УДК 546.881 **Yildirim A. K., Palabiyik A. A.**

THE INVESTIGATION OF EFFECTS OF ENDURANCE EXERCISES ON TOTAL OXIDANT AND TOTAL ANTIOXIDANT STATUS PARAMETERS IN THE RAT LIVER WITH EXPERIMENTAL THYROID DYSFUNCTION

Introduction. *Free radicals are highly reactive, short-lived and labile molecules that have one or more unpaired electrons. Oxygen-derived radicals are the most important free radicals in biological systems. Hyperthyroidism is the clinical condition that occurs due to excessive production of thyroid hormone. In this case, oxygen consumption increases due to high levels of the thyroid hormone while basal metabolic rate increases due to further stimulation of the energy metabolism and heat production.*

The ***purpose*** *of this research is to study the effects of regular endurance exercises on total oxidant status (TOS) and total antioxidant status (TAS) parameters in liver tissues of rats that are experimentally made to acquire hyperthyroidism.*

Methods. *In this study, a total of 24 male Sprague-Dawley rats that weigh between 200 to 220 grams were used. Experimental animals were randomly divided into four groups: Control (n=6), hyperthyroid (n=6), exercise (n=6), and hyperthyroid + exercise (n=6). TOS was measured using a commercially manufactured measurement kit (Total Oxidant Status Assay Kit, Product Code: RL0024, Rel Assay Diagnostics® Mega Tıp Ltd. Gaziantep, Turkey). Measurements were conducted in line with the recommendations of the manufacturing company. TAS was measured using a commercially manufactured measurement kit in line with the recommendations of the manufacturing company (Total Antioxidant Status Assay Kit, Product Code: RL0017, Rel Assay Diagnostics® Mega Tıp Ltd., Gaziantep, Turkey).*

Results. *Weights of the rats in the control and exercise groups increased by 19% and 12.9% on average during the experiment respectively, while weights of the rats in the hyperthyroid group decreased by 8.6% in the meantime. On the other hand, it was noteworthy that a weight gain of 8.6% on average was observed in the rats in the hyperthyroid + exercise group at the end of the 8 weeks.*

When we compared the liver TAS concentrations among study groups, the highest TAS value was observed in the exercise group. TAS concentration increased for the exercise group and decreased for the hyperthyroid + exercise group when compared to the control group. In addition, differences among groups were not statistically significant.

When we compared the liver TOS concentrations among study groups, the highest TOS value was observed in the exercise + hyperthyroid group. When compared to the control group, a statistically insignificant decrease was observed in the TOS measured in the exercise group. In addition, when compared to the exercise group, a significant increase of the TOS concentration was detected in the hyperthyroid + exercise group. When compared to the control group, even though the TOS levels for both the hyperthyroid and exercise groups demonstrated a declining trend, it is thought that the increase observed in the hyperthyroid + exercise group might be the result of a synergic effect.

Conclusion. *It is concluded that neither hyperthyroidism nor endurance exercises significantly affect TAS concentrations of the liver tissue; however, the total antioxidant status in a liver tissue increases in a statistically significant manner when rats with hyperthyroidism practice endurance exercises.*

Key words: *total oxidant status, total antioxidant status, hyperthyroidism*

Topicality of Research. Free radicals are highly reactive, short-lived and labile molecules that have one or more unpaired electrons. Oxygen-derived radicals are the most important free radicals in biological systems. Molecules that have an unpaired electron such as hydroxyl radical, superoxide, nitric oxide and lipid peroxide are examples for a free radical.

There is a balance, namely "oxidative balance", between the rate of formation of free radicals that are produced within metabolic processes of living organisms and the rate of neutralization of these radicals. As long as the oxidative balance is ensured within a biological

system, the organism is protected from a free radical damage. This balance can be upset by either an increase in the rate of free radical formation and/or a decrease in the rate of the neutralization of radicals [1, 2]. This is called an "oxidative stress" and it demonstrates the disrupted balance between free radical formation and antioxidant defence mechanisms and brings about structural and functional disturbance of many biomolecules and causes cell damage [1, 2].

