COMPARISON OF THE EFFECTS OF DIFFERENT TRAINING METHODS ON ARYLESTERASE ACTIVITY AND PARAOXONASE ACTIVITY LEVELS IN HOT ENVIRONMENT

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Abstract

The aim of this study was compare of different training method in hot environment to find which one of these methods more efficient on glutathione and malondialdehyde levels. Thirty voluntary male students whose average is participated in this study. None of the subjects had performed regular exercise. Study group were divided into two groups as continuous runnings (n=15) and interval runnings (n=15). The training program was carried out on training groups three times a week during 8 weeks. Blood samples collected at before and after the trainings during 8 weeks, were analysed for the determination of Arylesterase Activity (ARE) and Paraoxonase Activity (PON).

According to results of study, PON levels decreased in interval run group before and after the training, but this decrease was not significant as a statistically, in ARE in terms of ($P<0.01$) there was a significant decrease, in terms of ($P<0.05$) between before training and after training level of continuous runnig group there was a significant difference in PON, although ARE level have no difference as statistically. According to different training methods in heating area pre and last test results are compared in terms of antioxidant level and we cannot find any statistical difference.

As a result; it can be said type and duration of training characteristics of subjects an climatical conditions effects the glutathione and malondialdehyde levels of the subjects.

Key Words: Hot environment, Endurance training, Arylesterase Activity, Paraoxonase Activity

INTRODUCTION

Regular exercising is a protective factor against coronary heart diseases and diabetes (Ma et. al. 2003; Powers et. al. 2002). It improves antioxidant system, leads to significant changes in LDL and HDL composition (Benitez et. al. 2002) [3]. Paraoxonase (PON) enzyme also acts as an antioxidant due to its protective effect on LDL oxidatioın and neutrolizing effect on other radicals including hydrogen peroxide (Çelik et. al. 2005).

Blood accumulation in cutaneıus and subcutaneous areas during exercises done in the hot does not cause a problem in mild or moderate exercises. However blood distribution and fluid loss through sweating lead to severe heat injury risk in long-standing exercises (Ünal 2002).

Magnitude of the oxidative injury that may occur during physical exercises is determined by not only free radical production but also defense capacity of antioxidants (Selçuk 2003).

The effects of climate change are evident in every aspect of our lives. Increased temperatures associated with climate change may exacerbate the negative effects of outdoor sporting activities. The pre-season preparation period of many sports generally coincides with the summer months. Depending on the climatic conditions of this season, training carried in hot weather increases the importance of the issue. These training programs specifically include exercises that improve endurance. These endurance exercises use a range of different training models, which often use interval and continuous running methods. Also, the effects of the interval and continuous running methods on the antioxidants and oxidative stress, two of the physiological changes occurring in our body, in hot weather are subjects of debate (Tas 2011).

In the light of these data, the aim of this study is to compare the effect of continuous conditions and interval conditions model among the exercising methods for endurance done in hot environments, that should be developed during general preparation period on arylesterase (ARE) and paraoxonase (PON) activities and to evaluate the obtained data in the light of literature.

Materials and Methods

Subject Selection:

30 male students from Ataturk University Physical Education and Sports School participated in this study. The subjects were categorized into 2 groups, termed continuous running (CRG, n:15) and interval running (IRG, n:15). The subjects trained for three days per week for 8 weeks in a hyperthermic environment in which the average temperature was 29–34^{o}C. The training was carried out on the athletic field of Ataturk University Physical Education and Sports School.

The body weights of the subjects were measured (barefooted, wearing t-shirt and tights using a bascule with 0.01 kg sensitivity. Weather temperature and humidity were taken from official data from the Erzurum Provincial Directorate of State Meteorology.

Exercise Program:

The target number of heart beats of the subjects in the continuous running group was determined by means of the Karvonen method (Özer 2006) and the subjects followed 25 to 60-minute duration running exercises with 50-70% intensity on three days per week for 8 weeks. The maximal running times over distances of 250 m, 400 m, 650 m and 900 m were determined for each subject, and a

common interval training program was applied at 250, 400, 650 and 900 m; subjects were required to run using pyramidal loading method at an intensity of 60-80% (250, 400, 650, 900, 650, 400 and 250 m). The interval-training group also exercised for 3 days per week for 8 weeks. The exercise was applied until the heart rate reduced to 120-130 between the loadings. In order to make the subjects adapt to the training, the interval training program was applied as 1 set for the first two weeks, 2 sets from the third week to the seventh week and 3 sets in the last two weeks. Both groups completed warm up exercises for 5-10 minutes before starting the training and 5-10 minutes of cooling down exercises after the training.

