Progress in Nutrition 2022; Vol. 24, N. 3: e2022103 DOI: 10.23751/pn.v24i4.13273

Original article

Determination of Oxidative Stress Level and Antioxidant Enzyme Activities in Biathlon Athletes and Sedentary Athletes

Mürşit Ceyhun Birinci
Ondokuz Mayıs University, Yaşar Doğu Faculty of Sport Science, Department of Coaching Education, Samsun, Turkey

Abstract. *Study Objectives:* This study aimed to compare oxidative stress levels and enzyme activities in biathlon skiers and sedentary athletes. *Methods:* A total of 30 participants, 15 biathlon athletes, and 15 sedentary athletes, were included in the study. The athletes were not subjected to a training program but were asked to refrain from eating or drinking after 10:00 PM the day before the samples were taken. Blood samples were taken from the participants after they were informed about the tests. SOD, CAT, and GPx enzyme activities, which are among the lipid peroxidation products, MDA, and antioxidant enzymes, were evaluated spectrophotometrically. *Results:* It was showed that SOD ($p<0.001$), CAT ($p<0.01$), and GPx ($p<0.001$) enzyme activities were higher in the biathlon athletes compared to those of the sedentary athletes and that the level of MDA, an important lipid peroxidation product, was lower in the biathlon group than in the sedentary group ($p<0.001$). *Conclusion:* As a result, SOD, CAT, and GPx enzyme activities, which are among the lipid peroxidation products of MDA and antioxidant enzymes, were evaluated spectrophotometrically. It was determined that MDA level, which is among the important lipid peroxidation products, decreased in the biathlon group and that SOD, CAT, and GPx enzyme activities were increased in the biathlon group compared to the sedentary group.

Key words: antioxidants, biathlon, CAT, GPX, MDA, SOD

Introduction

The biathlon, an endurance sport that requires a high level of concentration, is an Olympic winter competition that combines cross-country skiing and rifle shooting (1, 2). High performance in this sport depends on skiing speed, shooting accuracy, and minimizing the time spent shooting (1-3). In addition, this tough endurance sport requires switching between various sub-techniques that require different relative degrees of the use of the upper and/or lower body while skiing on changing terrains. In addition to performance values and physiological parameters, a significant improvement has been observed in the quality of athletic performances in worldwide biathlon competitions, particularly in terms of biomechanical and motor control factors, such as postural control, rifle stability, shoulder strength, triggering or aiming strategies (4-10). It is known that certain enzymes, like MDA, SOD, CAT, and GPX, which are among the antioxidants important for metabolism, are affected by free radicals. In the light of this information, this study aimed to determine and compare the oxidative stress levels and antioxidant enzyme activities in biathlon athletes and sedentary athletes.

Materials and Method

The study included 30 athletes, 15 biathlon athletes, and 15 sedentary athletes. The athletes were

not subjected to a training program, and measurements were taken at rest after the participants were informed about the tests. The participants were asked to refrain from eating or drinking after 10:00 PM the day before the samples were taken. The blood samples were taken in the morning between 9:00 and 10:00 AM. A 5 ml blood (fasting) sample was taken from the forearm veins and placed into biochemistry tubes that were centrifuged at 5000 rpm for 10 minutes. The obtained serum samples were then placed into Eppendorf tubes and stored at -80 degrees until biochemical analysis. The blood samples were centrifuged, and their serums were separated in the biochemistry laboratory of the Faculty of Science at Yüzüncü Yıl University. Next, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), enzyme activity levels were determined spectrophotometrically in the biochemistry laboratory of Van Yüzüncü Yıl University, Faculty of Science, Department of Chemistry.

Determination of malondialdehyde (MDA) level

The level of malondialdehyde, a lipid peroxidation product formed as a result of the reaction of fatty acids with free radicals, was determined using a spectrophotometer at 532 nm. Results were expressed in μmol/L (11).