Hyperthyroidism is the clinical condition that occurs due to excessive production of thyroid hormone. In this case, oxygen consumption increases due to high levels of the thyroid hormone while basal metabolic rate increases due to further stimulation of the energy metabolism and heat production [3, 4]. The main source of production of free radicals in a cell is the mitochondria; therefore, the rate of formation of free oxygen radicals is directly related to the rate of mitochondrial oxygen consumption [5]. Experiments conducted on animals and clinical trials demonstrate that hyperthyroidism most likely results in oxidative stress due to mitochondrial oxygen consumption [6, 7].

It is reported in various studies that there is a link between the increasing oxygen consumption and the formation of free oxygen radicals during physical activity and that physical exercise may cause oxidative stress depending on its intensity and duration [8-10]. It is also reported, however, that while increasing the rate of free oxygen radicals, regular endurance trainings enhance antioxidant defence by stimulating the antioxidant systems in the body [11, 12].

The **purpose** of this research is to study the effects of regular endurance exercises on total oxidant status (TOS) and total antioxidant status (TAS) parameters in liver tissues of rats that are experimentally made to acquire hyperthyroidism.

Materials and methods

In this study, a total of 24 male Sprague-Dawley rats that weigh between 200 to 220 grams were used. The rats were obtained from the Medical Experimental Research and Application Centre of Ataturk University. During the experiment, rats were accommodated and fed in wire cages in an environment of approximately 22^0C and 50-60% moisture with a 12-hour day and 12-hour night setting. This study was approved by the Local Ethical Board of Animal Experimentation of Ataturk University and all experimental stages were conducted in accordance with ethical rules.

Experimental animals were randomly divided into four groups: Control (n=6), hyperthyroid (n=6), exercise (n=6), and hyperthyroid + exercise (n=6). Rats in the control group were administered 0.5 ml of subcutaneous isotonic NaCl solution on a daily basis and were made run on a treadmill for 5 days a week for 8 weeks at a speed of 2 m/min for 5 minutes.

During the experiment, 250 g/kg of subcutaneous L-thyroxin was injected into the rats in the hyperthyroid group on a daily basis [13]. Rats in the exercise group were administered 0.5 ml of subcutaneous isotonic NaCl solution on a daily basis and were made run on a treadmill for 5 days a week for 8 weeks at a speed of 23 m/min for 45 minutes. Rats in the hyperthyroid + exercise group were made to acquire hyperthyroidism by an injection of L-thyroxin, same as the second group of rats, and these rats were made to run on a treadmill for 5 days a week for 8 weeks at a speed of 23 m/min for 45 minutes.

TOS was measured using a commercially manufactured measurement kit (Total Oxidant Status Assay Kit, Product Code: RL0024, Rel Assay Diagnostics® Mega Tıp Ltd. Gaziantep, Turkey). Measurements were conducted in line with the recommendations of the manufacturing company.

TAS was measured using a commercially manufactured measurement kit in line with the recommendations of the manufacturing company (Total Antioxidant Status Assay Kit, Product Code: RL0017, Rel Assay Diagnostics® Mega Tıp Ltd., Gaziantep, Turkey).

The statistical analyses were made using the SPSS 18.0 (SPSS Inc., Chicago, IL) program. The normal distribution of data was analysed using the Kolmogorov–Smirnov test. One-Way ANOVA LSD Post Hoc test was used for intergroup comparison of normally distributed data. Differences that were $p<0.05$ were deemed statistically significant.

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Results and discussion

Weights of the rats in the control and exercise groups increased by 19% and 12.9% on average during the experiment respectively, while weights of the rats in the hyperthyroid group decreased by 8.6% in the meantime. On the other hand, it was noteworthy that a weight gain of 8.6% on average was observed in the rats in the hyperthyroid + exercise group at the end of the 8 weeks.

