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The Effect of Dark Chocolate on Oxidative Stress Parameters After High-Intensity Kickboxing Training

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Abstract

In our study, the impact of dark chocolate on oxidant and antioxidant parameters was examined. Dark chocolate intake has increased as a result of its beneficial benefits on human health. Twenty male kickboxers in the study. On the first day blood samples were collected. Then, two groups of 10 people were randomly divided into two groups. Only one of the groups received chocolate. At 18:00 at night, blood samples were collected and stored at -80 °C. To determine oxidant stress; malondialdehyde (MDA), to determine antioxidant capacity; Superoxide dismutase activity (SOD), glutathione peroxidase (GPx) and total antioxidant capacity (TAC) values were measured. After it was determined that the data showed normal distribution, the paired t test was performed. According to the Groups (Control Grup or Chocolate Receiving Group) the changes before and after the training were examined. When examined, it is seen that there is no significant difference in the GPx, MDA and TAC values of the oxidant and antioxidant parameters of the athletes in both groups before and after training. However, a statistically significant change was found in the SOD value of the athletes in the chocolate receiving group before training ($p<0.05$). In control Group, a statistically significant change was observed between "before and after training" in terms of "GPx and TAC" parameters ($p<0.05$). Our results showed that dark chocolate consumed acutely before intense exercise is important in terms of showing that it can prevent the increase of oxidative stress markers.
Keywords: Exercise, Dark chocolate, Oxidative stress, Antioxidant defense, Kick boxing

Yüksek Yoğunluklu Kickboks Antrenmanı Sonrası Bitter Çikolatanın Oksidatif Stres Parametrelerine Etkisi

Öz

Çalışmamızda, insan sağlığı üzerindeki olumlu etkileri nedeniyle tüketimi artan bitter çikolatanın oksidan ve antioksidan parametreler üzerindeki etkisi araştırılmıştır. Çalışmaya, milli takım kampına katılan 20 erkek kick boks sporcusu dahil edilmiştir. Kampın ilk günü tüm sporcuların boy ve kilo ölçümleri ile 10 cc venöz kan örnekleri alındı. Ölçümler alındıktan sonra sporcular, çikolata alan grup (ÇK) ve çikolata almayan kontrol grubu (KG) olmak üzere rastgele 10 kişilik 2 gruba ayrıldı. ÇK grubuna antrenman öncesi sabah saat 09:00'da %80 kakao içeren 40 gram siyah çikolata (SÇ) yedirildi. Tüm sporcular sabah ve öğleden sonra her biri 1 saat süren yüksek yoğunlukta kick boks antrenmanı yaptı. Akşam saat 18:00'de tekrar 10 cc kan örnekleri alınarak santrifüj edildi ve serumları ayrıştırılarak -80 °C'de saklandı. Oksidan stressi belirlemek için; malondialdehit (MDA), antioksidan kapasiteyi belirlemek için; superoksitdismutaz (SOD) aktivitesi, glutatyon peroksidaz (GSPH) ve total antioxidant kapasite (TAK) değerleri spektrofotometrik yöntem ile ölçüldü. Verilerin normal dağılım gösterdiği belirlendikten sonra eşleştirilmiş t testi yapıldı. Gruplara göre (Kontrol Grubu veya Çikolata Alan Grup) eğitim öncesi, sonrası değişimler incelenmiştir. İncelendiğinde her iki gruptaki sporcuların oksidan ve antioksidan parametrelerinin GPx, MDA ve TAC değerlerinde antrenman öncesi ve sonrası anlamlı fark olmadığı görülmektedir. Ancak çikolata alan gruptaki sporcuların antrenman öncesi SOD değerlerinde istatistiksel olarak anlamlı bir değişiklik bulundu ($p<0,05$). Oksidan ve antioksidan belirteçlerdeki değişimler "antrenman öncesi ve sonrası" gruplarda incelenmiştir. Kontrol Grubunda "GPx ve TAC" parametrelerinde "antrenman öncesi ve sonrası" arasında istatistiksel olarak anlamlı değişim gözlemlendi ($p<0.05$). Sonuçlarımız yoğun egzersiz öncesi akut olarak tüketilen bitter çikolatanın oksidatif stres belirteçlerinin yükselmesini engelleyebileceğini göstermiştir.
Anahtar kelimeler: Egzersiz, Siyah çikolata, Oksidatif stres, Antioksidan savunma, Kick boks

