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Myocardial and skeletal muscle aging and changes in oxidative stress in relationship to rigorous exercise training

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Abstract

Cardiac and skeletal muscle are very different functional tissues, and we would expect a variation in the ROS generation, in ageing and rigorous exercise-related in both tissues. We determined TBARS, total SOD, Cu, ZnSOD and MnSOD activities, and the patterns of SOD isoenzymes in skeletal muscle and heart of male Wistar rats, young and old, in rest and after rigorous exercise. There were no differences in the levels of lipoperoxidation in aged rest animals in both tissues, but the level was increased after exhaustion. The level of SOD activities was bigger in the heart than in skeletal muscle. Total SOD and Cu, ZnSOD activities were higher in old rest animals in the skeletal muscle than in young rest rats. This change did not occur in the heart. After rigorous exercise, the level of SOD activities was increased in young rats in both tissues. However, in old exhausted rats, the activities were only elevated in the heart. Different Cu, ZnSOD isoenzyme patterns showed in relation to tissues. In the skeletal muscle in old animals, the Cu, ZnSOD isoenzyme pattern was modified. The rigorous exercise did not change this pattern. The pattern of MnSOD isoenzyme was not varied in either tissue, age nor and exercise.

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1. Introduction

The damage accumulated throughout life by reactive oxygen species (ROS) production within mitochondrial respiration affects the efficiency of numerous cellular homeostatic mechanisms (Harman, 1972). One of these affected mechanisms is the balance between pro-oxidants and antioxidants, caused either by increased oxidants and/or decreased antioxidant defences (Sohal et al., 1995). Those tissues with few or no cellular division will be theoretically more susceptible in showing accumulative damage caused by ROS. As a result, in this study we selected the heart and skeletal muscle because both are fixed posmitotic tissues (Miquel, 1992, 1998).

The physical exercise of high intensity causes an important production of ROS. We considered that the capacity of elimination of free radicals produced during exhaustive exercise would be modified depending on the age of the experimental animals (Reznick et al., 1992). Cardiac muscle and skeletal muscle, both with no cellular renovation, are very different from a metabolic, histological and functional point of view. It would be expected that the ROS production level would not be the same in both tissues; therefore the skeletal muscle has a more predominant glicolitic metabolism, while the heart has a more oxidative metabolism. We propose that a lipoperoxide generation is increased in relationship to the rigorous exercise in old animals. The antioxidant SOD activities are probably modified with regard to age and exercise. However, the chronic metabolic differences in myocardial and skeletal muscle tissues allows us to suppose a different response in their antioxidant mechanisms.

2. Materials and methods

2.1. Animals in the experiments

We used 71 male Wistar rats for this study and they were classified into four groups: rest young (CY; $n = 16$), rest old (CO; $n = 24$), exhausted young (EY; $n = 16$) and exhausted old (EO; $n = 15$). Young rats were 3–5 months old, and aged rats were 24–27 months old. They housed two per cage. All animals were permitted food and water ad libitum, at room temperature (22–28°C), and a 12:12 h light–dark cycle.These rats were provided by the Central Service of Experimental Animals Department of the University of Cádiz. This study was approved by the University Committee for Use of Animals in Research, and followed guidelines established by the European Regulation on animal treatment submitted to experiment.

2.2. Protocol of rigorous exercise training

The rigorous exercise training was performed in a treadmill, with different speed and slope. The protocol was as follows: the exercise started at 10 m min^{-1}, 0% grade, followed by a gradual increases of treadmill speed and grade every 5 min up to 30 m min^{-1}, 15% grade. Average run time to exhaustion for the young rats (EY) was ~ 55 min, and the old rats (EO) time was ~ 27 min. The animals were considered exhausted when lactic acid in serum was more than 6.1 mM. The method of this analysis was according to Wahlefeld (1988).

2.3. Tissue preparation

The heart and soleus muscle were quickly excised after death of the animals by decapitation with both rest and exhausted rats. These tissues were immediately introduced into liquid N_2, and maintained at $-80°C$ until determination.

