of GPX activity by aging was expected due to the great increase of its mRNA expression, however this did not happen.

According to Franco et al. (1999), the response of antioxidant enzyme activities in muscle exposed to oxidative stress is less pronounced than the increases in their mRNA levels. This process may occur due to a decrease in translational

efficiency in cells under oxidative stress (Ho et al., 1996). Therefore, post-transcriptional inhibition of GPX was possibly induced by aging. In fact, the rates of whole body protein turnover and of total muscle protein synthesis decline with normal aging (Short et al., 2004). According to Schoneich (2006), more than 200 different post-translational modifications are induced by enzymatic and non-enzymatic processes, and modifications of specific proteins accompany the biological aging process.

In spite of the maintenance of GPX and Cu,Zn-SOD activities an increase of cytosolic ROS production has been observed in skeletal muscles with aging. The cytosolic sites for ROS production are: NADPH oxidase complex and XO system (Banerjee et al., 2003). Few studies have shown an aging associated increase of XO activity in rat kidney (Chung et al., 1999) and liver (Kim et al., 2005), and of NADPH oxidase activity in mitochondria of rat skeletal muscle (Bejma and Ji, 1999). Superoxide production by XO system is favoured by an accumulation of intracellular ADP (Ji, 1999). A depression of mitochondrial function observed in aging (Papa, 1996; Conley et al., 2000; Hepple et al., 2003; Mansouri et al., 2006) leads to an unbalance of ATP/ADP ratio in the cytosol and an associated increase of XO activity. The increase of XO activity in the soleus muscle during aging observed in this study probably leads to an increase in cytosol superoxide production contributing to the observed lipid peroxidation (Kang and Hamasaki, 2003), as indicated by the increase in TBARS content, a qualitative indicator of lipid peroxidation and oxidative stress.

The exercise training imposed promoted important changes in the activities of the antioxidant enzymes in the soleus muscle. In young rats, an increase of all antioxidant enzyme activities was observed, except for Cu,Zn-SOD activity that remained unchanged. In aged rats, the exercise training did not increase the antioxidant enzyme activities and their mRNA levels as also observed by others (Ji, 1995; Banerjee et al., 2003; Khassaf et al., 2003). However, TBARS content and XO activity of soleus muscle from aged rats showed a significant decrease by the exercise training, reaching the values observed in young rats. The marked decrease in lipid peroxidation and XO activity with no increase in antioxidant enzyme activities suggests a low production of ROS by soleus muscle of aged rats induced by the exercise training protocol.

In conclusion, the exercise training induced marked changes in the oxidative state of soleus muscle from both young and aged rats. In young rats, exercise training raised the activities of CAT, GPX, and Mn-SOD. In aged rats, exercise training did not increase the activities of these enzymes but decreased TBARS content and XO activity. The findings obtained in this study show that exercise training induces a significant decrease of oxidative stress in skeletal muscle from aged rats supporting the recommendation of aerobic exercise for prevention of sarcopenia and to improve the life quality of aged people.

Acknowledgments

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References

Adams, G.R., Haddad, F., Baldwin, K.M., 1994. Interaction of chronic creatine depletion and muscle unloading: effects on postural locomotor muscles. J. Appl. Physiol. 77, 1198–1205.

Aebi, H., 1984. Catalase in vitro. Meth. Enzymol. 105, 121–126.

Aksoy, Y., Yapanoglu, T., Aksoy, H., Demircan, B., Oztasan, N., Canakci, E., Malkoc, I., 2006. Effects of endurance training on antioxidant defense mechanisms and lipid peroxidation in testis of rats. Arch. Androl. 52, 319–323.

Aoi, W., Naito, Y., Takanami, Y., Kawai, Y., Sakuma, K., Ichikawa, H., Yoshida, N., Yoshikawa, T., 2004. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic. Biol. Med. 37, 480–487.

Baker, D.J., Betik, A.C., Krause, D.J., Hepple, R.T., 2006. No decline in skeletal muscle oxidative capacity with aging in long-term calorically restricted rats: effects are independent of mitochondrial DNA integrity. J. Gerontol. 61, 675–684.

Balagopal, P., Schimke, J.C., Ades, P., Adey, D., Nair, K.S., 2001. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am. J. Physiol. 280, E203–E208.