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INTRODUCTION

The interest in kickboxing is increasing worldwide due to its beneficial effects such as personal protection, increasing muscle strength and keeping the body in shape (Zazryn et al., 2003). Kickboxers need a high anaerobic threshold value during the competition, as kickboxing is a high - intensity sports activity, in which strong kicks are made with fast and hard techniques during the competition (Davis et al., 2014; El-Ashker & Nasr, 2012; Smith, 2006). Generally, oxidative phosphorylation is used as the dominant energy source in low-intensity movements in ring sports, while it is stated that ATP-PC (phosphagen system) and glycolysis provide energy for the explosion of defense and offensive movements (Beneke et al., 2004). By products of anaerobic glycolysis, namely H+, have detrimental effects on athletic performance therefore, reduction or neutralization of these by-products can improve performance and time to extinction (Chaabène et al., 2014).

In branches where high-level performance is expected such as kickboxing, the recovery process between training significantly affects the performance of the athletes during training and competition. The most important condition of being the winner in ring sports is to be able to train regularly, effectively, and efficiently. Various mechanisms cause the formation of ROS in skeletal muscles in response to intense exercise in sports such as kickboxing. Among them, NADPH oxidase-induced ROS generation can cause changes in the redox state of the muscles, which can lead to contractile muscle dysfunction, accelerated muscle fatigue, longer recovery time, and reduced exercise performance (Powers et al., 2011). Exercise can increase oxygen consumption (VO_2) up to 20 times resting values. In the mitochondria in muscle cells, this means 200 times greater use of oxygen followed by the production of large amounts of ROS (Peternelj & Coombes, 2011). ROS produced in this way can cause oxidative damage to mitochondria and muscle contraction proteins, and subsequently result in direct induction of muscle damage and fatigue after exercise (Peake et al., 2017). Increasing evidence of exercise-induced oxidative damage and impaired athlete performance has spurred extensive research on the evaluation of muscle protection by antioxidant supplementation in exercisers (Pyne et al., 2000).

Many studies have identified the potential antioxidant effect of polyphenols, a large group of natural compounds found in foods and beverages (Banerjee et al., 2003). It has been shown that the use of antioxidant supplements such as cocoa, dark chocolate and ocoa extract can reduce oxidative stress and muscle damage caused by exercise for athletes, as it is a food rich in polyphenols (González-Garrido et al., 2017). Cocoa, which is the main component of chocolate, contains polyphenols as well as important oils such as oleic acid, palmitic acid, stearic acid, cocoa butter (Rusconi et al., 2010). In general, the best characterized biological property of polyphenols is to scavenge ROS or inhibit enzymes involved in ROS production. They also increase natural antioxidant defenses, but do not act as antioxidants (Forte et al., 2016). Loffredo et al., 2016). Elena et al., (2018) studies have shown that dark chocolate, a dietary supplement rich in polyphenols, reduces exercise-induced oxidative stress and biomarkers of muscle injury in professional football players (Elena et al., 2018). The studies of José et al., (2017) on the other hand, showed that cocoa consumption causes a change in the redox state even in the presence of an adverse environment or physical stress factors; therefore,

it has been revealed that cocoa has a protective role and can reduce oxidative damage (José et al.,2017). Increasing evidence suggests that cocoa in the form of cocoa bean extract, chocolate drinks, chocolate bars or dark chocolate blocks has beneficial effects on the cardiovascular system (Pucciarelli, 2013). In addition, dietary flavanols found in natural cocoa powder have gained attention for their potential to reduce oxidative stress and aid muscle recovery after exercise (Mcbrier et al., 2010; Sathyapalan et al., 2010). Dietary recommendations for individuals who exercise intensely, such as in kickboxing, should emphasize a balanced diet or consumption of natural antioxidant-rich foods such as cocoa and chocolate, rather than taking antioxidant supplements. This "nutraceutical strategy" is increasingly recommended as a potentially viable tool to prevent or reduce oxidative stress and muscle damage during intense physical training. In addition to being especially high-energy foods, it is stated that chocolate, cocoa and cocoa products are a rich source of antioxidant, anti-inflammatory and metabolic properties and antioxidant polyphenols with proven health-promoting effects (Magrone et al., 2017).

