ELSEVIER

Atherosclerosis 167 (2003) 327–334

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women

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Received 5 July 2002; received in revised form 23 December 2002; accepted 9 January 2003

Abstract

Physical activity (PA) is associated with a reduced risk of coronary heart disease, and may favorably modify the antioxidant–prooxidant balance. This study assessed the effects of aerobic PA training on antioxidant enzyme activity, oxidized LDL concentration, and LDL resistance to oxidation, as well as the effect of acute PA on antioxidant enzyme activity before and after the training period. Seventeen sedentary healthy young men and women were recruited for 16 weeks of training. The activity of superoxide dismutase in erythrocytes (E-SOD), glutathione peroxidase in whole blood (GSH-Px), and glutathione reductase in plasma (P-GR), and the oxidized LDL concentration and LDL composition, diameter, and resistance to oxidation were determined before and after training. Shortly before and after this training period they also performed a bout of aerobic PA for 30 min. The antioxidant enzyme activity was also determined at 0 min, 30 min, 60 min, 120 min, and 24 h after both bouts of PA. Training induces an increase in GSH-Px (27.7%), P-GR (17.6%), and LDL resistance to oxidation, and a decrease in oxidized LDL (−15.9%). After the bout of PA, an increase in E-SOD and GSH-Px was observed at 0 min, with a posterior decrease in enzyme activity until 30–60 min, and a tendency to recover the basal values at 120 min and 24 h. Training did not modify this global response pattern. Regular PA increases endogenous antioxidant activity and LDL resistance to oxidation, and decreases oxidized LDL concentration; 30 min of aerobic PA decreases P-GR and B-GSH-Px activity in the first 30–60 min with a posterior recovery.

Keywords: Antioxidants; Physical activity; Free radicals; Lipoproteins

1. Introduction

Regular physical activity (PA) is associated with a reduced risk of coronary heart disease (CHD) [1], and consequently, physical inactivity has been considered a risk factor for CHD [2]. The mechanisms underlying this protective effect are not fully established.

On the other hand, oxidative stress has been found to be linked to the development of several chronic diseases including atherosclerosis [3]. The oxidation of low-density lipoprotein (LDL) components is a cornerstone of atherosclerosis [3]. The oxidative status is controlled by a wide spectrum of dietary exogenous antioxidants such as tocopherols, ascorbate, carotenoids, and phenolic compounds, and by endogenous antioxidants such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and catalase among others [4]. The balance between free-radical generation and antioxidant activity is critical in the pathogenesis of CHD. LDL resistance to oxidation, mainly dependent on its antioxidant content and lipid particle composition, is also an important factor limiting this process.

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0021-9150/03/$ - see front matter
doi:10.1016/S0021-9150(03)00018-2

One of the favorable effects of regular PA could be to modify the prooxidant/antioxidant balance, increasing endogenous antioxidant activity, and LDL resistance to oxidation. On the other hand, acute PA increases oxygen uptake and free-radical production, and consequently could induce lipid peroxidation [5,6].

The aims of this study were (1) to assess the effect of an aerobic PA training program on antioxidant enzyme activity, oxidized LDL concentration, and LDL resistance to oxidation in young healthy subjects; and (2) to assess the effect of a single bout of acute PA on antioxidant enzyme activity, before and after the training period.

2. Subjects and methods

2.1. Subjects

Seventeen sedentary healthy young volunteers, seven men and 10 women, were recruited among medical students. Subjects with previous personal history of cardiovascular disease, diabetes mellitus, dyslipemia, physical disability, or chronic respiratory disease, as well as those with a body mass index (BMI) over 30 kg/m^2, alcohol consumption greater than 40 g per day, or long-term medication use, including mineral or vitamin supplements, were excluded. Another exclusion criterion was regular PA for more than 2 h per week during the preceding 3 months. The local research ethics committee approved the protocol and all participants provided informed consent.