Determination of superoxide dismutase (SOD) level

Determination of superoxide dismutase enzyme activity was performed according to the method developed by Williams et al. The enzyme activity values were calculated by recording the enzyme activity changes at 505 nm. Results were expressed in U/m1 (12).

Measurement of catalase (CAT) enzyme activity

CAT enzyme activity levels were determined according to the method developed by Aebi et al. Serum samples were treated with Tris and HCl at pH 7.4. The absorbance change of the enzyme in the serum samples was measured at 240 nm with a spectrophotometer. Results were expressed in U/m1 (13).

Determination of Glutathione peroxidase (GPx) level

GPx levels were determined according to the method developed by Paglia and Valentine. Following the standard principle, the enzyme activity was calculated by measuring the change in absorbance at 340 nm with the decrease of NADPH. Results were expressed in U/L (14).

Statistical Analysis

Descriptive statistics were expressed as mean, standard deviation, and minimum and maximum values. The student t-test was used for the comparison of groups. The statistical significance level was taken as 5% in the calculations, and the SPSS 11th Statistical Package Program was used to perform the calculations.

Results

SOD, CAT, and GPX enzyme activities, which are among the lipid peroxidation products, MDA, and antioxidant enzymes, were evaluated spectrophotometrically. Evaluation of the results showed that the level of MDA, an important lipid peroxidation product, was lower in the biathlon group compared to that of the sedentary group ($p<0.001$) (Figure 1). SOD ($p<0.001$) (Figure 2), CAT ($p<0.01$) (Figure 3), and GPx ($p<0.001$) (Figure 4) enzyme activities were found to be higher in the biathlon group compared to those of the sedentary group.

Discussion

The current study found that the MDA levels in biathlon athletes were significantly lower than those in the sedentary group ($p<0.001$) (Figure 4). Previous studies have shown that MDA levels were higher in some diseases and lower in healthy individuals (17-19). Algül et al. (2018) reported low MDA levels in those who exercised (20). Results from previous studies on this subject generally support those found in the present study. SOD is an important enzyme that scavenges peroxide anion radicals and prevents lipid peroxidation

Table 1. Antioxidant Enzyme Levels of Sedentary Athletes and Biathlon Athletes

Parameters	Sedentary	Biathlon	p
MDA (µmol/L)	40.72 ± 6.21	13.89 ± 3.96	***
SOD (U/m1)	645.65 ± 306.62	1552.20 ± 327.21	***
CAT (U/m1)	1.12 ± 0.92	1.92 ± 1.00	**
GPx (U/L)	29.93 ± 5.61	45.09 ± 5.54	***

The data are expressed as mean ± standard deviation ** p<0.01. *** p<0.001

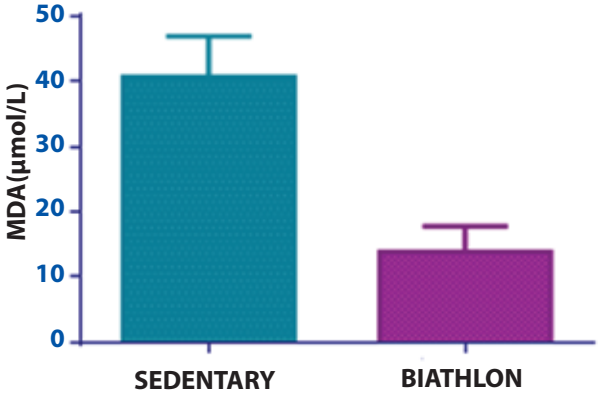

Figure 1. MDA levels of groups (*** p<0.001)

