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EFFECTS OF OXIDATIVE STRESS CAUSED BY ACUTE AND REGULAR EXERCISE ON LEVELS OF SOME SERUM METABOLITES AND THE ACTIVITIES OF PARAOXONASE AND ARYLESTERASE*

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Abstract. Regular exercise practice has a protective role on coronary heart disease and empowers antioxidant defense system, whereas acute exercise induces oxidative stress. The aims of this study were to investigate the effects of oxidative stress on the levels of serum paraoxonase (PON1) and arylesterase activities, HDL-cholesterol (HDL), malondialdehyde (MDA) and various lipid parameters in regular exercised individuals and to compare them with those of acute exercised and sedentary persons. The study was carried out on three groups called regular exercise group (REG), acute exercise group (AEG) and sedentary group (SG) that consisted of respectively 23 healthy individuals having 6.0±2.50 year sport age and regular physical and conditional sport activities 3 hours a day for last 6 months, 24 healthy subjects performing acute exercise 3 days a week for 3 months and 26 healthy men with no sport activity. The levels of PON1 and arylesterase activities and MDA in REG were 221.96±35.66 $U \cdot L^{-1}$, 103.85±28.93 $U \cdot ml^{-1}$ and 1.836±0.31 $nmol \cdot ml^{-1}$, respectively. The levels of serum creatine kinase (CK) (125.29±81.86 $U \cdot ml^{-1}$), MDA (1.215±0.32 $nmol \cdot ml^{-1}$) and PON1 activity (184.68±33.37 $U \cdot L^{-1}$) displayed statistically significant differences in AEG compared with REG ($p<0.001$). Serum arylesterase activity levels exhibit no significant difference in three groups ($p>0.05$). HDL levels in AEG and REG significantly increased ($p<0.001$) as compared to SG. Our results demonstrated that regular exercise caused an increase in PON1 activity, which shows that oxidative stress has a significant influence on this enzyme activity.

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Key words: Acute-regular exercise – Paraoxonase – Arylesterase – Malondialdehyde

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Introduction

It is well known that reactive free radicals, predominantly super oxide, hydroxyl ion and nitric oxide, are involved in initiation and development of many pathological events in human [20]. Regular exercise plays an important role on serum lipid profiles, blood pressure, insulin sensitivity and cardiovascular diseases, prevents coronary heart disease and enhances antioxidant systems, whereas acute exercise induces oxidative stress [3,21]. The effect of regular exercise on lipid profile leads to a decrease in triglyceride, LDL and VLDL-cholesterol levels but an increase in HDL-cholesterol levels [21]. Serum LDL levels in regular exercised individuals are lower than the values of sedentary individuals [18]. However, the progresses of oxidative stress that cause tissue damage because of increased oxygen consumption during acute exercise are thought to be the main disadvantages of the exercise [10,23]. Although exercise increases the sensitivity of LDL to oxidation [2], this harmful change has not been investigated in detail. There are contradictory data about the effect of oxidative stress on plasma lipoperoxidation levels after acute exercise even though it is proven that plasma concentrations of some antioxidant molecules such as bilirubin, uric acid and ascorbic acid increase after this exercise [1,2,4]. It is not well-understood whether acute exercise influences the qualitative characteristics of plasma lipoproteins via efficient oxidative stress or non-oxidative processes.

HDL contains PON1 that prevents the accumulation of lipid peroxidation products on LDL and protects it from oxidation. PON1 also hydrolyzes lipid peroxides in human atherosclerotic lesions and thus is thought as anti-atherogenic enzyme *in vivo* [17].

To our knowledge, the role of regular exercise on PON1 activity has also not been well-established yet. The aims of this study were to investigate the effects of oxidative stress on the levels of serum PON1 and arylesterase activities, HDL, MDA and various lipid parameters in regular exercised individuals and to compare them with those of acute exercised and sedentary persons.