2.2. Physical fitness assessment

All the participants underwent a continuous, incremental cycling test on an electromagnetically braked ergocycle (EC 1200; Marquette Hellige Medical Systems, Milwaukee, USA). The test began with a warm-up at 25 W (women) or 50 W (men) for 5 min, after which the power output was increased by 25 W every 2 min until exhaustion. During the test, oxygen uptake (VO_2), minute ventilation (VE), and respiratory exchange ratio (RER) were continuously measured using an Oxicon Alpha (Jaeger, Wuerzburg, Germany). The metabolic cart and volume instruments were calibrated with known calibration gases and with a 5-l calibration syringe, respectively, before each test. VO_2 max was determined by attaining at least two of the following criteria: RER above 1.1, heart rate over 90% of the predicted maximal rate, or a plateau in VO_2 despite an increase in power output. VO_2 max was calculated as the average of the three highest V_{O_2} values registered. The ventilatory anaerobic threshold (VT) was determined by the V-slope method [7]. The maximal power output (MPO) was defined as the highest achieved power completed for at least 1 min. The maximal aerobic power output (APO) was defined as the power achieved at VT level.

2.3. Intervention

A general schema of the intervention is presented in Fig. 1.

2.3.1. Training period

Participants followed an individualized and supervised aerobic PA training program during 16 weeks. Initially, the frequency of training sessions was four 30-min sessions per week. The frequency and duration of these sessions were progressively increased to 5 days per week and 50 min per day during the first 8 weeks, and continued at this level for another 8 weeks. The intensity of training was individualized and adapted to the basal physical fitness of each participant at 65–80% of his/her maximal oxygen consumption. The training was performed in the Physical Activity Service facilities of the Universitat Autònoma of Barcelona.

2.3.2. Bout of aerobic PA

All the participants performed a bout of aerobic PA before and after the training period. One week after completing a maximal effort test to assess fitness level, participants cycled in an ergometer for 30 min at a level corresponding to his/her maximal aerobic power output. This aerobic PA was performed 3 h after lunch. Participants warmed up for 5 min at 25 or 50 W (women and men, respectively), and achieved APO in the following 5 min. This power was then sustained for 25 min. The only dietary control was the requirement that a participant eat the same diet in the 3 days preceding each of the two bouts of PA.

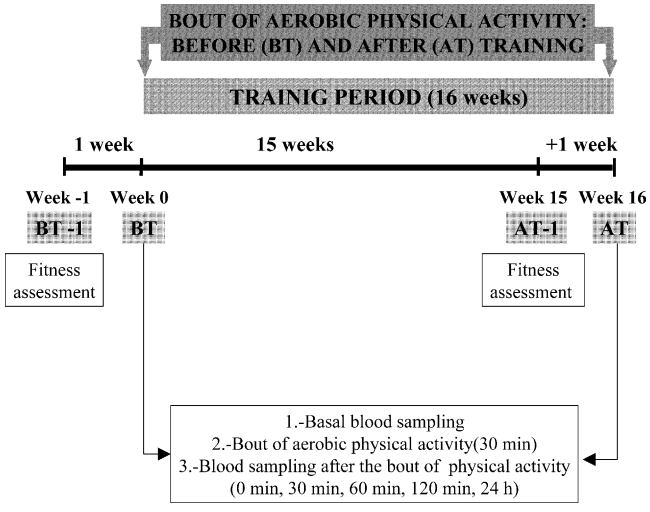

Fig. 1. General schema of the intervention.

2.4. Blood sampling

A blood sample was drawn (basal) prior to each bout of PA, immediately after exercising (0 min), at 30 min, and 1, 2, and 24 h thereafter.

2.5. Laboratory methods

2.5.1. Enzymatic assays

E-SOD activity in erythrocytes followed that of McCord and Fridovich [8] (Ransel RS 125; Randox Laboratories, Crumlin, UK), and was expressed in U/g of hemoglobin. B-GSH-Px activity was measured by a modification of the method of Plagia and Valentine [9] (Ransel RS 505; Randox Laboratories, Crumlin, UK), and expressed in U/l. The catalytic concentration of plasmatic glutathione reductase (P-GR) was measured by following the oxidation of NADPH to $NADP^+$ during the reduction of oxidized glutathione (Ransel GR 2368; Randox Laboratories, Crumlin, UK) [10], and expressed in U/l. Intra-run imprecision was 4.7, 3.6, and 3.5% for E-SOD, B-GSH-Px, and P-GR, respectively. Between-run imprecision was 5.6, 5.4, and 4.4% for E-SOD, B-GSH-Px, and P-GR, respectively.

