



The effect of testosterone consumption during resistance training on the expression of some genes related to heart damage of male Wistar rats

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ABSTRACT

Introduction: Many studies have addressed the side effects of anabolic steroid abuse in different tissues such as heart tissue. The purpose of this research is to study the effect of 8 weeks of resistance training and testosterone consumption on some indicators of heart tissue damage in male rats.

Methods: 21 adult male Wistar rats aged 8 weeks (220 ± 10 g) were randomly divided into 3 groups: control, training (resistance training) and training + testosterone groups. The intervention of resistance exercise and testosterone injection lasted for 8 weeks. Resistance exercises were performed 5 times a week in the form of climbing a step ladder with resistance applied by tying a weight to the tail (4 sets, 6 repetitions in each set). Testosterone enanthate was injected intramuscularly 3 days a week (20 mg/kg). One-way ANOVA and Tukey's post hoc test were used to compare variables.

Results: Creatine kinase-MB (CK-MB) expression increased significantly in the training + testosterone compared to the control group ($P < 0.05$). No significant difference was observed in troponin expression between the studied groups ($P > 0.05$).

Conclusion: It seems that testosterone enanthate administration during resistance training leads to cardiac tissue damage in laboratory rats.

Keywords:

Resistance training, CK-MB, troponin, Heart tissue, Testosterone enanthate.

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1. Introduction

Resistance exercises are one of the exercises that are very popular among young people and other age groups today, paying attention to body type with the increase in volume and muscle mass has increased the tendency to use energizing substances among them. Therefore, the use of anabolic steroids to increase energy and muscle mass has become a complex problem in sports with an undeniable role (1).

Anabolic androgenic steroids (AAS) are compounds derived from testosterone that have

anabolic or androgenic effects depending on the target tissue (2). The mechanism of action of anabolic androgenic steroids may differ according to their composition due to changes in the steroid molecule and their affinity to androgen receptors (3).

Following the increase in long-term abuse of anabolic steroids, concerns about its side effects are increasing. The biggest concern in people who use steroid drugs is related to their effects on the liver, heart and blood vessels, kidneys, hormonal system, reproduction and nervous

system (4). The use of these compounds has many health risks. Various cases of sudden death have been reported in people using anabolic steroids, and histological studies show extensive changes in the heart tissue in these people (5, 6). The harmful effects of using anabolic steroids can be associated with a wide range of side effects, as well as the occurrence of heart and liver diseases.

Testosterone was first prepared artificially by Boutennet and colleagues, and after that, the use of this hormone to treat some specific diseases and general and specialized strengthening became common (7). The medicinal type of testosterone is known as testosterone enanthate, which unfortunately is widely available to athletes to increase muscle mass (8). Hayward showed that steroid hormones can cause changes in the structure and function of heart muscle cells (9).

Cardiac troponin indicators Cardiac troponin-I (CtnI) and cardiac troponin T (CTnT), Creatine kinase-MB (CK-MB) or creatine kinase isoenzyme MB are used in the examination of heart muscle cell damage (10). Among cardiac biomarkers, cardiac troponin I is a new and promising indicator for predicting the condition of patients with acute coronary syndromes. Heart problems increase cardiac troponin I (11). Kaess (2017) showed in his research that high cardiac troponin I predicts cardiovascular risks and is a predictor for the risk of heart attack (12).

Troponins are a set of three proteins bound to tropomyosin, which include: troponin T, troponin I, and troponin (C), which regulate the action of actin and myosin through calcium (13). Troponin C in skeletal muscle and heart, troponin T and troponin I are present only in the myocardium (Marston et al. 2020). Elevation of serum troponin I is clinically significant and highly specific for myocardial damage (14).

Creatine kinase (CK) is also a dimeric molecule consisting of two subunits named M and B. The combination of these subunits forms CK-MB (in muscle), CK-MB (in heart) and CK-BB (in brain) isozymes. A significant concentration of the CK-MB isozyme is found almost exclusively in the myocardium (about 15-25% of CK), and the appearance of high levels of CK-MB in the serum is very specific and sensitive for myocardial cell wall damage (15). Troponin I and CK-MB are very sensitive and special tools for recognizing heart cell necrosis and are also used to evaluate the possible damage

of heart muscle cells in athletes (16).

Some studies have reported an increase in indicators of heart muscle damage such as CK-MB, troponin I following AAS abuse (17, 18). In the study of Kolaksis et al (2019) in his study, he showed an increase in LDH and troponin indices after 30 minutes of swimming training and testosterone consumption (17). Also, in Karbasi's study, by examining the effect of 8 weeks of testosterone enanthate consumption and resistance training on the myocardium of Wistar rats, he reported an increase in CK-MB index and mild hypertrophy in the group of resistance training and training and injection compared to the control (18). In addition, mild hypertrophy occurred in the myocardium in these two groups. The higher levels of heart damage indicators in the mentioned groups can indicate the synergistic effect of exercise and medication.