Materials and Methods

The study included three groups, regular exercise group (REG), acute exercise group (AEG) and control or sedentary group (SG). REG consisted of 23 healthy men having professional sport activities for 6.0±2.50 years and doing physical and tactics condition studies for 3 hours per day and running on an average of 7.0-10.4 km in a week for last 6 months. AEG included 24 healthy men having aerobic-step

and jogging-race exercises for 1 hour in 3 days a week for last 3 months and carrying out slowly intensified exercise that gave rise to 130-140/minute pulsation within 3 months period. SG consisted of 26 healthy male subjects with no sport activity.

The subjects had no special diets but had a fasting period for 12 hours and no vitamin supplement intake at least 1 week before blood sampling. The blood samples were drawn into simple tubes prior to exercise in the morning, stored at 4°C and analyzed within 1 week, with regard to the parameters shown in Table 1 using an auto analyzer (Olympus AU 600, Japan).

Table 1
The comparison of physical and biochemical data of research groups

	Regular exercise persons (n=23)	Acute exercise persons (n=24)	Sedentary persons (n=26)	Significance
Age (year)	25.86±3.27	26.21±4.98	27.12±2.5	ns
Height (cm)	178 ± 6.02^{b}	171 ± 6.47^{ab}	173 ± 5.33^{a}	*
Weight (kg)	72.06 ± 11.43^{b}	75.78 ± 7.48^{b}	78.19 ± 4.60^{a}	**
BMI (kg/m^2)	22.66 ± 2.32^{b}	26.62 ± 2.55^{a}	26.71 ± 2.54^{a}	***
Paraoxonase ($U\cdot L^{-1}$)	221.96 ± 35.66^{a}	184.68 ± 33.37^{b}	136.27 ± 24.42^{c}	***
Arylesterase ($U\cdot ml^{-1}$)	103.85±28.93	104.83±31.40	111.01±19.31	ns
Total cholesterol ($mg\cdot dl^{-1}$)	168.09 ± 31.72^{b}	190.21 ± 27.41^{a}	200.27 ± 16.60^{a}	***
Triglyceride ($mg\cdot dl^{-1}$)	66.78 ± 15.94^{b}	131.14 ± 27.90^{a}	144.6 ± 41.79^{a}	***
HDL-C ($mg\cdot dl^{-1}$)	49.74 ± 4.90^{a}	43.21 ± 7.12^{b}	41.08 ± 5.04^{b}	***
LDL-C ($mg\cdot dl^{-1}$)	105.13 ± 26.28^{b}	$121.50\pm18.77^{a,b}$	126.92 ± 29.25^{a}	*
VLDL-C ($mg\cdot dl^{-1}$)	23.26 ± 6.10^{b}	31.5 ± 10.95^{a}	32.27 ± 8.28^{a}	***
MDA ($nmol\cdot ml^{-1}$)	1.836 ± 0.31^{a}	1.215 ± 0.32^{b}	0.497 ± 0.13^{c}	***
Creatine kinase ($U\cdot ml^{-1}$)	433.65 ± 245.13^{a}	125.29 ± 81.86^{b}	93.38 ± 29.77^{b}	***

ns - no significance; *$p<0.05$, **$p<0.01$, ***$p<0.001$
a,b,c - differences between mean of the groups in same line that contains different letters are significant

PON1 activities were determined spectrophotometrically at 412 nm with 4-nitrophenol formed after an addition of 100 µl serum sample into the paraoxon solution as substrate (2 mM paraoxon, 2 mM $CaCl_2$, 100 mM Tris-buffer [pH: 8]) [8]. The enzyme quantity that disintegrates 1 µmol paraoxon substrate in 1 minute was taken one Unit PON 1. For measuring the activity of arylesterase, phenylacetate was used as substrate and the formed phenol measured spectrophotometrically at 217 nm after the addition of 50-fold diluted serum sample in arylesterase activity measurements [7]. MDA level was determined with thiobarbituric acid (TBA) with a method modified from Satoh and Yagi [19] using Schimadzu UV-1201 spectrophotometer at 532 nm.

