

## Effects on the antioxidant immune system of intensive training period of female athletes and female ski runners

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### Abstract:

This study is designed to investigate the effects of physical activity on antioxidant system parameters. Subjects were composed of 26 elite level runners who regularly train in Agri including 9 female athletes, 9 female ski runners and 8 sedentary between 17 – 18 years of age. The research was conducted during a season by making athletes cardio training 3 days a week and cross training 4 days a week. The blood samples were analyzed with respect to the results of antioxidant system parameters MDA (Malondialdehyde), SOD (superoxide dismutase), CAT (Catalase), CA (carbonic anhydrase), GSH (glutathione peroxidase) enzyme activation. An increase has been observed in SOD enzyme activity and level of MDA after training according to the resting state and this increase was statistically significant ( $p < 0.05$ ).

In this study, no significant difference has been found in CA, CAD and GSH levels after training. Determined decrease in CA, CAD and GSH levels suggests that reducing the free radicals and consequent lipid peroxidation as a result of the strengthening of the antioxidant defense system by a workout. These results may indicate that aerobic training improves the ability of adaptation to oxidative stress.

**Keywords:** antioxidant system, skiing, athletics

### Introduction

Normally, there is a delicate balance between oxidant and antioxidant systems in the organism. Imbalance can cause lipid peroxidation. The effect of endurance training on antioxidants and lipid peroxidation levels of individuals who are having sedentary living and regular physical activity (trained) is being determined.

According to the studies, increases were reported in various antioxidant enzymes with regular training. However, there is not a consensus that which enzyme/enzymes and under what conditions can be activated in the antioxidant defense.

Like many organisms in nature, people need oxygen in order to survive. Superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $OH^\cdot$ ) is formed during the metabolism of oxygen that enters the body. These substances are called as "free oxygen radicals". Extremely active these radicals damage many tissues, first cell membrane and DNA (Clark, et al. 1985). They have been shown to play a role in the pathogenesis of many diseases (Alessio & Goldfarb, 1988).

There are strong evidence that severe physical exercise increases oxygen consumption and hence the formation of free oxygen radicals dramatically (Alessio & Goldfarb, 1988). In the last 20 years, especially in experimental animals, there have been many studies examining the effects of exercise and training at various time and intensity on the antioxidant defense system (Alessio & Goldfarb, 1988). Generally, it has been observed that there is an adaptation between training and antioxidant enzymes in the skeletal muscle. While many researchers have showed that there is an increase in SOD enzyme with training (Higuchi et al, 1985). some researchers have not determined any change (Powers et al, 1993). During physical exercise, the rate of metabolism is increased in proportion to the intensity of muscular activity. Exercise can lead to oxidative stress depending on the severity and duration. Accordingly, it is believed that the lipid peroxidation occurs if the increase in the level of free oxygen radicals during exercise exceeds the antioxidants in the defense capacity of the cell. Malondialdehyde (MDA) which is one of the substances that arise as a result of 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, when exercise is performed regularly and at a certain intensity, it strengthens the antioxidant defense (Leaf, D.A et al., 1997). As a result of hematological tests, it was observed an increase in some blood parameters after acute exercise. Although, only the leukocyte was increased within normal limits, this was not statistically significant. The obtained values are compatible with the results of the studies which have reported an increase in blood cells. During exercise, in connection with the intensity muscular activity, there is an increase in the amount of erythrocytes in the circulation, in the circulation rate and in the arteriovenous oxygen difference; that is, in the amount of oxygen released active muscle and in the