In this study, the effects of dark chocolate, whose consumption has increased in recent years due to its positive effects on human health, on oxidant stress parameters were investigated. There are limited studies on the effect of dark chocolate on anaerobic exercises that require explosive power. For this reason, it is aimed to reveal the effect of dark chocolate on oxidant and antioxidant parameters that may develop due to high-intensity kickboxing training.

METHOD

Research Model

This research is a study conducted in an experimental design with pre-test post-test paired control group.

Research Groups

Study group was formed from kickboxers in the National Team camp. Kickboxers originated from athletes with comparable physical characteristics and ages. The study included 20 male athletes with mean ages of 22.3 1.4 years, heights of 181.4 1.6 cm, and weights of 78.6 1.8 kg for the chocolate receiving group (RG) and 23.4 1.3 years, heights of 179.6 1.2 cm, and weights of 79.1 1.7 kg (mean SD) for the control group (CG). Those with disability, those who use nutritional support supplements and those who are afraid of giving blood were excluded from the study.

Ethical Approval

Ethics committee approval was obtained for the study from the Ethics Committee Clinical Research of Erciyes University with the number 2017/456. The Declaration of Helsinki was respected in the study. After the necessary information was given to all volunteers during the research process, their written consent was obtained.

Data Collection Tools

Height and weight measurements of all athletes were taken on the first day of the camp. To determine oxidant stress, malondialdehyde (MDA), to determine antioxidant capacity,

Superoxide dismutase activity (SOD), glutathione peroxidase (GPx) and total antioxidant capacity (TAC) values were measured by spectrophotometric method.

Collection of Data

Athletes were randomly divided into 2 groups of 10 people as Chocolate Receiving Group (RG) and Control Group (CG). While the RG group was fed 40 grams of dark chocolate (DC) containing %80 cocoa at 09:00 in the morning before the training, the CG was not given chocolate. Due to the fact that the athletes trained twice daily and because the recommended dosage in the literature was 40 grams, the athletes received this amount of chocolate (Massaro et al., 2019). All the athletes did high-intensity kickboxing training for 1 hour each in the morning and afternoon. No food supplement was given at breakfast, lunch and dinner, except standard food and water. At 18:00 in the evening, 10 cc blood samples were taken again, centrifuged, and the serums were separated and stored at -80 °C. Blood samples were taken by health professionals, kept in the cold and brought to Erciyes University, Sports Medicine laboratory.

Analysis of Data

In calculating the sample size of our study, Power was determined by taking at least 0.80 and Type 1 Error 0.05 for each variable. The Shapiro-Wilk test ($n<50$) was used to determine whether the means of continuous variables in the study were normally distributed. The Mann-Whitney U test was used to compare the variation between blood samples according to groups (who took chocolate and those who did not). The changes in the blood samples before and after the training were analyzed with the Wilcoxon Test, separately in the groups. The statistical significance level was taken as (α) %5 in the calculations and the SPSS (IBM SPSS for Windows, ver.24) statistical package program was used for the calculations.

RESULTS

The characteristics of the 20 kick box athletes participating in the study were examined and has also been given Table.1.

Table 1. Descriptive statistics of kick boxers

	Chocolate Receiving Group	**Control Group**
Variables	**X±S**	**X±S**
Age	22,3 ± 1,4	23,4 ± 1,3
Height	181,4 ± 1,6	179.6± 1,2
Weight	78,6 ± 1,8	79,1 ± 1,7

When examined Table.1., it was seen that the age of the athletes who were given chocolate was 22.3, height was 181.4 and weight was 78.6, while in the control group, the average age 23.4, and height was 179.6, kilograms was 79.1. The results analyzed with the Whitney-U test has also been given Table.2.

Table 2. Intergroups comparison GPx, MDA, SOD, TAC values of group before and after training

	GROUP								
	Control Group				Chocolate Receiving				
Variables	**Med.**	**X±S**	**Min.**	**Max.**	**Med.**	**X±S**	**Min.**	**Max.**	**p**
GPx(BT)	69,47	68,23±12,28	48,59	85,13	76,43	76,72±20,19	49,46	115,59	,314
GPx(AT)	107,76	97,81±19,97	65,99	116,46	86,00	80,59±22,30	51,20	119,07	,266
MDA (BT)	3,81	5,07±2,34	3,10	8,76	4,87	6,52±4,38	3,10	17,43	,457
MDA (AT)	4,34	4,41±,93	3,45	6,11	4,69	5,30±1,81	3,63	9,12	,288
SOD (BT)	15,09	14,68±1,43	12,50	15,95	12,36	12,59±5,25	4,96	24,70	,039*
SOD (AT)	14,37	15,83±4,74	10,64	25,13	14,80	15,96±4,94	10,35	24,84	,791
TAC (BT)	,51	,50±,06	,43	,57	,50	,49±,07	,35	,57	,560
TAC (AT)	,56	,56±,05	,49	,64	,57	,58±,06	,50	,72	,153