The data were presented as the mean ±SD and evaluated with one-way ANOVA, t-test and linear regression analysis using SPSS statistic package program.

Results

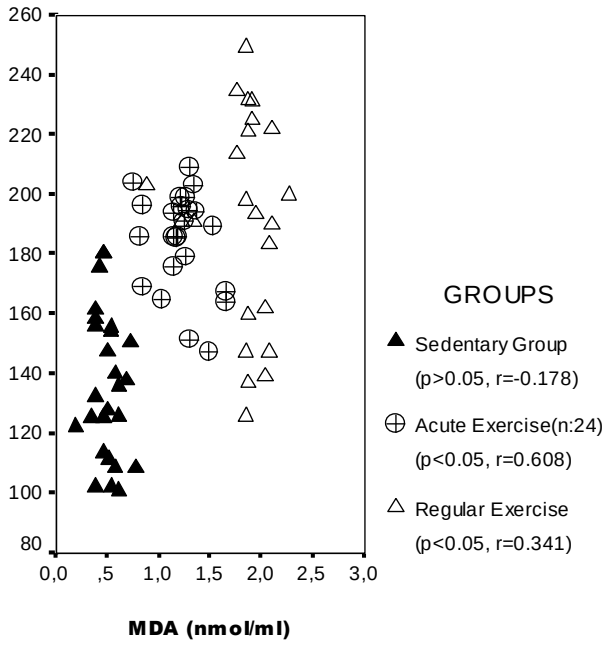

Fig. 1
The relationship between PON activity and MDA levels in subjects of acute exercise, regular exercise and sedentary groups

The clinical characteristics of the subjects presented in Table 1 show that the relationship between groups with regard to age variation is not significant ($p>0.05$) whereas that between groups regarding to height ($p<0.05$), weight ($p<0.01$) and body mass index (BMI) ($p<0.001$) is significant. While serum PON1 activity ($p<0.001$), CK ($p<0.001$), MDA levels ($p<0.001$) increased, LDL ($p<0.05$), TG ($p<0.001$) and total cholesterol ($p<0.001$) levels decreased significantly in REG as compared with two other groups (Table 1).

There was a significant positive correlation between PON1 and MDA levels in AEG ($p<0.05$; $r=0.608$) and REG ($p<0.05$; $r=0.341$) groups (Fig. 1). When the data of MDA and arylesterase activities were compared, it was detected a significant ($p<0.001$) change in MDA but a non-significant change ($p>0.05$) in arylesterase activity between groups (Fig. 2).

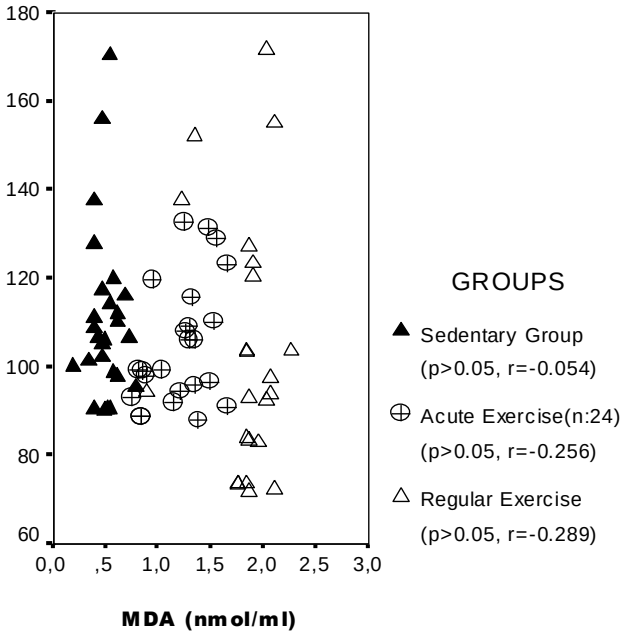

Fig. 2
The relationship between arylesterase activity and MDA levels in subjects of acute exercise, regular exercise and sedentary groups

Discussion

Regular exercise is a protective factor against coronary heart disease and diabetes mellitus, enhances antioxidant systems [5,11,12,16] and causes significant changes in LDL and HDL compositions [2,4]. However, the effective biochemical mechanisms that prevent tissue damage from free radicals produced as a result of a marked increase in MDA in regular exercise have not been well-understood yet.