Banerjee, A.K., Mandal, A., Chanda, D., Chakraborti, S., 2003. Oxidant, antioxidant and physical exercise. Mol. Cell. Biochem. 253, 307–312.

Bejma, J., Ji, L.L., 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J. Appl. Physiol. 87, 465–470.

Borges, F., Fernandes, E., Roleira, F., 2002. Progress towards the discovery of xanthine oxidase inhibitors. Curr. Med. Chem. 9, 195–217.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Caillaud, C., Py, G., Eydoux, N., Legros, P., Prefaut, C., Mercier, J., 1999. Antioxidants and mitochondrial respiration in lung, diaphragm, and locomotor muscles: effect of exercise. Free Radic. Biol. Med. 26, 1292–1299.

Catani, M.V., Savini, I., Duranti, G., Caporossi, D., Ceci, R., Sabatini, S., Avigliano, L., 2004. Nuclear factor kappaB and activating protein 1 are involved in differentiation-related resistance to oxidative stress in skeletal muscle cells. Free Radic. Biol. Med. 37, 1024–1036.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Chung, H.Y., Song, S.H., Kim, H.J., Ikeno, Y., Yu, B.P., 1999. Modulation of renal xanthine oxidoreductase in aging: gene expression and reactive oxygen species generation. J. Nutr. Health Aging 3, 19–23.

Conley, K.E., Jubrias, S.A., Esselman, P.C., 2000. Oxidative capacity and ageing in human muscle. J. Physiol. 526 (Pt 1), 203–210.

Dalton, T.P., Shertzer, H.G., Puga, A., 1999. Regulation of gene expression by reactive oxygen. Annu. Rev. Pharmacol. Toxicol. 39, 67–101.

Delp, M.D., Duan, C., 1996. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. J. Appl. Physiol. 80, 261–270.

Dufloth, D.L., Morris, M., Michelini, L.C., 1997. Modulation of exercise tachycardia by vasopressin in the nucleus tractus solitarii. Am. J. Physiol. 273, R1271–R1282.

Fano, G., Mecocci, P., Vecchiet, J., Belia, S., Fulle, S., Polidori, M.C., Felzani, G., Senin, U., Vecchiet, L., Beal, M.F., 2001. Age and sex influence on oxidative damage and functional status in human skeletal muscle. J. Muscle Res. Cell Motil. 22, 345–351.

Fitts, R.H., 2003. Effects of regular exercise training on skeletal muscle contractile function. Am. J. Phys. Med. Rehabil. 82, 320–331.

Flohe, L., Otting, F., 1984. Superoxide dismutase assays. Meth. Enzymol. 105, 93–104.

Franco, A.A., Odom, R.S., Rando, T.A., 1999. Regulation of antioxidant enzyme gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. Free Radic. Biol. Med. 27, 1122–1132.

Frontera, W.R., Hughes, V.A., Fielding, R.A., Fiatarone, M.A., Evans, W.J., Roubenoff, R., 2000. Aging of skeletal muscle: a 12-yr longitudinal study. J. Appl. Physiol. 88, 1321–1326.

Fulle, S., Protasi, F., Di Tano, G., Pietrangelo, T., Beltramin, A., Boncompagni, S., Vecchiet, L., Fano, G., 2004. The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp. Gerontol. 39, 17–24.

Gianni, P., Jan, K.J., Douglas, M.J., Stuart, P.M., Tarnopolsky, M.A., 2004. Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. Exp. Gerontol. 39, 1391–1400.

Gomez del Arco, P., Martinez-Martinez, S., Calvo, V., Armesilla, A.L., Redondo, J.M., 1997. Antioxidants and AP-1 activation: a brief overview. Immunobiology 198, 273–278.

Greenlund, L.J., Nair, K.S., 2003. Sarcopenia—consequences, mechanisms, and potential therapies. Mech. Ageing Dev. 124, 287–299.

Gregorevic, P., Lynch, G.S., Williams, D.A., 2001. Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. Eur. J. Appl. Physiol. 86, 24–27.

Gul, M., Demircan, B., Taysi, S., Oztasan, N., Gumustekin, K., Siktar, E., Polat, M.F., Akar, S., Akcay, F., Dane, S., 2006. Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 143, 239–245.

