



RESEARCH ARTICLE

DETERMINATION OF CHANGES IN ANTIOXIDANT LEVELS OF ELITE GRADE MALE ATHLETES AND SKI RUNNERS DOING ENDURANCE TRAINING

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ARTICLE INFO

Article History:

Received 29th November, 2015

Received in revised form

05th December, 2015

Accepted 05th January, 2016

Published online 14th February, 2016

Key words:

Athletics,
Endurance,
Antioxidant,
Cross-Country Skiing.

ABSTRACT

This study was performed to investigate the effect of the activity at high-altitude on carbonic anhydrase (CA), catalase (CAT), erythrocyte glutathione peroxidase (GSH), methylenedioxy-methylamphetamine (MDA), superoxide dismutase (SOD) enzyme levels. 8 sedentary males, 9 male athletes and 9 male skiers between the ages of 17 – 19, 26 healthy volunteers took part in this study. Athletes doing athletics and the sport of skiing in the experimental group have been selected among athletes who are doing long duration endurance training, training for 2 hours per day and 7 days a week. In addition, they were provided cardio practice for 3 days a week. The sedentary group has been chosen from non-elite athletes doing 3 or 4 day soft training in a week. SPSS 16 program was used to evaluate data and analysis were made by Wilcoxon test. CA, CAT and GSH enzyme levels of the male control group, male athletes and male skiers have been determined after taking blood samples and a significant difference has not been observed among values according to $p < 0,05$. However, when looking at the MDA and SOD values, a significant correlation has been observed among research groups according to $p < 0,05$. The results achieved in this study yielded meaningful outcomes on the antioxidant defence of MDA and SOD of athletes doing endurance training. Based on these results; the requirement of consideration of changes in the MDA and SOD values of endurance sport commissioned individuals can be expressed as the recommendation of our work.

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Citation: Cemil Tuğrulhan ŞAM, Metin BAYRAM and Vahit DOĞAR, A. 2016. "Determination of changes in antioxidant levels of elite grade male athletes and ski runners doing endurance training", *International Journal of Current Research*, 8, (02), 26261-26265.

INTRODUCTION

The endurance of the whole organism is the ability to resist fatigue in a long-lasting sporting exercise or the ability to resume very high intensity load in a long time. In another approach, endurance is defined as the strength of the athlete on physical and physiological fatigue (Gunay and Yuce, 1996). During physical exercise, the rate of metabolism increased in proportion to intensity of muscular activity. Exercise can be expected to result in oxidative stress depending on the intensity and duration. Accordingly, it is believed that lipid peroxidation occurs while the increase in the level of free radicals during exercise exceeds antioxidants in the defence capacity of the cell. Malondialdehyde (MDA) that is one of the effluent

resulting with lipid peroxidation is used as an indicator of oxidative stress. The extent of damage formed in the body would affect the duration of the regeneration in athletes. However, exercise strengthens the antioxidant defence when performed by regularly and at certain intensity (Leaf et al., 1997; Schroder et al., 2000; Turgut et al., 1999). The metabolic rate increases in proportion to the intensity of muscle activity during physical exercise (Leaf et al., 1997). Intense physical exercises constitute a rapid increase in oxygen in the entire body, especially skeletal muscle. This induces oxidative stress and free radical formation in the body. Antioxidants are molecules which respond to free radicals, stop or completely destroy the radical chain reaction. Thus, they prevent damage to the vital components of the body (Clarkson, 2000). Training can be expected to result in oxidative stress depending on the severity and duration. Accordingly, a lipid peroxidation is thought to occur if the increase in free oxygen radicals during exercise passes the antioxidants in the defence capacity of the cells (Turgut, 1999).