2.5.2. LDL preparation, isolation, and composition

Blood from volunteers was collected in tubes containing 1 g/l of EDTA. Plasma was separated by centrifugation at $1000 \times g$ at 4 °C for 15 min. LDL isolation was performed by sequential flotation ultracentrifugation [11]. Native LDL was desalted by chromatography of molecular size exclusion in a G-25 Sephadex column (Pharmacia, Uppsala, Sweden) with 2.7 ml of 0.01 M phosphate buffered saline, pH 7.4. Protein content was determined by the red pyrogallol method (Sigma, St. Louis, MO). Total and free cholesterol, triglyceride, apoB (Roche Diagnostics), and phospholipid (Wako Chemicals GmbH) content of LDL were determined by commercial methods in a Hitachi 911 autoanalyzer, and the results were expressed as percentage of LDL mass.

2.5.3. Copper-mediated LDL oxidation

Dialyzed LDL (0.05 g protein/l) was incubated with cupric sulfate (5 μM) in PBS at a final volume of 1 ml. Absorbance at 234 nm was continuously monitored at 2 min intervals for 5 h at 30 °C [12] using a spectrophotometer (Hewlett-Packard, Palo Alto, USA). The diene versus time profile was divided into three consecutive phases: lag phase, propagation phase, and decomposition phase [12]. Intra-run imprecision was 2.3, 5.3, and 1.5% for lag phase, oxidation rate, and maximum amount of dienes, respectively. Between-run imprecision was 7.1, 7.9, and 7.0%, respectively.

2.5.4. Antioxidant content

α-Tocopherol, α-carotene, β-carotene, and lycopene content in LDL were quantified by reverse-phase HPLC (Ultrasphere ODS $4.6 \times 25 \times 5$ mm^3 column, System Gold, Beckman) with a diode-array detector (Detector168, Beckman), as described previously [13].

2.5.5. LDL size

LDL particle size was determined by polyacrilamide gradient gel electrophoresis (2–16%) according to Nichols et al. [14] with modifications.

2.5.6. Oxidized LDL

Oxidized LDL concentration was determined in serum by an enzyme-linked immunosorbent assay using two antibodies against the antigenic determinants of oxidized apolipoprotein B molecule (ox-LDL; Mercodia AB, Uppsala, Sweden). Values were expressed in U/l. The intra- and inter-assay coefficients of variation were 2.8 and 10.7%, respectively.

Antioxidant enzyme activity and oxidized LDL concentration were adjusted for plasma volume changes, as described elsewhere [15].

2.6. Other measurements

Weight and height were measured on a calibrated balance. BMI was calculated as the weight (in kilogram) divided by the squared height (in meters). Smokers were defined as those smoking one or more cigarettes per day during the previous 3 months.

2.7. Sample size

Accepting an α risk of 0.05 and a β risk of 0.20 in a two-sided test, the sample of 17 subjects allows us to recognize as statistically significant a difference greater than or equal to $0.70 \times$ standard deviation units between the initial and the final measurements.

2.8. Statistical analysis

Paired Student's t-test was used to compare means of continuous variables before and after the training period; when the variables departed from normality, the Wilcoxon rank test was used instead. To assess changes of antioxidant enzymes or lipid peroxides after the bout of acute PA with respect to the basal, paired Student's t-test was also used, taking into account, however, the Bonferroni correction for multiple comparisons. Linear general models for repeated measures were used to assess the effect of training, acute aerobic PA, and the modifier effect of training on antioxidant enzyme activity.

A P-value lower than 0.05 was considered as statistically significant. The SPSS program was used for statistical analysis.