Gunduz, F., Senturk, U.K., Kuru, O., Aktekin, B., Aktekin, M.R., 2004. The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats. Physiol. Res. 53, 171–176.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine. Oxford University Press Inc., New York.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

Hatao, H., Oh-ishi, S., Itoh, M., Leeuwenburgh, C., Ohno, H., Ookawara, T., Kishi, K., Yagyu, H., Nakamura, H., Matsuoka, T., 2006. Effects of acute exercise on lung antioxidant enzymes in young and old rats. Mech. Ageing Dev. 127, 384–390.

Hepple, R.T., Hagen, J.L., Krause, D.J., Jackson, C.C., 2003. Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O_2 delivery. J. Appl. Physiol. 94, 744–751.

Higuchi, M., Cartier, L.J., Chen, M., Holloszy, J.O., 1985. Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J. Gerontol. 40, 281–286.

Higuchi, R., Dollinger, G., Walsh, P.S., Griffith, R., 1992. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY) 10, 413–417.

Ho, Y.S., Dey, M.S., Crapo, J.D., 1996. Antioxidant enzyme expression in rat lungs during hyperoxia. Am. J. Physiol. 270, L810–L818.

Hollander, J., Bejma, J., Ookawara, T., Ohno, H., Ji, L.L., 2000. Superoxide dismutase gene expression in skeletal muscle: fiber-specific effect of age. Mech. Ageing Dev. 116, 33–45.

Hollander, J., Fiebig, R., Gore, M., Ookawara, T., Ohno, H., Ji, L.L., 2001. Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. Pflugers Arch. 442, 426–434.

Jemiolo, B., Trappe, S., 2004. Single muscle fiber gene expression in human skeletal muscle: validation of internal control with exercise. Biochem. Biophys. Res. Commun. 320, 1043–1050.

Jenkins, R.R., 1993. Exercise, oxidative stress, and antioxidants: a review. Int. J. Sport. Nutr. 3, 356–375.

Ji, L.L., 1995. Oxidative stress during exercise: implication of antioxidant nutrients. Free Radic. Biol. Med. 18, 1079–1086.

Ji, L.L., 1999. Antioxidants and oxidative stress in exercise. Proc. Soc. Exp. Biol. Med. 222, 283–292.

Ji, L.L., Leeuwenburgh, C., Leichtweis, S., Gore, M., Fiebig, R., Hollander, J., Bejma, J., 1998. Oxidative stress and aging. Role of exercise and its influences on antioxidant systems. Ann. NY Acad. Sci. 854, 102–117.

Joksic, G., Pajovic, S.B., Stankovic, M., Pejic, S., Kasapovic, J., Cuttone, G., Calonghi, N., Masotti, L., Kanazir, D.T., 2000. Chromosome aberrations, micronuclei, and activity of superoxide dismutases in human lymphocytes after irradiation in vitro. Cell Mol. Life Sci. 57, 842–850.

Kakarla, P., Vadluri, G., Reddy, K.S., Leeuwenburgh, C., 2005. Vulnerability of the mid aged rat myocardium to the age-induced oxidative stress: influence of exercise training on antioxidant defense system. Free Radic. Res. 39, 1211–1217.

Kang, D., Hamasaki, N., 2003. Mitochondrial oxidative stress and mitochondrial DNA. Clin. Chem. Lab. Med. 41, 1281–1288.

Khassaf, M., McArdle, A., Esanu, C., Vasilaki, A., McArdle, F., Griffiths, R.D., Brodie, D.A., Jackson, M.J., 2003. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. J. Physiol. 549, 645–652.

Kim, Y.J., Yokozawa, T., Chung, H.Y., 2005. Suppression of oxidative stress in aging NZB/NZW mice: effect of fish oil feeding on hepatic antioxidant status and guanidino compounds. Free Radic. Res. 39, 1101–1110.

Lagranha, C.J., de Lima, T.M., Senna, S.M., Doi, S.Q., Curi, R., Pithon-Curi, T.C., 2005. The effect of glutamine supplementation on the function of neutrophils from exercised rats. Cell Biochem. Funct. 23, 101–107.

Lawler, J.M., Powers, S.K., Van Dijk, H., Visser, T., Kordus, M.J., Ji, L.L., 1994. Metabolic and antioxidant enzyme activities in the diaphragm: effects of acute exercise. Respir. Physiol. 96, 139–149.