2.4. Biochemical assays

The tissues were homogenized with a Tempest Virtis homogenizer in sodium phosphate buffer 200 mM (pH 7.8) at 4°C, and the assays were rapidly made. The lipoperoxidation level was determined as thiobarbituric acid-reactive substances (TBARS) (Buege and Aust, 1978). The total and mitochondrial SOD (EC 1.15.1.1) activities were analyzed using the method by Elstner et al. (1988). The Cu, ZnSOD activity was defined as the difference between total and MnSOD activities. The protein concentration was measured by the methods of Lowry et al. (1951).

The isoforms of citoplasmic (Cu, ZnSOD) and mitochondrial (MnSOD) isoenzymes SOD were determined by isoelectrofocusing, which was carried out by Phastsystem Equipment (Pharmacia), followed by in situ staining for SOD activity (using nitroblue–tetrazolium, riboflavin and TEMED) (Beauchamp and Fridovich, 1971).

2.5. Statistical analysis

The determinations were expressed as mean $\pm$ standard deviation (S.D.). The differences between the values in the different groups were analyzed by Student' s t-test. The significance was considered when $P = 0.05$.

3. Results

3.1. The lipoperoxidation level

TBARS levels were used as the lipoperoxidation index. There were no differences between the animals in rest, and we found an increase in TBARS levels after exhaustive exercise both in young and in old animals in the skeletal muscle (Table

Table 1
TBARS levels and total Cu, ZnSOD and MnSOD activity in skeletal muscle in control young (CY), control old (CO), exhausted young (EY) and exhausted old (EO) groups[a]

Groups	TBARS (μmol/g tissue)	SOD total (U SOD/g prot)	MnSOD (U SOD/g prot)	Cu, ZnSOD (U SOD/g prot)
CY ($n=16$)	23.94 ± 5.27	150.41 ± 12.92**	75.99 ± 5.37	74.41 ± 16.71[c]
CO ($n=24$)	23.78 ± 5.44	234.63 ± 24.30	76.75 ± 12.12	157.99 ± 32.24
EY ($n=16$)	33.14 ± 10.94	291.50 ± 42.89**	99.12 ± 16.20*,[b]	192.50 ± 45.51[c]
EO ($n=15$)	45.91 ± 14.61**,[d]	218.42 ± 20.35	81.56 ± 7.93	136.68 ± 20.96

[a] Values are expressed as means ± S.D. Values of TBARS levels and total SOD published by Navarro-Arévalo and Sánchez-del-Pino (1998).
[b] $P=0.01$ EY versus CY.
[c] $P=0.01$ CY versus CO, CY versus EY, and EY versus EO.
[d] $P=0.05$ EO versus CO.
* $P=0.05$ EY versus EO.
** $P=0.01$ CY versus CO, CY versus EY and EY versus EO.

1). The aged exhausted rats group (EO) showed higher values than in the other groups, with significant differences when we compared them to exhausted young (EY) ($P=0.01$) and to rest old (CO) ($P=0.05$) (Table 1).With respect to the heart, this tissue showed lower TBARS levels than the skeletal muscle. The exhausted old animals (EO) presented higher levels of TBARS than the other groups (Table 2), with significant differences compared to the control group (CO) and the exhausted young group (EY) ($P=0.01$) (Table 2).

3.2. The total SOD, Cu, ZnSOD and MnSOD antioxidant enzymatic endogenous activities

In skeletal muscle, the rest old rats showed a larger total SOD activity than the rest young (CY versus CO) ($P=0.01$) (Table 1). The exhausted young animals

Table 2
TBARS levels and total Cu, ZnSOD and MnSOD activity in the heart in control young (CY), control old (CO), exhausted young (EY) and exhausted old (EO) groups[a]