Studies show that following the use of steroids, it can be associated with an increase in the amount of CK-MB enzyme and pathological hypertrophy in the heart muscle (17,19). In another study in rats that practiced swimming, the use of AAS in these rats caused an increase in CK and CK-MB enzymes and pathological hypertrophy of the myocardium (20). The studies conducted in connection with the use of anabolic steroids show that the use of these compounds can have positive effects on the physical condition and physical strength of athletes, but this so-called positive effect only exists as long as the use of the drug continues. After stopping the use of the drug, the resulting effects disappear to a considerable extent (21), however, despite the potential risk of steroid use, they have a significant prevalence throughout the world. Therefore, it is very important to study the effects of these compounds on different organs of the body, including the heart. On the other hand, considering the limitations of studies related to the effects of using these substances on the expression of genes related to myocardial damage, in this study, the effect of eight weeks of resistance training and consumption of testosterone enanthate on the indicators of heart tissue damage in rats is investigated.

2. Materials and Methods

In this experimental research, 21 male Wistar rats aged 8 weeks with (220 ± 10 grams) were prepared from Pasteur Institute of Iran and

transferred to the laboratory. Then they adapted to the conditions of the laboratory for a week. The animals were kept in washable polycarbonate cages under standard conditions with 45-55% humidity, 12:12 light-dark cycle, and 23°C temperature. They also had free access to food and water. Air conditioner, thermometer and hygrometer were used to ensure control of suitable environmental conditions and monitoring of temperature and humidity changes.

Exercise protocol and testosterone injection: One week after familiarization with the laboratory environment, familiarization exercises were performed in order to get familiar with climbing the ladder for resistance training of rodents. After that, the animals were divided into three equal groups: 1) control, 2) training, 3) training + testosterone. Rats in training and training + testosterone groups did resistance exercises for eight weeks (5 days/weekly) (21). This exercise consisted of 4 sets with 6 repetitions in each set in the form of climbing a 1-meter 26-step ladder and tying weights to the rats' tails. So climbing the stairs was done with 60% body weight in the first week and 20% was added to the weights every week. Rest between sets increased to 90 seconds and rest between repetitions increased to 30 seconds in the final week. In the combine group, in addition to exercise, testosterone enanthate (Iran Hormone Company, Iran, serial number 0069, 20 mg/kg) was injected intramuscularly 3 times a week (22). 48 hours after the last training session, all rats were anesthetized by intraperitoneal injection of mixture of 10% ketamine (50 mg/kg) and 2% xylosin (10 mg/kg) after 10-12 hours of fasting. Then their heart tissue was extracted and washed in physiological serum and immediately frozen in liquid nitrogen and transferred to freezer-80 to measure the variables.

Tissue extraction and gene expression: Real time-PCR method was used to measure the expression of studied genes. To determine the quality and quantity of the extracted RNAs, the OD of all samples was measured with a nanodrop device. Then cDNA synthesis was done by TAKARA kit (TAKARA cat NO. 6130) according to the manufacturer's instructions. RNA extraction was performed according to the instructions of the manufacturer of the kit. From each sample, 2 micrograms of mRNA were used to synthesize the first strand of DNA. The relative

amount of gene expression in the studied genes was measured with the help of their specific primers. The absorption ratio of 260 to 280 nm for all extracted samples was 1 to 2.8. Electrophoresis and 1% agarose gel were used to check the quality of extracted RNA. And the comparative Ct method was used to determine the relative expression level of target genes (24). GAPDH was used as the control gene. The sequence pattern of the primers are shown in table 2.

Table 2: The sequence pattern of the primers

Genes	Primer sequence
Troponin	For: CTCTGCCAACTACCGACCTAT
	Rev: CTTCCATCTCCTGCTTCGCAA
CK-MB	For: GCTCATTGACGACCACTTCCTC
	Rev: CCTCCTCGTTAATCCACACCAG

Statistical analysis: All statistical studies were done using SPSS/Win version 24 software. Shaperovic's test was used to ensure the normal distribution of the data. One-way ANOVA and Tukey's post hoc test were used to compare variables. Changes of less than 5% were considered significant.

3. Results

Based on the results of the ANOVA test, a significant difference was observed in the expression of the CK-MB gene between the studied groups ($P = 0.024$). On the other hand, the findings of Tukey's post hoc test revealed that the expression of CK-MB in the training + testosterone group is significantly higher than the control group ($P=0.032$). In other words, testosterone injection during resistance training led to a significant increase in CK-MB expression compared to the control group. However, no significant difference was observed in the training and control groups ($P=0.353$). Also, there was no significant difference in the expression of CK-MB between the training and training + testosterone groups ($P=0.741$) (Fig. 1).