Lawler, J.M., Powers, S.K., Visser, T., Van Dijk, H., Kordus, M.J., Ji, L.L., 1993. Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am. J. Physiol. 265, R1344–R1350.

Leeuwenburgh, C., Fiebig, R., Chandwaney, R., Ji, L.L., 1994. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am. J. Physiol. 267, R439–R445.

Leeuwenburgh, C., Hollander, J., Leichtweis, S., Griffiths, M., Gore, M., Ji, L.L., 1997. Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. Am. J. Physiol. 272, R363–R369.

Lexell, J., 1995. Human aging, muscle mass, and fiber type composition. J. Gerontol. 50 Spec No, 11–16.

Linnane, A.W., Marzuki, S., Ozawa, T., Tanaka, M., 1989. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 1, 642–645.

Linz, W., Jessen, T., Becker, R.H., Scholkens, B.A., Wiemer, G., 1997. Long-term ACE inhibition doubles lifespan of hypertensive rats. Circulation 96, 3164–3172.

Liu, W., Saint, D.A., 2002. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Anal. Biochem. 302, 52–59.

Locke, M., Atkinson, B.G., Tanguay, R.M., Noble, E.G., 1994. Shifts in type I fiber proportion in rat hindlimb muscle are accompanied by changes in HSP72 content. Am. J. Physiol. 266, C1240–C1246.

Luhtala, T.A., Roecker, E.B., Pugh, T., Feuers, R.J., Weindruch, R., 1994. Dietary restriction attenuates age-related increases in rat skeletal muscle antioxidant enzyme activities. J. Gerontol. 49, B231–B238.

Mahoney, D.J., Carey, K., Fu, M.H., Snow, R., Cameron-Smith, D., Parise, G., Tarnopolsky, M.A., 2004. Real-time RT-PCR analysis of housekeeping genes in human skeletal muscle following acute exercise. Physiol. Genomics 18, 226–231.

Mannervik, B., 1985. Glutathione peroxidase. Meth. Enzymol. 113, 490–495.

Mansouri, A., Muller, F.L., Liu, Y., Ng, R., Faulkner, J., Hamilton, M., Richardson, A., Huang, T.T., Epstein, C.J., Van Remmen, H., 2006. Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging. Mech. Ageing Dev. 127, 298–306.

Mastaloudis, A., Yu, T.W., O'Donnell, R.P., Frei, B., Dashwood, R.H., Traber, M.G., 2004. Endurance exercise results in DNA damage as detected by the comet assay. Free Radic. Biol. Med. 36, 966–975.

McArdle, A., Vasilaki, A., Jackson, M., 2002. Exercise and skeletal muscle ageing: cellular and molecular mechanisms. Ageing Res. Rev. 1, 79–93.

Meydani, M., Evans, W., Handelman, G., Fielding, R.A., Meydani, S.N., Fiatarone, M.A., Blumberg, J.B., Cannon, J.G., 1992. Antioxidant response to exercise-induced oxidative stress and protection by vitamin E. Ann. NY Acad. Sci. 669, 363–364.

Morley, J.E., Baumgartner, R.N., Roubenoff, R., Mayer, J., Nair, K.S., 2001. Sarcopenia. J. Lab. Clin. Med. 137, 231–243.

Muller, J.M., Rupec, R.A., Baeuerle, P.A., 1997. Study of gene regulation by NF-kappa B and AP-1 in response to reactive oxygen intermediates. Methods (San Diego, CA) 11, 301–312.

Navarro-Arevalo, A., Sanchez-del-Pino, M.J., 1998. Age and exercise-related changes in lipid peroxidation and superoxide dismutase activity in liver and soleus muscle tissues of rats. Mech. Ageing Dev. 104, 91–102.

Pajovic, S.B., Joksic, G., Kasapovic, J., Pejic, S., Kanazir, D.T., 2000. Role of antioxidant enzymes in radiosensitivity of human blood cells. J. Environ. Pathol. Toxicol. Oncol. 19, 325–331.

Pansarasa, O., Bertorelli, L., Vecchiet, J., Felzani, G., Marzatico, F., 1999. Age-dependent changes of antioxidant activities and markers of free radical damage in human skeletal muscle. Free Radic. Biol. Med. 27, 617–622.

