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REGULAR PHYSICAL TRAINING REDUCED OXIDATIVE STRESS CAUSED BY EXERCISE IN RAT'S BLOOD

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ABSTRACT

The aim of this study was to investigate the effect of endurance training and exhaustive exercise on oxidative stress and evaluate whether endurance training reduces exercise induced oxidative stress.

Male Wistar rat (n=40), body weight 154±2 kg were divided into two groups randomly: A) trained (n=20) and B) untrained (n=20). Training group participated in eight weeks training program, five days per week, consisted of treadmill running that began speed was eight m/min for 10 min and with gradually increase in speed and time reaching the speed of 30 m/min for 1.5 h per day at 8th week.

At the end of 8th week each group further divided into groups where the rats were studied at rest and after exhaustive exercise. For acute exhaustive exercise graded treadmill running was conducted reaching the speed of 35 m/min and was continued until exhaustion, then animals were anesthetized and blood sample were collected for measurements of catalase and SOD activity in red blood cell and plasma MDA. The results indicated that acute exhaustive exercise increased the MDA level in sedentary but not in trained rat. The catalase activity was elevated significantly in trained group but was unchanged by exhausting exercise and acute exhaustive exercise decreased erythrocyte SOD activity in the sedentary rats but increased the activity of this enzyme in the trained rats.

Results of the present study indicated that regular physical exercise can strengthens the antioxidant system and suppress oxidative stress.

Keywords: Training, Antioxidant system, MDA, SOD, Rat

1. INTRODUCTION

Oxidative damage or oxidative stress refers to a condition in which homeostatic balance between oxidant and antioxidant capacity of biological systems is disrupted, and imbalance occurs between the production of oxidants and their removal by antioxidants [Alessio 1993& Radák 2000]. Reactive oxygen species are highly reactive due to presence of unpaired electron in their structure ,there for can damage proteins, lipids, and nucleic acids in the body and damage to the cells in different parts of the body [Radák 2000].

Inside the body, there are particular systems to deal with the damage caused by free radical, which are known as antioxidant defense system. When more free radicals produced than the antioxidant defense system can deal with, they cause damage to the cellular components. Some components of the antioxidant defense system such as superoxide dismutase enzymes, glutathione peroxidase, catalase, glutathione, and uric acid are produced inside the body; however, some like vitamin E, vitamin C, and beta-carotene should be supplied through diet [Radák 2000].

During physical activity, oxygen consumption rises. Acute physical activity can increase oxygen consumption up to 100 times, which leads to an increase in production of free radicals. The increased consumption of oxygen and the created conditions during exercise can cause oxidative damage, and some biomarkers of oxidative damage like MDA that is biomarker of damage to lipids increase. Production of free radicals during exercise plays a role in causing muscular damage and creating and developing inflammation after the exercise, they can also influence increased cellular damage [Castell et al. 2007&Miyazaki et al. 2001]. Increasing in lipid peroxidation after acute and exhaustive endurance training has been reported [Castro et al. 2009&Miyazaki et al. 2001&Oztasan 2004].

On the other hand, it has been observed that regular physical activity can cause positive regulation of antioxidant systems of the body [Revan and Erol 2011]. Previous studies show that endurance training prevents some symptoms of production of free radicals, and the damage to the free radicals, in turn, improves antioxidant defense of the tissue by increasing the activity of antioxidant substances like glutathione peroxidase, superoxide dismutase, and catalase [Oztasan 2004& Radák 2000]. However, this issue is not supported by all studies and different tissues can respond differently. It seems that production of free radicals stimulates the antioxidant system to a certain extent, which can be considered as a cellular defense mechanism. If the production of reactive oxygen species is very high, it can weaken the antioxidant system of the body. Acute aerobic exercise can cause oxidative stress more than other exercises [Radák 2000]. Different studies have indicated that the extent of antioxidant system influence, depends on the type, duration, and intensity of the exercise [Margaritis et al. 2003&Show, 2006].

Previous studies have presented different results for the effect of endurance training and one session of exhaustive exercise. For example, some studies have reported that regular endurance training can lead to an increase in the activity of catalase antioxidant enzyme and superoxide dismutase and improvement of antioxidant system [Somani, 1995]. It has also been indicated that endurance training can decrease harmful effects of acute exercise [8] , and some studies, have reported that it is not effective in prevention of oxidative damage [Miyazaki et al. 2001 &Senturk et al. 2004]. The results of the present study can determine the effects of prolonged and acute exercise on the antioxidant system and the level of oxidative stress and respond to this question whether a period of aerobic exercise can compensate for harmful effects of a session of exhaustive exercise or not. Therefore, better programs can be devised in order to figure ht the destructive effects of the free radicals.

