

Effects of Alpine Ski Trainings on Oxidative Stress and
Antioxidant Levels*

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ORIGINAL ARTICLE

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Abstract

The aim of this study was to examine the oxidant and antioxidant levels that could be observed during a single unit slalom and giant slalom trainings routinely practiced by alpine skiers by measuring the Total Oxidant Capacity (TOC) and Total Antioxidant Capacity (TAC). 18 male athletes, aged between 18 and 29 and experienced in the international alpine ski competitions, participated voluntarily in the study. Initially, body weight, height, body mass index, and VO₂max values of the volunteers were determined as required. Next, the volunteers underwent giant slalom and slalom training, lasted 2.5 hours with five days interval, on the competition slope that complies with the international standards. Venous blood samples of the volunteers were collected before and after both training applied and so TOC and TAC values were found out accordingly. While no significant change was observed in TOC values after both slalom and giant slalom applied, a significant increase was detected in TAC values (p<0.05). The reason for not observing any significant increase in TOC values though a significant increase has been detected in TAC values, may be due to the increased antioxidant enzymes which compress any increase in oxidant enzymes. Furthermore, the fact that alpine ski competition and training loads have been both in short-term and occurred at medium and high intensities rather than maximal intensity may have resulted with an antioxidant system which has been more activated than the oxidant system.

Keywords: Total Oxidant Capacity, Total Antioxidant Capacity, Athletic Performance, Alpine Skiing.

Alp Kayağı Antrenmanlarının Oksidatif Stres ve
Antioksidan Düzeylerine Etkisi

Öz

Bu çalışmada alp kayakçılarının rutin olarak gerçekleştirdikleri tek bir birim slalom ve büyük slalom antrenmanları süresince oluşabilecek olan oksidan ve antioksidan seviyelerinin total oksidan seviye (TOS) ve total antioksidan seviye (TAS) ölçümleri ile incelenmesi planlandı. Çalışmaya, uluslararası alp disiplini yarışmaları deneyimine sahip, 18-29 yaş aralığında 18 erkek sporcu gönüllü olarak katıldı. İlk olarak gönüllülerin vücut ağırlığı, boy uzunluğu, beden kitle indeksi ve MaxVO₂ değerleri tespit edildi. Daha sonra, gönüllülere uluslararası standartlara uygun olan yarışma pistinde, beş gün arayla 2.5 saat süren büyük slalom ve slalom antrenmanları uygulandı. Her iki antrenman uygulaması öncesinde ve sonrasında gönüllülerden venöz kan örnekleri alınarak TOS ve TAS değerleri tespit edildi. Hem slalom hem de büyük slalom sonrasında TOS değerlerinde anlamlı bir değişim gözlenmezken, TAS değerlerinde anlamlı bir artış tespit edildi (p<0.05). TAS değerlerinde anlamlı bir artış gözlenmesine rağmen TOS değerlerinde anlamlı bir artış gözlenmemesinin nedeni, artış gösteren antioksidan enzimlerin oksidan enzimlerdeki artışı baskılaması olabilir. Ayrıca, alp kayağı yarışma ve antrenman yüklenmelerinin hem kısa süreli olması hem de maksimal şiddetten ziyade orta ve yüksek şiddette gerçekleşmesi, antioksidan sistemin oksidan sisteme oranla daha fazla aktive olmasına neden olmuş olabilir.

Anahtar kelimeler: Total Oksidan Seviye, Total Antioksidan Seviye, Atletik Performans, Alp Kayağı.

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Introduction

Free radicals are too much proactive molecules with more than one unpaired electrons, short lifespan, and low molecular weight (Halliwell, 2006). The initiation of oxidative stress is the imbalance between the production of radical species and antioxidant defense systems, which can damage cellular biomolecules, including lipids, proteins and DNA. (Juan et al., 2021). Human organism guards itself against oxidative harms due to the enzymatic and non-enzymatic antioxidant systems (Halliwell, 2006). Oxidative stress causes considerable cell damages (Netzer et al., 2015). Furthermore, it is stated that free radicals can affect energy production (Yoshiko et al., 2024), muscle contractility (Powers et al., 2020), and eventually athletic performance (Thirupathi et al., 2021). Hypoxia and training are pointed out as the fundamental factors that disrupt the equilibrium of prooxidant and antioxidant (Williamson-Reisdorph et al., 2021).

