

Structural changes of cardiac tissue in response to L-carnitine supplementation during endurance training in Wistar male rats toxicated by steroid anabolic hormone

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Original Article

Abstract

Introduction: The aim of this study was to investigate the structural changes of cardiac tissue in response to L-carnitine supplementation and endurance training in Wistar male rats toxicated by steroid anabolic hormone.

Methods: 36 male Wistar rats aged 8-12 weeks (weight 195 ± 7.94 g) were randomly divided into six groups: Control, Non-treated, Boldenone (5 mg/kg), L-carnitine (100 mg per kg), Endurance Training-L-carnitine and Sham group. Injection was conducted deep in the hamstring once a week on an appointed day. The endurance training Protocol with moderate intensity (50-55% maximal oxygen consumption) was performed for six weeks and five times a week. After anesthesia, autopsy was performed and the cardiac tissue was isolated.

Results: The results showed that boldenone causes damage to the heart tissue muscle and heart muscle cells. Also, boldenone caused mild necrosis and congestion in the heart tissue. However, the results showed that L-carnitine supplementation with endurance training can decrease to normal levels and damage the heart tissue muscle and heart muscle cells.

Conclusion: According to the findings, it seems that boldenone causes some structural changes in the heart tissue, and possibly endurance training with L-carnitine supplementation can reduce these changes.

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Introduction:

Over the past decades, anabolic-androgenic steroids have found great popularity among athletes for improving their performance. Misuse of anabolic-androgenic steroids is not limited to elite

athletes; this action has been proven among many amateur and recreational athletes (1). Among several approved toxic and hormonal effects of anabolic-androgenic steroids, cardiovascular effects have been particularly considered in recent years (2). An increase in blood pressure and peripheral

vascular resistance has been shown in experimental studies (3,4). Also, their effects have been confirmed on the heart muscle, especially on left ventricular hypertrophy and limited diastolic function (5,6). Boldenone is a 1-dehydro derivative of testosterone that is banned due to its adverse effects on the human body (7). This steroid is used directly to build muscle. However, there is little information on its adverse effects on different body tissues such as heart tissue structure.

Studies have shown that long-term use of high doses of anabolic-androgenic steroids can lead to an increase in ventricular wall thickness, cardiomyopathy, arrhythmia, heart failure and sudden heart death in strength athlete (8-10). On the other hand, exercise and endurance training can cause significant changes in heart tissue morphology, including increasing the size of the left ventricular cavity, wall thickness and increase in heart mass, which is known as athlete's heart (11).

Physiological balance and adjustment are reversible and are followed by required and new angiogenesis, because muscle hypertrophy occurs as a result of heart muscle adaptation in response to increased physical activity (12).

Testosterone and its synthetic derivatives have been used to increase muscle mass and improve physical performance. Administration of exogenous anabolic-androgenic steroids induces heart hypertrophy, in vitro and in vivo (13,14). In addition, administration of anabolic-androgenic steroids at high doses in conjunction with intense exercise in rats leads to heart hypertrophy, increased levels of inflammatory cytokines and significantly stimulation of sympathetic nervous system (15). Thus, the combination of these factors may predispose individuals to myocardial injury.

However, heart muscle adaptation to exposure to high doses of anabolic-androgenic steroids and supplement use and its association with physical activity have been poorly understood. It has been shown that L-carnitine supplementation inhibits inflammation (16). L-carnitine is the biologically active form of carnitine, an endogenous non-essential branched-chain amino acid, which has a vital role in energy production. L-carnitine plays an important role in energy production in the myocardium and passes free fatty acids into the mitochondria, increasing the preferred substrate for

oxidative metabolism in the heart (17). It has been demonstrated that exogenous supplementation with L-carnitine restores depleted reserves of heart carnitine and improves heart and left ventricle metabolism (18). Studies have shown that L-carnitine plays potential therapeutic role in several chronic diseases, including cardiovascular and inflammatory diseases (19).

In almost all studies, side effects of using anabolic-androgenic steroids have been investigated in a short term or within a few weeks to a month (20). However, medium-and long-term effects are still controversial issues. Reviews show that the studies have been designed to show the effects of high doses of anabolic-androgenic steroids on the heart muscle tissue, with or without training (9,15,21,22). The present study was conducted to better understand the effects of high dose of anabolic-androgenic steroids on the heart muscle tissue morphology combined with training and supplements within a six-week period.