2. METHODOLOGY

2.1. Animals and groups

Forty two-months male Wistar rats with average weight of 154±2 gr that were kept in the animals, house of Tarbiat Modarres University and fed with standard laboratory food were used in this study. After the veterinarian confirmed the rats' health, they were randomized into two groups of 20, an endurance training group and a non-training group. Each of them were divided into two subgroups of 10 at the end of the period.

2.2. The program of endurance training and exhaustive exercise

The exercise protocol for the endurance training group included eight weeks and five times per week. After the rats were familiarized with the treadmill, at the beginning they were given an exercise of 10 minutes running eight m/min, speed and duration were increased gradually and reached 30 m/min for 1.5 hours in the 8th week [Somani, 1995]. Three rats that could not run well were crossed out from the study. In the end of the training period, the training rats and half of the non-training rats selected randomly were chosen for exhaustive exercise. For the exhaustive exercise group without endurance training, before carrying out the exhaustive protocol, five days for 15 minutes at the speed of 16 m/min was considered in order to make sure that the rats can endure the exhaustive exercise. The protocol of exhaustive exercise included running at the pace of 16 m/min with zero slope in the first 10 minutes and gradual increase of speed up to 35 m/min and lasted until the rat got tired and exhausted and could not continue running.

2.3. Blood sampling and evaluating biochemical indices

At the end of the 8th week, blood sampling was conducted on the subjects. For the training group, blood sampling was carried out 24 hours after the last exercise. And for the two groups that had done exhaustive exercise, blood samples were collected after the exhaustive exercise. The blood samples were placed inside tubes containing EDTA and simple tubes that had been assigned before.

At the end of the experiment, the animals were anaesthetized and the blood was collected by cardiac puncture after thoracotomy. The activity of erythrocyte catalase was assessed by measuring the degradation rate of the substrate H2O2 and via spectrophotometer assay at 240 nm. The MDA level was estimated by thiobarbituric acid reactivity. MDA, an end-product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbency at 532 nm. CuZn-superoxide dismutase (SOD) activity was determined by inhibition of the reduction of nitroblue tetrazolium by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system [14].

2.4. Statistical methods

First of all, normality of data distribution was examined through Kolmogorov-Smirnov test. ANOVA and LSD post hoc test were used to analyze the data collected from the four groups.

2.5. RESULTS

- **a)** There was a significant difference between the two endurance training groups and the control group in regard with their catalase activity.
- **b)** There was a significant difference between the two groups of endurance training and one session of exhaustive exercise and the non-training group with one session of exhaustive exercise in terms of their catalase activity (**Figure 1**).
- **c)** There was no significant difference in MDA level between the endurance group and endurance training and exhaustive exercise group but the MDA level in the exhausted trained group was significantly higher compared with control group (Figure 2).
- **d)** Acute exhaustive exercise decreased erythrocyte SOD activity in the sedentary rats but increased the activity of this enzyme in the trained rats (Figure 3).