** Mann-Whitney Test, *p<.05 BT(Before Training), AT(After Training)*

(MDA BT: malondialdehyde before training ; MDA AT: malondialdehyde after training; SOD BT:superoxide dismutase activity before training , SOD AT:superoxide dismutase activity after training; GPxBT :glutathione peroxidase before trainig; GPxAT :glutathione peroxidase after trainig ;TAC BT:total antioxidant capacity before training; TAC AT:total antioxidant capacity after training)

In the table above, the comparison results of the blood parameters according to the Groups (Control Grup or Chocolate Receiving Group) are given. Here, the changes before and after the training according to the groups were examined. Table.2. When examined, it is seen that there is no significant difference in the GPx, MDA and TAC values of the oxidant and antioxidant parameters of the athletes in both groups before and after training. However, a statistically significant change was found in the SOD value of the athletes in the chocolate receiving group before training ($p<0.05$).

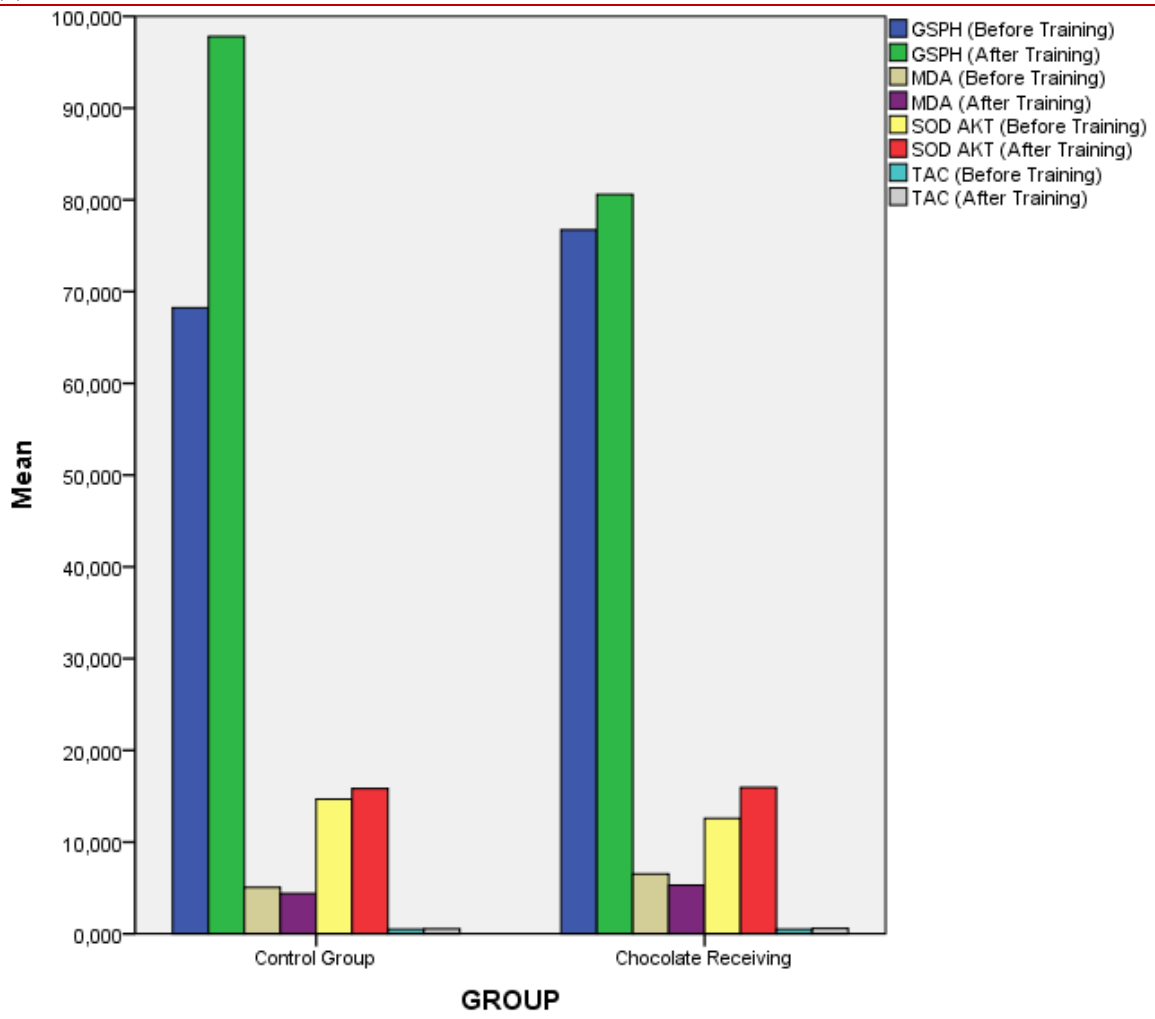
Table 3. In-groups pre-test post -test GPx, MDA, SOD, TAC values of the groups at the before and after training