3. Results

Seventeen participants were enrolled in the study. Mean age was 19.5 (standard deviation: 1.2). Five (29.4%) were smokers, and mean BMI was 23.6 kg/m^2 (standard deviation: 2.1). No statistically significant differences between men and women, and between smokers and non-smokers, were detected in antioxidant enzyme activity, LDL resistance to oxidation, or oxidized LDL concentration. Men had a higher VO_2 max than women, 44.5 (5.7) and 32.9 (4.2) ml/kg, respectively ($P < 0.01$). Women had higher high-density lipoprotein (HDL) cholesterol than men, 54.0 (13.1) versus 40.4 (11.4) mg/dl, respectively ($P = 0.04$).

3.1. Effects of PA training program

The observed changes in physical fitness parameters and lipid levels are presented in Table 1. Statistically significant increases in VO_2 max, anaerobic threshold, and aerobic power output were observed, along with a marginally significant increase in maximal power output. No statistically significant changes in total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol were observed.

In Table 2, changes in antioxidant enzyme activity, LDL resistance to oxidation, LDL particle characteristics (components, antioxidant content, and diameter), and oxidized LDL concentration are presented. A statistically significant increase in the activity of B-GSH-Px and P-GR was observed, ranging from 15 to 23% with respect to the basal level. No statistically significant change in E-SOD activity was observed after the training period.

A significant increase in LDL resistance to oxidation was observed, with an increase in the lag-time duration, a decrease in the maximal oxidation rate, and a marginally significant decrease in the maximal amount of produced dienes in LDL.

No changes were observed in LDL characteristics (components, antioxidant content, and diameter). However, a significant decrease in oxidized LDL concentration after training was observed.

3.2. Effects of a bout of aerobic PA before and after training on antioxidant enzyme activity

The effect of 30 min of aerobic PA practice on different antioxidant enzymes (P-GR, E-SOD, and B-GSH-Px), before and after the training program, is presented in Figs. 2–4. Before training, a late (24 h) and significant increase in E-SOD activity was observed after the bout of PA. After training, no significant changes in E-SOD activity were observed. E-SOD activity was significantly higher after than before training at 30, 60, and 120 min, and 24 h.

Before training, B-GSH-Px activity showed an abrupt increase just after finishing PA, followed by a decrease at 30 and 60 min, with a posterior steep tendency to normalization (Fig. 3). After training, the pattern presented a similar decrease at 30 and 60 min, with a posterior normalization, but without the initial increase in GSH-Px activity. A higher GSH-Px activity was observed after training at times basal, 120 min, and 24 h.

Before training, P-GR showed an abrupt and significant activity decrease 30 min after finishing PA; a slow tendency to recover the basal value was observed afterwards (Fig. 4). After the training period, a different pattern was observed with a light and non-significant decrease in P-GR activity after the acute PA bout. Consequently, higher P-GR activity at time basal and 30 min was statistically significant when comparing before and after training status.

Table 1
Physical fitness and lipids before and after the training period (mean and standard deviation)

	Before training	After training	%Change	P-value
Physical fitness				
VO_2 max (ml/kg)	37.43 (7.66)	46.19 (11.02)	23.41	<0.001
VT (%VO_2 max)	63.36 (13.39)	77.02 (8.48)	19.33	<0.001
APO (W)	131.25 (46.99)	159.37 (63.82)	21.43	0.009
MPO (W)	206.25 (55.15)	220.31 (72.01)	6.82	0.057
BMI (kg/m^2)	23.51 (2.07)	23.47 (2.51)	−0.01	0.875
Lipids				
Total cholesterol (mmol/l)	4.41 (0.73)	4.08 (0.84)	−7.45	0.125
HDL cholesterol (mmol/l)	1.25 (0.36)	1.31 (0.34)	4.69	0.390
LDL cholesterol (mmol/l)	2.54 (0.67)	2.23 (0.81)	−12.48	0.177
Triglycerides (mmol/l)[a]	1.02 (0.87–1.40)	1.15 (0.83–1.35)	−11.36	0.756

VO_2 max, maximal oxygen uptake; VT, ventilatory anaerobic threshold; APO, aerobic power output; MPO, maximum power output.
[a] Median (25–75 percentile). Wilcoxon rank test.