metabolic rate (Ji & Leichtweis, 1997). They have reported that the antioxidant enzyme activities were not affected by the exercise and the half-marathon. In the literature, there are studies indicating that SOD levels were not affected with acute or chronic exercise (Kanter, 1993). He has not determined any increase in SOD levels during the 80km race (Ortenblad et al, 1997), in volleyball players (Duthie et al, 1990), in the half marathon runner (Ohno et al, 1988), in sedentary students have not been found any change in SOD activity (Marzatiko et al, 1997), have found an increase in SOD levels in half-marathon runners and sprinters (Balakrishnan, 1998), has also showed an increase in SOD level (Turgut et al, 1999), have determined an increase in SOD level after 800 m of freestyle swimming (Zergeroğlu et al, 1997), have also determined an increase in SOD level in cyclist and in another study on endurance training (Gönenç, 1995), has found a significant increase in SOD level after 4 weeks of swimming lessons. In this study, an increase in SOD activity has been determined. This increase was statistically significant. In this study, CAT activity showed a slight increase within normal limits after acute exercise according to the resting state. However, this increase was not statistically significant. This is thought to be caused enzymes located on the second stage antioxidant defense at a level beyond their capacity in the applied to the exercise program. Because superoxide radicals are converted to H<sub>2</sub>O<sub>2</sub> by SOD enzyme catalysis in the first stage of antioxidant defense (Mena et al, 1991), have found a decrease in CAT values in cyclists (Zergeroğlu et al, 1997), have also found a decrease in CAT value in their study on cyclists.

Although, there was a confliction in evidence of the CAT enzyme, general reviews obtained from the literature are no changes in the CAT level. In a survey conducted by Şıktar (2009), the average of exercised rats CA levels were significantly lower than the average of sedentary group CA levels (Şıktar, 2009). In another study in high-intensity exercise performed rats, it has been shown a decrease in the CA level in high-intensity exercise. Therefore, a decrease in CA level of a group doing more intensive training may be associated with a decrease in CA levels induced with high-intensity exercise. In a study that examined the effects on antioxidant enzymes of the exercise done in high altitude with long-distance athletes, it has been observed an increase in the CAT level of athletes after exercise at high altitude but it has been stated that this increase was not significant (Bayram, 2013). In another survey conducted by Kıyıcı et al., the increase in CAT levels after exercise was not found significant. Referring to studies, an increase in CAT levels after exercise was observed but this increase was not statistically significant (Kıyıcı & Kışalı, 2010). In the study that we conducted, MDA and SOD levels of elite female athletes and female ski runners doing high-intensity training were significant but CAT, GSH and CA levels were not significantly different. These values are found to be low and show the similarity of other studies can be associated with the different load of training in studies. Similarly, previous studies conducted on rats and mice have stated that formaldehyde exposure has caused a decrease in testicular SOD and GSH-Px values and an increase in MDA levels. (Turgut et al., 1999). Besides this, it was reported in studies that formaldehyde has also caused oxidative damage in other tissues (Zararsız et al., 2006). Chronically confrontation with moderate levels of oxidative stress has been reported to potentiate the antioxidant defense. Therefore, moderate intensity and regularly performed exercises are strengthening the antioxidant defense. Researchers have reported that some elements of the antioxidant defense increased with regular training. The general belief is that exercises could alter the antioxidant enzyme activities.

These increases in antioxidant enzymes are thought to be a positive adaptation to training. In another study conducted on trained rats, SOD and Se independent GPx has been found high but a significant increase in SE dependent GPx has not been reported.

The aim of this study was to investigate the effect of acute loading on the antioxidant defense system in mainly aerobic loading athletes and ski runners.

## Materials and methods

**Selection of subjects:** 9 female national athletes, 9 female national ski runners and 8 females from a control group who have not any health problems, are age close to each other and don't use any of antioxidant supplementation have been selected for this study.

**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 have been studied in Biochemistry Research Laboratory of Yüzüncü Yıl University.

**Biochemical Analysis:** 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.

**Statistical Analysis:** The "SPSS 11.0 for Windows" statistical software was used for analysis on the results of biochemical parameters (MDA, SOD, CAT, CAT and GSH). The distribution of the groups was evaluated by one-sample Kolmogorov-Smirnov test that is one of the non-parametric tests. Since the groups have normal distribution, Wilcoxon test that is a parametric test and LSD that is one of the ANOVA and Post Hoc

tests were used for comparison of values. Values around  $p < 0.05$  were considered as significant for statistical significance.

**Findings**

**Table 1:** When looking at MDA values of female athletes engaged in athletics and skiing, a meaningful correlation has been found in the MDA values of female control and female athletics; female control and female skiing groups according to  $p < 0.05$ .

**Table 2:** When looking at SOD values of female athletes engaged in athletics and skiing, a meaningful correlation has been found in the SOD values of female control and female athletics; female control and female skiing; athletics and skiing groups according to  $p < 0.05$ .