	GROUP									
	Control Group					Chocolate Receiving				
Variables	**Med.**	**X±S**	**Min.**	**Max.**	**p**	**Med.**	**X±S**	**Min.**	**Max.**	**p**
GPx(BT)	69,47	68,23±12,28	48,59	85,13	,028*	76,43	76,72±20,19	49,46	115,59	,635
GPx(AT)	107,76	97,81±19,97	65,99	116,46		86,00	80,59±22,30	51,20	119,07	
MDA (BT)	3,81	5,07±2,34	3,10	8,76	,612	4,87	6,52±4,38	3,10	17,43	,953
MDA (AT)	4,34	4,41±,93	3,45	6,11		4,69	5,30±1,81	3,63	9,12	
SOD (BT)	15,09	14,68±1,43	12,50	15,95	,735	12,36	12,59±5,25	4,96	24,70	,173
SOD (AT)	14,37	15,83±4,74	10,64	25,13		14,80	15,96±4,94	10,35	24,84	
TAC (BT)	,51	,50±,06	,43	,57		,50	,49±,07	,35	,57	,028*
TAC (AT)	,56	,56±,05	,49	,64	,046*	,57	,58±,06	,50	,72	

** Wilcoxon Test, *p<.05 BT(Before Training), AT(After Training)*

(MDA BT: malondialdehyde before training; MDA AT: malondialdehyde after training; SOD BT:superoxide dismutase activity before training , SOD AT:superoxide dismutase activity after training; GPxBT :glutathione peroxidase before trainig; GPxAT :glutathione peroxidase after trainig ;TAC BT:total antioxidant capacity before training; TAC AT:total antioxidant capacity after training)

In the table above, the changes in oxidant and antioxidant markers "before and after training" were examined separately in the groups. According to this; In the Control Group, a statistically significant (significant) change was observed between "before and after training" in terms of "GPx and TAC" parameters ($p<0.05$). Similarly, the difference between "before and after training" was statistically significant in terms of "TAC" parameter in the "chocolate group" ($p<0.05$)



Graph 1. GPx, MDA, SOD, TAC values of the groups at the before and after training

(MDA BT: malondialdehyde before training ; MDA AT: malondialdehyde after training; SOD BT:superoxide dismutase activity before training , SOD AT:superoxide dismutase activity after training; GPxBT :glutathione peroxidase before trainig; GPxAT :glutathione peroxidase after trainig ;TAC BT:total antioxidant capacity before training; TAC AT:total antioxidant capacity after training)

DISCUSSION and CONCLUSION

Recently, there has been a great deal of research interest in the potential beneficial effects of cocoa, due to the potent antioxidant properties of polyphenols, of which cocoa is an abundant source. Polyphenols are amphipathic, exert their antioxidant effects in both lipid and aquatic environments, and act on cellular molecular targets in vivo by various mechanisms. It is stated that dietary antioxidant supplements are effective in reducing the magnitude of exercise-induced oxidative stress. In addition, some studies have reported that dietary antioxidant supplements have no effect on exercise-induced oxidative stress and even have negative effects (Davison et al., 2012).

