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Effect of Boldenone Administration on Some Organ Damage Markers in Trained Rats

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Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited.

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Abstract

The aim of this study was to examine the effects of Boldenone administration on skeletal muscle, liver and heart organs in exercised rats. Rats were divided into 4 groups as Control(C), Exercise (E), Boldenone(B) and Boldenone + Exercise (BE). There are 6 rats in the C and E groups and 7 rats in the other groups. The rats in groups E and BE were given a 45-minute treadmill exercise 5 days a week at a speed of 1.5km/hour for 8 weeks. In the statistical evaluation of the data, the results were given as mean±SD using the SPSS 22 package program. ANOVA and Duncan tests were used to compare the data between groups. At the end of the 8-week study, blood samples taken from rats were found to be significantly higher in AST and CK-MB values in B and BE groups than in C and E groups as a result of the statistical analysis (p0,05), it was observed that the mean values of the B and BE groups were higher than the mean values of the C and E groups in both parameters. LDH values were significantly higher in E, B and BE groups compared to C group ($p<0.05$). With the result obtained from the findings; It has been observed that the use of AAS increases liver enzyme levels (ALT, AST, ALP, LDH) and CK-MB levels, which is a marker of heart muscle damage. With these findings, it can be said that the use of Anabolic Androgenic Steroids has negative effects on the heart and liver. In addition, the fact that the mean values of the BE group were higher than the other groups in all parameters, as exercise did not reduce the amount of these negative side effects of AASs, indicates that exercise may increase these side effects even more.

Keywords: Anabolic androgenic steroids, Boldenone, Exercise, Heart, Skeletal muscle.

Boldenon Uygulamasının Egzersiz Yaptırılan Sıçanlarda Bazı Organ Hasarı Belirteçlerine Etkileri

Özet

Bu çalışmanın amacı boldenon uygulamasının egzersiz yaptırılan sıçanlarda iskelet kası, karaciğer ve kalp organlarına etkilerini incelemektir. Ratlar, kontrol (C), egzersiz (E), boldenon (B) ve boldenon + egzersiz (BE) olmak üzere 4 gruba ayrıldı. C ve E gruplarında 6, diğer gruplarda ise 7'şer sıçan vardı. E ve BE gruplarındaki sıçanlara 8 hafta boyunca 1.5km/saat hızda olacak şekilde haftada 5 gün 45 dakikalık koşu egzersizi yaptırıldı. Buna ek olarak B ve BE grubundaki sıçanlara haftada 10mg/kg dozda Boldenon uygulaması yapıldı. Verilerin istatistiki değerlendirilmesinde SPSS 22 paket programı kullanılarak sonuçlar ort±SS olarak verildi. Verilerin gruplar arası karşılaştırılmalarında ANOVA ve Duncan testi uygulandı. 8 Haftalık çalışmanın sonunda sıçanlardan alınan kan örneklerinde Yapılan istatistiki inceleme sonucunda Aspartat Aminotransferaz (AST) ve Kreatin fosfokinaz (CK-MB) değerlerinin B ve BE gruplarında C ve E gruplarına göre anlamlı ölçüde yüksek çıktığı görülmüştür ($p<0,05$). Alanin Aminotransferaz (ALT) ve Alkalen Fosfataz (ALP)

değerlerinin ise diğer gruplarla kıyaslandığında E grubunda anlamlı ölçüde düşük çıktığı gözlemlenmiştir ($p<0,05$), her iki parametrede de B ve BE grubu ortalama değerlerinin C ve E grubu ortalama değerlerinden daha yüksek çıktığı görülmüştür. Laktat dehidrogenaz (LDH) değerleri ise E, B ve BE gruplarında C grubuna göre anlamlı ölçüde yüksek çıkmıştır ($p<0,05$). Bulgulardan elde edilen sonuçla; Anabolik-androjenik steroid (AAS) kullanımının karaciğer enzim seviyelerini (ALT, AST, ALP, LDH) ve kalp kası hasarının belirteci olan CK-MB seviyelerini artırdığı gözlemlenmiştir. Bu bulgularla AAS kullanımının kalp ve karaciğer üzerine olumsuz etkileri olduğu söylenebilir. Ayrıca egzersiz yapmanın AAS'lerin bu olumsuz yan etkilerinin miktarını azaltmadığı gibi, BE grubunun ortalama değerlerinin tüm parametrelerde diğer gruplardan daha yüksek çıkması, egzersizin bu yan etkileri daha da artırıyor olabileceğini gösterebilmektedir.