Groups	TBARS (μmol/g tissue)	SOD total (U SOD/g prot)	MnSOD (U SOD/g prot)	Cu, ZnSOD (U SOD/g prot)
CY ($n=16$)	1.70 ± 0.36	347.87 ± 54.22	98.42 ± 20.7	243.03 ± 56.36
CO ($n=24$)	1.71 ± 0.01	341.93 ± 65.63	115.51 ± 20.38	233.15 ± 53.18
EY ($n=16$)	1.81 ± 0.71	407.59 ± 30.30**	123.30 ± 16.74**	286.98 ± 32.62*
EO ($n=15$)	4.45 ± 0.72**,[b]	431.75 ± 56.82**	136.93 ± 14.04*	289.26 ± 32.79*

[a] Values are expressed as means ± S.D.
[b] $P=0.01$ EO versus EY.
* $P=0.05$ CY versus EY and CO versus EO.
** $P=0.01$ CY versus EY and CO versus EO.

(EY) had the activity level higher than the other study groups, and they presented significant differences compared to rest young (CY) and to exhausted old (EO) ($P = 0.01$) (Table 1). The Cu, ZnSOD activity in the skeletal muscle was higher in old rest rats than in young rest rats (CY versus CO) ($P = 0.01$) (Table 1). After rigorous exercise, the exhausted young group increased this activity, and presented significant differences between rest young and exhausted old rats (CY versus EY) (EY versus EO) ($P = 0.01$) (Table 1). The MnSOD activity increased after rigorous exercise both in the young and in the old rats. The significant differences were shown when we compared the rest young rats with respect to the exhausted young group (CY versus EY) ($P = 0.01$), and on comparing exhausted young animals with respect to exhausted old rats (EY versus EO) ($P = 0.05$) (Table 1).

We found that the heart showed a higher total SOD, citoplasmic SOD (Cu, ZnSOD) and mitochondrial (MnSOD) antioxidant activities than the skeletal muscle. In the heart, the exhausted animals had higher total SOD, Cu, ZnSOD and MnSOD antioxidant activities than the rest rats (Table 2). When we compared the different groups, the total SOD activity was different between rest young and exhausted young, and this difference was significant (CY versus EY) ($P = 0.01$). There were similar differences between old rats (CO versus EO) ($P = 0.01$) (Table 2). The behaviour of Cu, ZnSOD activity was similar to total SOD activity (CY versus EY) (CO versus EO) ($P = 0.05$) (Table 2). With respect to MnSOD activity, there were significant differences on comparing rest young and exhausted young (CY versus EY) ($P = 0.01$) and when we compared rest old to exhausted old (CO versus EO) ($P = 0.05$) (Table 2).

3.3. Patterns of Cu, ZnSOD and MnSOD isoenzymes

The pattern of Cu, ZnSOD isoenzyme in skeletal muscle presented three isoforms in the old animal groups with pI 5.6, 5.2 and 4.9, respectively (Fig. 1). The differences were appreciable in the young animals where we observed another isoform with pI 5.1 (Fig. 1). The patterns were equal after rigorous exercise in young and old animals.

The pattern of Cu, ZnSOD isoenzyme in the heart presented only three isoforms in all animal groups. There were no differences between old and young rats. The pI were 5.6, 5.2 and 4.9 (Fig. 2).

No differences were appreciable in the MnSOD pattern between tissues, nor with the age or exercise groups. Only one band was shown with pI 5.8.

4. Discussion

4.1. Influence of aging

In our hypothesis, we did not expect to find one important modification in the level of lipoperoxidation in aged animals, neither heart nor skeletal muscle, and our results were in this sense just as we postulated in our first hypothesis. However, a

