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Full Length Research Paper

Comparison of two different endurance training methods on glutathione s-transferase level

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This study compared the effects of different training methods (continuous and interval) carried out in a hot environment on glutathione s-transferase level (GST). The study group comprised 30 male volunteers who do not exercise regularly. The participants were categorized into 2 groups, continuous running (CRG, n: 15) and interval running (IRG, n: 15). The participants followed a training program on 3 days per week for 8 weeks. Comparison of the pre- and post-training samples showed a statistically significant increase in GST value (P < 0.01) within the interval running group (IRG) and a significant decrease in GST value (P < 0.01) within the continuous running group (CRG). GTS values were compared on the basis of the pre-test and post-test results of various training methods carried out in a hot environment. In this scope, statistically significant differences were observed between the groups in the pre-tests (p < 0.05) and the post-tests (p<0.01). As a result, it can be said that the CRG in a hot environment had a greater effect on oxidative stress by increasing lipid peroxidation, and the IRG prevented the formation of free radicals by producing a positive increase in the glutathione s-transferase level.

Key words: Hot environment, endurance training, glutathione s-transferase, oxidative stress.

INTRODUCTION

Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS) (Gülçin et al., 2003a, 2004a and b; Oktay et al., 2003). They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. ROS include free radicals such as superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals (OH•) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Gülçin, 2006a, and b, 2007). ROS are continuously produced during normal physiological events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides (Gülçin; 2010; Gülçin and Dastan, 2007; Balaydın et al., 2010; Şerbetçi and Gülçin, 2010; Gülçin et al., 2010a; 2010b). ROS are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions. ROS have been implicated in more than 100 diseases (Halliwell and Gutteridge, 1990; Gülçin et al., 2004c, 2005a and b, 2006a and b).

All aerobic organisms have antioxidant defences, including antioxidant enzymes and antioxidant food constituents, to remove or repair the damaged molecules. Antioxidant compounds can scavenge free radicals and oxidative damage (Ak and Gülçin, 2008; Gülçin et al., 2007a, 2009a, 2010c; Talaz et al., 2009).

The level of oxidative damage which can occur during physical training is not only determined by the formation of free radicals, but also by the defence capacity of antioxidants. While it is expressed that especially acute, intensive training may cause oxidative stress, it is suggested that regular endurance training can reduce oxidative stress and muscle damage after the exercise,

Abbreviations: ROS, Reactive oxygen species; **ETDA,** ethylenediaminetetraacetic acid; **ELISA,** enzyme-linked immunosorbent assay; **MDA,** malondialdehyde; **CRG,** continuous running group; **IRG,** interval running group; **GST,** glutathione s-transferase.

and develop antioxidant defence capacity (Finaud et al., 2006; Galassetti et al., 2006; Elosua et al., 2003).

Physical exercises require new arrangements in the organism. However, physiological adaptations becomes more important if the organism, which is in a more intensive arrangement compared with the resting position, is exposed to stress resulting from a different sources. Thus, the adaptations of organisms to altitude, underwater, in hot and cold environments, the physiological stresses and the precautions are discussed (Goto et al., 2003; Durstine et al. 2001; Stein and Colditz 2004).

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 out 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.

In the light of the earlier information, the purpose of the present study is to compare the effects of continuous running and interval running models, two training methods aimed at improving endurance, which are often carried in a hot environment during pre-season preparation, on glutathione s-transferase level and to assess the results within the framework of the literature.

MATERIALS AND METHODS

Subject selection

Thirty male students from Physical Education and Sports School, Atatürk University, 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 to 34°C. The training was carried out on the athletic field of Physical Education and Sports School, Atatürk University.

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 min duration running exercises with 50 to 70% intensity on three days per week for 8 weeks. The maximal running times over distances of 250, 400, 650 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 to 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 to 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 to 10 min before starting the training and 5 to 10 min of cooling down exercises after the training.