The effects of Reactive Oxygen Species (ROS) in top-level athletes denote variables from metabolism of antioxidants, extreme uric acid release, formation of lipid peroxidation products, levels of autoantibodies that show changes against oxidized low-density lipoproteins to DNA damages (Mastaloudis et al., 2001; Subudhi et al., 2001). The effects aforespecified damage skeletal muscles and can therefore lead to adverse impacts on physical performance (Schippinger et al., 2009). The antioxidant defence mechanisms of many living beings have the ability to adapt to oxidants to which they are exposed chronically. The extent of oxidative stress that may occur during physical exercises is comprehended not only through production of free radicals, but also via the defensive capacities of antioxidants (Powers, 2016).

During alpine skiing, skiers are exposed to vibration and ischemic conditions may happen in active muscles due to the isometric contraction of muscles during the turning phase (Ferguson, 2010). As a result of high levels of muscle contraction during alpine skiing, therefore, intramuscular pressure increases (Sejersted et al., 1984), and such a situation restrains or even stops the muscle blood flow (Sjøgaard et al., 1988). Consequently, a decrease is observed in oxygen delivery to active muscles and in tissue oxygenation, and muscles are exposed to the ischemic conditions. In a study accomplished, a decrease has been reported in oxygen saturation of quadriceps muscle during giant slalom (GS) and slalom (SL) (Szmedra et al., 2001). All of the relevant circumstances may lead to the formation of oxidative stress during alpine ski training. In addition to these, the factors such as altitude-related hypobaric hypoxia, low ambient temperature, and increased UV exposure may have a role in oxidative stress for alpine skiers (Chao et al., 1999). Hypobaric hypoxia can cause the organism to produce excess free radicals, which can damage deoxyribonucleic acid (DNA), cell

membranes, and enzymes. This damage can negatively affect human performance (Li et al., 2024). Such an oxidative stress exposure may adversely influence the performance of athletes. (Schipfinger et al., 2009).

Being aware of the extent of the effects of these factors on the formation of oxidative stress during alpine skiing is crucial for both coaches and athletes. In the present study, it was planned, therefore, to examine the oxidant and antioxidant levels that may be observed during a single unit of GS and SL training routinely accomplished by alpine skiers, by measuring Total Oxidant Capacity (TOC) and Total Antioxidant Capacity (TAC).

Materials and Methods

Research Model

In the study, the results of two different training sessions applied to a single group were examined using the pre-test post-test experimental research model.

Population Sample / Research Group

The study was conducted together with 18 male volunteer athletes between the ages of 18 and 29, experienced in the international alpine skiing competitions. The criteria for inclusion in the study were determined as actively participating in international ski competitions, not having any health problems, and not using regular medication. All participants were informed about the purpose of the study and signed a voluntary consent form. Volunteers were asked not to participate in any physical activity for at least 48 hours before the study.

Data Collection Tools

Body weight, height, body mass index, and VO₂max values, about which the measurement procedures were submitted below, were determined accordingly. Next, the volunteers underwent Giant Slalom training and Slalom training with five days interval on the competition slope at Erciyes Ski Resort, which complies with the international standards.

5ml of blood was collected from each subject's antecubital vein 20 minutes before the giant slalom training in order to find out the total oxidant and antioxidant levels. Then, the volunteers performed a 2.5-hour giant slalom training on the giant slalom slope set up in compliance with the regulations imposed by the International Ski Federation (FIS) as detailed below. 5 minutes after finishing the giant slalom applied, 5 ml of blood was recollected from the antecubital veins of each volunteer in order to determine the total oxidant and antioxidant levels.

Then, the volunteers weren't allowed to practice any exercise for 5 days. Five days after, 5

ml of blood was collected from the antecubital vein 20 minutes before slalom training to determine the oxidant and antioxidant levels. Next, the volunteers performed a 2.5-hour slalom training on the slalom slope which was set up, as detailed below, in accordance with the regulations imposed by the International Ski Federation (FIS). 5 minutes after the volunteers completed slalom practices, 5 ml of blood was recollected from their antecubital veins to find out the total oxidant and antioxidant levels. The samples collected hereby were forwarded to the Department of Medical Biochemistry, Faculty of Medicine, Erciyes University to determine the TAC and TOC levels.