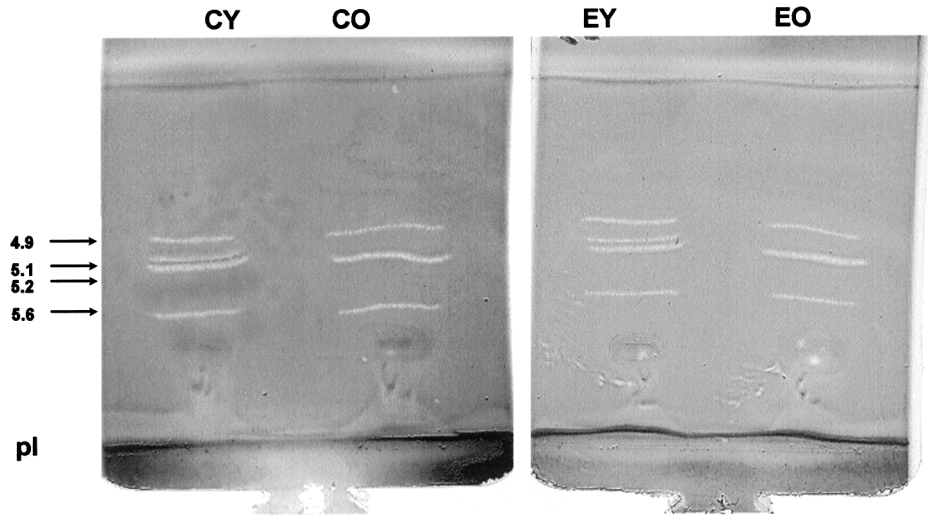

Fig. 1. Pattern of Cu, ZnSOD isoenzyme in skeletal muscle in control young (CY), control old (CO), exhausted young (EY) and exhausted old (EO) groups.

higher concentration of TBARS levels was shown in the skeletal muscle than in the heart, both in young and old rest animals. These results were in accordance with Cand and Verdetti (1989), who assumed that the ratio of unsaturated/saturated fatty acids decreased, and that this would reduce the amount of substrate for the

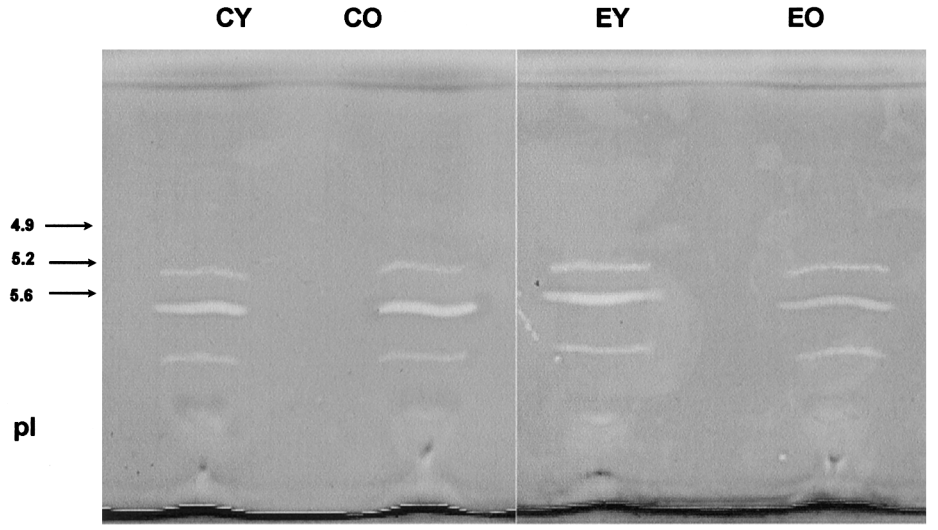

Fig. 2. Pattern of Cu, ZnSOD isoenzyme in the heart in control young (CY), control old (CO), exhausted young (EY) and exhausted old (EO) groups.

lipoperoxidation. Moreover, these authors considered that the lipid and protein copolymerisation was increased. Muscari et al. (1990) proposed that the variation of the TBARS levels would be caused because the final products of lipoperoxidation are metabolized in vivo in the mitochondria: it would have the lipoperoxidation but end products would be undetectable. In further studies, these authors showed several damages in the aged heart caused by free radical generation (Muscari et al., 1996), such as lipofuscin accumulation, decreased phospholipid unsaturation index, greater formation of both hydrogen peroxide and 8-hydroxy-2′-deoxyguanosine.

