

# Investigating the effect of testosterone supplement on heart and muscle damage in rats applied with swimming exercise

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## Abstract

Anabolic Androgenic Steroids which are a synthetic derivative of testosterone are known to be used by some sportsmen in order to improve performance. The present study was conducted to investigate the effect of testosterone supplement on heart and muscle damage in rats applied with swimming exercise. The study was conducted on 30 day-old 27 (Wistar, male) rats that were supplied from "Selçuk University Experimental Medicine Research and Application Center". The rats were divided into 4 groups. The rats in control groups (n:6) were fed during the study without doing any application. The rats in testosterone group (n:7) were supplied with a 10 mg/kg dose of testosterone 5 days a week for 3 weeks. The rats in exercise group (n:7) did freestyle swimming exercise for 30 minutes a day for 5 days a week for 3 weeks. The rats in testosterone + exercise group (n:7) did freestyle swimming exercise for 30 minutes and were administered with 10 mg/kg dose of testosterone supplement for 5 days a week for 3 weeks. At the end of the trial, necessary biochemical parameters were measured in serums obtained by drawing blood from tails of the rats. While levels of Serum, LDH, CK-MB, and CK were studied by using an autoanalyzer, Troponin I level was analyzed by using ELISA kit. SPSS 15.0 packaged software was used in statistical evaluation of the data and the results were given as mean±SE. Since the data were convenient with the normal distribution, ANOVA test was applied for between groups comparison and Duncan test was used to identify from which group the difference was resulted. When between-groups values of troponin I, ck-MB, and LDH, which are indicators of heart damage, and enzyme CK, which is an indicator of muscle damage, for control, exercise, testosterone, and testosterone+exercise groups were compared, a statistically significant difference was determined between control, exercise, testosterone, and testosterone+exercise groups ( $p<0.05$ ). As a result of statistically analysis performed on all groups, the difference was found to be caused by testosterone and testosterone+exercise groups. Because testosterone production is naturally increased in the body during the exercise, swimming exercise and testosterone administration led to significant occurrence of troponin I, ck-MB, LDH, which are heart damage indicators in rats, and CK value, which is an indicator of muscle damage, in the blood.

**Keywords:** Heart, Muscle Damage, Swimming Exercise, Testosterone

## INTRODUCTION

AASs have been used by athletes for many years to increase strength, speed and durability. Although its effect seems to be positive at first, it is known that it has many physiological effects in human body (4). Because of the great effect on the body's muscle structure, testosterone is used to increase muscle performance in athletes (testosterone or synthetic androgen instead of testosterone) (6,7). Because AASs provide hypertrophy (10,11) to increase muscle mass by athletes because of its provision of hypertrophy (16), it is physically good in young people that the recovery period is fast (2,3,15,16).

It has profound effects on the human body of the hormones secreted by the endocrine system.

Therefore, it is known that any change in the endocrine system may affect the blood level and also affect human metabolism (32).

The hormone that determines gender-specific attitudes and behaviors in humans, especially in men, is testosterone. Testosterone is a hormone that easily passes the blood brain barrier with a steroid structure produced by cholesterol (1). Testosterone is more present in the body than other androgenic hormones. However, most testosterone is converted to dihydrotestosterone, which is more active in target tissues (21). Testosterone and dihydrotestosterone are all steroid compounds. Testosterone is the hormone, which is generally responsible for the formation of a distinct male sex character of the human body. In the life of the fetal

life, the testes are stimulated with the presence of the gingival gonadotropins in the placenta and secrete moderate testosterone. This hormone is present in the body during the fetal development period and even 10 or more weeks after birth. Therefore, testosterone is not produced until the age of 10-13 years. Then, testosterone production increases rapidly with the stimulation of anterior pituitary gonadotropic hormones during puberty (29).

When side effects of AAS were examined, it was stated that it causes sudden heart attack in the cardiovascular system, cerebrovascular diseases in the brain and nervous system, disrupting the function of the liver, causing hepatitis, increasing the risk of cancer and in the blood LDL and HDL (17). Another side effects of AAS are gender. Long-term use of steroids causes women to exhibit masculine behavior, excessive hair formation in certain regions, thickening of the vocal cords, deformity of the breasts, irregularity in the period and hair loss. In males, it is known that testosterone level may cause decrease in sperm quality and number and abnormal reduction in testes (33). In addition, it can affect the formation of pregnancy by increasing the internal structure of the uterus in the womb and causing an abnormal expansion of the testosterone intake (8).

The aim of this study was to investigate the effect of testosterone supplementation on heart and muscle damage in swimming rats.

## MATERIALS & METHODS

The study was performed on 27 rats (Wistar, male), 30 days from Selcuk University Experimental Medicine Research and Application Center. The study was approved by the Ethics Committee of the Center for Experimental Research and Application at Selcuk University (number of decisions: 2016-29). Rats were fed and housed in the experimental animal unit, plastic rat cages,  $23 \pm 2$  °C at room temperature,  $50 \pm 10\%$  humid environment, 12/12 day / night light period, ad-libitum. The rats were provided with daily fresh water (~ 50 ml / day / rat) that they could drink at any time. Animals were divided into 4 groups as follows.

