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The Effects of Thyme Tea Supplement on Free Radicals Formation and Antioxidant System of Elite Wrestlers

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Abstract: The intensive acute exercises results in too much oxygen consumption and hence produce excessive free radicals and damage of tissues. This study was conducted to investigate the effects of thyme tea on free radical formation and antioxidant system. Eighteen elite volunteer wrestlers joined the study. The wrestlers were randomly divided into two groups to from experiment and control groups. The subjects wrested five times during the study period according to F.I.L.A rules. The measurement are performed before and after the thyme tea loading. Study groups drunk thyme tea three times a day in 35 day period before loading after first measurement. The subjects participated in the study pre-contest (Comp. Before), immediately after the competition (Comp. After), 24 hours after the competition (Comp. after 24 h), 48 hours after the competition (Comp. After 48 h), blood samples were collected from forearm vein by 5cc total of 2 tubes. Tubes, centrifuge immediately after serums and transferred to tubes Eppendorf Total of Antioxidant Capacity (TAC), Malondialdehyde (MDA) and total sulfhydryl group (RSH) stored at the 75°C until determination of analysis. The results showed that no difference was noted between the values of the groups before the thyme loading process, a meaningful increase ($p<0.05$) was determined in the level of TAC, after thyme loading, for the experimental group when it was compared to the pre-loading levels. Therefore, the decrease observed in the level of MDA after thyme loading process was meaningful statistically ($p<0.01$). The numerical decrease observed in the experimental group, meanwhile, in the levels of RSH wasn't meaningful ($p>0.05$). Whereas significant differences were found in the levels of MDA and TAC, after thyme loading, for the experimental group when compared to the control group ($p<0.05$), no meaningful difference was observed for the level of RSH. it is determined in the study that wrestling competition causes significant oxidant stress in wrestlers and increase total antioxidant capacity. However, it is detected that it makes nonenzymatic antioxidant capacity fall.

Key words: Wrestling, oxidant stress, thyme, antioxidant capacity

INTRODUCTION

Although many researches have been carried out recently on beneficial effects of physical activities, it is possible to meet some studies, though they are in few numbers, on negative effects of the physical activities (Günay *et al*., 2006). The training performed at high intensities causes much oxygen to be consumed and also free radicals to be constituted of (Yilmaz, 2002; Wootton, 1988). The muscle contractions which increase during physical activity and training cause some energy consumption and metabolic activity to increase considerably. It results with, in parallel to the consumption of gradually increasing level of oxygen, production of free radical (Yilmaz, 2002; Wootton, 1988; Fiçicilar, 1991). On the other hand, some derivatives of free oxygen, mainly as superoxide (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) are formed at all cases using molecular oxygen during metabolic activity and including the electron transport. The amount of these derivatives is primarily related to the level of metabolic activity (Selçuk, 2003).

The cells contain various enzymatic and nonenzymatic antioxidant systems of defense so that the cell organelles and membranes can be protected against harmful effects of the free radicals. Spoiling of such a balance causes some pathological changes to occur. The oxidation products of muscle, lipid peroxidation to be formed as a result of the effects of Reactive Oxygen Species (ROS) (Wootton, 1988; Clark, 1986). and carbonhhydrate cause some modifications in amino acid contents of the proteins and also cause some increases in the content of carbonyl of plasma protein during training sessions (Selçuk, 2003).

The defense systems of many mammals have a capability of to be adapted to the antioxidants to which they are exposed chronically. Therefore, the level of the

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oxidative damage to be occurred during physical exercises is related to not only to the production of free radical but also to the capacity of antioxidant defense (Wootton, 1988; Selçuk, 2003; Clark, 1986).

Whereas it is well known that the biomarkers of free radical activities have been influenced by the type, intensity and time intervals, their levels aren't known precisely. It is claimed that the biomarkers (TBARS) increased by 120% for the exercises at high intensities and the increase was by 68% in the muscles of skeleton following an exercise session at moderate intensity. (Ünlü, 2001). Although we have some data claiming that the antioxidants cause the lipid peroxidation to decrease, there is no certainty relating to in what amounts of the antioxidants consumed indicate such positive effects (Ünlü, 2001; Craig, 1999; Lai *et al*., 2004).