Anahtar Kelimeler: Anabolik androjenik steroidler; Boldenon; Egzersiz; İskelet kası; Kalp.

INTRODUCTION

Anabolic androgenic steroids (AAS), is a very wide group of androgen molecules that contain testosterone and its synthetic derivatives (1). AAS's are widely used worldwide to achieve an aesthetic appearance by promoting muscle growth and to enhance the performance of athletes (2). Also, AAS increases the thickness of muscle fibers and causes an increase in muscle strength (3). For this reason, the use of AAS, which was initially common only among bodybuilders, has become widespread among athletes over time (4). AAS, particularly due to its long-term detrimental effects on the cardiovascular system, poses adverse impacts on all body organs, tissues, bodily functions, and fertility. Consequently, the usage of AAS is considered a matter of public health concern (5, 6). Diseases of the cardiovascular system are the most prevalent kind of health threats to humans and represent a societal health concern (7, 8).

Boldenone is an anabolic steroid widely used in the sports world. It was first developed for animals, especially horses, but over time it has also begun to be used by humans for bodybuilding and performance enhancement. Boldenone is a compound with anabolic and androgenic effects. Its anabolic effects are manifested by properties such as increased muscle mass, increased protein synthesis, and increased nitrogen retention. Its androgenic effects are associated with the development of male sexual characteristics. Boldenone use can lead to many potential side effects. These include acne, hair loss, deepening of the voice, changes in libido, and cardiovascular problems. In addition, long-term and high-dose use can have negative effects on liver and kidney health (4,9).

Creatine kinase MB (CK-MB) is the enzyme that plays a pivotal role in generating ATP, the primary energy source within the heart and skeletal muscles. CK-MB facilitates energy supply to the cell by transforming creatine in the muscle into creatine phosphate as required. Hence, CK-MB assumes a critical role particularly for high-energy-demand tissues like muscles. Given that CK-MB constitutes a key cellular component of the heart and skeletal muscle, any damage inflicted on these tissues could potentially lead to a surge in the serum CK-MB levels within the heart and skeletal muscles (10). Troponin I and Troponin T are distinct isoforms present in cardiac and skeletal muscles, respectively. Troponin I is located within the cardiac muscle, whereas Troponin T is incorporated into the skeletal muscle's architecture (11). Just as with CK, Troponins can also exhibit a rise in serum levels as a result of tissue damage (12). Creatine Kinase is a form of protein primarily located in cardiac and skeletal muscles. It's recognized that CK concentrations rise following circumstances that inflict damage on the muscle, like muscle traumas and physical exertion (13, 14). Enzymes in the liver, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma glutamyl transpeptidase (GGT), serve as crucial indicators for diagnosing liver damage. Reports suggest that in individuals who consume AAS, these enzymes can elevate to levels that are 2-3 times higher than the standard values (15, 16). Despite the serious and permanent damage that AAS can cause to human health, they are used unconsciously and widely among athletes to enhance performance. The current study is important in terms of revealing the disadvantages of these substances being used for doping by athletes and contributing to the field of sports sciences. The aim of this study was to examine the effects of Boldenone administration on skeletal muscle, liver and heart organs in exercised rats.