1. Group: C (Control group, n: 6), Standard rat feed and drinking water ad libitum were given during the study period.

2. Group T (Testosterone group, n: 7), Standard rat feed and drinking water ad libitum were given during the study period. Testosterone (sustanon) was administered at a dose of 10 mg / kg / rat diluted 100 ml in peanut oil and administered intraperitoneally for 5 days and 3 weeks.

3. Group: E (Exercise group, n: 7), Standard rat feeding and drinking water ad libitum were given during the study period. The rats in this group were given swimming training for 3 weeks and 5 days a week for 30 minutes.

4. Group: TE (Testosterone + Exercise group, n: 7), Standard rat feed and drinking water ad libitum were given during the study period. Testosterone (sustanon) was administered to this group by intraperitoneal injection at a dose of 10 mg / kg for 5 days per week and was included in the exercise program 1 hour before the start of the exercise.

The rats in all groups, including the control group, were weighed by weighing with a precision scale (Ohaus CS 200 Compact scala, Mexico) once a week for the duration of the experiment and before the experiment, and the mean weight was measured weekly. The required dose adjustment was carried out weekly in terms of the live weight of the rats belonging to testosterone treated groups.

Exercise Program: Swimming exercises were performed in swimming pool for 3 weeks, 5 days 30 min. The water tank was filled with water with a temperature of 25 °C and rested for 1 hour and the water temperature was 22-25 °C. For adaptation to water at the start of exercise, the rats were kept free in water for 10 minutes and then a swimming exercise program was applied. At the end of the exercise time, the rats were removed from the water tank and dried with a towel.

Measurements and Analysis: At the end of the experiment, the necessary biochemical parameters were obtained from the sera of the rats. Serum, LDH, CK-MB, CK levels were determined in autoanalyser (Ilab 300 Plus, Milan, Italy) and Troponin I levels were determined by ELIZA kit.

Statistical Analysis: Statistical data were analyzed using SPSS 15.0 (SPSS 15.0 for Windows / SPSS® Inc., Chicago, USA). ANOVA and Duncan test were used to compare data between groups.

## RESULTS

Table 1. The levels of control (c), exercise (E), testosterone (T) and testosterone + exercise (TE) were compared with blood troponin I, creatinine kinase (ck), ck-MB and LDH values. (Mean  $\pm$  SE)

Groups	Troponin I (ng/mL)	ck-MB (ng/mL)	LDH (mg/dL)	Creatine Kinase (ck) (U/L)
Control	13.25 $\pm$ 6.71 <sup>b</sup>	342.68 $\pm$ 105.46 <sup>c</sup>	277.16 $\pm$ 76.28 <sup>bc</sup>	266.50 $\pm$ 40.61 <sup>b</sup>
Exercise	10.88 $\pm$ 1.53 <sup>b</sup>	280.33 $\pm$ 32.36 <sup>c</sup>	170.33 $\pm$ 40.56 <sup>c</sup>	184.50 $\pm$ 18.98 <sup>b</sup>
Testosterone	16.17 $\pm$ 2.64 <sup>b</sup>	616.66 $\pm$ 105.59 <sup>b</sup>	648.16 $\pm$ 132.70 <sup>b</sup>	401.33 $\pm$ 102.15 <sup>ab</sup>
Tes+Exercise	34.32 $\pm$ 7.50 <sup>a</sup>	954.16 $\pm$ 45.03 <sup>a</sup>	1078.16 $\pm$ 198.60 <sup>a</sup>	590.33 $\pm$ 115.02 <sup>a</sup>

Different letters in the same column (a, b, c) are statistically significant (Duncan test,  $p < 0.05$ ).

When C, E, T and TE groups were compared between the groups. the values of troponin I protein were determined as C (13.25  $\pm$  6.71 ng/mL), E (10.88  $\pm$  1.53 ng/mL), T (16.17  $\pm$  2.64 ng/mL) and TE (34.32  $\pm$  7.50 ng/mL) were found to be statistically significant differences ( $p < 0.05$ ). Post hoc test was used to determine which group the difference was caused and it was seen that the difference was caused by TE group.

When the values of C, E, T and TE groups of creatine kinase-MB (ck-MB) were compared in the determination of heart injury. C (342.68  $\pm$  105.46 ng/mL), E (170.33  $\pm$  40.56 ng/mL), T (616.66  $\pm$  105.59 ng/mL) and TE (954.16  $\pm$  45.03 ng/mL) groups were found to be statistically significant ( $p < 0.05$ ). Post hoc test was used to determine which groups caused the difference and it was seen that the difference was caused by T and TE groups.