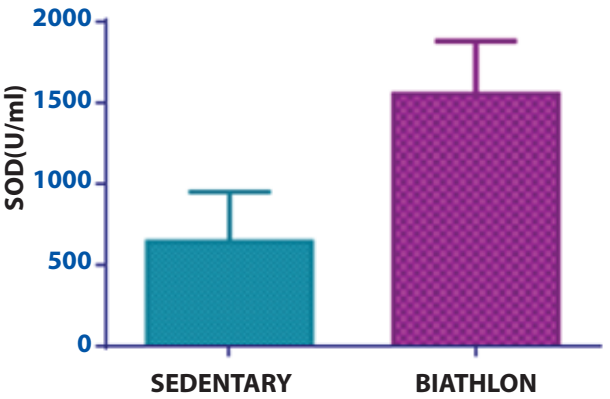

Figure 2. SOD levels of groups (*** p<0.001)

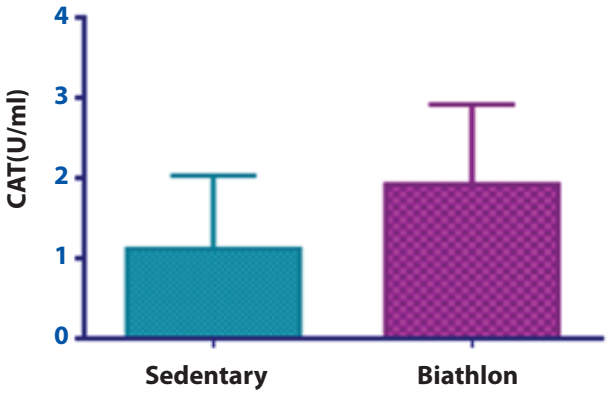

Figure 3. CAT levels of groups (** p<0.01)

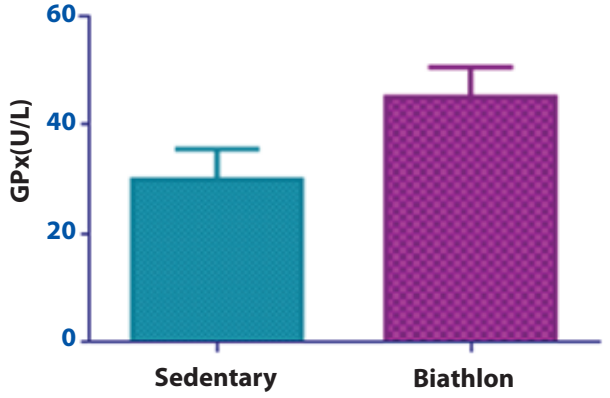

Figure 4. GPx levels of groups (*** p<0.001)

due to free radicals (15-21). The present study found that SOD (p<0.001) enzyme activities were higher in the sedentary group compared to those of the biathlon group (Figure 2). Yavuz et al. (2018), in their study, observed higher SOD enzyme activity in the patient group compared to that of the control group (22), a finding consistent with that found in the present study. CAT is among the important enzymes that prevent free radical accumulation and lipid peroxidation (16). Catalase is an enzyme that can be used for common and analytical purposes as a compound of hydrogen peroxide or glucose biosensors to remove hydrogen

peroxide, or as an oxidizer or bleach for sterilization purposes (23). Evaluation of CAT ($p<0.01$) enzyme activity in the present study showed that it was higher in the biathlon group compared to that of the sedentary group (Figure 3). Kaplan et al. (2013) reported in their study on antioxidant enzyme levels and the effect of exercise on syndrome X and the slow coronary flow phenomenon that exercise increased CAT enzyme activity (24). In the study by Kıyıcı et al. (2010) investigating blood antioxidant levels after speed exercises in alpine skiers, it was reported that CAT enzyme activity levels increased after exercise (25). The related findings reported in the literature support the results of the present study. GSH-Px plays an important role in cell defense against reactive oxygen species (15) It was found in the present study that GPx ($p<0.001$) (Figure 4) enzyme activities were higher in the biathlon group than in the sedentary group. Li et al. (2012) determined in their study on the effects of salidroside on extensive exercise-induced oxidative stress in rats that GSH-Px enzyme activities increased in the rats after exercise, a finding that supports the results observed in the present study (26).