Giant Slalom Trainings and Slope

The giant slalom slope was established in compliance with the regulations imposed by FIS. The altitude difference of the slope was 300 meters, and 36 gates that correspond 12% of the altitude difference were set up. The distance between these gates was set to be 28 meters long. The training period lasted 2.5 hours.

Slalom Trainings and Slope

The slalom slope was set up in accordance with the regulations instituted by FIS. The altitude difference of the ski slope was 120 meters, and 42 gates, which correspond to 35% of the altitude difference, were prepared accordingly. The distance between horizontal gates was set to be 10 meters, and the distance between vertical gates was 5.5 meters. The training period lasted 2.5 hours.

Height, Body Weight, and Body Mass Index Measurement

Height measurement of the athletes was accomplished with bare feet, and their body weight was measured with bare feet and wearing only shorts and T-shirts. Height measurement was accomplished using a tape measure with a precision of 0.1cm. Body weight was measured using an electronic scale with a sensitivity of 0.1kg. Body mass index values were calculated, on the other hand, by dividing the body weight value by the square of height in meters (kg/m^2).

Aerobic Capacity Measurement

An exercise protocol of gradually increased intensity was applied in order to find the $\text{VO}_{2\text{max}}$ values of the athletes. The test was initiated with a running speed of 7 km/h on a 0% slope and afterwards the speed was increased by 1 km/h per minute, allowing the athletes to continue their practices till they were exhausted. Several criteria, accepted for reaching $\text{VO}_{2\text{max}}$, were that an athlete's to attain the maximal heart rate during the test (220-years), the ratio of expired carbon dioxide (VCO_2) and oxygen intake (VO_2) the respiratory exchange ratio (RER) expressed as instantaneous values to increase above 1.10, and oxygen intake which was remained at a plateau despite the increase in exercise intensity (Howley et al., 1995). The highest 15-second oxygen uptake

value, where at least two of the aforesaid criteria occurred simultaneously, was accepted as VO₂max (ml/kg/min). The exhaustion time was determined as the total duration of this test.

Total Oxidant Capacity (TOC) Measurement

The serum TOC levels were measured using the TOC kit, brand Rel Assay Diagnostics (Ref No: RL0024). The measurement method is based upon the oxidants in the sample which shall oxidate the ferrous-ion chelator complex in solution provided in kit to ferric ions. The oxidation reaction was extended by the “enhancer” molecules contained abundantly within the reaction medium. The density of the coloured complex formed by the resulted ferric ions with the chromogen in the acid medium was measured spectrophotometrically in proportion to the amount of oxidant in the medium. The method was calibrated with hydrogen peroxide and the result was expressed as the micromoles of hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Equiv./L).

Total Antioxidant Capacity (TAC) Measurement

The serum TAC levels were measured by means of TAC kit, brand Rel Assay Diagnostics (Ref No: RL0017). The measurement method is based on the principle that antioxidants in the sample shall reduce the dark blue-green ABTS radical to colourless ABTS form. The process of absorbance change measured at 660 nm was in correlation with the total antioxidant level of sample. The method was calibrated with a stable antioxidant standard solution containing Trolox (a water-soluble vitamin E analog). The results obtained were expressed as millimol Trolox equivalent per liter (mmol Trolox Equiv./L).

Data Evaluation

First, the descriptive statistical calculations of the data collected in the study were completed. Then, Shapiro-Wilk test, values of skewness and kurtosis, and graphs of histogram, Q-Q, and P-P were utilised to find whether the data were distributed normally, and it was determined hereby that the data did not indicate a normal distribution. Wilcoxon Signed Ranks Test was applied to compare the TAC and TOC values before and after the relevant training sessions. Significance level was accepted as $p < 0.05$.

Ethics of Research

Prior to starting the present study, the approval required was received from the Clinical Research Ethics Committee of the Erciyes University with date 20.01.2017 and decision number 2017/32. During the current research, the "Higher Education Institutions Scientific Research and Publication Ethics Directive" was followed. Furthermore, the study was endorsed by the Scientific Research Projects Coordination Unit of Erciyes University with Project No. TYL-2017- 7493.

Results

The values of age, height, body weight, BMI and VO₂max of the volunteers were given in Table 1 below. Average age of the volunteers was determined as 23.28 years, average height 176.11 cm, average weight 82.39 kg, average BMI 26.55 kg/m², and average VO₂max 50.46 ml/kg/min.