On the other hand, the results of the ANOVA test showed no difference in troponin expression between the studied groups ($P = 0.154$). In other words, resistance training alone and the use of testosterone enanthate during resistance training did not lead to changes in troponin expression (Fig 2).

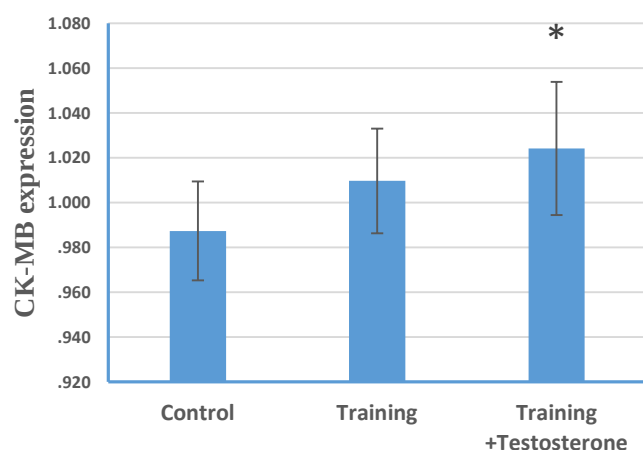


Fig 1: The pattern of changes in CK-MB expression following resistance training and testosterone injection compared to the control group (mean $\pm$ -SD, * $p < 0.05$ compared to control).

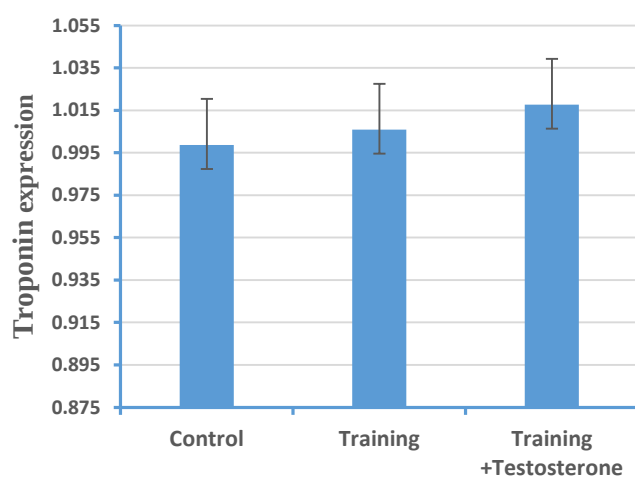


Fig 2: The pattern of changes in Troponin expression following resistance training and testosterone injection compared to the control group (mean $\pm$ -SD)

4. Discussion

The increase in CK-MB expression in the training + testosterone group compared to the control group is the main finding of the study. Based on statistical results, despite the upward trend of troponin and CK-MB expression in response to resistance training, this increase was not statistically significant. Some studies in line with the study conducted in the field of the effect of exercise training on heart muscle damage indicators, show an increase in the levels of these indicators, among others, we can mention the study by Abdulahi et al (2017) that the effect of 8 weeks of resistance training and has investigated the effect of troponin, myoglobin and CK-MB on non-athlete men. The results showed that in the resistance training group, all three measured factors were associated with a significant increase after 8 weeks (25). The researcher states that the

increase in the mentioned indicators can be caused by the intensity of the exercises. Considering that troponin is considered as an indicator of cardiac necrosis, it may be overshadowed in the conditions of intense training and lead to heart tissue damage (25). Also, in Rejaei et al.'s study (2013) by comparing three models of resistance, endurance and combined training on the damaging indicators of heart cells of active people, they showed that troponin levels increased insignificantly after performing sports activities, but these values in resistance training And there was more endurance. The researcher stated that this increase is due to the type of exercises and the possible damage caused by the intensity of the activities performed (26).

In addition, in a review study, in the review of three studies on men's triathlon, a total of 69% of subjects reported an increase in troponin. Participating in men's triathlon often leads to a gradual increase in troponin levels, and an increase in troponin has been associated with echocardiographic evidence of abnormal left ventricular function, which should be considered as the long-term consequences of such damage to the heart (27).

Some studies that have reported increases in troponin or CK-MB levels after exercise indicate that these values approach baseline values after a few days. So, in the study of Sedaghat et al (2015), the effect of acute resistance training on changes in biochemical markers and myocardial damage (cTnT, cTnI, CK-MB) in non-athletes showed that troponin T and CK-MB levels in 24 and 48 hours returned to baseline values after exercise. The increase in troponin in exercise can be caused by the increase in the permeability of the heart muscle cell membrane and the release of free cytoplasmic cardiac troponin, and not due to the necrosis of cardiac cells and the release of troponin bound to myofilaman (28).