Due to the widespread use of anabolic-androgenic steroids by athletes and their side effects on the heart tissue structure as well as with respect to wide prescription of these drugs without any supervision by unqualified individuals to athletes and youth, the results of this research could contribute to a better understanding of athletes about the effects of these drugs. Furthermore, there is little information about the effects of anabolic-androgenic steroids on the responses of heart tissue structure during endurance training and nutrition (supplements). According to the above-mentioned issues, this study was performed to evaluate the changes of the heart tissue structure in response to L-carnitine over a period of endurance training in boldenone-intoxicated male Wistar rats.

Methods:

The subjects consisted of male Wistar rats provided by Damghan Institute of Applied Science and Technology, Iran; among the sample, 36 rats aged between 8 and 12 weeks with initial weight of 195 ± 7.94 g were selected as statistical samples.

The statistical samples in this research were obtained using purposive-selective sampling method according to weight and age; then, they were divided randomly into six groups, with six rats in

each group. First, all groups began to use 7-week high-dose steroids (5mg/kg) (9). Groups were divided as follows: After that,

1. one group died after steroid intake (control group (C));
2. The group without treatment did not use any substance and did not perform any activity (without treatment group (WT));
3. One group that continued to use steroids (boldenone group (B));
4. One of the groups used only L-Carnitine (100 mg per kg) (L-Carnitine group (LC));
5. The other group used carnitine and performed training (L-Carnitine-training (LCT));
6. The sham group used olive oil because steroids were diluted with oil (5 mg/kg) (SH).

The study groups were kept in PVC cages for rodents with metal mesh cap and floor covered by clean wood shavings. Room temperature was $22 \pm 1.4^\circ\text{C}$ with humidity of 65-75%. The samples were maintained in accordance with 12-hour sleep-wake cycle with access to water and food. They used ready and compressed food for rats (prepared from Gorgan feed industry) and urban treated water in PVC drinking cup. Graduated insulin syringes were used to inject medication in the posterior thigh muscle by depth injection in a certain time and hour (11 am) once a week and on an appointed day of the week. The control group received a physiological saline solution or sodium chloride 0.09.

Endurance training protocol

In the present study, moderate intensity (50-55% moderate intensity training) and physiologically efficient training was used, so that training groups were exposed to moderate-intensity treadmill training for five days per week during six weeks. Speed and duration of training on a treadmill increased gradually from 10 meters per minute for 10 minutes in the first week, 10 meters per minute for 20 minutes in the second week, 14-15 meters per minute for 20 minutes in the third week, 14-15 meters per minute for 30 minutes in the fourth week to 17-18 meters per minute for 30 minutes in the fifth week. All training variables were kept constant in the final week to reach steady

adaptations (23). Low-voltage electrical was used to stimulate the rats for running.

Sampling procedures and measuring structural changes in the heart muscle tissue

At the end of the training sessions (after 56 days) the animals were held fasting for 12 hours. The samples were weighed and anesthetized for sampling. Anesthesia was performed using closed glass chamber (desiccator) containing cotton soaked in chloroform (Merck, Germany). The animals were placed in good anesthesia after 40 to 50 seconds. After anesthesia, the dissection was performed by fixing the animal on rodent surgery board, and the heart tissue was removed immediately. The samples were fixed in 10% formalin, and they were then prepared for routine histological methods. After performing the routine procedures, 5-micron tissue sections were obtained. The sections were stained with hematoxylin and eosin. The tissue sections were evaluated using a microscope, and images were taken from all slides. The heart tissue samples were prepared from six groups after the intervention of the independent variables.

Structural changes in the heart tissue were studied and then compared with one another. Ethical principles were considered in this study about how to work with laboratory animals, including access to food and water, proper living conditions and absence of coercion in training. All tests were carried out according to Helsinki declaration, and were reviewed and approved by the ethics committee of Islamic Azad University, Ayatollah Amoli Branch, Iran.

How to measure structural changes in heart tissue.

Variables evaluated in the heart tissue present in the samples included overall changes in heart tissue, muscle cells, necrosis, inflammation and congestion. The severity and degree of these changes were based on microscopic observation and photomicrographs. At least four fields were observed in each slide under the microscope (400X) and imaging was carried out.