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CA, carbonic anhydrase enzyme makes the low pH of the bicarbonate buffer system suitable for body and produces carbonic acid from carbon dioxide and water. The reaction is bi-directional, so there is a balance in the reaction and carbonic acid is split into hydrogen ions and bicarbonate. So, CA plays a very important role in making bicarbonate secretion or lumen hydrogen in the gastrointestinal system. Additionally the great majority of the carbon dioxide is transported in the blood as erythrocyte hydrogen and bicarbonate by this enzyme (Arslan, 1996). Another importance is the task taken on bone resorption. Because osteoclasts perform this procedure and the main ingredient of resorption are the hydrogen ion. Osteoclasts provide hydrogen ions by combining carbon dioxide and water by means of carbonic anhydrase-2 enzyme. If there is a lack of carbonic anhydrase-2, bone resorption decreases and this arises a disease called Osteopetrosis in which the bones harden, becoming denser and also the bones become less dense and more brittle, or the bones soften (Chakraborty, 1988).

Optimal O₂ uptake increases significantly with regular and increasingly controlled training. Not only VO₂ max increases, also maximum respiratory minute volume and maximum cardiac output increase by affecting each other. Of course, this should be considered as a metabolic response against aerobic workout. As it has been seen that all three physiological values are interrelated. A high aerobic capacity is converted into positive anaerobic capacity (Akgun, 1994). Antioxidant molecules and enzymes use very effective antioxidant enzymes such as CAT and SOD to reduce the oxidative stress and throw free radicals from the body. (Clarkson PM, 2000) If the level of free radicals exceeds the antioxidant capacity; oils, proteins and other cell components are oxidized (Smith, 2000). Malondialdehyde (MDA) that is one of the resulting material of lipid peroxidation is known to be an indicator of oxidative stress. The extent of the damage that occurs in the body may affect the duration of the regeneration of the athletes. However, specific intensity and regular training can strengthen the antioxidant defences (Celik, 2001). GSH-Px determination: it was studied according to the GSH-Px activity method. GSH-Px, in the presence of hydrogen peroxide catalyses the oxidation of glutathione (GSH) and oxidized glutathione (GSSG). GSSG formed by GSH-Px in the presence of hydrogen peroxide is reduced to GSH with the assistance of glutathione reductase and NADPH. GSH-Px activity is calculated by reading the absorbance decrease at 340nm during the oxidation of NADH to NADP⁺ and it is indicated in the form of tissue protein as units /gram (IU /g) (Agila DE, 1967).

MATERIALS AND METHODS

Selection of subjects: 9 national male athletes, 9 national male ski runners and 8 healthy male sedentary who were doing continuous endurance training have participated voluntarily in this study. Athletes who participated in this study have made about 2 hours practice on 7 days a week. Before the test, by providing not to take any medications that would affect antioxidant defence, an attention has been paid to the diet of athletes. In the study, blood samples of athletes were taken from the antecubital vein at rest and after exercise right within

5 minutes for SOD, CAT, CA, GSH, MDA activities and measurements of hemogram values.

Taking Blood Samples: CA, CAT, GSH, MDA and SOD levels in generalized blood samples taken from the antecubital place were determined. Blood samples were kept in EDTA and normal test tubes. The samples disrupted for 3-5 min. Shaped elements precipitated by centrifugation for 5 minutes at 3500 rpm after standing 5-10 minutes at room temperature. The supernatant plasma was stored to the Eppendorf tubes at -80 °C until the day of the analysis. All blood analysis has been studied in Biochemistry Research Laboratory of Yüzüncü Yıl University.

Biochemical Analysis: 80 °C blood samples (serums) were taken -20 °C then +4 °C and slow thawing was provided. SOD determination was made by the method of Sun *et al.* MDA determination was made by the method described by Jain *et al* (1989). GSH-Px determination: it was studied according to the GSH-Px activity method. GSH-Px, in the presence of hydrogen peroxide catalyses the oxidation of glutathione (GSH) and oxidized glutathione (GSSG). GSSG formed by GSH-Px in the presence of hydrogen peroxide is reduced to GSH with the assistance of glutathione reductase and NADPH. GSH-Px activity is calculated by reading the absorbance decrease at 340nm during the oxidation of NADH to NADP⁺ and it is indicated in the form of tissue protein as units /gram (IU /g) (Uglia & Valentine, 1967).