In the study of Savukoski et al (2015), after resistance training, cTn values were significantly higher than before training, but returned to baseline levels after 3 days. The researcher states that the examination of troponin changes related to exercise should be carefully examined as part of the evaluation of "normal cTn". Studies show that the higher the intensity and duration of exercise, the higher the increase in troponin. The reason for the contradiction between these results

and the results of the present study can be the measurement time, which in our study was done 48 hours after the last training session, and in the aforementioned study, the values returned to baseline levels 72 hours after the training (29).

Some studies have also reported a decrease in the mentioned indicators. For example, in the study of Rangraz et al (2018), the effect of resistance training for 8 weeks led to a decrease in troponin I in elderly men (30). Also, in the study of Linden et al (2024), 24-week resistance training led to a significant decrease in plasma cTnT in elderly people (31), it seems that the reason for the different response in these elderly groups, in addition to the lower intensity of the activity, is the role of exercise. It is a resistance in preventing skeletal and cardiac muscle atrophy and preventing sarcopenia by reducing higher basal troponin levels.

The results of the present study regarding the effect of exercise + testosterone on the CK-Mb index showed a significant increase of this index in the combined group compared to the control group. Although the changes in troponin expression showed a non-significant increase in the combined group compared to the control group in the rats of the present study. In relation to the effect of exercise and testosterone on the mentioned indicators, limited studies have been conducted, the results of the present study in this regard are in line with the results of the study of Karbasi et al (2017) (18). In Karbasi's study with the aim of determining the effects of 8 weeks of testosterone enanthate consumption and resistance training on the myocardium of Wistar rats, the results showed that the activity of CTnI and CK-MB was significantly higher in the resistance training and training + testosterone groups compared to the control group. . Also, mild hypertrophy occurred in the myocardium in these two groups. The higher levels of heart damage indices in the mentioned groups can indicate the synergistic effect of exercise and testosterone.

In the study of Kulaksız et al (2019), who investigated the role of anabolic androgenic steroids on rat heart muscle damage. The levels of lactate dehydrogenase (LDH), CK-MB and CK troponin I were higher in the groups that took testosterone. The researcher states that the use of testosterone can increase the indicators of heart damage (17). Also, Mazji et al (2022)

investigated the effect of eight weeks of exercise training on the structural and biochemical changes in the heart tissue of male Wistar rats receiving steroids, and did not show any significant difference between the groups in the cardiac troponin index. Also, the amount of testosterone in the plasma of rats receiving steroids, both in the therapeutic dose and in the abuse, significantly decreased compared to the training groups (32). The results of this research showed that aerobic training can prevent the decrease of endogenous testosterone, but it has no effect on the amount of cardiac troponin and the weight of rats. The difference observed in this study could be due to the type of steroid used (Boldenone) and the nature of the exercise (aerobic) in the Mazji study.

In general, the physiological levels of androgenic steroids can have positive effects on the coronary arteries of the heart through the release of nitric oxide and the inhibition of smooth muscle tone, but the abuse of steroids can reverse this vasodilating response and lead to apoptosis (33). These effects are likely to be modulated by secondary membrane receptor signaling cascades that increase intracellular calcium ion flux and calcium ion recall from the sarcoplasmic reticulum (34). An increase in calcium ions affects mitochondrial permeability and leads to the release of apoptogenic factors such as cytochrome C and caspase 9 (35). These findings may confirm clinical observations that the expression of anabolic androgenic steroids without coronary thrombosis can lead to death or atherosclerosis of the heart arteries (34).

On the other hand, according to the studies, steroid abuse can increase the pathological hypertrophy of heart muscle cells (36), and the increase of CK-MB enzyme can also increase the cross-sectional area of heart cells and their hypertrophy (19, 37,38) which is associated with euchromatinization of the size of the nucleus of heart muscle cells, which is a sign of high activity of the nucleus and hypertrophy of heart muscle cells (39). As mentioned, hypertrophy can cause an increase in CK-MB and a sign of heart muscle cell damage (37). Although examining the expression of troponin and CK-Mb indices in the heart tissue along with exercise and testosterone consumption is one of the strengths of this study, but since the increase of injury indices can cause inflammation and myocarditis, the lack of

examination of myocarditis indices such as TGF β gene expression and telomerase activity of myocarditis, as well as lack of heart histology in this research, are among its other limitations.

In the present study, myocardial histopathology studies that show the degree of hypertrophy of heart cells were not performed, which is one of the limitations of the present study.

5. Conclusion

In general, according to the findings of the present study, it is concluded that resistance training does not cause changes in the CK-MB and troponin expression as indicators of heart tissue damage in rats. But the abuse of testosterone along with intense resistance training can lead to an increase in the expression of some indicators of heart damage. Therefore, in order to prevent damage and reduce heart function, it is suggested not to use supra-physiological doses of anabolic steroid compounds with resistance exercises.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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