**Table 3:** A significant correlation has not been found in the CA value of the control and experimental group of female athletes doing athletics and skiing according to  $p < 0.05$

**Table 4:** A significant correlation has not been found in the CAT value of the control and experimental group of female athletes doing athletics and skiing according to  $p < 0.05$

**Table 5:** A significant correlation has not been found in the GSH value of the control and experimental group of female athletes doing athletics and skiing according to  $p < 0.05$ .

Table 1: Comparison of MDA Values

	N	Average	Standard Deviation (+/-)	Z	p*
Female Control	8	,3910	,18138	-2,521	0,012*
Female Athletics	9	1,4278	,74783		
Female Control	8	,3910	,18138	-2,380	0,017*
Female Skiing	9	,9100	,43415		
Female Athletics	9	1,4278	,74783	-1,599	0,110
Female Skiing	9	,9100	,43415		

\*  $p < 0.05$ ; there is a meaningful difference between the averages.

When looking at MDA values of female athletes engaged in athletics and skiing, a meaningful correlation has been found in the MDA values of female control and female athletics; female control and female skiing groups according to  $p < 0.05$ .

Table 2: Comparison of SOD Values

	N	Average	Standard Deviation (+/-)	Z	p*
Female Control	8	18,4737	,88632	-2,100	<b>0,036*</b>
Female Athletics	9	16,1844	1,92855		
Female Control	8	18,4737	,88632	-2,240	<b>0,025*</b>
Female Skiing	9	17,3673	,33259		
Female Athletics	9	16,1844	1,92855	-1,540	0,123
Female Skiing	9	17,3673	,33259		

\*  $p < 0.05$ ; there is a meaningful difference between the averages.

When looking at SOD values of female athletes engaged in athletics and skiing, a meaningful correlation has been found in the SOD values of female control and female athletics; female control and female skiing; athletics and skiing groups according to  $p < 0.05$ .

Table 3: Comparison of CA Values

	N	Average	Standard Deviation (+/-)	Z	p*
Female Control	8	0,3606	0,12695	-0,980	0,327
Female Athletics	9	0,2679	0,19949		
Female Control	8	0,3606	0,12695	-1,400	0,161
Female Skiing	9	0,3054	0,19180		
Female Athletics	9	0,2679	0,19949	0,000	1,000
Female Skiing	9	0,3054	0,19180		

\*  $p < 0.05$  there is no significant difference between the averages.

In CA values of the control and experimental group of female athletes doing athletics and skiing, there is no significant correlation according to  $p < 0.05$ .

Table 4: Comparison of CAT Values

	N	Average	Standard Deviation (+/-)	Z	p*
Female Control	8	0,0018	,00171	-1,260	0,208
Female Athletics	9	0,0038	,00247		
Female Control	8	0,0018	,00171	-,840	0,401
Female Skiing	9	0,0047	,00635		
Female Athletics	9	0,0038	,00247	-,178	0,859
Female Skiing	9	0,0047	,00635		

\* 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 female athletes doing athletics and skiing, according to p<0,05.

Table 5: Comparison of GSH Values

	N	Average	Standard Deviation (+/-)	Z	p*
Female Control	8	,1640	,01020	-,702	0,483
Female Athletics	9	,1582	,01496		
Female Control	8	,1640	,01020	-,085	0,932
Female Skiing	9	,1572	,03822		
Female Athletics	9	,1582	,01496	-1,008	0,314
Female Skiing	9	,1572	,03822		

\* 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 female athletes doing athletics and skiing, according to p<0,05