The changed cells were counted in each field, and changes in cells and tissues were graded from 0 to 3 (Grade 0: no change, Grade 1: mild, Grade 2: moderate, and Grade 3: severe). Observations on

the cellular changes included changes in cell morphology, size, color and amount of cytoplasm, color and shape of nucleus, and necrotic changes (degree of cell death). The presence of inflammatory cells in the area was studied for inflammation variable.

Results:

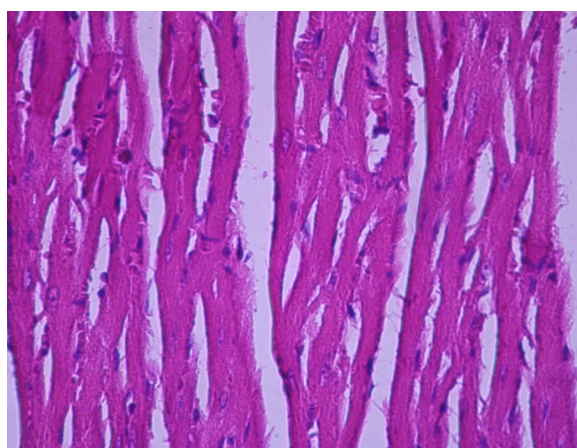
Table 1 shows separation and grading of changes in the variables evaluated in the heart tissue in different groups. The results indicated that

supplementation with boldenone caused damage to the heart muscle tissue and cells as well as mild congestion and necrosis in the heart tissue. The results demonstrated that L-carnitine supplementation could reduce damage in the heart muscle tissue and heart muscle cells to some extent (Table 1). In addition, L-carnitine supplementation along with endurance training can reduce damage in the heart muscle tissue and heart muscle cells at normal level (Table 1).

Table 1. Separation and grading of changes in variables evaluated in heart tissue in different groups

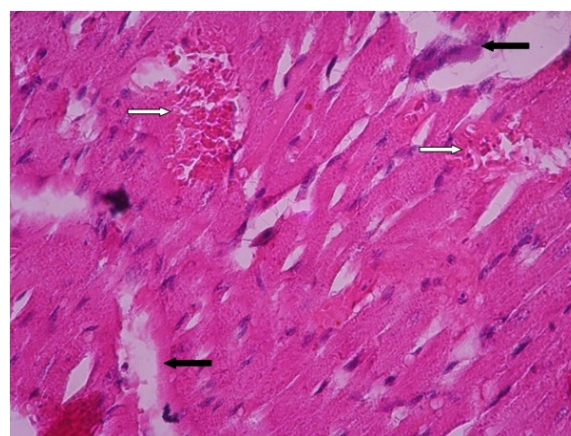
Groups	Changes in heart muscle tissue	Muscle cells	Necrosis	Inflammation	Congestion
Control	0	0	0	0	0
Sham	0	1	0	0	1
Boldenone (5 mg/kg)	2	2	1	0	1
L-Carnitine	0	1	0	0	1
Training -L-Carnitine	0	0	0	0	1
Without treatment	1	1	0	0	1

Histological analysis related to photomicrographs obtained from samples of heart in experimental groups is presented in Figures 1 to 6. The photomicrographic results obtained from rat hearts in the control group showed that the cells have normal ramifications, regular connection, acidophilic cytoplasm and clear nucleus, as well as cell death or distortion cannot be seen in the tissue (Figure 1).



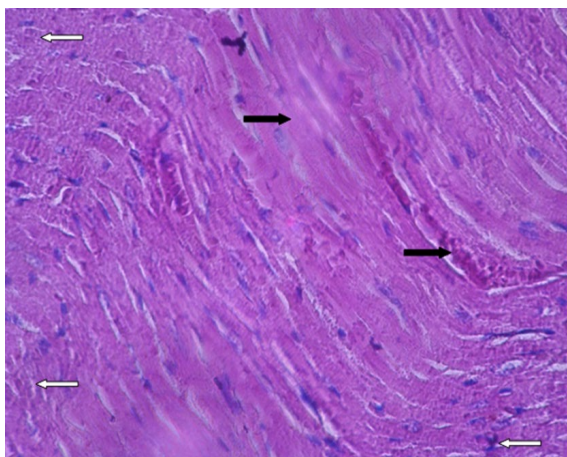
**Figure 1. Photomicrography produced by cardiac rats. (H&E × 400)
Control group**

Most of the heart muscle cells have normal characteristics in the sham group, but congestion (white arrows), some detachment and distortion (black arrows) are visible in the tissue (Figure 2).