In this study the effect of dark chocolate on oxidant stress parameters that may develop due to high-intensity kickboxing training was investigated. Allgrove et al. (2011) associated daily consumption of dark chocolate (40 g/day) for 2 weeks with a reduction in exercise-induced oxidative stress (Allgrove et al., 2011). It is frequently emphasized in some studies that the

extent of exercise-induced oxidative stress decreases with dietary antioxidants (Alessio et al.,1997; Davison & Gleeson, 2007). However, in some studies, it is stated that there is no difference, and that antioxidant supplements negatively affect exercise-induced oxidative stress (Nieman et al., 2004). In particular, the effects of short-term acute use and long-term chronic use of dietary antioxidants on oxidative stress and antioxidant defense mechanisms constitute special areas of study and reveal conflicting results. Two weeks of cycling exercise and regular consumption of dark chocolate rich in cocoa polyphenols it should be at the end of sentence have been reported to reduce markers of oxidative stress (Allgrove, 2011). Nieman et al. (2004) emphasized that triathletes using vitamin E for 2 months had higher IL-6 and oxidative stress responses compared to placebo (Nieman et al., 2004). Alessio et al. (1997) showed that acute (1 day) vitamin C supplementation was more effective at blunting exercise-induced oxidative stress compared to a 2-week daily supplementation period (Alessio, Goldfarb and Cao, 1997). It is stated that acute antioxidant supplementation may be more effective because long-term supplementation may cause an adaptive decrease in other (endogenous) antioxidant defenses in vivo (Davison et al.,2007). Another study found that acute pre-exercise dark chocolate consumption increased plasma antioxidant concentrations compared to exercise alone emphasizing that it improved plasma concentrations of antioxidant markers after exercise, thereby reducing oxidative atress (Davison et al., 2012). Superoxide dismutase (SOD) is the first detoxification enzyme in the cell and the strongest antioxidant. It is an important endogenous antioxidant enzyme that acts as a component of the first-line defense system against reactive oxygen species (ROS) (Ighodaro & Akinloe, 2017). It is the main free radical scavenger that can significantly reduce free radical damage in the body. It is an active substance produced by living organisms that can eliminate harmful substances such as free radicals produced during normal oxygen metabolism (Marks, 2014).

The comparison results of the blood parameters according to the Groups (Control Grup or Chocolate Receiving Group) the changes before and after the training were examined. Table.2. When examined, it is seen that there is no significant difference in the GPx, MDA and TAC values of the oxidant and antioxidant parameters of the athletes in both groups before and after training. However, a statistically significant change was found in the SOD value of the athletes in the group given chocolate before training ($p<0.05$). The athletes in our study group are the athletes who take part in a camp period that includes a very intense dual training program that starts at 09:00 in the morning and 18:00 in the evening, which lasts for about an hour. Cavarretta et al. (2018) found an increase in antioxidant levels in elite athletes using dark chocolate after 30 days of dark chocolate intake (Cavarretta et al., 2018). The relationship between exercise and oxidative stress is extremely complex and it is stated that it mainly depends on the fore frequency, intensity, and duration of the exercise (Cavarretta et al.,2018). Tonkonogi et al. (2000) found that endurance training performed for half an hour a day for 6 weeks did not affect the SOD activities of male and female athletes (Tonkonogi et al., 2000). On the other hand, Fauzi et al. (2007) found a significant increase in SOD enzyme activity immediately after and 24 hours after acute exercise after a 5-week exercise program (Fauzi et al., 2007). It has been shown that regular training eliminates the harmful effects of oxidative stress by increasing the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase.

In many studies, it has been reported that acute exercises of unusual intensity increase lipid peroxidation in untrained groups (Branth, et al.,2009; Fisher-Wellman & Bloomer,2009;