## Discussion

In this study, when considering the MDA values of athletics and skiing female athletes, a significant relationship has been found between control female – athletics female and control female – skiing female according to p<0,05. When considering the SOD values of athletics and skiing female athletes, also a significant relationship has been observed in SOD values between control female – athletics female and control female – skiing female according to p<0,05. During surveys conducted in several studies, it has been reported an increase in various antioxidant enzymes with regular training. Clark et al. 1985, have stated that people require oxygen to sustain their lives like many organisms in nature. Alesio et al. 1988, have found strong evidence that severe physical exercise increases oxygen consumption and hence the formation of free oxygen radicals dramatically. In the last 20 years, Alesio and Goldfarb, 1988 especially in experimental animals there have been many studies examining the effects of exercise and training at various time and intensity on the antioxidant defense system. These studies have shown results parallel to the study that we have done. Alessio ve Goldfarb, 1988 have observed that there is an adaptation between training and antioxidant enzymes in the skeletal muscle. While many researchers have showed that there is an increase in SOD enzyme with training Higurashi et al, 1985 have not determined any change. This increase in the SOD enzyme has strengthened our study. According to Powers et al. 1993, the lipid peroxidation occurs if the increase in the level of free oxygen radicals during exercise exceeds the antioxidants in the defense capacity of the cell. Malondialdehyde (MDA) which is one of the substances that arise as a result of lipid peroxidation is used as an indicator of oxidative stress. Leaf and D.A et al. 1997 have indicated that the extent of damage formed in the body would affect the duration of the regeneration in athletes. However, if exercise is performed regularly and at certain intensity it strengthens the antioxidant defense. The obtained values in the studies of Ji, L.L., Leichtweis, S., 1997 are compatible with the results of the studies which have reported an increase in blood cells. During the exercise, there is an increase in the amount of erythrocytes in the circulation, in the circulation rate and in the arteriovenous oxygen difference; that is, in the amount of oxygen released active muscle and in the metabolic rate in connection with the intensity muscular activity.

Kanter, 1993 has reported that the antioxidant enzyme activities were not affected by the exercise and the half-marathon. There are studies indicating that SOD levels were not affected with acute or chronic exercise in the literature. Qrtenblad et al. 1997, have not determined an increase in SOD levels during the 80km race. Duthie et al. 1990 in volleyball players, Marzatiko et al. 1997 in the half marathon runner, Ohno et al. 1988 in sedentary students have not been found any change in SOD activity. However, in another study conducted by Balakrishnan, 1998, an increase in SOD level has been observed. Turgut et al, 1999 has also determined an increase in SOD levels. In a study performed by Zergeroğlu et al. 1997, they have determined an increase in SOD level after 800 m of freestyle swimming. They have also determined an increase in SOD levels in cyclist and in another study on endurance training. And also Gönenç, S. 1995 has found a significant increase in SOD level after 4 weeks of swimming lessons.

Zergeroğlu et al, 1997 have also observed a decrease in CAT value in their study on cyclists. There was a confliction in evidence of the CAT enzyme but general reviews obtained from the literature are no changes in the CAT level. In a research conducted by Şıktar, average of exercised rats CA levels were significantly lower than the average of sedentary group CA levels. Also, in another study in high-intensity exercise performed rats, he has shown a decrease in the CA levels in high-intensity exercise. Therefore, in this study, a decrease in the CA levels of the group that are doing more intensive training can be associated with the highly intensive training induced decrease of CA levels. This study shows results parallel to our study.

In a study that examined the effects on antioxidant enzymes of the exercise done in high altitude with long-distance athletes conducted by Bayram, 2013, it has been observed an increase in the CAT level of athletes after exercise at high altitude but he has stated that this increase was not significant. In another research performed by Kızılcı et al., 2010, the increase in CAT levels after exercise was not found significant. When considering the studies conducted, an increase in CAT levels after exercise was observed but this increase was not statistically significant.

In this study, MDA and SOD levels of elite level female athletes and female ski runners doing high-intensity training were significant but CAD, GSH and CA levels were not significantly different. We can associate with the different load of training in studies that these values are found to be low and show the similarity of other studies. Similar to that, Turgut et al., 1999, have stated that formaldehyde exposure has caused a decrease in testicular SOD and GSH-Px values and an increase in MDA levels in previous studies conducted in rats and mice. Besides this, Zararsız et al., 2006 have reported that formaldehyde has also caused oxidative damage in other tissues. As a result, in this study, MDA and SOD levels were found high after training in female athletes and female skiers engaged in intensive training. However, a meaningful change has not occurred. These results suggest that aerobic training improves the ability of adaptation to oxidative stress and this may indicate that it reduces lipid peroxidation levels.

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