Statistical Analysis: 26 healthy volunteers including 8 sedentary males, 9 male athletes and 9 male skiers between the ages of 17-19 have been enrolled to determine the effect of exercise on antioxidant enzymes. SPSS 16 software was used in analysing the data and analyses were performed by Wilcoxon test.

RESULTS

Table 1: A significant correlation has not been found in the CA value of the control and experimental group of male athletes doing athletics and skiing according to $p < 0,05$.

Table 2: A significant correlation has not been found in the CAT value of the control and experimental group of male athletes doing athletics and skiing according to $p < 0,05$.

Table 3: A significant correlation has not been found in the GSH value of the control and experimental group of male athletes doing athletics and skiing according to $p < 0,05$.

Table 4: When looking at MDA values of male athletes engaged in athletics and skiing, a meaningful correlation has been found in the MDA values of male control and male athletics; male control and male skiing groups according to $p < 0,05$.

Table 5: When looking at SOD values of male athletes engaged in athletics and skiing, a meaningful correlation has been found in the SOD values of male control and male athletics; male control and male skiing; athletics and skiing groups according to $p < 0,05$.

Table 1. Comparison of CA Values

	N	Avarage	Standard Deviation (+/-)	Z	p*
Male Control	8	0,5400	0,39984	-1,820	0,069
Male Athletics	9	0,2286	0,09685		
Male Control	8	0,5400	0,39984	-1,680	0,093
Male Skiing	9	0,3343	0,18552		
Male Athletics	9	0,2286	0,09685	-1,244	0,214
Male Skiing	9	0,3343	0,18552		

*p<0,05 there is no significant difference between the averages.

In CA values of the control and experimental group of maleathletes doing athleticsandskiing, there is no significant correlation according to p<0,05.

Table 2. Comparison of CAT Values

	N	Avarage	Standard Deviation (+/-)	Z	p*
Male Control	8	0,0035	,00392	-,980	0,327
Male Athletics	9	0,0049	,00418		
Male Control	8	0,0035	,00392	-,980	0,327
Male Skiing	9	0,0063	,00764		
Male Athletics	9	0,0049	,00418	-,533	0,594
Male Skiing	9	0,0063	,00764		

*p<0,05 there is no significant difference between the averages.

There is no significant correlation in the CAT values of the control and experimental group of maleathletes doing athleticsandskiing, according to p<0,05.

Table 3. Comparison of GSH Values

	N	Avarage	Standard Deviation (+/-)	Z	p*
Male Control	8	,1646	,01218	-1,540	0,123
Male Athletics	9	,1569	,01660		
Male Control	8	,1646	,01218	-,700	0,484
Male Skiing	9	,1611	,01162		
Male Athletics	9	,1569	,01660	-,415	0,678
Male Skiing	9	,1611	,01162		

*p<0,05 there is no significant difference between the averages.

A significant correlation has not been found in the GSH values of the control and experimental group of maleathletes doing athleticsandskiing, according to p<0,05.

Table 4. Comparison of MDA Values

	N	Avarage	Standard Deviation (+/-)	Z	p*
Male Control	8	,3163	,07352	-2,521	0,012*
Male Athletics	9	1,5802	,99416		
Male Control	8	,3163	,07352	-2,521	0,012*
Male Skiing	9	,9179	,39655		
Male Athletics	9	1,5802	,99416	-1,718	0,086
Male Skiing	9	,9179	,39655		

*p<0,05; there is a meaningful difference between the averages.

When looking at MDA values of maleathletesengaged in athletics and skiing, a meaningful correlation has been found in the MDA values of male control and male athletics; male control and male skiing groups according to p<0,05.