Blood sample

Blood uptake and haemolyzate preparation were performed as in previous studies (Beydemir et al., 2003; Gülçin et al., 2004d; Beydemir and Gülçin, 2004; ArasHisar et al., 2004; Hisar et al., 2005a and b). Blood samples were taken 2 days before and 2 days after the two different training programs, each of which lasted for 8 weeks, for comparison. The samples were taken into normal biochemistry and ethylenediaminetetraacetic acid (ETDA) tubes. The samples taken into the ETDA tubes were inverted 3 to 5 times. After the samples in the biochemistry tubes were left at room temperature for 20 min, they were stored at -80°C prior to analysis. For the analysis, the samples were centrifuged at 3500 rpm for 5 min to precipitate the shaped particles (Gülçin et al., 2005b, 2008a, 2009; Çoban et al., 2008, 2009; Beydemir et al., 2005; Şentürk et al., 2008; 2009; Öztürk Sarıkaya et al. 2010).

Glutathione s-transferase level (GST) activity assay

GST assay reactive was adjusted at 36.20 ml 0.1 M K-phosphate buffer and pH was taken as 6.5 on the day of measurements. At that time, the microplate which was sample pipetted was covered up and kept at 25°C, the assay reactive was mixed slightly after being incubated at 37°C for 3 min and its kinetic reading was carried out in an enzyme-linked immunosorbent assay (ELISA) Reader at 340 nm. The results were printed out as GST (U/L) after being calculated from the standard graph (Donald et al., 1967).

Statistical analysis

The data analysis was carried out using the Statistical Package for the Social Sciences (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

This study compared the effects of two different endurance-training programs (interval and continuous running), conducted in hot conditions, on glutathione s-transferase enzyme. The study was carried out in an average temperature of 29.40 ± 1.49°C and average humidity level was 50.71 ± 8.46%. The average and

Table 1. Average and standard deviation of continuous and interval running groups before and after exercises.

Variables		CRG[§]	IRG[§]
Age (year)		22.73 ± 3.51	24.27± 2.71
Height (cm)		1.73 ± 0.06	1.75 ± 0.06
Weight (kg)	Pre-test	71.99 ± 10.70	75.35 ± 7.14
	Post-test	70.30 ± 12.57**	73.46 ± 6.89*

*p < 0.05, **p < 0.01, [§]: Mean ± SD, IRG: Interval running group, CRG: Continuous running group.

Table 2. Comparison of pre-test and post-test GST level of the interval running group.

Variable	Interval running group (IRG)				
	Pre-test		Post-test		Z
	X	SS	X	SS	
GST (U/L)	10.80	1.08	12.94	0.48	-3.181**

GST: Glutathione S-transferaze; **p < 0.01.

Table 3. Comparison of pre-test and post-test GST level of the continuous running group.

Variables	Continuous running group (CRG)				
	Pre-test		Post-test		Z
	X	SS	X	SS	
GST (U/L)	12.38	2.03	10.13	2.05	-3.408**

GST: Glutathione S-transferaze; **p< 0.01.

standard deviation of continuous and interval running groups before and after exercises is given in Table 1. The results in Table 2 shows that after training was carried in a hot environment, there was a significant increase in the level of GST of the IRG (p < 0.01). The results in Table 3 show that, after training in a hot environment, there was a significant decrease in GST value of the CRG group (p < 0.01).