The tendency for consuming tea plants has increased too much for last 2 decades worldwide. The antioxidant levels of some of these plants were identified and some positive effects were obtained after inspecting their effects against oxidative harms on sportsmen (Wootton, 1988). When the antioxidant levels of some plants (black tea, sage tea, linden tea, green tea and thyme) found in Turkiye were inspected, the thyme was determined to be the plant with the highest antioxidant level among all. The thyme is under the same family as rosemaries. Its main Antioxidant Compound (AOC) was identified as phenolic glycoside. Muskat contains 2-allilfenols and several lignans. These compounds were found to have strong antioxidant activity. Additionally, the capsaicin, a new and strong AO, was separated from the above mentioned spicy and the antimicrobial effect of the thyme plant, therefore, was pointed out too (Ünlü, 2001).

The performance levels of sportsmen have made it possible for them to obtain incredible degrees recently. Meanwhile, intensified applications of exercises are required so that they can obtain such high scores. Therefore, the wrestling is one of the sports which requires too much physical activity (Gökdemir, 2000).

This study aims to investigate the effects of using thyme on forming free radical and also on the antioxidant system in eligible sportsmen.

MATERIALS AND METHODS

Selection of study groups: In this study the experimental groups selected from 18 male wrestler students of Nigde University Physical Education and Sports School, this experimental group that entered the Turkish National Team youth and adults category or students took the top three in the Turkish Championships, experimental group don't have any bad habits (cigarette, drug, alcohol), any disease and injury. The experimental group randomly divided two different groups, each test and control groups kept 9 students. The experimental group received and signed the form of volunteer participation after that all of the details information about the study subjects were explaining to the experimental group and The World Medical Association Declaration of Helsinki ethical principles applied in medical research involving human subjects were reading to the experimental group.

Measurements of height and body weight: The subjects height measured by seca brand height measures (0.01m, sensitivity) with type of cm. The subjects were made to make a bare feet, the body fully erect and chin parallel to the ground. The subjects body weight was measure with wrestling trunks on them only by seca brand scales (0.01kg, sensitivity) with type of kg.

Preparation of thyme tea: Thyme plants collected from mountains of Central Anatolia and after dried in the shade. Thyme Tea measured by JOMECTA ES-120 J4 brand analytical measures and was put for 1 grams each bag. After collecting the first data, the experimental group consumed the thyme tea after every third meals during the 35 days and 150 cm^3 thyme tea was put in to the boiled water and steeped for 10 minutes.

Diet controls: The study subjects who involved in this study groups recorded three meals of dietary habits, during that time the students not allowed to take vitamins and the study subjects has been used same nutritional programs.

Exercise protocol: The study subjects have made of 5 wrestling matches with the rules of F.I.L.A association and each student half an hour 2 circuits within given 30 seconds rest between circuits. The study subjects have setted their own warm-up times and their severity. Two days before the match were not allowed to do any exercise. 35 days after the operation was repeated.

Measurements: All measurements are related to study in the same time period and measurements have been made on with the F.I.L.A regulations appropriate wrestling mat in the indoor sports hall. Blood samples were analyzed in the lab.

Collection of data: The subjects participated in the study pre-contest (Comp. Before), immediately after the competition (Comp. After), 24 hours after the competition (Comp.after 24), 48 hours after the competition (Comp. After 48), blood samples were collected from forearm vein by 5cc total of 2 tubes. Tubes, centrifuge immediately after serums and transferred to tubes ependorf Total of Antioxidant Capacity (TAC), Malondialdehyde (MDA) and Total Sulfhydryl Group (RSH) stored at the 75 Celcius until determination of analysis.

Experimental design: The tournament consisted of five matches scheduled according to the official regulations of the FILA. Each match (three 2-min rounds, 30-s breaks between rounds) was formally refereed and scored. Each wrestler has made 5 matches (40-50 minutes of rest between matches) the subjects regulated their own warm-up times and their severity. The first match started at 9.00 am.

RESULTS

Some meaningful increases were determined for MDA measurement values as in favor of after the competition between before the competition and after the competition ; in favor of 24 hours after the competition between before the competition and 24 hours after the competition and between before the competition and 48 hours after the competition. Therefore, no meaningful increase has been found between other measurements. It could be seen that the MDA levels approaches to the basic level for the measurements taken 48 hours after the competition.

When the TAC measurement values those representing the antioxidant capacities of the subjects were taken into consideration, some meaningful increases were determined between values as in favor of just after the competition between before the competition and just after the competition; an increase in favor of 24 hours after the competition between before the competition and 24 hours after the competition; an increase in favor of 48 hours after the competition between before the competition and 48 hours after the competition and an increase in favor of just after the competition between just after the competition and 24 hours after the competition.