Table 1

Descriptive Information of the Volunteers Participated in The Study

	n	$\bar{X} \pm SD$	Minimum	Maximum
Age (years)		23,28 ± 5,33	18	29
Height (cm)		176,11 ± 4,39	170	184
Body Weight (kg)	18	82,39 ± 9,04	68	103
BMI (kg/m²)		26,55 ± 2,61	22,78	33,25
VO₂max (ml/kg/min)		50,46 ± 2,51	46,35	53,58

Table 2

Comparison of TOC Values Before and After Giant Slalom Training

	n	Median (25% - 75%)	z	p
Before Training (µmol)		7.94 (7.50 - 8.58)		
After Training (µmol)	18	8.42 (7.54 - 9.22)	- 0.893	0.372

Table 2 shows the TOC values of the volunteers before and after giant slalom training. While the median TOC values of the volunteers before training were 7.94 (7.50 - 8.58), the median TOC values obtained after training were 8.42 (7.54 - 9.22). The difference between TOC values acquired both before and after training was not statistically significant ($p > 0.05$).

Table 3

Comparison of TOC Values Before and After Slalom Training

	n	Median (25% - 75%)	z	p
Before Training (µmol)	18	6.88 (6.26 - 8.02)	-0.237	0.813

After Training (µmol) 7.14 (6.70 – 7.72)

Table 3 gives the TOC values of the volunteers before and after slalom training. The median TOC values of the volunteers prior to training were 6.88 (6.26 - 8.02), while the median TOC values after training were 7.14 (6.70 - 7.72). The difference between TOC values both before and after training was not statistically significant ($p>0.05$).

Table 4
Comparison of TAC Values Before and After Giant Slalom Training

	n	Median (25% - 75%)	z	p
Before Training (mmol)	18	1.37 (1.32 – 1.44)	-2.788	0.005*
After Training (mmol)		1.43 (1.37 – 1.55)		

* $p < 0,05$

Table 4 above provides the TAC values of the volunteers both before and after giant slalom training. While the median TAC values of the volunteers prior to training were 1.37 (1.32 - 1.44), the median TAC values after training were 1.43 (1.37 - 1.55). The difference between the TAC values before and after training was found to be significant ($p<0.05$).

Table 5
Comparison of TAC Values Before and After Slalom Training

	n	Median (25% - 75%)	z	p
Before Training (mmol)	18	1,34 (1,30 - 1,43)	-2,817	0,005*
After Training (mmol)		1,44 (1,33 - 1,69)		

* $p < 0,05$

Table 5 presents the TAC values of the volunteers before and after slalom training. While the median TAC values of the volunteers prior to training were 1.34 (1.30 - 1.43), the median TAC values after training were 1.44 (1.33 - 1.69). The difference between the TAC values before and after training was statistically significant ($p<0.05$).

Discussion and Conclusion

Although physical activities are known to have beneficial effects on health issues, many research results have revealed that some exercises cause oxidative stress by increasing the amount of

reactive oxygen. Even though exercise causes oxidative stress by increasing free oxygen radicals, it can also improve the defence mechanism against oxidative stress by increasing antioxidant enzyme activity (Meng and Su, 2024).

During alpine skiing trainings or competitions, some increases are realized in intramuscular pressure due to the need for contractions at high strength and long-term (Sejersted et al., 1984). Such a condition reduces or even halts the blood flow to the active muscles. Eventually, the amount of oxygen getting to the active muscles decreases and so tissue oxygenation is reduced, resulting with muscle ischemia (Ferguson, 2010). Upon completion of muscle contraction, meanwhile, perfusion is restored since the localized microvasculature is no longer compressed. This condition may increase the production of reactive oxygen species in skeletal muscles by inducing the activation of xanthine oxidase and NADPH oxidase, and electron leakage from the mitochondrial electron transport chain (Kalogeris et al., 2012). It has also been reported that the amount of oxidants and antioxidants to be released during exercises may vary due to the intensity of exercises (Radák et al., 2000).