Table 2
Antioxidant enzyme activity, LDL resistance to oxidation, LDL main components and characteristics, and oxidized LDL concentration, before and after the training period (mean and standard deviation)

	Before training	After training	%Change	*P*-value
Antioxidant enzyme activity				
E-SOD (U/g Hb)	859.25 (155.68)	995.69 (337.78)	15.88	0.100
B-GSH-Px (U/l)	5531.69 (1160.04)	6802.12 (1313.60)	22.97	0.016
P-GR (U/l)	48.47 (9.95)	55.56 (11.97)	14.64	0.033
LDL resistance to oxidation				
Lag-phase duration (min)	111.53 (13.81)	126.80 (12.57)	13.69	0.007
Maximal oxidation rate (μmol/(min/g))	11.28 (2.29)	8.18 (2.45)	−27.51	0.002
Maximal amount (μmol/g)	825.42 (177.22)	717.67 (159.84)	−13.05	0.073
LDL mass components				
Free cholesterol (%)	9.00 (0.57)	9.13 (0.52)	1.44	0.404
Sterified cholesterol (%)	30.51 (1.95)	30.95 (1.77)	1.44	0.404
Triglycerides (%)	6.56 (1.03)	7.08 (0.83)	7.98	0.080
Phospholipids (%)	27.41 (2.19)	26.41 (1.70)	−3.67	0.245
Apoprotein B (%)	26.52 (1.02)	26.43 (0.90)	−0.33	0.785
LDL antioxidant content				
Lycopene (mol/mol apoB)	0.28 (0.18)	0.45 (0.26)	58.79	0.062
α-Carotene (mol/mol apoB)	0.07 (0.04)	0.07 (0.06)	3.90	0.874
β-Carotene (mol/mol apoB)	0.31 (0.13)	0.30 (0.16)	0.74	0.947
Tocopherol (mol/mol apoB)	7.77 (0.85)	7.78 (1.23)	0.04	0.988
LDL diameter (nm)	26.64 (0.58)	26.72 (0.58)	0.32	0.102
Oxidized LDL (U/l)	48.80 (16.21)	41.06 (10.55)	−15.9	0.043

E-SOD, erythrocytary superoxide dismutase; B-GSH-Px, blood glutathione peroxidase.

3.3. Global effects of training, acute PA, and the interaction between training and acute PA on antioxidant enzyme activity

The effects of training, acute PA, and the interaction between them on antioxidant enzyme activity were assessed by general linear models for repeated measures (MANOVA) (Table 3). Considering not only the basal activity, but all measures after the bout of PA, training increased the activity of E-SOD, B-GSH-Px, and P-GR. On the other hand, acute PA significantly reduced the activity of B-GSH-Px and P-GR.

Training did not significantly modify the global response of antioxidant enzyme activity to acute aerobic PA.

4. Discussion

This experimental study found an increase in physical fitness, B-GSH-Px activity, P-GR activity, and LDL resistance to oxidation, and a decrease in oxidized LDL concentration after 16 weeks of aerobic training. The effect of 30 min of aerobic PA on antioxidant enzyme

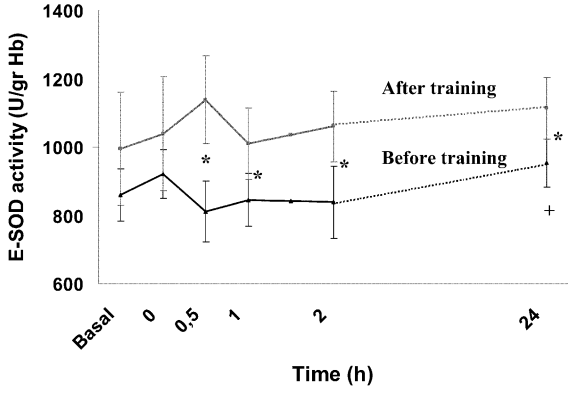

Fig. 2. Time response pattern of catalytic activity of SOD in erythrocytes to acute aerobic PA before and after training. Adjusted for plasma volume changes. $^{*}P < 0.05$ when comparing before and after training activity; $^{+}P < 0.05$ when comparing to basal value.