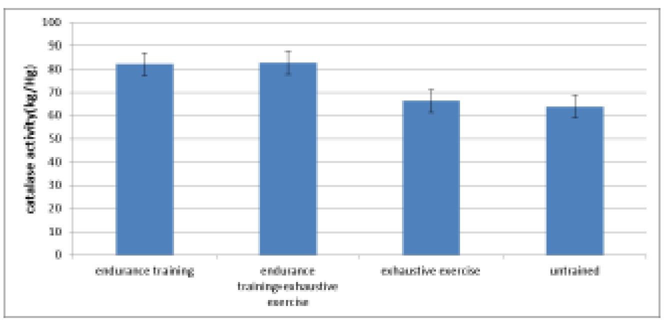

Figure 1: Erythrocyte Catalase (CAT) activity (e

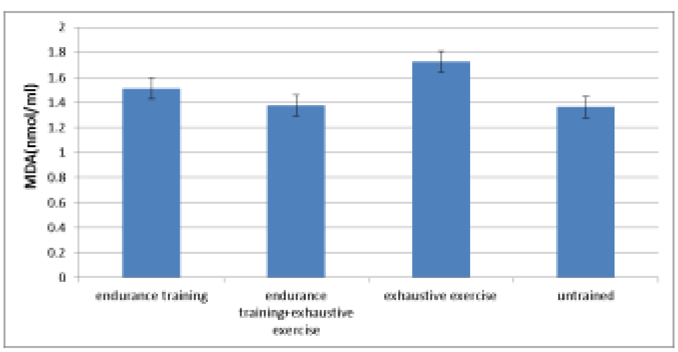

Figure 2: Serum malondialdehyde (MDA) levels (f

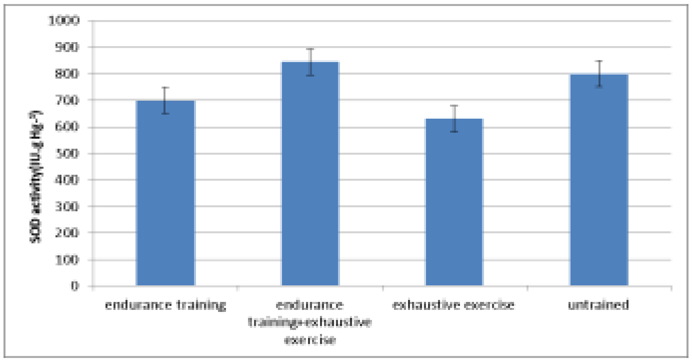

Figure 3: Erythrocyte superoxide dismutase (SOD) activity

The performance of Networks-on-Chip (NoC) is highly dependent on throughput and latency properties of the on-chip routers. Routing strategies have a key role on communication and performance in on-chip interconnection networks and several efforts have been done attempting to improve the performance of them in on-chip interconnection networks. In [Glass and Ni 1992], a partially adaptive routing algorithm, called turn model which is based on prohibiting certain turns during routing packets to prevent deadlock is presented. In [Chiu 2000] a routing algorithm called odd-even was proposed based on turn model. It restricts some locations where turn can be taken so that deadlock can be avoided. In comparison with previous methods, the degree of routing adaptiveness provided by the model is more even for different source destination pairs. A routing scheme called DyAD was proposed in [Hu and Marculescu 2004]. This algorithm is the combination of a deterministic routing algorithm and an adaptive routing algorithm. The router can switch between these two routing modes based on the network's congestion. Another adaptive routing named DyXY along with an analytical model based on queuing theory for a 2D mesh has been proposed [Li et al. 2006]. The authors claim that DyXY ensured deadlock-free and livelock-free routing and it can achieve better performance compared with static XY routing and odd-even routing. In [Pirretti et al. 2004] and [Dumitras et al. 2003] some fully fault tolerant routing algorithms are explained, one of them is named directed flooding algorithm. In this algorithm a message is sent to each outgoing link with probability p which is not fixed but varies based on the destination of the packet. In [Asad et al. 2009] a source routing algorithm called *Predominant Routing* was proposed which exploits the advantages of both deterministic and adaptive routing algorithms. Also in [salehi and Dana 2010] a routing algorithm for avoiding congested areas using a fuzzy-based routing decision is proposed.

3. DISCUSSION

MDA that is one of the most important markers of oxidative damage to lipids was investigated in the present study. After a period of endurance training, a significant difference was observed in MDA level of the endurance training group and the control one, which is in agreement with the results of the study conducted by Oztasan et al (2004) [Oztasan 2004] and San Turk et al (2001) [Senturk et al. 2004]. Mustafa Gul et al (2002) reported a decrease in TBARS in some tissues of diabetic male rats after an 8-week endurance training period [Gul et al. 2002]. Therefore, the utilized training program in this study had appropriate intensity, which increased the antioxidant capacity while did not change oxidative damage. As the results of research have indicated, MDA level increases as a result of exhaustive exercise. Oztasan et al (2004) who utilized a protocol similar to that of the present study have also observed an increase in MDA level [Oztasan 2004].Voces et al investigated the effect of one session of exhaustive exercise along with utilization of ginseng extract on rats and observed a reduction in TBARS level in some tissues of the rats after exhaustive exercise. One of the reasons for this reduction is utilization of ginseng extract due to its antioxidant qualities [Voces et al. 1999]. Although studies have reported contradicting results, most of them have indicated that one session of exhaustive exercise or acute physical activity leads to lipid peroxidation in different tissues. Oxygen consumption in exhaustive exercise highly increases, which can increase production of reactive oxygen species. This increasing in ROS can overcomes the antioxidant system and causes damage to the tissues. In fact, it is recommended that balance is not constructed between the damage exerted to the lipids and the antioxidant system, and plasma MDA in the training group has increased.