Pansarasa, O., Castagna, L., Colombi, B., Vecchiet, J., Felzani, G., Marzatico, F., 2000. Age and sex differences in human skeletal muscle: role of reactive oxygen species. Free Radic. Res. 33, 287–293.

Papa, S., 1996. Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications. Biochim. Biophys. Acta 1276, 87–105.

Parise, G., Phillips, S.M., Kaczor, J.J., Tarnopolsky, M.A., 2005. Antioxidant enzyme activity is up-regulated after unilateral resistance exercise training in older adults. Free Radic. Biol. Med. 39, 289–295.

Pereira, B., Costa Rosa, L.F., Safi, D.A., Medeiros, M.H., Curi, R., Bechara, E.J., 1994. Superoxide dismutase, catalase, and glutathione peroxidase activities in muscle and lymphoid organs of sedentary and exercise-trained rats. Physiol. Behav. 56, 1095–1099.

Pithon-Curi, T.C., De Melo, M.P., Curi, R., 2004. Glucose and glutamine utilization by rat lymphocytes monocytes and neutrophils in culture: a comparative study. Cell Biochem. Funct. 22, 321–326.

Powers, S.K., Criswell, D., Lawler, J., Ji, L.L., Martin, D., Herb, R.A., Dudley, G., 1994. Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am. J. Physiol. 266, R375–R380.

Prajda, N., Weber, G., 1975. Malignant transformation-linked imbalance: decreased xanthine oxidase activity in hepatomas. FEBS Lett. 59, 245–249.

Quindry, J., French, J., Hamilton, K., Lee, Y., Mehta, J.L., Powers, S., 2005. Exercise training provides cardioprotection against ischemia-reperfusion induced apoptosis in young and old animals. Exp. Gerontol. 40, 416–425.

Ramakers, C., Ruijter, J.M., Deprez, R.H., Moorman, A.F., 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. 339, 62–66.

Rinaldi, B., Corbi, G., Boccuti, S., Filippelli, W., Rengo, G., Leosco, D., Rossi, F., Filippelli, A., Ferrara, N., 2006. Exercise training affects age-induced changes in SOD and heat shock protein expression in rat heart. Exp. Gerontol. 41, 764–770.

Rodriguez, C., Mayo, J.C., Sainz, R.M., Antolin, I., Herrera, F., Martin, V., Reiter, R.J., 2004. Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal. Res. 36, 1–9.

Sambrook, J., Russel, D.W., 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Schoneich, C., 2006. Protein modification in aging: an update. Exp. Gerontol. 41, 807–812.

Sen, C.K., 2001. Antioxidant and redox regulation of cellular signaling: introduction. Med. Sci. Sports Exerc. 33, 368–370.

Short, K.R., Vittone, J.L., Bigelow, M.L., Proctor, D.N., Nair, K.S., 2004. Age and aerobic exercise training effects on whole body and muscle protein metabolism. Am. J. Physiol. 286, E92–E101.

Srere, P.A., Brazil, Gonen, 1963. The citrate condensing enzyme of pigeon breast muscle and moth flight muscle. Acta Chem. Scand. 17, 127–134.

Trappe, S., Williamson, D., Godard, M., Porter, D., Rowden, G., Costill, D., 2000. Effect of resistance training on single muscle fiber contractile function in older men. J. Appl. Physiol. 89, 143–152.

Vincent, H.K., Powers, S.K., Demirel, H.A., Coombes, J.S., Naito, H., 1999. Exercise training protects against contraction-induced lipid peroxidation in the diaphragm. Eur. J. Appl. Physiol. Occup. Physiol. 79, 268–273.

Wei, Y.H., Lee, H.C., 2002. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. Exp. Biol. Med. (Maywood) 227, 671–682.

Wendel, A., 1981. Glutathione peroxidase. Meth. Enzymol. 77, 325–333.

Winterbourn, C.C., Gutteridge, J.M., Halliwell, B., 1985. Doxorubicin-dependent lipid peroxidation at low partial pressures of O_2. J. Free Radic. Biol. Med. 1, 43–49.

Zhou, L.Z., Johnson, A.P., Rando, T.A., 2001. NF kappa B and AP-1 mediate transcriptional responses to oxidative stress in skeletal muscle cells. Free Radic. Biol. Med. 31, 1405–1416.