Conclusion

As a result, SOD, CAT, and GPX enzyme activities, which are among the lipid peroxidation products, MDA, and antioxidant enzymes, were evaluated spectrophotometrically. It was determined that the level of MDA, an important lipid peroxidation product, was lower in the biathlon group compared to the sedentary group and that SOD, CAT, and GPx enzyme activities were higher in the biathlon group than in the sedentary group.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethical Approval: For this study, voluntary informed consent was obtained from the athletes prior to their participation in the study. Ethical approval to perform the study was obtained from the Siirt University Non-Invasive Clinical Research Ethics Committee (dated 08.01.2021; numbered 438).

References

1. Ateş, B. The Evaluation of Somatotype, Body Composition and Motoric Characteristics of Turkish National Biathlon Team. Celal Bayar Üniversitesi Beden Eğitimi ve Spor Bilimleri Dergisi / Celal Bayar University Journal of Physical Education and Sport Science 2017; 12 (2), 108-116. Retrieved from https://dergipark.org.tr/tr/pub/cbubesbd/issue/33059/338004.
2. Carlson, R. Talent Detection and Competitive Progress in Biathlon - A National Example. Polish Journal of Sport and Tourism 2012; 18(4) 290-295. https://doi.org/10.2478/v10197-011-0023-5.
3. Hoffman MD, Gilson PM, Westenburg TM, Spencer WA. Biathlon shooting performance after exercise of different intensities. Int J Sports Med 1992 Apr;13(3):270-3. doi: 10.1055/s-2007-1021265. PMID: 1601564.
4. Rundell KW., Bacharach DW. Physiological characteristics and performance of top U.S. biathletes. Med Sci Sports Exerc 1995; 27(9), 1302–1310.
5. Stöggl T, Bishop P, Höök M, Willis S, Holmberg HC. Effect of carrying a rifle on physiology and biomechanical responses in biathletes. Med Sci Sports Exerc 2015 Mar;47(3):617-24. doi: 10.1249/MSS.0000000000000438. PMID: 25003775.
6. Laaksonen MS, Andersson E, Jonsson Kårström M, Lindblom H, McGawley K. Laboratory-Based Factors Predicting Skiing Performance in Female and Male Biathletes. Front Sports Act Living 2020 Aug 5;2:99. doi: 10.3389/fspor.2020.00099. PMID: 33345089; PMCID: PMC7739653.
7. Groslambert A, Candau R, Hoffman MD, Bardy B, Rouillon JD. Validation of simple tests of biathlon shooting ability. Int J Sports Med 1999 Apr;20(3):179-82. doi: 10.1055/s-1999-970286. PMID: 10333095.
8. Baca A, Kornfeind P. Stability analysis of motion patterns in biathlon shooting. Hum Mov Sci 2012 Apr;31(2):295-302. doi: 10.1016/j.humov.2010.05.008. Epub 2010 Aug 2. PMID: 20675002.
9. Heinrich A, Stoll O, Cañal-Bruland R. A Biopsychosocial Framework to Guide Interdisciplinary Research on Biathlon Performance. Front Psychol 2021 Apr 29;12:671901. doi: 10.3389/fpsyg.2021.671901. PMID: 33995230; PMCID: PMC8116492.
10. Pelin F., Lungociu I., Pelin Gh. Antrenamentul de tir pentru biathlon / Biathlon shooting training. Bucureşti: Printech, 2007.
11. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995 Dec;41(12 Pt 2):1819-28. PMID: 7497639.
12. Woolliams JA, Wiener G, Anderson PH, McMurray CH. Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Res Vet Sci 1983 May;34(3):253-6. PMID: 6878874.