The pre-test and post-test GST values were compared according to the different training methods used in a hot environment. Statistically significant differences between the groups were seen in the pre-tests (p < 0.05) in comparison with the post-test results (p < 0.01)

Tissue damage can occur during sports activities, depending on muscle damage, thermal temperature and ischemia reperfusion. The sports scientists depend on the ROS production during sports activities and some elements such as mitochondrial electron transfer chain, xanthine oxidase system, the metal catalyzed reactions and activated neutrophiles (Peake and Suzuki, 2004). It is thought that malondialdehyde (MDA) causes oxidative stress in direct proportion to the intensity and the duration of the exercises and increases lipid peroxidation reactions. In situations in which oxygen use is low, the superoxide radical and its derivatives are deactivated by the antioxidant defence. However, in an exercise in which oxygen consumption increased considerably, these defence mechanisms may not keep up with the formation of free radicals and this may result in cell damage (Cheeseman and Slater, 1993). If the level of free radicals exceeds the antioxidant capacity, the fats, proteins and other parts of the cells will be oxidized (Clarkson and Thompson, 2000; Smith and Miles, 2000). Endurance exercises were used in most of the experimental studies examining the oxidative stress which results from exercise and its exact effect on plasma antioxidants or fat peroxidation (Sanchez et al., 1995).

Instances of heat exhaustion occurring during and after exercise cause the increase of reactive oxygen species (Salo et al., 1991). In a study by Osorio et al. (2003), it was suggested that exercise under thermal stress increased the effectiveness of antioxidants; also, although the metabolic responses continued, a mother was exposed daily to extreme heat stress, which had a dangerous effect on the baby and it was necessary, when swimming, to avoid hot water, since it could damage foetal development

In the study carried out in a hot environment, it was observed that while there was a significant increase in the GST value of the IRG before and after the training (p < 0.01 level, Table 2), there was a significant decrease in

Table 4. Comparison of pre-test and post-test GST values between groups.

Variable		Group comparison[§]		Z
GST(U/L)	Pre-test	11.59	1.79	-2.178 *
	Post-test	11.54	2.19	-3.505**

GST: Glutathione S-transferaze; *p < 0.05; **p < 0.01; [§]: Mean ± SD.

the values of the CRG before and after the training (p < 0.01, Table 3). When the values were compared according to the pre-test and post-test results of the different training methods, it was seen that there are statistically significant differences between the groups at p < 0.05 and < 0.01 levels (Table 4).

Many previous studies stated that a regular training program clearly increased the GST levels (Melikoğlu et al., 2008). In this study, it is seen that the interval training method shows parallel results to the findings of previous studies. Gül et al. (2006) examined the effects of endurance training and acute exhaustive exercises on the antioxidant defence mechanisms in rats. The training group exercised on a treadmill for 1.5 h per day, 5 days a week for 8 weeks. Both the training and the control groups then carried out exhaustive exercises. It was found that the GST level in the trained and untrained rats was not affected by the acute exhaustive exercise.

Kaldırımcı (2010) conducted a study that involved basketball training at 60 and 75% max VO_2 on 3 days per week for 12 weeks. There was a significant increase in the GST values of the basketball players and, as a result, this increase raised the antioxidant activities of the physical exercise, which continued for long periods and caused adaptations in the antioxidant defense. In another study, it was observed that although acute exercise caused a decrease in the GST activities of the liver tissue of untrained rats, this decrease was also seen in trained rats. It was stated that the GST activity in the livers of the rats doing endurance exercise was not affected. Moreover, acute exercise-induced GST-reduction is prevented in trained rats and ROS production is probably reduced. These are partially responsible for the positive effects of the endurance training. Also, it was determined that there was a decrease in the fat tissue around the testicles of rats doing endurance training and this decrease might affect the body composition, depending on the use of fats rather than carbohydrates as the source of energy during the endurance training (Öztaşan et al., 2007).

In the present study carried out in a hot environment, it was seen that there was a statistically significant increase in the interval running group, a statistically significant decrease in the continuous running group and statistically significant differences in the comparison of the pre-test and post-test values between the groups. Consequently, it can be said that the continuous running training in a hot environment affected the oxidative stress much more by increasing lipid peroxidation, while the interval running prevented the formation of free radicals by providing a positive increase in the glutathione s-transferase level.

In conclusion, it can be suggested that athletes and their trainers should generally apply the interval exercise program in order to reduce oxidative stress to a simpler state while doing endurance training in a hot environment.

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