In the present study, it was observed that MDA level in the endurance training group did not change after the exhaustive exercise, and compared to the rest state it remained the same, this finding is in agreement with Oztasan et al [Oztasan 2004] whose study used a similar exhaustive exercise protocol. However, San Turk et al (2001) observed an increased level of TBARS as an indication of damage to the lipids in the training group after exhaustive exercise [Senturk et al. 2004]. A probable reason for this matter can be the shorter period of the endurance training of San Turk's study which was 4 weeks. This training period might failed to provide necessary adaptations in order to prevent oxidative damages. Kramer et al studied the effect of exhaustive exercise on well-trained runners and observed a decrease of plasma TBARS [Radák 2000].

As was observed in the present study and many other ones, acute and exhaustive exercise can cause oxidative damage; however, endurance training can have a preventive effect against this damage by activating and boosting the antioxidant systems.

Catalase is one of the antioxidant enzymes and plays a defensive role against reactive oxygen species. As one of the antioxidant enzymes, it plays a role in eliminating hydrogen peroxide and converting it into water and oxygen and defends the cells against disruptive and harmful effects of oxidant damage. Different investigations have indicated that catalase activity can be influenced by physical activities and their intensity. According to the results of the present study, it was concluded that the mean of catalase activity in the training group has increased compared to the control group, and this increase was significant in the catalase activity of erythrocytes in blood. This finding is in line with that of some studies [Somani, 1995]. However, Miyazaki et al (2001) concluded that a period of endurance training had no effect of catalase activity although they observed an increase in activity of other antioxidant enzymes like superoxide dismutase and glutathione peroxide after a period of endurance training [Miyazaki et al. 2001]. The results of the present study indicated that catalase activity can be affected and enhanced by endurance training, and this increase is a highly useful adaptive activity which has preventive effects against increased free radicals. Regular endurance training causes permanent slight oxidative stress that affects and over-regulates the antioxidant system. This regular exercise can be likened to a special vaccine that regulates antioxidant system. Mechanism of increasing the activity of the antioxidant enzymes is unknown. However, a probable mechanism for this increased activity can be an increase in adenosine level caused by ATP consumption that can result in adaptation through its regulatory effects [Somani, 1995]. Another possible mechanism for increased catalase activity is gene regulation; however, there is limited information on this mechanism. In a study, it was observed that adding hydrogen peroxide can enhance mRNA catalase in the cell [Somani, 1995]. Therefore, such an increase in activity of an antioxidant enzyme can be resulted from gene regulation.

In the present study, a session of exhaustive exercise did not created a significant change in catalase activity. This finding is in agreement with that of Miyazaki et al (2001) [Miyazaki et al. 2001]. The study conducted by Somani et al indicated a decrease in catalase activity after exhaustive exercise; however, this reduction was not significant [Somani, 1995]. Researchers have stated that increased level of catalase is an indication for defense mechanism against harmful effects of H2O2.

In the present study, after exhaustive exercise in the non-training group, a decrease in SOD activity was observed, which is not in line with the San Turk's finding (2001) that indicated lack of change in the activity of this enzyme [Senturk et al. 2004]. On the other hand, in the present study the endurance training group experienced an increase in SOD activity after the exhaustive exercise, which is in agreement with some studies [Oztasan 2004 &Senturk et al. 2004]. This change can also be seen among trained individuals after acute exercise [Miyazaki et al. 2001].

An increase in superoxide ion caused by acute activity can be one of the reasons for the increased activity of this enzyme in the trained group. Activity of SOD enzyme had less reduction in the trained group while they were relaxing. However, studies have reported no change for this enzyme [Miyazaki et al. 2001 &Senturk et al. 2004]. Such difference in the results of different studies can be attributed to different methods of training, different samples, and different studied tissues.