13. Aebi H. Catalase in vitro. Methods Enzymol 1984;105: 121-6. doi: 10.1016/s0076-6879(84)05016-3. PMID: 6727660.
14. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967 Jul;70(1):158-69. PMID: 6066618.
15. Akkoyun HT., Bengü AŞ., Ulucan A., Bayramoğlu-Akkoyun M., Arıhan O. Protective Effect of Ellagic Acid Against Carbon Tetrachloride (Ccl4) - Induced Oxidative Braın Injury In Rats. Fresenius Environmental Bulletin 2018; 27(5), 3148-3155.
16. Akkoyun HT., Bengü AŞ., Ulucan A., Bayramoğlu-Akkoyun M., Arıhan O. Protectıve Effect Of Astaxanthın Agaınst Alumınum Induced Lıver Oxıdatıve Damage. Fresenius Environmental Bulletin 2017; 26(10), 6159-6165.
17. Asker S, Asker M, Sarikaya E, Sunnetcioglu A, Aslan M, Demir H. Oxidative stress parameters and their correlation with clinical, metabolic and polysomnographic parameters in severe obstructive sleep apnea syndrome. Int J Clin Exp Med 2015 Jul 15;8(7):11449-55. PMID: 26379962; PMCID: PMC4565345.
18. Demir CY, Kocak OF, Bozan N, Ersoz ME, Demir H. Is There a Role for Oxidative Stress in Temporomandibular Joint Disorders? J Oral Maxillofac Surg 2018 Mar;76(3):515-520. doi: 10.1016/j.joms.2017.11.004. Epub 2017 Nov 21. PMID: 29216476.https://doi.org/10.1016/j.joms.2017.11.004.
19. Radak Z., Chung HY., Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 2008; 44(2), 153–159. https://doi.org/10.1016/j.freeradbiomed.2007.01.029.
20. Algül S., Ugras S., Kara M. Comparative Evaluation of MDA Levels During Aerobic Exercise in Young Trained and Sedentary Male Subjects. Eastern J Med 2018; 23(2), 98-101. https://doi.org/10.5505/ejm.2018.40469.
21. Escobar JA, Rubio MA, Lissi EA. Sod and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic Biol Med 1996;20(3):285-90. doi: 10.1016/0891-5849(95)02037-3. PMID: 8720898.
22. Yavuz, İ. H., Özaydın Yavuz, G., Güneş Bilgili, S., Demir, H., & Demir, C. Deri kanseri hastalarının serum oksidatif stres parametrelerinin değerlendirilmesi / Evaluation of Serum Oxidative Stress Parameters of Skin Cancer Patients. Bozok Tıp Dergisi 2018; 8 (4), 134-138. DOI: 10.16919/bozoktip.452100.
23. Alptekin, Ö., Yıldırım, D., Özyılmaz G., & Tokel, S., Florosile immobilize edilmiş katalaz enziminin immobilizasyon koşullarının optimizasyonu / Optimization of immobilization conditions of fluorosylated immobilized catalase enzyme, XVIII. National Chemistry Congress, proceeding book, S. 498, Kars, 2004.
24. Kaplan O, Meriç M, Acar Z, et al. The effect of exercise and antioxidant enzyme levels in syndrome X and coronary slow flow phenomenon: an observational study. Anadolu Kardiyol Derg 2013;13(7):641-6. doi: 10.5152/akd.2013.186. Epub 2013 Jul 31. PMID: 23912786.
25. Kıyıcı, F. & Kishalı, N. F. Alp disiplini kayakçılarında sürat egzersizleri sonrası kan antioksidan düzeylerinin incelenmesi / Assessment of some antioxidant levels in alpineskiers after sprint exercises. Beden Eğitimi ve Spor Bilimleri Dergisi, 2011; 12 (1), 0-0. Retrieved from https://dergipark.org.tr/tr/pub/ataunibesyo/issue/28841/308553.
26. Xu, J., Li, Y. Effects of salidroside on exhaustive exercise-induced oxidative stress in rats. Mol Med Rep. 2012; 6(5), 1195–1198. https://doi.org/10.3892/mmr.2012.1060

Correspondence:
M. Ceyhun Birinci
Yaşar Doğu Sport Science Faculty, Ondokuz Mayıs University, Samsun, Turkey
E-mail: ceyhun.birinci@hotmail.com.tr