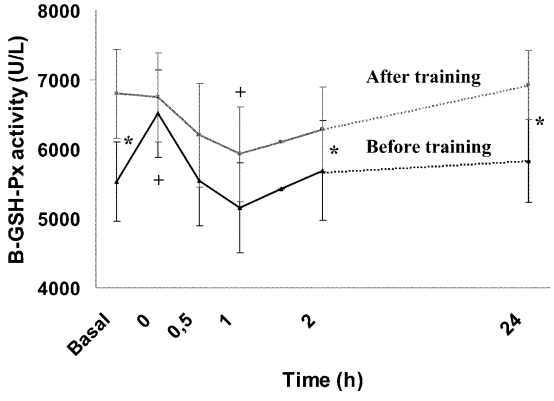

Fig. 3. Time response pattern of catalytic activity of GSH-Px in whole blood to acute aerobic PA before and after training. Adjusted for plasma volume changes. $^{*}P < 0.05$ when comparing before and after training activity; $^{+}P < 0.05$ when comparing to basal value.

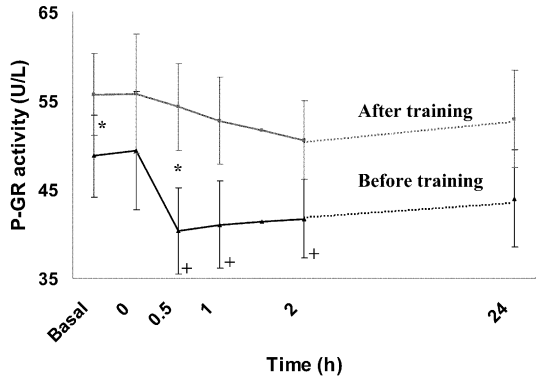

Fig. 4. Time response pattern of catalytic activity of P-GR to acute aerobic PA before and after training. Adjusted for plasma volume changes. $^{*}P < 0.05$ when comparing before and after training activity; $^{+}P < 0.05$ when comparing to basal value.

Table 3
P-value result of the general linear models assessing the effect of training, acute aerobic PA, and the interaction between them on antioxidant enzyme activity

	E-SOD	B-GSH-Px	P-GR
Training	0.002	0.007	0.022
Acute PA	0.102	0.001	0.003
Interaction between training and acute PA	0.093	0.085	0.542

E-SOD, erythrocytary superoxide dismutase; B-GSH-Px, blood glutathione peroxidase.

activity, and the modifier effect of training status, was also assessed.

4.1. Effects of aerobic training on antioxidant enzyme activity and LDL resistance to oxidation

Although aerobic training increases antioxidant activity in animal models [16,17], the effects of aerobic training on antioxidant activity in human are controversial. Higher levels of antioxidant enzyme activity have been observed in trained subjects than in sedentary ones [18,19]. Some experimental studies report an increase of the antioxidant enzyme activity after training [20,21], while others have documented no changes [22] or even a decrease [23] in circulating antioxidants. In this study, after 16 weeks of controlled aerobic training, the increase in B-GSH and P-GR was statistically significant, and although that of E-SOD did not reach statistical significance ($P = 0.100$), the difference was substantial. This activity increase may result from endogenous antioxidant production related to the repeated increases of free radicals acting as inductors of gene transcription after each PA session [24].

On the other hand, cross-sectional studies have shown a higher LDL resistance to oxidation in aerobically trained subjects than in sedentary ones [13,25]. In this study, a significant increase in LDL resistance to oxidation was produced by aerobic training, and lag time increased, and oxidation rate decreased. The same effect was observed in an experimental study after a 3-week diet and PA program [26], although the differential effect of diet and PA could not be assessed.

The mechanism involved in this phenomenon is not clear. In this study, no changes in LDL diameter, measured LDL fatty components, and LDL antioxidant content were observed. Similar results were reported by Sanchez-Quesada et al. [13] in a cross-sectional study. The increase in LDL resistance to oxidation after training might be related to increased endogenous antioxidant enzyme activity. These antioxidants could preserve other antioxidants (polyphenols, flavonoids, etc.) linked to LDL [27] and protect LDL polyunsaturated fatty acids from oxidation [28]. Unmeasured changes in non-esterified fatty acids [29] and other qualitative properties of the LDL particle (e.g. glycation) [30] might also account for the observed effect.