Our results are in disagreement with other authors (Nohl et al., 1979; Ji et al., 1991), who found that TBARS levels increased in the heart, thus affecting the membranes and being responsible for the age-related changes. However, Lewin and Timiras (1984) found changes in the phospholipids and cholesterol content when they compared myocardiac mitochondrial membranes of the young and old rats, and they interpreted these results as indicative of an age-related decrease in fluidity and energy transduction of mitochondrial membranes in the heart of aged rats. It might be responsible, in part, for the decrease in cardiac function with aging.

The increase with age in ROS production is currently accepted (Gershman, 1954; Harman, 1956), and ROS are related to mitochondrial damage (Miquel, 1998), and also with a decrease in the cellular reparation mechanism and a homeostatic capacity leakage (Nohl, 1993), Thus, a greater production of superoxide anions were found in mitochondrial aged heart than in young (Nohl and Hegner, 1978; Sohal et al., 1995). Therefore, an increase in the endogenous antioxidant activity in the old animal tissues is expected. However, in this sense, the data in the bibliography are controversial, depending on the tissue type and/or the animal study (Hammeren et al., 1992; Ji, 1993; Sohal et al., 1995).

Our results only showed a marked increase in total SOD activity in the skeletal muscle of the aged rest rats, and this increase is caused by the high level of Cu, ZnSOD. Myocardic total SOD activity was not modified. Ji et al. (1990) found an increase in the citoplasmic SOD in skeletal muscle in relation to age, but the mitochondrial SOD was increased too. With respect to the heart, our results are in agreement with Cand and Verdetti (1989), and only partially in agreement with Ji et al. (1991), because they did not find modifications in myocardial Cu, ZnSOD when they compared young and old rest rats, but they found an increase in MnSOD activity with age.

It is interesting to underline that the total SOD activity is a lot more superior in the heart than in the skeletal muscle, both in young and in old animals, and this is in agreement with the differences in TBARS levels in both tissues. These results are in agreement with Vertechy et al. (1989), but in disagreement with Kanter et al. (1993). In skeletal muscle, a pattern of Cu, ZnSOD isoenzyme showed three bands in the old animals and four in the young animals. Therefore, there is an age-related difference in this tissue. In the heart, three bands were shown in old and young study groups, hence there were no differences with respect to age. In young animals, the Cu, ZnSOD pattern was different in both tissues. The MnSOD isoenzyme pattern was equal in both tissues and in all animal groups. The different pattern in

skeletal muscle in young rats would be explained by the increase in the TBARS level in this tissue. This proposal is sustained by Pedrajas et al. (1998). These results suggest that malondialdehyde leads to changes in SOD pattern.

As the cardiac muscle and the skeletal muscle are two very different tissues from a metabolic point of view, our results could show that the modification of SOD antioxidant activity could be caused by glicolitic metabolism of the skeletal muscle. According to our data, the increase in the ROS generation during aging is compensated, in rest, by endogenous antioxidant capacity, because the lipoperoxides levels were maintained constant.

4.2. Influence of rigorous exercise

High intensity exercise increases the ROS production in the skeletal muscle (Davies et al., 1982; Packer, 1986) and in the heart (Boveris and Chance, 1973; Ohkuwa et al., 1997). The tissue consumption of oxygen increases during exercise, but the generation of the ROS at the electron transport chain is only 2–4% of total oxygen (Alessio, 1993; Tiidus and Houston, 1995). This increase in the ROS production will produce tissue damage and it will occur in fibre I (Ebbeling and Clarkson, 1989). We found a lipoperoxidation increase after strenuous exercise in old animals, both in the skeletal muscle and in the heart. The increase in lipoperoxidation will affect the fluidity and the permeability membranes. In skeletal muscle, our results are in agreement with Jenkins and Goldfarb (1993), Alessio and Goldfarb (1988), Ji and Fu (1992). However, in the heart we can not compare our data because we did not find a similar protocol in old rats in the bibliography.

It has been proposed that exhaustive exercise would increase antioxidant activity by the induction of several enzymes (Dekkers et al., 1996). However, in previous results, we found that this induction depends on age, type, intensity and duration of exercise (Navarro-Arévalo and Sánchez-del-Pino, 1998).