In general, the results of the present study indicated that endurance training of 8-weeks 5 days per week can strengthens the antioxidant system and enhance catalase activity to a remarkable extent. Moreover, the extent of oxidative damage following endurance training experienced no increase, which indicates that the intensity of the training protocol was appropriate because appropriate physical exercise should not enhance oxidative damage. In the present study, it was observed that holding one session of exhaustive exercise can increase the rate of oxidative damage, which should be noticed by the individuals who are involved with sudden and heavy sports. However, endurance training can reduce harmful effects of exhaustive exercise and the extent of oxidative damage caused by one session of exhaustive exercise. In fact, endurance training reduces the harmful effects of sudden oxidative stress by boosting and up-regulating the antioxidant system.

REFERENCES

[Alessio 1993] H.M. Alessio," Exercise-induced oxidative stress", Med Sci in Sports Exerc, 25, PP: 218-22, 1993.

[Castell et al. 2007] L. Castell, L. Gough, M. Johnston, K. Armstrong, J. Knight, R. Godfrey, "Antioxidant capacity of serum, saliva and urine before and after acute exercise: A pro-oxidant reaponse". 12th Annual congress of the ECSS. 11-14 July, Finland, 2007.

[Castro et al. 2009] De Castro, MAC. Calvacanti. , Neto, FF, Lima, LMC. DaSilva, FM. de Oliviera, RJ. Zanesco, A. "Production of free radicals and catalase activity during acute exercise training in young men". Biol Sport; 26, PP: 113-118. 2009,

[Gul et al. 2002] Gul, D.E. Laaksonen, M. Atalay, L. Vider and O. Hanninen, "Effects of endurance training on tissue glutathione homeostasis and lipid peroxidation in streptozotocin-induced diabetic rats", Scand. J. Med. Sci. Sports 12, PP: 163–170, 2002.

[Leelarugrayubl et al. 2005] N. Leelarugrayubl, T. Sutabhaha, P. Pothongsunun, N. Chanarat, "Exhaustive Exercise test and oxidative stress response in athletic and sedentary subjects". CMU Journal, 4(2), PP: 183-5. 2005.

[Margaritis et al. 2003] I. Margaritis,S. Palazzetti, AS. Rousseau, MJ. Richard, A. Favier. " A antioxidant supplementation and tapering exercise improve exercise- induced antioxidant response". J Am Coll Nutr. 22(2), PP: 147-56. 2003.

[Miyazaki et al. 2001] H.Oh. Miyazaki, S. ishi, T. Ookawara, T. Kizaki, K. Ha. S. Toshinai, S.Ji LL. Haga, H. Ohno,"Strenuous endurance training in humans reduces oxidative stress following exhausting exercise".Eur J Appl Physiol. Jan-Feb;84(1-2),PP:1-62001 .

[Oztasan 2004] N. Oztasan," Endurance training attenuates exercise – induced oxidative stress in erythrocytes in rat".Eur. J.Appl.Physiol.91.PP: 622-627. 2004.

[Radák 2000] Radák, Zsolt. "Free Radicals in Exercise and Aging". August 7. 2000.

[Revan and Erol 2011] S. Revan,A. Erol,"Effects of endurance training on exhaustive exercise induced oxidative stress markers.”African journal of pharmacy and pharmacology, Vol.5, No.3, PP: 437-441,2011.

[Senturk et al. 2004] UK. Senturk , F. Gunduz, O. Kuru, MR. Aktekin, D. Kipmen, O. Yalcin, B. Kucukatay, M,Yesilkaya. A, Baskur.”Exercise-induced oxidative stress affect erythrocyte in sedentary rat but not exercise-trained rat”.J Appl Physiol 91, PP: 1999- 2004.

[Show, 2006] Y.Show,” Free radical scavenging capacity and antioxidant enzyme activity in deerberry (vacciniumstamineuml)”. J Agricultrual Food Chemistry. 58, PP: 233-241. 2006.

[Somani, 1995] SM. Somani, S. Frank, P. Rybak,”Response of antioxidant system to acute and trained exercise in rat heart subcellular fractions”. Pharmacology Biochemistry and Behavior, Vol.51, No.4, PP: 627-634. 1995

[Sun 1988] Y. Sun, L.W. Oberley, Y. Li.” A simple method for clinical assayof superoxide dismutase”. Clin Chem 34, PP: 497–500. 1988.

[Voces et al. 1999] J. Voces , , A.I. Alvarez, L. Vila, A. Ferrando, C. Cabral de Oliveira, J.G. Prieto.”Effects of administration of the standardized Panax ginseng extractG115 on hepatic antioxidant function after exhaustive exercise”.Comparative Biochemistry and Physiology Part C 123, PP: 175–184. 1999.