Some meaningful increases were determined statistically in favor of measurement values before the competitions for the RSH values which are one of the indications of non-enzymatic antioxidant values between before the competition and just after the competition; before the competition and 24 hours after the competition. and before the competition and 48 hours after the competition. Whereas no difference was noted between the values of the groups before the thyme loading process, a meaningful increase ($p<0.05$) was determined in the level of TAC, after thyme loading, for the experimental group when it was compared to the pre-loading levels. Therefore, the decrease observed in the level of MDA after thyme loading process was meaningful statistically ($p<0.01$). The numerical decrease observed in the experimental group, meanwhile, in the levels of RSH wasn't meaningful ($p>0.05$). Whereas significant differences were found in the levels of MDA and TAC, after thyme loading, for the experimental group when compared to the control group ($p<0.05$), no meaningful difference was observed for the level of RSH.

Groups in age, height and weight the total scores

Variables	N		Age (years)	Height (m)	Weight (kg)
Experimental group	9	Min.	18.00	1.63	60.00
		Max.	28.00	1.76	92.00
		Mean	21.66	1.71	74.11
		SD	3.16	3.82	9.42
Control Group	9	Min.	19.00	1.70	65.00
		Max.	26.00	1.78	82.00
		Mean	21.00	1.73	73.22
		SD	2.06	2.61	5.60
Significance (t value)			0.604	0.214	0.811

There were not a significant difference between the total scores.
SD: Standard deviation

Some meaningful increases were determined for MDA measurement values as in favor of just after the competition between before the competition and after the competition ; in favor of 24 hours after the competition between before the competition and 24 hours after the competition ; and between before the competition and 48 hours after the competition. Therefore, no meaningful increase has been found between other measurements. It could be seen that the MDA levels approaches to the basic level for the measurements taken 48 hours after the competition.

When the TAC measurement values those representing the antioxidant capacities of the subjects were taken into consideration, some meaningful increases were determined between values as in favor of just after the competition between before the competition and just after the competition; an increase in favor of 24 hours after the competition between before the competition and 24 hours after the competition; an increase in favor of 48 hours after the competition between before the competition and 48 hours after the competition and an increase in favor of just after the competition between just after the competition and 24 hours after the competition.

The numerical decrease observed in the experimental group, meanwhile, in the levels of RSH wasn't meaningful ($p>0.05$).

DISCUSSION

The study examines the effect of thyme loading to elite wrestlers at consequent wrestling competitions on free radical formation and antioxidant system. Meaningful differences between before the competition and just after the competition, before the competition and 24 hours after the competition, before the competition and 48 hours after the competition for MDA measures have been found. The increases have been detected for Just after the competition, 24 hours after the competition, 48 hours after the competition There has been no meaningful difference between other measures. In the measures which were conducted 48 hour later after the competition, MDA levels were found to be near to basal level. MDA is one of the most important indicators of