When we compared the values of C, E, T and TE groups of LDH enzyme in determination of heart damage. C (277.16  $\pm$  76.28 mg/dL), E (170.33  $\pm$  40.56 mg/dL), T (648.16  $\pm$  132.70 mg/dL) and TE (1078.16  $\pm$  198.60 mg/dL) were found to be statistically significant differences ( $p < 0.05$ ). Post hoc test was used to determine which groups caused the difference and it was seen that the difference was caused by T and TE groups.

When the values of C, E, T and TE groups were compared between C, E, T and TE groups. C (266.50  $\pm$  40.61 U/L), E (184.50  $\pm$  18.98 U/L), T (648.16  $\pm$  132.70 U/L) and TE (401.33  $\pm$  102.15 U/L) groups were found to be statistically significant differences ( $p < 0.05$ ). Post hoc test was used to determine which groups caused the difference and it was seen that the difference was caused by T and TE groups.

## DISCUSSION

Anabolic androgenic steroids have many side effects in both young and adults (22). Nandrolone Decanoate application of the effects of the application of Nandrolone Decanoate on the heart

muscle was investigated in rats. It was reported that the use of Nandrolone Decanoate had negative effects on the heart muscle and could negatively affect the benefits of exercise (14).

Troponin I protein is an important protein in the determination of heart damage in the body (5). During exercise, the body naturally increases testosterone production. In some studies, uncontrolled exercise can also reduce testosterone production. Exercises that are appropriate and specific at the rhythm can provide better production and functioning of the testosterone hormone secreted by the body. On the contrary, it is stated that overdose cardio exercise may decrease the testosterone level (12,18). When testosterone supplementation is taken with the level of testosterone increased by exercise, it causes more harm than good in the body (16). The most important of these is the Troponin I protein, which has a high blood value in heart failure (25). In the study, which investigated the effects of AAS use on swimming-exercised rats, it was reported that the use of AAS impaired cardiac hypertrophy and adversely affected the benefits of aerobic exercise (26).

The creatine kinase-MB (CK-MB) enzyme is an enzyme found in skeletal muscle, brain, heart and other organs in the human body (9). When CK-MB enzyme rises in the blood, it can cause heart attack. The enzyme used as a marker of heart damage is secreted from the damaged cell and then mixed with the blood to increase the level of CK-MB in the blood. (27). Exercise can automatically increase the CK-MB level in the blood. Therefore, careful planning of exercise programs is necessary. When testosterone is supplemented with exercise, the CK-MB level rises rapidly in the blood in a short time (34). In the present study, CK-MB enzyme was higher in T and TE groups than the other groups. In addition, the value of the enzyme was measured at the highest level in the group where the swimming exercise and testosterone supplement were

administered. CK-MB enzyme can be a significant marker of heart damage when taken with exercise and testosterone supplementation similar to the effect of Troponin I protein.

Another important marker of heart damage is the LDH enzyme. LDH is an enzyme necessary for use in the production of energy normally used. It is found in all the body cells. In case of any damage, the level of the blood rises rapidly (20). Troponin I, CK-MB and LDH enzymes should be considered together to determine heart damage (3). During heart attack, LDH enzyme increases in blood. In severe and compelling exercises, it may cause elevated levels of LDH in the blood (34). In the present study, the LDH enzyme in the T and TE groups given testosterone was higher in blood than E and K groups. The highest number of heart damage occurred in the TE group, who received both testosterone and swimming exercises. From this finding of the study, it can be concluded that the testosterone hormone is more harmful to the body when used with exercise. Taşgın et al (30) in their study of testosterone and nandrolone combined with the increase in LDH level and the difference was reported to be statistically significant.

Muscle damage markers as blood viewed creatine kinase (CK) enzyme derived from creatine is necessary for muscle contraction and body is an enzyme used to produce energy quickly (Kanda et al 2013). In the event of any damage, muscle cells may cause CK to be mixed. Elevated CK levels may be a marker of muscle damage (3). A high level of CK in the blood may be a sign of muscle damage. A person exercising regularly may experience less muscle

damage than a person starting a new exercise. While severe exercises lead to significant increases in CK level, the increase in lighter exercises is not so much (19). In the present study, testosterone given T and TE groups showed higher levels of CK enzyme in the blood than exercise and control groups. The highest muscle damage was seen in the TE group, who received both testosterone and swimming exercises. From this finding of the study, it can be concluded that the testosterone hormone is more harmful to the body when used with exercise. In the study of Taşgın et al (30), testosterone and nandrolone were reported to be elevated in CK level and the difference was statistically significant.

## CONCLUSIONS

In the study conducted to investigate the effect of testosterone supplementation on heart and muscle damage in swimming rats. It is seen that young people tend to use banned substances in order to improve their physical appearance and to gain more performance in competitions by increasing their muscle mass and strength. In general, when the use of prohibited substances raises many problems in the organism and all the negative results of this study are taken into consideration; It can be said that it causes mainly heart and muscle damage in the body and these results can make a significant contribution to the awareness of athletes.

## ACKNOWLEDGEMENTS

This study is a portion of master thesis and is supported by Selcuk University Scientific Research Projects Coordinator (SUBAPK, 16202029).

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