Blood analysis:

Blood samples were taken both 2 days before and 2 days after the two different training programs, each of which lasted 8 weeks, for comparison. The samples were taken into normal biochemistry and ETDA tubes. The samples taken into the ETDA tubes were inverted 3-5 times. After the samples in the biochemistry tubes were left at room temperature for 20 minutes, they were stored at -80^{o}C prior to analysis. For the analysis, the samples were centrifuged at 3500 rpm for 5 minutes to precipitate the shaped particles Gülçin et. al. 2009; Şentürk et. al. 2008). For paraoxonase activity, absorbances were read at 37 °C, 412 nm for 5 min at microplate reader. After reading had been finished, absorbance change emerging from hydolysis product, p-nitrophenol (ΔA/min) was recorded.

Paraoxonase activity was calculated using the following Formula (Karakoç 2008; Gülcü 2003).

U/mL (nmol/min/mL)= (ΔA/min x 10^{9} /ε) x SF

Measurement of arylesterase activity was used by diluting serum samples so as to be total 400 fold in reaction environment. 1410 µL buffer + 40 µL phenylacetate solution were added into a clean quartz cuvette. The cuvette was turned upside down, absorbance of the device was reset at 270 nm by placing into spectrophotometer (device was blinded). 1265 µL buffer + 145 µL sample (diluted in the ratio of 1/40) + 40 µL phenylacetate solution was added, cuvette was turned upside down. Minut phenol formation absorbance was measured by recording absorbance increase at 270 nm. 1 unit was stated as the amount of enzyme converting 1 µmol phenylacetate to product per minute. ARE activity was calculated using molar absorbtivity coefficient 1310 (ε) (Karakoç 2008; Gülcü 2003).

Statistical Analysis:

The data analysis was carried out using the SPSS (version 15.0) statistical analysis program. The arithmetic averages and the standard deviations of the data were calculated and given as descriptive statistics. The Mann-Whitney U test, which is a nonparametric test, was used to examine the differences between independent groups, and the Wilcoxon test, which is a nonparametric test, was used to examine the differences between the dependent groups. Values of $p<0.01$ and $p<0.05$ were taken as the significance level

Results

In this study carried out with the aim of comparing the effects of different endurance exercising methods (interval and continuous running) on arylesterase activity and paraoxonase activity levels under mean temperature of 29,40±1,49°C or mean humidity of %50,71±8,46, mean ages of the subjects were found as 22,73±3,51 years and 24,27±2,71 years in continuous runnings and interval runnings, respectively. Pre-test and post-test maxVO$_2$ values of the subjects were 31,73±3,10–44,41±4,68 ml/kg/min in continuous running group and 31,95±3,28– 44,99±6,08 ml/kg/min in interval running group and when pre and post-training maxVO$_2$ values were compared, while a statistically significant difference was detected in continuous and interval running group ($p<0.01$), a significant difference was not observed in comparison of pre and post-tests between groups.

Table 1: Comparison of PON and ARE pre-post test in interval running group

Variables	IRG				
	PRE-TEST		**POST-TEST**		**Z**
	X	**SS**	**X**	**SS**	
ARE (U/mL)	73,25	19,36	60,01	20,86	**-3,107***
PON (U/mL)	232,66	86,05	217,16	74,74	-1,392

ARE:Arylesterase activity, PON:Paraoxonase activity, IRG:Interval running group *(P<0.01)

In interval running training, while a reduction is seen in PON values before and after the training although statistically insignificant, a significant reduction is seen in ARE compared to PON ($p<0.01$) (Table 1).

Table 2: Comparison of PON and ARE pre-post test in continuous running group

Variables	**CRG**				
	PRE-TEST		**POST-TEST**		**Z**
	X	**SS**	**X**	**SS**	
ARE (U/mL)	80,37	21,66	72,58	25,56	-1,193
PON (U/mL)	210,47	77,47	183,83	69,60	**-2,442****

ARE:Arylesterase activity, PON:Paraoxonase activity, CRG:Continuous running group **P<0.05)

In continuous running training performed in a hot environment, while a significant difference was found between pre and post-training values of PON ($p<0.05$), a statistically significant difference was not found in ARE (Table 2).

Table 3. Comparison of pre-post test antioxidant values of interval and continuous running trainings

Variables	IRG (PRE-TEST)		CRG (PRE-TEST)		Z	IRG (POST-TEST)		CRG (POST-TEST)		Z
	X	SS	X	SS		X	SS	X	SS	
ARE (U/mL)	73,25	19,36	80,37	21,66	-,602	60,01	20,86	72,58	25,56	-1,246
PON (U/mL)	232,66	86,05	210,47	77,47	-,456	217,16	74,74	183,83	69,60	-1,141

ARE:Arylesterase activity, PON:Paraoxonase activity, IRG:Interval running group, CRG:Continuous running group

ARE and PON values were compared according to pre-post test results of different training methods in a hot environment and a statistically significant difference was not seen between groups (Table 3).