METHOD

Experimental Animals

The research was conducted on 27 rats (Male, Wistar) that were 30 days old (106.8 g), obtained from the Experimental Medicine Research and Application Center of Selcuk University (The study was completed with 26 rats due to the death of 1 rat in the Exercise group). The trial period lasted 8 weeks. The procurement, care, feeding, and experimental application of the rats were carried out at the Experimental Medicine Research and Application Center of Selcuk University. The rats were housed in plastic rat cages in the animal trial unit, at a room temperature of 23±2°C, in an environment with 50±10% relative humidity, under a 12/12 night/day light period, and were fed ad libitum. Fresh water, which the rats could always drink, was kept in front of them and refreshed daily. The animals were grouped as follows.

Group C (Control group) (n:6): The rats in this group were given standard rat feed and drinking water ad libitum throughout the study period.

Group E (Exercise group) (n:7): Rats in this group were given standard rat feed and drinking water ad libitum throughout the study. They were made to exercise on a treadmill at a speed of 25m/min for 45 minutes a day, 5 days a week, for 8 weeks. As a result of the death of 1 (one) rat in this group, the study continued with 6 rats

Group B (10 mg Boldenone group) (n:7): The rats in this group will be given standard rat feed and drinking water ad libitum throughout the study period. Boldenone undecylenate has been administered at a dose of 10 mg/kg/rat, diluted in 100 µl of peanut oil, intraperitoneally once a week for a duration of 8 weeks.

Group BE (10 mg Boldenone + Exercise group) (n:7): The rats in this group will be given standard rat feed and drinking water ad libitum throughout the study period. This group received an intraperitoneal injection of Boldenone undecylenate at a dose of 10 mg/kg, diluted in 100 µl of peanut oil, once a week, administered one hour before starting exercise. The rats in this group have been exercised for a duration of 8 weeks.

Boldenone supplementation: For 8 weeks, rats in the B and BE groups were administered Boldenone (EQUIPOISE (Boldenona-E) Boldenone undecylenate 200 mg/ml, SP Laboratories) at a dose of 10 mg/kg/rat (17), diluted in 100 mcl of peanut oil, intraperitoneally. The body weights of the rats were measured at the beginning of the study and every week for the following 8 weeks on the same day, and the weekly dose (10 mg/kg/rat) for Boldenone application was adjusted

Exercise program: An 8-lane treadmill, specifically designed for rats, was used for the exercise application. Following a 1-week (5 days) acclimatization period, the groups to which exercise would be applied were made to exercise on the treadmill for 45 minutes at a speed of 25m/min (1.5 km/hour) (17), 5 days a week, for 8 weeks.

Warm-up protocol:

Day 1: 10 m/min, 10 min

Day 2: 20 m/min, 10 min

Day 3: 25 m/min, 10 min

Day 4: 25 m/min, 20 min

Day 5: 25 m/min, 30 min

Measurements: At the end of the trial, the necessary biochemical parameters were measured from the serum obtained by taking blood from the hearts of the rats. The levels of serum LDH, AST, CK, ALT, and ALP were determined on an autoanalyzer (Ilab 300 Plus, Milan, Italy), while the levels of Troponin I and CK-MB were determined with an ELISA (BT LAB) kit. The biochemical analyses in the study were conducted at Konya System Laboratory, a competent laboratory.

Data Analysis: For the statistical evaluation of the research data, the SPSS 22.0 (SPSS 22.0 for Windows/ SPSS® Inc, Chicago, USA) package program was used. The data of the study were evaluated in a computer environment, using mean and standard deviation for descriptive statistics, and the One Way ANOVA test was

used for comparing the average scores between groups. The Duncan test was preferred as a post hoc test to find out which groups the difference originated from. The level of statistical significance was accepted as $p<0.05$.

Ethical approval and institutional permission

In order to conduct the research, ethical approval was received from Selçuk University Experimental Medicine Application and Research Center Animal Experiments Ethics Committee (Decision number: 2021-52, Meeting Date: 30.07.2021). In addition, the researcher had a Certificate for the Use of Experimental Animals approved by the university.

FINDINGS

Table 1. Comparison of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), and Alkaline Phosphatase (ALP) Biochemical Parameters of the C (Control), E (Exercise), B (Boldenone), and BE (Boldenone+Exercise) Groups.