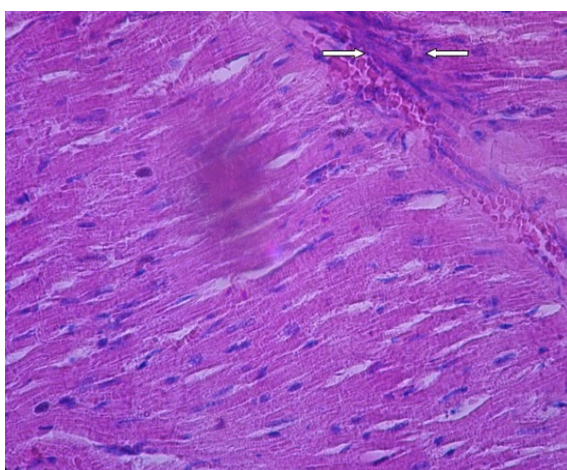
**Figure 2. Photomicrography produced by cardiac rats. (H&E × 400)
Sham group**

In the boldenone group (5mg/kg), tissue specifications show destruction so that most of the cells have no clear nucleus and cytoplasm. The nucleus is dark and cytoplasm is non-uniform and pale. In some areas (white arrows), cytoplasm has vacuole and abnormal articles. There are also signs of congestion and intracellular destruction (black arrows) (Figure 3).



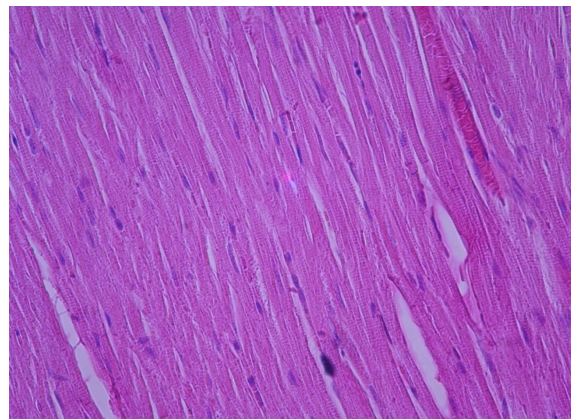
**Figure 3. Photomicrography produced by cardiac rats. (H&E × 400)
Boldenone group (5 mg / kg)**

In Group carnitine, distortions and increased density are observed (white arrows) in some areas of the tissue, the congestion is also evident. Specifications of nucleus and cytoplasm are also slightly abnormal (Figure 4).



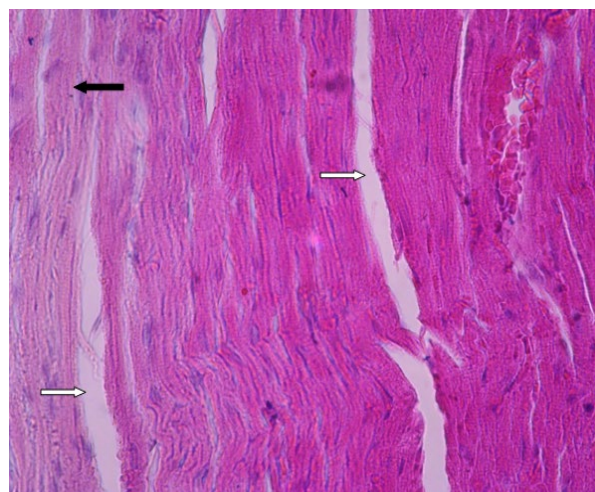
**Figure 4. Photomicrography produced by cardiac rats (H&E × 400)
L-carnitine group**

Tissue characteristics are normal in most samples in the heart of carnitine-endurance training group and there is only congestion. The cells are regular with no abnormal profile (Figure 5).



**Figure 5. Photomicrography produced by cardiac rats. (H&E × 400)
Endurance training- L-carnitine group**

The important point in the non-treated group is pale cytoplasm and unclear nucleus (black arrows), indicating abnormal areas of tissue. In addition, most cells are regular, but there is detachment and tissue torsion in some areas. Congestion can be seen in the tissue (Figure 6).