The final result of this increase in endogenous antioxidant activity and in LDL resistance to oxidation is a lower concentration of oxidized LDL. Lower-oxidized LDL in trained young girls [31] and in middle-aged men and women after a 10-month PA program [32] has been previously reported.

4.2. Effects of aerobic PA before and after training on antioxidant activity

The effect of acute aerobic PA on antioxidant enzyme activity was assessed at different time intervals, before and after training, to determine the time response pattern.

Concerning antioxidant enzymes, SOD is the first defense against superoxide radicals. SOD dismutases superoxide radicals (O_2^-) to form hydrogen peroxide (H_2O_2) and O_2. The glutathione system is another important antioxidant defense. GSH-Px catalyzes the decomposition of H_2O_2, producing glutathione disulfide (GSSG). Intracellular GSSG may be reduced to GSH by GR.

Gohil et al. [33] examined in eight moderately trained male volunteers the effect of PA at 65% of peak oxygen uptake during 90 min on the glutathione system. They observed a 100% increase in blood GSSG, and decrease in GSH, after 15 min of PA, that was stable during the rest of PA. Sixty minutes after PA, the values were similar to the basal ones. Similar results on GSH have been recently reported [34]. In our study, we did not measure GSSG or GSH levels, although we measured the activity of the enzymes involved in its metabolism. The observed increase in B-GSH-Px activity shortly after the bout of PA has also been reported in two other studies, where the GSH-Px activity rose by approxi-

mately 14% [35] and 18% [34]. In our study, the increase was approximately 13%. After this initial increase, a maximum B-GSH-Px activity decrease was observed 60 min after PA. On the other hand, an abrupt decrease in P-GR activity was observed 30 min after PA, with a posterior slow recovery during 24 h.

These results are compatible with an induction of enzyme activity by PA free-radical production, followed by enzyme inactivation as a consequence of enzyme consumption in the free-radical scavenger activity, with a posterior recovery that begins 30–60 min later when the basal GSH levels are achieved [33]. The differential sustained decrease in GSH-Px activity at 60 min may be explained by the fact that this enzyme not only detoxifies H_2O_2, but also converts lipid hydroperoxide to non-toxic alcohols, thus acting as a chain-breaking antioxidant of lipid peroxidation [36]. This antioxidant activity decrease coincides with an increase in oxidized LDL concentration, as previously reported [37].

Training did not modify the global response pattern of antioxidant enzyme activity to acute aerobic PA. Nevertheless, the activity was higher after training in most instances, suggesting a better antioxidant defense system in trained subjects. Importantly, the PA intensity for each subject corresponded to the aerobic threshold before and after training (similar metabolic stress), which corresponds to a mean power of 131 W before and 159 W after training (different performance).

4.3. Limitations of the study

One limitation of the study is that diet during the training period was not controlled. However, study did require that a participant follow the same diet in the 3 days preceding each blood sampling, and no significant changes in measured LDL components were observed.

Smokers were included in the study and smoking could be considered a confounder variable. However, after adjusting for smoking, no significant differences were observed.

The lack of a control group is another limitation of this study. The best design would have been a randomized clinical trial.

5. Conclusions

Regular PA increases endogenous antioxidant activity and LDL resistance to oxidation, and decreases oxidized LDL concentration. On the other hand, a decrease in P-GR and B-GSH-Px activities was observed in the first 30–60 min after 30 min of aerobic PA, with a posterior recovery. The global time response pattern to acute aerobic PA seems to be quite similar before and after training, although the enzymatic activities were higher after training than before.

Acknowledgements

This study was partially supported by Bayer Grant 1999 (Spanish Society of Cardiology) and by CIRIT 2001SGR 00408. Fitó M. was supported by Fondo de Investigación Sanitaria (FIS) grant (98/9562 FPI). We thank the Servei d'Activitat Física of the Universitat Autònoma of Barcelona and the study participants for their cooperation, and Stephanie Lonsdale and Elaine Lilly for the revision of the English manuscript.

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