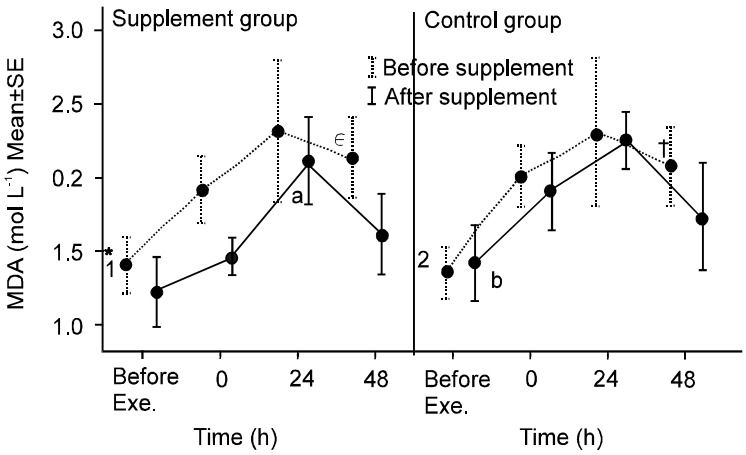

Fig. 1: Before and after the experimental measurement values of MDA

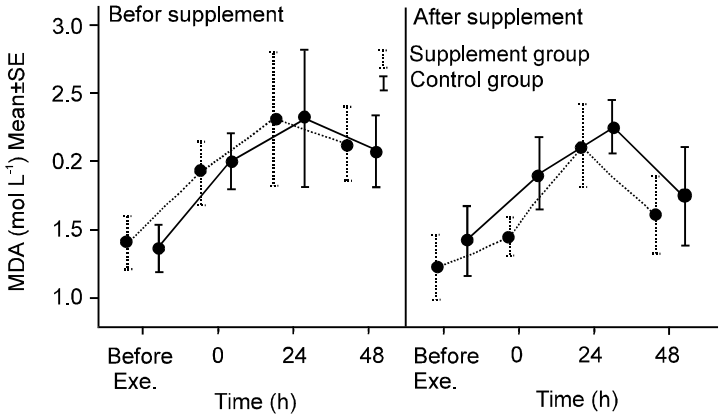

Fig. 2: Comparison of the measurement times before and after measurement

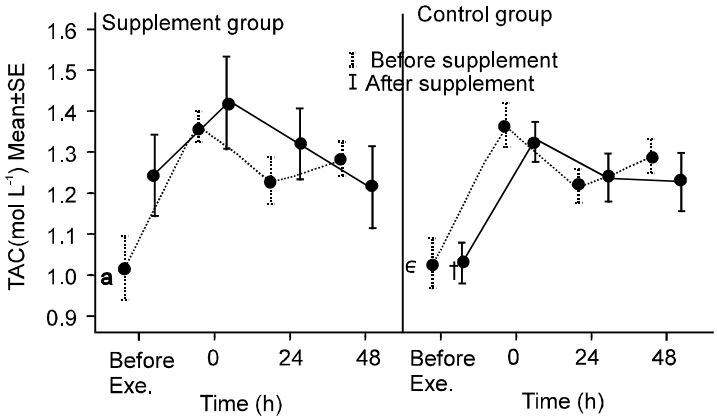

Fig. 3: Groups before and after the experimental measurement values of TAC

oxidant stress and the most important product of lipid peroxidation is malondialdehide (MDA). MDA forms in peroxidation of fat acids which contain three or more compound pairs. Forming MDA affects ion exchange at cell membranes and it causes cross binding. This causes negative results such as enzyme activity change and ion permeability. Because of this feature, MDA may react with DNA's nitrogen-alkalies so it is mutagenic and also genotoxic and carcinogenesis for cell cultures (Kalender *et al*., 2002; Porter, 1984). MDA measures reflecting lipid oxidation are often conducted to determine oxidative stress.

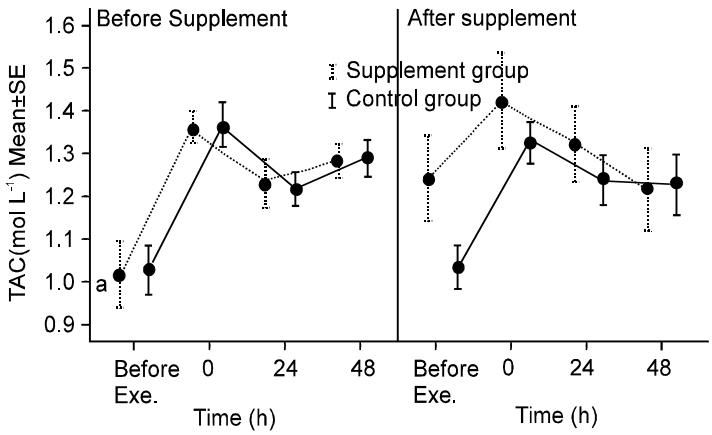

Fig. 4: Comparison of the measurement times before and after measurement

It is reported in many studies that exercise causes oxidative stress (Goldfarb *et al*., 2005; Ji *et al*., 1992; Marzatico *et al*., 1997; Kanter *et al*., 1985; Inal *et al*., 2000). Tauler *et al*. (2006) detected in a study which was on mountain cyclists that MDA levels three hours after competition was very high. Lovlin *et al*. (1987) showed that lipid peroxidation of plasma and erythrocyte membrane increased in acute exercise with bicycle ergometer. Gleeson *et al*. (1987) found that when they compared three groups, namely, subjects doing exercises irregularly, middle and elite athletes, erythrocyte MDA during resting of subjects doing exercises irregularly is very high than those of other two groups. Magalhaes *et al*. (2007) showed in the study on 14 men platform climbers that MDA and uric acid levels increased significantly just after the exercise and an hour after exercise but sulfhydryl groups' level decreased meaningfully. Groussard *et al*. (2003) stated that anaerobic exercise caused oxidative stress just after wingate test on sprinters and 40-minute of resting after. Anuradha *et al*. (1998) found in the study on rats that MDA level increased after exercises.