Table 5. Comparison of SOD Values

	N	Avarage	Standard Deviation (+/-)	Z	p*
KontrolErkek	8	20,0250	,56929	-2,521	0,012*
AtletizmErkek	9	14,3922	,60776		
KontrolErkek	8	20,0250	,56929	-2,524	0,012*
Kayak Erkek	9	17,2178	,32752		
AtletizmErkek	9	14,3922	,60776	-2,666	0,008*
Kayak Erkek	9	17,2178	,32752		

*p<0,05; there is a meaningful difference between the averages.

When looking at SOD values of maleathletesengaged in athletics and skiing, a meaningful correlation has been found in the SOD values of male control and male athletics; male control and male skiing; athletics and skiing groups according to p<0,05.

DISCUSSION AND CONCLUSION

During physiological processes occurring in the body or in a pathological process, oxidative damage is a result of the balance between the resulting free radicals and antioxidant systems crossing to the side of free radicals. Organism protects itself against oxidative damage to enzymatic and non-enzymatic antioxidant systems and molecules. SOD and GSH-Px are antioxidant enzymes that are effective at the cellular level (Akyol, 1994). It has been reported that chronically confrontation with moderate levels of oxidative stress enhances the antioxidant defence (Kanter, 1985). Therefore, moderate intensity and regularly performed exercises are strengthening the antioxidant defence (Ji, 1993). Researchers have indicated that some elements of the antioxidant defence increased with regular training (Alessio, 1988). The widespread belief is that antioxidant enzyme activities could be changed by exercise. However, which enzymes located in the antioxidant defence and under which conditions these enzymes could be activated are controversial. While there are very few changes with exercise in the liver and myocardial enzyme system in rats, it has been reported that exercise may cause the adaptive increase in skeletal muscle antioxidant enzymes (glutathione peroxidase enzymes in particular) (Li, 1993). Similar results were found in rats performed 12 weeks of training. (Laughlin MH1990) Kanter *et al.* have shown that CAT, GPx, SOD levels rose in the blood of 9 and 21 week swimming training performed rats but at the end of 21 weeks of training GPx and CAT level of liver increased (Kanter, 1985). In this study we have determined that SOD and MDA enzyme values of athletes, skiers and sedentary group increased but CA, CAT and GSH levels decreased. As a result, the data we have obtained in biochemical level strengthen the antioxidant defence in person engaged in endurance sports. These increases in antioxidant enzymes are thought to be a positive adaptation to training. In another study, it has been reported that SOD and Se independent GPx was found higher and a significant increase was found in selenium-dependent GPx in trained rats. (Vani, 1990) In humans, there are limited data on the effects of physical exercise on antioxidant enzymes. Ohno *et al.* have found no significant change in the antioxidant enzymes by 30 min. Sub maximal intensity exercises (Ohno, 1985). Athletes' plasma Mn-SOD levels were significantly higher. The Cu-Zn-SOD levels were not significantly different when compared with sedentary (Ohno, 1992).

However, in another study it has been reported that there was a significant change for both isoenzymes of SOD after 3 months of training (Ohno, 1993). In our study, training, MDA and changes while SOD. Indeed, in their study on subject animals, Alessio and Goldfarb (1988) have observed that endurance training has the effect of increasing on antioxidant defence and reducing on lipid peroxidation. Cao and Chen (1991) also had similar findings. Mena *et al.* have investigated the antioxidant enzymes in three groups; control, amateur and professional cyclists. In the case of relaxing, SOD value of amateur group was higher than the control group and SOD, GPX, CAT values of the professional group were significantly higher than control group. (Mena, 1991) When we look at the SOD values, this is in line with our study. After exercise of moderate intensity, compared with the resting level, they didn't find different

MDA levels in muscle and liver tissue. These results reveal that lipid peroxidation levels are associated with the intensity of exercises. In another study, it has been found that in MDA levels in skeletal muscle, there was an increase of 120% following excessive running exercise and an increase of 68% while moderate running (Alessio, 1993).