DISCUSSION AND CONCLUSION

Proteins on HDL show enzymatic activity (usually hydrolytic). In the controlled study of Durrington et al. conducted with the aim of realizing the hypothesis that PON is responsible for degrading lipid peroxides before accumulation on LDL occurs, they observed that purified PON was highly effective for preventing lipid peroxidation of LDL (Durrington et. al. 2002).

Arslan et al. detected an elevation in PON activity after regular exercise they applied, in conclusion, high serum PON activity levels were shown to reduce cardiovascular disease risk and prevented elimination of beneficial lipoprotein profile, in addition that study indicated that elevated PON activity following regular exercising was associated with HDL levels. They reported that the reason for this elevation in PON activity was evaluated as the response of the organism against oxidative stress developing during exercise (Arslan et. al. 2005).

Tomas et al. reported that PON activity reduced before aerobic exercise applied to 17 healthy young volunteers for 16 weeks and a statistically significant elevation occured after the exercise (Tomas et. al. 2002).

Global antioxidant activity developed with exercise studies may indirectly reduce PON inhibition caused by acute exercise effect oxidative stress and as the result, basal levels of PON

activity may be obtained in trained conditions. Besides these effects, exercise study may directly affect PON protein or PON transporting lipoprotein. PON enzyme is mainly related with HDL. However, there is not a correlation between HDL levels and PON activity in exercising group or control group at any time point. Thus, they reported that HDL changes is not likely to explain changes in PON activity (Tomas et. al. 2002).

In another study, well trained rugby (American football) players with high PON activities and sedentary individuals with low PON levels were compared. However they reported that making a comparison got difficult as the values were quite different (Brites et. al. 2000).

Brites et al. compared PON values of 18 sportsmen and 18 sedentary individuals following a 2-week exercising program 24 hours at each. When an assessment was done without taking polymorphism into consideration, they found no significant differences between exercising and sedentary individuals (Brites et. al. 2006).

Following a 4-week exercising at a constant temperature of 23 ± 2°C, Demirayak reported a reduction in PON activity of both exercise and control group although insignificant (Demirayak 2007).

In the study conducted in hot environment, a reduction was shown in PON values of both interval and continuous running groups however the reduction in continuous running group was found to be statistically significant , no significant difference was seen when groups were compared. As the result of this reduction in continuous running group, it may be stated that lipid peroxidation and LDL increased and led to cell injury and this arised from reduction in PON activity caused by elevation in MDA values of continuous running group.

ARE is accepted as the indicator of the main protein that is not affected from changes in PON (Çelik et. al. 2005).

In a study of Goldhammer et al. , serum ARE activities of 37 patients with ischemic heart diseases were measured following a 12-week exercise program and ARE level was seen to increase 16,7% compared to pre-exercise program (Goldhammer et. al. 2007).

Arslan et al. reported no difference in ARE activity following regular and acute exercise (Arslan et. al. 2005).

Brites et al. compared ARE activities of 18 sportsmen and 18 sedentary individuals following a 2-week exercise program 24 hours at each. They reported no changes in ARE values between exercising or sedentary individuals (Brites et. al. 2006).

Demirayak reported a reduction in tissue activities of the group that exercised for 4 weeks at 23 ± 2°C constant temperature although insignificant, ARE activities of exercising group reduced

significantly in liver, kidney and heart tissues, in conclusion enzyme expression could have reduced by rat metabolisms' adapting to exercise following one month exercise program and that could lead to a reduction in ARE enzyme activity (Demirayak 2007).

In some studies, while ARE activites are seen not to change following regular exercise (Arslan et. al. 2005; Brites et. al. 2006), they are seen to elevate in some others (Goldhammer et. al. 2007).

In this study conducted at a temperature of 29,40±1,49°C, and humidity of 50,71±8,46, ARE values of both interval and continuous running groups were seen to reduce however the reduction in interval runing group was seen to be statistically significant and no significant differences were found when groups were compared. This reduction in interval running group may be stated to arise from thermal stress's reducing enzyme activities (Demirayak 2007).

In conclusion, it was considered that type, duration and intensity of training programs, type of the subjects, durations of pre and post-program measurements, methods' to be different and conducting the research in different environments are effective on antioxidant levels of the subjects.

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