**Figure 6. Photomicrography produced by cardiac rats. (H&E × 400)
No treatment group (5 mg / kg)**

Conclusion:

The results of the present study showed that boldenone caused damage to the heart muscle tissue

and cells, necrosis and mild congestion in the heart tissue. In the existing literature, there are a set of data regarding the chronic effects of the misuse of anabolic-androgenic steroids on the functioning and structure of many organs (24-26). Similarly, the misuse of steroids is associated with changes in the structure of the heart, size and thickness of ventricle, as well as the content of heart connective tissue (26-32,33). The results of this study are consistent with previous findings. In the current study, damage to the heart muscle tissue, heart muscle cells, necrosis and mild hyperemia were observed in the boldenone-toxicated groups. Physiological and pharmacological mechanisms of the influence of anabolic-androgenic steroids on the structure and functioning of the heart have not yet been clearly identified. Anabolic-androgenic steroids bind towards androgen receptors in the heart and major arteries, and physiological levels (e.g., testosterone) may have a positive impact on coronary artery by releasing endothelial nitric oxide and inhibit vascular smooth muscle tone (33).

Animal studies have shown that the misuse of anabolic-androgenic steroids such as nandrolone at high doses may reverse the vasodilator response, increase growth effects on the heart tissue as hypertrophic cardiomyopathy, and consequently cause cell apoptosis (34). These effects are adjusted most likely by cascade of secondary membrane receptors that increase the intracellular Ca^{2+} and Ca^{2+} recall from sarcoplasmic reticulum (35).

Increased Ca^{2+} affects the mitochondrial permeability and leads to the release of apoptogenic agents such as cytochrome C, apoptosis-inducing factor, and caspase-9. It is noteworthy that the dose of anabolic-androgenic steroids regarding sudden heart death, MI, cardiomyopathy and ventricular reconstruction is related to apoptosis (36).

Also, the results of the present study showed that L-carnitine supplementation with endurance training could reduce damage in the heart muscle tissue and heart muscle cells to some extent. The role of L-carnitine in energy metabolism and fatty acid oxidation is very important in the heart (37), because the heart provides 70 percent of its energy through the catabolism of fatty acids (38).

In this study, the role of carnitine concentrations and duration of administration were shown to be critical for heart tissue damage resulting from

boldenone. The results of this study showed that carnitine injection along with endurance training could reduce the myocardial tissue damage caused by boldenone. Previous studies have demonstrated the protective effects on the heart due to supplementation with L-carnitine (39-42). In this regard, Sada et al. (2015) showed that L-carnitine supplementation significantly increased left ventricular function (42). Koeth et al. found a significant association between plasma L-carnitine levels and risk of coronary artery disease, peripheral vascular disease, and heart risk factors in heart disease (43).

In the present study, reduction of heart damage after heart intoxication was observed after L-carnitine supplementation combined with exercise activities. Researchers have confirmed that the steroids, with or without training, influence the thickness of the heart with a dose-dependent method, but they have often examined the effects of high doses (from 1 to 10 mg/kg/week) on the heart (9,15,21,22,44). However, due to the lack of similarity in dose of anabolic-androgenic steroids, training programs and duration of experimental treatment in various studies, it is difficult to draw strong conclusions about the real effects of specific doses of steroids on the heart tissue. In the current study, high-dose steroids were used, which caused some changes in the heart tissue. Given that most parts of the heart wall consist of heart muscle cells, the findings of this study are consistent with the results of measuring cell diameter in a research by Sretenovic et al. (2016) who confirm these findings (44). Ren et al. (2012) also found that nandrolone induces heart hypertrophy (44). Overall, the present study demonstrates the side effects of androgenic steroid of boldenone on the heart morphology. Therefore, high doses of androgenic steroids used by some athletes during training and competition can lead to heart tissue damage and destruction. The limitations of this study can be seen in not measuring other factors related to the structure and morphology of the heart tissue. Also since it is that the administration of steroids during training affects the heart in dose-dependent manner (44), therefore, administration of different over dosage physiologic doses can help to better understand the effects of androgenic anabolic steroids.

In conclusion, the results of this study indicated that boldenone causes damage to the heart muscle tissue, heart muscle cells, necrosis and mild congestion in heart tissue. According to the findings of this research, it seems that endurance training along with L-carnitine supplementation could possibly reduce the structural changes in the heart tissue of boldenone-intoxicated rats. However, since few studies have been conducted in this research area, studies on the relationship among training activities, anabolic steroids and structural changes in the heart tissue clearly require further elucidation.

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