Studies show that low intensive exercises as well as intensive exercises increased free radical production and therefore, oxidant stress. However, it is also expressed that the metabolic rate amount of free radical increases proportionally (Goldfarb *et al*., 2005; Ji *et al*., 1992; Marzatico *et al*., 1997; Kanter *et al*., 1985; Inal *et al*., 2000; Tauler *et al*., 2006; Lovlin *et al*., 1987; Gleeson *et al*., 1987; Magalhaes *et al*., 2007; Groussard *et al*., 2003; Anuradha *et al*., 1998). In hypoxic conditions, the situation becomes intensified (Vasankari *et al*., 1997; Wozniak *et al*., 2001). When free radical forming which depends on speeding up metabolic processes due to increased oxygen consumption exceeds antioxidant defense capacity, oxidant stress emerges. Therefore cell damage may be developed (Katch *et al*., 1994; Jenkins *et al*., 1993; Supinski, 1998; Wozniak *et al*., 2001). The size of oxidative damage which may emerge during physical exercises is not only related to free radical production but also defense capacity of antioxidants (Ji, 1999). Metabolic rate increases differently according to type and intensity of exercise. Heavy endurance exercises can increase oxygen consumption of all humans 10-20 times. But maximum oxygen consumption can be 100 times bigger at muscle febrile level. This situation may induct oxidative stress and cause excessive free radical production (Wozniak *et al*., 2001; Katch *et al*., 1994; Jenkins *et al*., 1993; Supinski, 1998).

In this study, it is shown that wrestlers experience oxidant stress after wrestling competition and oxidant stress vanishes 48 hour after competition. When TAC measure values are examined, it is found that meaningful differences between before the competition and Just after the competition, before the competition and 24 hours after the competition, before the competition and 48 hours after the competition for TAC measures have been found. The increases have been detected for Just after the competition, 24 hours after the competition, 48 hours after the competition.

TAC values reach maximum levels just after competition and the high level still continues 48 hour after competition. It is thought that this increase in TAC values is a respond to oxidant stress caused by wrestling competition. A regular physical exercise has many useful effects. During exercise, enzymatic and non enzymatic oxidants increase. Exercises done at regular intervals can increase resistance against oxidative stress and antioxidant level may lower tiredness (Hollander *et al*., 1999). The antioxidant defense system of most mammals shows that they have the ability to adopt themselves against oxidants they are exposed to chronically (Selçuk, 2003).

It is shown that during acute phase of exercises, in most biological tissues such as heart, liver, lungs and skeleton muscles, Superoxide Dysmutase (SOD) activity, one of antioxidant enzymes, increases. The increased SOD activity may be the indicator of Superoxide radical which increases during exercises (Ji,