Spirlandeli et al.,2014). Ookawara et al. (2003) stated that training did not have a significant effect on the MDA level at rest, while acute exercise significantly increased the MDA level even after the training period (Ookawara et al., 2003). However, regular exercises reduce the level of lipid peroxidation. Oztaşan et al. (2004) stated that acute exhaustion exercise applied after 8 weeks of endurance training significantly increased the erythrocyte MDA level in the sedentary group but did not cause a significant change in the training group (Oztasan et al., 2004). It is thought that the contradictions in the results of the study may be due to the fact that ach study has different variables such as intensity, sample quality, and exercise type. It is stated that the increase in MDA is due to an increase in the level of oxygen intake rather than short-term ischemia and reperfusion, infiltration of phagocytic cells or an imbalance of calcium homeostasis (Tauler et al., 2006). Studies have generally found that antioxidant supplementation does not increase performance, but improves antioxidant status (Finaud et al.,2006). The age, nutritional and activity status of the people participating in the study may affect the results. Antioxidant restriction has been shown to reduce exercise performance in animals. Compared to animals with adequate vitamin E, the exercise capacity of animals with vitamin E restriction was decreased by 40% (Davies et al., 1982). It was found that six weeks of vitamin E and C supplementation prevented lipid peroxidation caused by endurance exercise but had no effect on inflammatory markers (Mastaloudis et al., 2004). Contrary to these results, there are also studies claiming that 2.5 hours of cycling exercise does not affect oxidative stress markers expressed by F2 - isotroptane levels in adults taking high doses of vitamin E and C supplements in recent years (Lee C-yung & Man-Fan Wan, 2000). It has been shown that acute vitamin C intake has limited effects on immunoendocrine changes during oxidative stress after prolonged exercise (Nieman et al., 2002; Nieman et al., 2004). It is often stated that endurance exercises increase antioxidant capacity. Studies have shown that regular exercises increase antioxidant enzyme activity, while long-term exercises cause an increase in oxidants in skeletal muscles. (Sen et al., 2000). It is emphasized that acute adaptation to exercise is an incomplete adaptation that can easily lead to oxidative damage. Therefore, it is very important to provide the body with adequate rest after exercise to restore balance (Radak et al., 2001).

In our study, when the groups were compared separately in order to see the effects of both exercise and chocolate on oxidant and antioxidant parameters in the athletes who were in the camp period and trained twice a day, it was seen that there was a significant increase in GPx and TAC parameters in the control group after training compared to pre-training. In addition, although we predicted more changes in antioxidant markers in the chocolate group, there was only an increase in TAC values. GPx activity is an important component of glutathione homeostasis. Studies show that regular endurance exercises increase GPx activity in skeletal muscles according to the intensity and duration (Lu et al., 2021). Davison et al., (2012) found that eating 100 g dark chocolate containing 70% cocoa solids to 14 healthy individuals led to high plasma antioxidant capacity and decreased oxidative stress markers 2 hours after cycling exercise for 2.5 hours (max VO_2 60%). Indicating a significant reduction in oxidative stress markers compared to our study, Davison et al., (2012) conflicts with. This difference may be due to the higher dose of DC and the training programs applied. In another study, it was stated that obese boys who consumed 30 g dark chocolate containing 83% cocoa for 6 weeks had five sessions of 40 min/day jump rope exercise for 6 weeks, and they obtained positive results regarding oxidative stress and health (Moghadam et al., 2021). Fraga et al., (2005)

demonstrated that consumption of 105 g chocolate (containing 168 mg of flavonols) for 14 days reduced oxidative stress markers in football players.

Our study shows that consumption of dark chocolate 2 hours before intense anaerobic exercise shows small increases in antioxidant capacity and a slight decrease in the oxidative stress marker MDA. Regular training eliminates the harmful effects of oxidative stress by increasing the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. In addition to suppressing the oxidative stress caused by exercise, aerobic training also stimulates antioxidant production. Repeated exercises of sufficient intensity and duration are very important. In our study, the athletes are those who train regularly and apply a very intense training program during the camp period. Therefore, the duration, intensity of the training programs and sample size are important parameters that affect the results. In addition, the high polyphenol content in chocolate, the dose of chocolate, the duration of consumption are important factors that determine the effect on oxidative stress and antioxidant markers. It is unclear whether the changes in this study were due to exercise or to consumption of dark chocolate. Detailed studies on exercise and dark chocolate are needed.

As a result, we would like to state that regular exercise is more effective than dark chocolate in improving antioxidant capacity.

Suggestions

- It is recommended to develop the sample group.
- It is recommended to apply different training programs.
- It is recommended to carry out different studies on the dose and consumption time of the chocolate to be consumed.

Conflicts of Interest: The author/authors of the article do not have any personal or financial conflicts of interest within the scope of the study.

Authors' Contribution: All authors read and approved the final manuscript.

Ethical Approval

Ethics Committee: Ethics Committee Clinical Research of Erciyes University.

Date/Protocol number: 2017/456.

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