In the present study, we observed in the skeletal muscle a significant increase in total SOD activity in exhausted young animals with respect to the rest young, and we are in agreement with Lammi-Keefe et al. (1984), Ji (1993), Criswell et al. (1993), and in disagreement with Alessio and Goldfarb (1988), Atalay et al. (1996), Laughlin et al. (1990). These discrepancies could be caused by the different composition of the muscle, because the type of muscular fibre could influence the antioxidant enzymatic activity. Therefore, tissues with a high oxidative capacity show more antioxidant activity. It is possible that muscular fibre I has different recruitment patterns (Dekkers et al., 1996). Thus, it is necessary to realize that previous training to extenuation decreases total SOD enzymatic activity in the skeletal muscle (Alessio and Goldfarb, 1988; Navarro-Arévalo and Sánchez-del-Pino, 1998), and we consider that the increase in the mitochondrial volume could serve in decreasing the stimulus for antioxidant induction.

A marked increase in citosolic and mitochondrial SOD isoenzymes activities was shown in skeletal muscle in exhausted young rats. However, total SOD and isoenzymes SOD activities were not modified in old animals after rigorous exercise. These results are partly in agreement with Ji et al. (1990), because they only

observed increased Cu, ZnSOD, and not MnSOD. This discrepancy could be explained because the determinations were made 48 h after the exhaustive exercise, when the superoxide anions had disappeared, while we analyzed the tissues immediately after extenuation, and at this moment the superoxide anions were present and could induce MnSOD activity. Other authors found a high level of MnSOD in the skeletal muscle in young animals after extenuation (Somani et al., 1995). Oh-ishi et al. (1997) observed an increase in Cu, ZnSOD but not in MnSOD. However, after adequate previous training, mRNA of MnSOD were elevated and not mRNA of Cu, ZnSOD.

As previously mentioned, we did not observe modifications in the antioxidant activity in exhausted old rats. These data are in agreement with the increment of lipoperoxides in the skeletal muscle of old animals in our experiments. Our results in this tissue could show that the young animal is capable of inducing the SOD antioxidant capacity, while the old animal does not reach the induction of SOD with this type of exercise, and thus increases the damage.

In the myocardial muscle tissue, as with the skeletal muscle, we found an increase of total SOD and isoenzyme Cu, Zn and MnSOD activity in exhausted young rats compared to the rest of the animals. However, in exhausted old animals, an increase in total SOD and in isoenzyme Cu, ZnSOD and MnSOD activity appeared in the heart while this did not occur in the skeletal muscle.

There are few data in the bibliography with respect to Cu, ZnSOD and MnSOD activity in both young and old animals submitted to rigorous exercise training. We only found a similar research by Ji et al. (1991), and the results of the citosolic isoenzymes are similar to ours. However, they did not find an increase in the mitochondrial isoenzyme activity after extenuation, and it is possible because they obtained high levels of isoenzymes in rest.

We did not find differences with respect to the patterns of Cu, ZnSOD and MnSOD isoenzymes in relation to rigorous exercise, in both tissues.

According to our results, we wonder why the myocardial muscle in the old animal is capable of increasing SOD antioxidant activity, but not the skeletal muscle. We think that the heart has a different metabolism than the skeletal muscle, and moreover that the heart is a special muscle because it continues contracting without pause throughout life and, in this sense we could consider it as a long-training muscle, while the skeletal muscle in old animals was only contracted in a concrete period, in exhaustive exercise and not submitted to previous training. For this reason, the data of the heart are in agreement with previous results inn our group, when we analyzed the SOD activity in skeletal muscle of old animals trained during half their life (Navarro-Arévalo and Sánchez-del-Pino, 1998). Hence, we think that rigorous exercise will not produce an increase in the SOD antioxidant activity in the old animals if a previous induction caused by the training does not exist.

In spite of the increase in SOD activity in the heart, we can not forget the damage caused by the ROS as a result of rigorous exercise in the old animals being manifested by an increase in lipoperoxides levels in this group. We think that it is necessary to study other antioxidant systems, thus it is probable that some of them are not elevated after exhaustion in old animals.

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