This study also showed the same results with our study. Studies investigating the relationship between lipid peroxidation and exercise in humans are, scarce (Jenkins, 1988). (Kanter *et al.* 1988) have been published that one's blood TBARM concentration increased 77% following the excessive exercise of running compared with the resting (Kanter, 1989). Likewise, (Marzatico *et al.* 1997) have not observed changes in the erythrocyte CAT activity in runners who were doing sprint exercise. However, they have observed an increase in CAT activity of long-distance runners from 24 to 28 hours after the endurance exercise. In our study, all of oxidative stress may be the sign of a strong antioxidant defence system of athletes. On the other hand, long-term endurance training has increased antioxidant enzymes (SOD and MDA) of athletes. However, it can be considered that oxidative stress measurements CAT, GPx, GSH reduce free radicals in the training. While it has been declared that MDA levels increased in cycling ergometer in sedentary people doing maximal intensity exercise, Vani *et al.* have not observed any changes in MDA levels by the same method. Birites *et al.* have observed high levels of plasma SOD activity in their research on football players (Marzatico F, 1997). Marzatico *et al.* have seen a significant rise in the erythrocyte SOD activity in their study on sprinters and marathoners (Vani, 1990). While Vani *et al.* have not determined any changes in the work they have carried out on MDA enzymes, (Marzatico *et al.* 1977) have seen noteworthy changes in SOD activity in the work they have done on sprinters and marathoners. This study showed similar results with our study.

REFERENCES

- Aglia DE, Valentine WN. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. *J Lab Clin Med.*, 1967; 70: 158-169.
- Akgün, N. 1994. Egzersiz Fizyolojisi. 2. Cilt, Ege Üniversitesi Basımevi, Bornova, İzmir
- Akyol, Ö. 1994. Beyin tümörlerinde SOD, CAT ve GSH-Px aktiviteleri. *Uzmanlık tezleri. Ankara Üni. Tıp Fakültesi Biyokimya Anabilim Dalı. Ankara*, 12-18.
- Alessio HM; "Exercise-induced oxidative stress". *Med Sci Sports Exerc.*, 25(2): 218-224, 1993
- Alessio, H. M., & Goldfarb, A. H. 1988. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *Journal of Applied Physiology*, 64(4), 1333-1336.
- Brites, F. D., Evelson, P. A., Christiansen, M. G., Nicol, M. F., Basilio, M. J., Wikinski, R. W., & Llesuy, S. F. 1999. Soccer player under regular trainings how oxidative stress but an improved plasma antioxidant status. *Clinical Science*, 96, 381-385.
- Chakraborty, M., Ghosal, J., Biswas, T. & Datta, A. G. 1988. Effect of erythropoietin on membrane lipid peroxidation, superoxide dismutase, catalase, and glutathione peroxidase