It is known that there is a direct link between the metabolic effects of the thyroid hormone and the increase of ROS (Reactive Oxygen Species) production and oxidative stress. One of the metabolic effects of the thyroid hormone is to increase the rate of basal metabolism by stimulating both catabolic and anabolic reactions [3,4]. This causes energy consumption, mobilization of molecules that provide energy, oxidation of molecules in order to acquire energy, increase in oxygen consumption, increase in electron transport chain (ETC) reactions and generation and emission of thermal energy. An increase in ETC reactions also increases the production of ROS that originate from the mitochondria [5,7]. Thyroid hormones also cause extra-mitochondrial ROS production by altering the expression of the genes having the codes for the enzymes that produce and neutralize ROS [14].

Table 1

Changes in the weights of the rats observed during the experiment

Groups	**Percentage of change compared to initial body weight**
Control (n=6)	Increase by 19%
Hyperthyroid (n=6)	Decrease by 8.6%
Exercise (n=6)	Increase by 12.9%
Hyperthyroidism + Exercise (n=6)	Increase by 8.6%

Since we could not find any research in the literature that studies the effect of endurance exercises on TAS and TOS parameters of the liver tissue of a rat that was experimentally made to acquire hyperthyroidism, we were unable to compare the results of our study. Therefore, we deemed it would be fitting to evaluate the subject by making use of hyperthyroidism-exercise studies that analyse antioxidant molecules such as SOD (Superoxide Dismutase), GPX (Glutathione Peroxidase), CAT (Catalase), and GSH (Glutathione) instead of TAS and an oxidative stress parameter such as MDA (Malondialdehyde) instead of TOS.

Djordjevic et al. compared pro-/anti-oxidant levels in the blood of athletes and non-athletes before and after an acute exercise. It was reported that during resting, the NO (nitric oxide), reduced GSH and SOD and CAT activities of athletes were higher while their thiobarbituric acid reactive substances (TBARS), which are products of lipid peroxidation, were lower compared to non-athletes [15]. An increase in the levels of NO, $O_2^{\cdot -}$ and H_2O_2 was observed in non-athletes after an acute exercise. On the other hand, it was identified that

trained athletes experienced a decrease in reactive oxygen species after an acute exercise and that regular physical exercise balanced redox homeostasis [15].

Messarah et al. studied the effects of a dysfunction of the thyroid gland on lipid peroxidation and antioxidant parameters in liver tissues and serum samples of rats that were experimentally made to acquire hypothyroidism and hyperthyroidism and measured GPX, SOD and CAT activities and GSH, TAS and TBARS, which are products of lipid peroxidation, levels for this purpose. They reported that compared to the control group, both TBARS levels and antioxidant enzyme activities of rats with hyperthyroidism increased in a statistically significant way and the serum TAS levels dropped and as a result, hyperthyroidism caused oxidative stress in rats [16].

Choi et al. studied the effects of physical exercise and oxidative stress on antioxidant activity in Sprague-Dawley rats. A group of rats were put to moderate-intensity physical exercise and made to run (10° incline, 0.5-0.8 km/h) for 30 minutes a day for 4 weeks on a treadmill. After the exercise, while plasma CAT and liver SOD activities increased in a statistically significant manner, the liver GSH/GSSH rate and MDA levels decreased. In the conclusion of the study, the authors reported that moderate-intensity exercise activates the antioxidant defences system [17].