Grups	ALT (μ/L)	AST (μ/L)	LDH (μ/L)	ALP (μ/L)
C (n=6)	25,00±3,28 [b]	41,16±6,73[a]	305,83±118,84 [a]	164,66±34,94 [b]
E (n=6)*	21,75±1,03 [a]	42,33±3,28[a]	363,50±52,56 [b]	134,41±24,82 [a]
B (n=7)	25,57±2,50 [b]	47,85±4,77[b]	351,07±92,43 [b]	174,85±21,69 [b]
BE (n=7)	26,78±4,59 [b]	55,14±8,37[b]	392,71±116,51 [b]	187,42±34,23 [b]
Test value, p	F:2,870 p:0,030	F:7,034 p: 0,001	F: 2,017 p: 0,02	F: 3,755 p: 0,026

Different letters (a, b) in the same column are statistically significant ($p<0.05$). F: One Way ANOVA

*: As a result of the death of one rat in the E (Exercise) group, the study continued with 6 rats.

When comparing the liver enzyme levels (ALT, AST, LDH, and ALP) between groups, a statistically significant difference was found in the AST and LDH levels of the B and BE groups compared to the C group ($F=7.034$; $P<0.001$; $F=2.017$; $P<0.02$). While no statistical difference was found in any parameter between the B and BE groups ($P>0.05$), it was found that the ALT and ALP levels of the E group were statistically different when compared with other groups ($F=2.870$; $P<0.03$; $F=3.755$; $P<0.026$). In addition to these results, when the B and BE groups were compared with the C and E groups, a non-statistical difference was found in all parameters ($P>0.05$).

Table 2. Comparison of CK (Creatine Kinase), CK-MB and cTn-I Biochemical Parameter of C (Control), E (Exercise), B (Boldenone), and BE (Boldenone+Exercise) Groups.

Groups	CK-MB	cTn-I (Troponin-I)	CK (μ/L)
C (n=6)	6,30±0,76[a]	291,07±39,86[a]	263,75±84,35[a]
E (n=6)*	6,55±0,45[a]	298,67±17,79[a]	236,85±79,95[a]
B (n=7)	7,56±0,64[b]	317,39±43,65[a]	306,33±111,86[a]
BE (n=7)	7,63±0,90[b]	334,35±56,88[a]	320,00±132,62[a]
Test value, p	F: 5,831 p: 0,004	F: 1,343 p: 0,28	F:1,203 p:0,319

Different letters (a, b) in the same column are statistically significant ($p<0.05$). F: One Way ANOVA

When comparing the CK, CK-MB, and cTn-I parameters between groups, no statistically significant difference was found in the levels of CK and cTn-I ($F=1.203$; $P=0.319$; $F=1.343$; $P=0.28$). However, when looking at the levels of CK-MB, it was observed that the B and BE groups were statistically significantly higher than the C and E groups ($F=5.831$; $P=0.004$).

DISCUSSION AND CONCLUSION

In this study, 26 rats were subjected to running exercise for 8 weeks and treated with Boldenone at a dose of 10 mg/kg. The effects on the heart, skeletal muscle, and liver were examined after the application. It was found that the levels of AST and LDH, which are biomarkers of liver damage, were higher in the B and BE groups compared to the C group, and this difference was statistically significant ($p<0.05$).

Similarly to the findings in the current study, Karbasi et al. (2018) reported in their study with 28 adult male rats that the use of testosterone enanthate for 8 weeks increased the levels of AST and LDH in the rats' blood (19). In their study examining bodybuilding athletes who have been using AAS for at least 1 year, Urhausen et al. (2004) reported that AAS use caused an increase in AST levels (20). In another study demonstrating the effects of AAS on the liver, Kulaksız (2017) found that the LDH levels of rats injected with AAS were significantly higher compared to the control group (21).