Cells continuously produce free radicals and reactive oxygen species as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes and numerous non-enzymatic antioxidants [15]. The present study examined how the qualitative change of HDL levels in regular exercise could affect PON 1 activity and how PON 1 could prevent the harmful effects of MDA level. Serum PON 1 activity level was found to increase significantly in REG subjects ($p<0.001$, Table 1) which is contrary to the results of Benitez *et al.* [2] suggesting no change in HDL-associated PON 1 activity after acute exercise.

It was seen that in REG, serum MDA, HDL and CK levels increased but, triglyceride, total cholesterol, LDL, VLDL levels decreased significantly ($p<0.001$). The decrease in serum triglyceride, VLDL and LDL levels and increase in HDL level after regular exercise are consistent with previous reports [18]. However, no significant difference in groups regarding the serum arylesterase activity level was detected ($p>0.05$).

Increased O_2 consumption in skeletal muscle during exercise causes oxidative stress [4]. We demonstrated that MDA level increased 3-4 folds in AEG, which conform to the results of some other studies [2,6,18,22,24]. We also demonstrated that an increase in MDA levels was parallel to that in PON1 activity in both REG and AEG individuals ($p<0.05$) (Fig. 1). However, increased PON 1 activity determined in AEG subjects of this study was not been recorded in another study [2] investigating the same parameter. The disagreement between these two studies may be related to the difference between the duration or intensity of exercise performed. The increase in serum antioxidant capacity in REG is also confirmed by other studies [5,12,23]. There is a common opinion that serum antioxidant capacity increases after exercise as a result of the increase in plasma uric acid concentration [23]. It was reported that acute exercise induce glutathione to be released from muscle and liver to plasma as a response to oxidative stress [9]. This study found the presence of a negative correlation between arylesterase activities and MDA level in acute and regular exercises (Fig. 2; $p>0.05$), which could be interpreted as the decrease in arylesterase protein activity as a result of increase in MDA levels

(Fig. 2). For this reason, the results of previous studies and the present one could not support the observations in the study of Benitez *et al.* [2] as they had not seen any changes in lipoperoxides or lipophilic antioxidants in lipoproteins.

Some studies indicated that oxidative stress may not be the main factor of the increased oxidizability and electronegativity of LDL. The changes in LDL composition together with the loss of the protective capacity of HDL after exercise may be the factor contributing to the oxidizability characteristic of LDL. HDL is the major carrier of lipid peroxides in human plasma [4] and plays an important protective role in the oxidation of LDL due to the antioxidant activity of PON 1 [14]. In addition, an increase in HDL levels in exercising individuals can enhance the resistance of LDL to oxidation in these individuals [10].

Regarding to the lipoprotein composition, it was reported that low free cholesterol and/or high protein content (small dimension or high density LDL) enhance the oxidation of LDL [23]. The most attractive compositional change observed in REG was the increase in PON1 activities. High serum PON1 activity level decreases cardiovascular disease risk [13] and also prevents the elimination of beneficial lipoprotein profile. The effects of PON1 on lipoprotein profile must be investigated attentively especially in individuals with cardiovascular pathology and in obese individuals that daily exercise is proposed.

In conclusion, the present study showed that marked increase in PON1 activity after regular exercise was associated with that in HDL levels. The cause of this increase in PON1 activity could be interpreted as a response of the organism to oxidative stress generated during exercise.

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