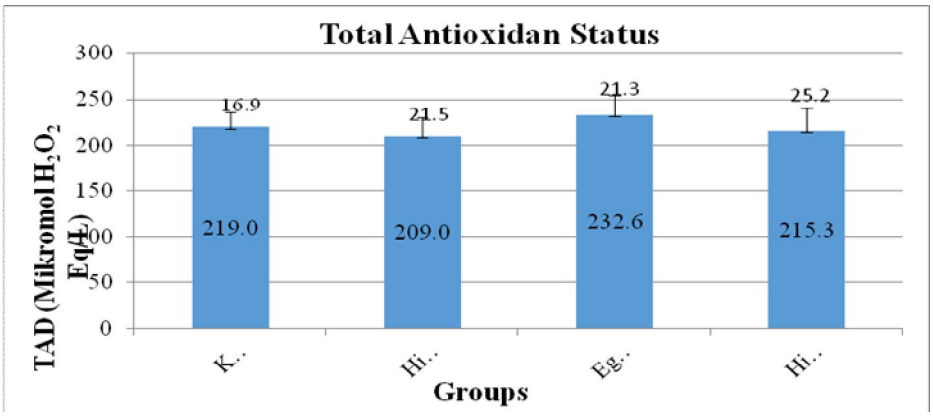

Figure 1. Total Antioxidant Status, TAS (Micromole H_2O_2 Eq/L): Control, Hyperthyroid, Exercise, Hyperthyroidism+Exercise, Groups TAS concentrations measured in the study groups. The results are indicated as average ± standard deviation.

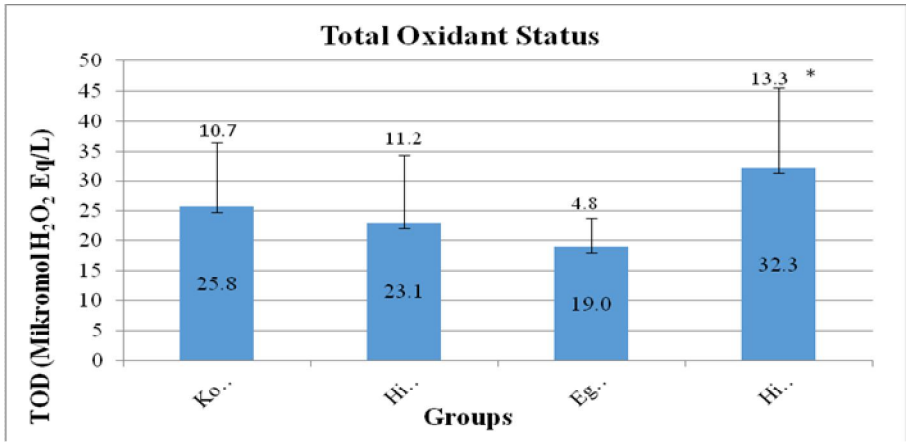

Figure 2. Total Oxidant Status (TOS) (Micromole H_2O_2 Eq/L), Control, Hyperthyroid, Exercise, Hyperthyroidism+Exercise). TAS concentrations measured in the study groups. The results are indicated as average ± standard deviation.

When we compared the liver TAS concentrations among study groups, the highest TAS value was observed in the exercise group. TAS concentration increased for the exercise group and decreased for the hyperthyroid + exercise group when compared to the control group. In addition, differences among groups were not statistically significant.

When we compared the liver TOS concentrations among study groups, the highest TOS value was observed in the exercise + hyperthyroid group. When compared to the control group, a statistically insignificant decrease was observed in the TOS measured in the exercise group. In addition, when compared to the exercise group, a significant increase of the TOS concentration was detected in the hyperthyroid + exercise group. When compared to the control group, even though the TOS levels for both the hyperthyroid and exercise groups demonstrated a declining trend, it is thought that the increase observed in the hyperthyroid + exercise group might be the result of a synergic effect.

Conclusions

It is concluded that neither hyperthyroidism nor endurance exercises significantly affect TAS concentrations of the liver tissue; however, the total antioxidant status in a liver tissue increases in a statistically significant manner when rats with hyperthyroidism practice endurance exercises.

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Анотація. Ялдірін А. К., Палабіїк А. А. Дослідження впливу вправ на витривалість на загальні параметри окислювача та загального антиоксидантного статусу в печінці щурів з експериментальною дисфункцією щитовидної залози.

Досліджували вплив вправ на витримку на загальний окисний статус і загальний антиоксидантний статус у тканинах печінки щурів, для яких експериментально створювали гіпертиреоз.

Встановлено, що ні гіпертиреоз, ні вправи на витривалість істотно не впливають на загальний антиоксидантний статус тканини печінки; однак, загальний оксидантий статус у тканині печінки достовірно зростає, коли пацюки з гіпертиреозом навантажувались вправами на витривалість.

Ключові слова: *загальний оксидантний статус, загальний антиоксидантний статус, гыпертиреоз.*

Ataturk University, Erzurum,Turkey

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