- of rat RBC. *Biochemical Medicine and Metabolic Biology*, 40(1), 8-18.
- Clarkson PM, Thompson HS. Antioxidants: what role do they play in physical exercise and health? *Am J Clin Nutr.*, 2000; 72: 637-646.
- Clarkson, P. M., & Thompson, H. S. 2000. Antioxidants: what role do they play in physical activity and health, *The American Journal of Clinical Nutrition*, 72(2), 637-646.
- Günay, M. & Yüce, A. İ. 2008. *Futbol antrenmanının bilimsel temelleri*. Gazi Kitabevi.
- Jain, S. K., McVie, R., Duett, J., & Herbst, J. J. 1989. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes*, 38(12), 1539-1543.
- Jenkins, R. R. 1988. Free radical chemistry. *Sports Medicine*, 5(3), 156-170.
- Ji, L. L. 1993. Antioxidant enzyme response to exercise and aging. *Medicine and science in sports and Exercise*, 25(2), 225-231.
- Ji, L. L. 1993. Antioxidant enzyme response to exercise and aging. *Medicine and science in sports and exercise*, 25(2), 225-231.
- Kanter MM, Lesmes LA, Kaminsky LA, LaHamsalger J, Nequin ND. 1988. "Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race". *Eur J Appl Physiol.*, 57: 60-83.
- Kanter, M. M., Hamlin, R. L., Unverferth, D. V., Davis, H. W., & Merola, A. J. 1985. Effect of exercise training on antioxidant enzymes and cardio toxicity of doxorubicin. *Journal of Applied Physiology*, 59(4), 1298-1303.
- Kaya Çelik, A. 2001. *Akut egzersizin futbolcularda antioksidan sistem parameter lerine etkisi* (Doctoral dissertation, Ege Üniversitesi).
- Laughlin, M. H., Simpson, T., Sexton, W. L., Brown, O. R., Smith, J. K., & Korhuis, R. J. 1990. Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training. *Journal of Applied Physiology*, 68(6), 2337-2343.
- Leaf, D. A., Kleinman, M. T., Hamilton, M. I. C. H. E. L. L. E., & Barstow, T. J. 1997. The effect of exercise intensity on lipid peroxidation. *Medicine and science in sports and Exercise*, 29(8), 1036-1039.
- Marzatico, F., Pansarasa, O., Bertorelli, L., Somenzini, L., & DellaValle, G. 1997. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *The Journal of Sports Medicine and Physical Fitness*, 37(4), 235-239.
- Mena, P., Maynar, M., Gutierrez, J. M., Maynar, J., Timon, J., & Campillo, J. E. (1991). Erythrocyte free radicals scavenger enzymes in bicycle professional racers. Adaptation to training. *International Journal of Sports Medicine*, 12(6), 563-566.
- Ohno, H., Kayashima, S., Nagata, N., Yamashita, H., Ookawara, T., & Taniguchi, N. 1993. Changes in immune reactivemanganese-superoxide dismutase concentration in human serum after 93 h strenuous physical exercise. *Clinica Chimica Acta*, 215(2), 213-219.
- Ohno, H., Sato, Y., Yamashita, K., Doi, R., Arai, K., Kondo, T. & Taniguchi, N. 1986. The effect of brief physical exercise on free radical scavenging enzyme systems in human red blood cells. *Canadian Journal of Physiology and Pharmacology*, 64(9), 1263-1265.
- Ohno, H., Yamashita, H., Ookawara, T., Saitoh, D., Wakabayashi, K., & Taniguchi, N. 1992. Training effects on concentrations of immune reactive superoxide dismutase iso-enzymes in human plasma. *Acta Physiologica Scandinavica*, 146(2), 291-292.
- Ozensoy, O., Arslan, O. & Sinan, S. O. 2004. A new method for purification of carbonic anhydrase isozymes by affinity chromatography *Biochemistry (Moscow)*, 69(2), 216-219.
- Schroder, H., Navarro, E., Tramullas, A., Mora, J., & Galiano, D. 2000. Nutrition antioxidant status and oxidative stress in professional basketball players: effects of a three compound antioxidant supplement. *International Journal of Sports Medicine*, 21(2), 146-150.
- Smith, L. L. & Miles, M. P. 2000. Exercise Induce Muscle Injury and Inflammation. *Exercise and Sport Science (William E., Garrett Jr., Ed.)*, 401-410.
- Sun, Y. I., Oberley, L. W. & Li, Y. 1988. A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*, 34(3), 497-500.
- Turgut, A., Özgürbüz, C., Azboy, O., Akyüz, F., İnal, M., Göktürk, E. & Seber, S. 1999. Yüzcülerde aerobik ve anaerobik ağırlıklı yüklenmelerde oksidatif stresin karşılaştırılması. *Spor Hekimliği Dergisi*, 34, 1-10.
- Vani, M., Reddy, G. P., Reddy, G. R., Thyagaraju, K., & Reddanna, P. 1989. Glutathione-S-transferase, superoxide dismutase, xanthine oxidase, catalase, glutathione peroxidase and lipid peroxidation in the liver of exercised rats. *Biochemistry International*, 21(1), 17-26.