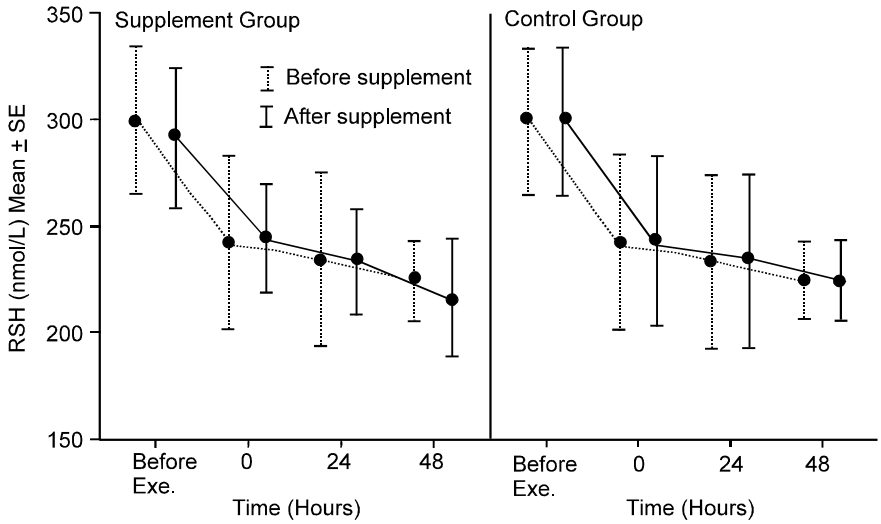

Fig. 5: Groups before and after the experimental measurement values of RSH

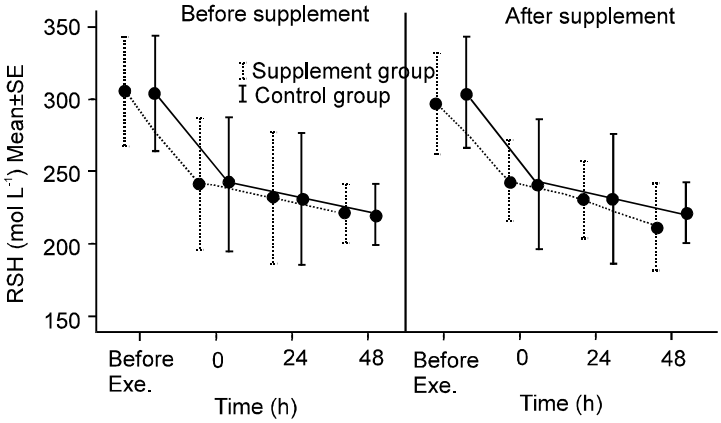

Fig. 6: Comparison of the measurement times before and after measurement

1995; Margaritis *et al*., 1997). The size of oxidative damage during physical exercise is determined not only by free radical production but also defense capacity of antioxidants (Margaritis *et al*., 1997).

Robertson *et al*. (1991) reported that erythrocyte SOD activity is lower in sedentary people than that of athletes and this may be related to metabolic speed and oxygen radical production. Inal *et al*. (2000) reported in a study on 19 swimmers that catalase (CAT) and glutathione peroxidase (GPx) activities increase just after exercise decrease at 20. and 40. minute but do not return to basal level and glutathione (GSH) levels decrease significantly just after exercise but increase at 20. and 40. minute again. Child et al detected sulfhydryl and TAC groups increase in muscles as a result of the study done on 9 healthy young men on eccentric exercise (Child *et al*., 1999). This study has similar results as indicated in literature. Those antioxidant level increases just after exercises and still being high 48 hour after exercise show that antioxidant capacity develops as response to oxidant stress caused by wrestling competition.

Significant differences has been found in favor of measurement for RHS values, one of nonenzymatic antioxidant indicators between Before the competition and Just after the competition, Before the competition, 24 hours after the competition and before the competition and 48 hours after the competition hour later. The measures conducted before the competitions were found to have been higher than those which were conducted after the competition. Decreasing figures have been found during measurements conducted within 48 hour after the competition finished.

Kahraman *et al*. (2003) detected in the study conducted on women wrestlers that the total sulfhydryl level which is one of RSH sub-group has been found much lower than that of control group. Anuradha *et al*. (1998) found in a study on rats that antioxidant nonenzymatic activity decrease after 6-week aerobic exercises. Seo *et al*.

(2006) showed in another study on rats that total sulfhydryl and glutathione groups are significantly lower in older rats than those of young ones at the end of aerobic exercises. Jerca *et al.* (2005) determined in a study conducted on patients with moderate hypertension that MDA increases whereas glutathione, uric acid, total sulfhydryl groups (G-SHT) and non protein sulfhydryl group (G-SHNP) decrease after exercise. After the exercises which continued for three months, they did not detect any increase in oxidative stress.

In the studies done, it is reported that TAC increases but RSH which represents nonenzymatic antioxidants decreases (Anuradha *et al.*, 1998; Kahraman *et al.*, 2003; Seo *et al.*, 2006; Jerca *et al.*, 2005). The function of nonenzymatic antioxidants reduces as a result of interaction with oxidant (Basaga *et al.*, 1997). It is thought that this decrease is due to the function of antioxidant. Mimic-Oka *et al.* (1999) attributed in a study on chronic kidney disease this decrease to oxidation of proteins after the accumulation of destroyer productions such as malondialdehyde and lipid peroxide radicals.

It is seen in this study that TAC increases whereas RSH decreases. The increase of TAC can be explained with the affect of exercise on antioxidant capacity. It is thought that decrease in RSH is linked to oxidants stress used during various process to make them harmless.

Conclusion: It is determined in the study that wrestling competition causes significant oxidant stress in wrestlers and increase total antioxidant capacity. However it is detected that it makes nonenzymatic antioxidant capacity fall. Therefore, it can be thought that supporting wrestlers with vitamins and other ergogenic which have nonenzymatic antioxidant effect before competition can help decrease oxidant stress.

As a result, the study shows that wrestling causes oxidative stress; thyme increases total antioxidant capacity and it prevents cell damage by hindering lipid peroxidation. However, it is detected that thyme has no effects on nonenzymatic antioxidant structures.

It may be useful to load people exercising with antioxidant supplements so as to lessen oxidative stress and increase antioxidant capacity.

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