Total Oxidant Capacity is a measurement that provides the total value of free oxygen radicals (Ghiselli et al., 2000). In the present study accomplished, although a slight increase was observed in TOC values of the athletes after both giant slalom and slalom training, the increase was not statistically significant ($p>0.05$). Such a condition may arise from suppression of oxidative enzymes to be resulted from antioxidant enzymes which are increased pretty much compared to oxidant enzymes during slalom and giant slalom training. However, it has been reported that while the damage-causing oxidant system is much more activated during acute exhaustive and high intensity exercises (Banerjee et al., 2003), regular and short-term non-maximal exercises far more activate the antioxidant systems (White et al., 2001). In measurements conducted on snow with alpine skiers, it was reported that athletes used 74.96% of their VO_{2max} during giant slalom (Polat, 2016). Spirk et al. (2012) reported, in another study, the relevant value as 73.5% during again giant slalom. The amount of blood lactate produced after giant slalom was found as 74% of the amount of blood lactate measured after the maximal test in laboratory (Polat, 2016). Spirk et al. (2012) reported furthermore that the amount of blood lactate accumulated after giant slalom was 69% of the values accumulated after maximal exercise test. The data aforespecified indicate that alpine skiing competitions and trainings involve short-term and high intensity loads, but not maximum level loads. The fact that no statistically significant difference was observed in TOC levels after both slalom and giant slalom training, but a significant difference was observed in TAC measurements in the present study may arise from the reasons mentioned above.

In our study, a significant increase was observed in TAC values after both giant slalom and slalom training ($p<0.05$). Total Antioxidant Capacity (TAC) is an assessment that submits the total

value of antioxidant status, which is the body's defence materials against free oxygen radicals, that is oxidants (Ghiselli et al., 2000). The antioxidant defence mechanisms can be enzymatic or non-enzymatic. Frequent encountered non-enzymatic antioxidants are vitamins A, C and E, glutathione, alpha lipoic acid, mixed carotenoids, coenzyme Q10, various bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), and cofactors such as folic acid, uric acid, albumin, and vitamins B1, B2, B6, and B12. Enzymatic antioxidants are consisted, on the other hand, of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), and glutathione reductase (GSH-RD) (Maritim and Sanders, 2003).

Subudhi et al. (2001) examined the oxidative stress markers in elite alpine skiers by performing 4 days of land training, followed by one day off, and then 5 days of ski training at 2200m to 2900m altitude. While maximal and near to maximal loadings could be realized during the land training performed in the study, medium and high intensity loads, which were not at maximal intensity due to the nature of skiing, could be applied in ski training. At the end of study, it was reported that the lipid hydroperoxide (LOOH) values after ski training were significantly lower than the values after land training ($p<0.05$) and the oxidative stress level after ski training was less than that after land training. They reported, meanwhile, a statistically significant change in antioxidant markers TEAC, UA, α -tocopherol, γ -tocopherol, and SOD values after ski training sessions ($p<0.05$). Schippinger et al. (2009) reported in a study conducted with distinguished alpine skiers that peroxide values did not change significantly after a single race ($p>0.05$), but there was a significant increase in uric acid values ($p<0.05$) and a significant change in the level of γ -tocopherol, one of the antioxidant markers ($p<0.05$). The results obtained in the aforesaid studies demonstrate similarities with the results acquired in the present study.

As a result, a significant increase was observed in TAC values after both giant slalom and slalom training in the present study. Although an increase was observed, meanwhile, in TOC values, it was not statistically significant. The reasons for the lack of significant increase in TOC values are considered specifically to be the significantly increased antioxidant enzymes which suppress any increase in oxidant enzymes. In addition, since the loads of alpine ski competition and training are both short-term and occur at moderate and high intensities rather than maximal intensity, the rate of increases realized in antioxidant system may have been higher than the rate of increase in the oxidant system due to the reasons discussed in the text.

Ethics Committee Approval Information

Ethics Committee: Erciyes University Clinical Research Ethics Committee

Date of Ethical Approval: 20.01.2017

Approval Number: 2017/32

Authors' Contribution Statement

The hypothesis and study design were determined by all authors. Duran Demiryürek and Metin Polat conducted the training implementations and collected blood samples with the help of healthcare personnel. The TAS and TOS measurements from the blood samples were performed by İnayet Güntürk. The statistical analysis and reporting of the research were carried out by Metin Polat.

Conflict of Interest Statement

The authors declare no conflict of interest related to this research.

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