In the current study, when the effects of exercise and boldenone application on AST, ALT, ALP, and LDH enzymes, which are significant biomarkers of liver damage, were examined, it was found that the ALT and ALP levels in the E group were significantly lower than the C group, while no significant difference was observed in AST levels. In addition, the LDH levels in the E group were significantly higher than the C group. When academic studies investigating the effects of exercise on liver enzymes were reviewed, they found that these effects vary depending on the intensity of the exercise. There are academic studies showing that exercise intensity, which causes oxidative stress, leads to an increase in liver enzymes, while exercises at lower intensities do not have an effect on liver enzymes (22, 23, 24, 25, 26). In the current study, the fact that there is no clear increase or decrease in liver enzymes between the E group and the C group, and that no significant result was found in any parameter between the B and BE groups, may indicate that the exercise applied is not intense enough to cause oxidative stress. In future studies where the effects of exercise on the liver will be examined, importance should be given to ensure that the intensity of the applied exercise is sufficient to cause oxidative stress.

In the current study, no significant difference was found in CK levels among the groups. However, it was observed that the average CK levels of the E and BE groups were numerically higher compared to the C and B groups. When the literature is reviewed, there are many studies indicating that the use of AAS in conjunction with exercise leads to an increase in CK levels (14, 27, 28, 29). On the other hand, while there are many experimental studies reporting that AASs cause an increase in the CK parameter measuring skeletal muscle damage, there are also studies showing the opposite (27, 30). When these studies are reviewed, it can be said that the effects of AASs and exercise on CK levels vary depending on the dose of AASs used and the intensity of the exercise performed.

In the current study, while no statistically significant difference was observed in cTn-I levels among the groups, it was found that the average cTn-I values of the B and BE groups were higher compared to the C and B groups. Similar to these findings, some studies have shown that the use of AAS increases cTn-I levels (19, 28).

In the current study, when CK-MB levels were compared between groups, it was observed that the CK-MB levels in the B and BE groups were significantly higher than those in the C and E groups. When looking at other studies examining the effect of AAS use on CK-MB levels, it has been observed that AAS use increases CK-MB levels, which is a significant indicator of heart muscle damage (19, 29)

In the current study, it was observed that the application of boldenone at a dose of 10mg/kg per week for 8 weeks increased the levels of biomarkers of liver, heart, and skeletal muscle damage. Based on these results, it can be said that the use of AAS causes damage in the heart and liver. Furthermore, the fact that no significant difference was observed in any parameter between the B and BE groups indicates that exercise does not reduce the negative effects of AAS. The majority of academic studies conducted in this field, similar to the findings in our study, report that the use of AAS may cause vital damage to human health, primarily the cardiovascular system. (8, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,30). Afshin et al (2009) reported that treadmill exercises and steroid use caused heart muscle damage in rats (30). Studies on treadmill exercises have reported that running exercises are related to exercise intensity and duration, and that heart damage is greater with additional steroid use (31,32,33).

AAS's are used indiscriminately and widely among athletes to enhance performance, despite causing serious and permanent damage to human health. These doping substances, which are used in low doses as a drug in the treatment of many different diseases, have been widely researched in the field of Health Sciences for their side effects. However, it has been observed that academic studies in the field of Sports Sciences regarding the side effects of these drugs at high doses are not at an adequate level. Majority of the academic studies in this field are conducted in the field of Health Sciences, analyzing the side effects resulting from the

use of Anabolic Androgenic Steroids (AAS) as a drug to combat diseases caused by testosterone deficiency. The AAS dose used in these studies is significantly lower than the AAS dose used by athletes. Especially in the field of Sports Sciences, studies conducted using AAS at higher doses and in conjunction with exercise should contribute to the literature. Athletes, coaches, and scientists should be enlightened about the side effects of the use of AAS at supraphysiological doses, and societal awareness should be developed about the harms of AAS.

Limitations of the Study

The fact that this study was conducted on rats indicates that although rats have many similarities to human physiological processes, there are also fundamental differences. For example, factors such as metabolic rate, organ size, immune system responses, and hormonal regulations may differ between rats and humans. These differences may make it difficult to directly apply the results obtained to humans. This situation also shows the limitations of the study.

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