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THE EFFECTS OF TESTOSTERONE, A PROHIBITED SUBSTANCE, ON THE BODY AND ORGAN WEIGHTS OF PUBESCENT RATS

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Abstract
The aim of the present study was to investigate the effects of testosterone on certain organs and the macro-anatomical changes induced by this hormone in pubescent male and female rats.
The trial was conducted in 32 Sprague-Dawley rats, 16 of which were male and 16 female, at the premises of the Experimental Animals Unit of Selçuk University, Faculty of Veterinary Medicine. The study protocol was approved by the Ethics Board of the same faculty.
The male and female rats were allocated to two groups, one which constituted the control group and the other the experimental group. The average body weight of each animal was calculated. The experimental group was injected subcutaneously with 5 mg/kg of testosterone 5 days a week for a period of 10 weeks. At the end of the 10^{th} week, the animals were euthanized; and their average body weight and the weight of the testes, liver, spleen, heart and kidneys were measured. The values of the group, which was administered with testosterone, were compared with those of the control group.
The weight of the heart, right kidney, left kidney and spleen of male experimental and control rats did not differ from each other significantly ($P>0.05$). However, it was determined that testosterone administration led to a statistically significant reduction in the weight of the right and left testes and the liver ($p<0.05$) in male rats.
In result, in the present study, it was demonstrated that testosterone caused certain morphometric changes in the organs of pubescent experimental rats. The results of the present study could be indicative of the effects of testosterone in young athletes, who use anabolic androgenic steroids. The results of the present study may contribute to raising awareness among athletes on the adverse effects of anabolic androgenic steroids.
Key words: Organ, morphometry, rat, testosterone

Introduction

Although the use of doping agents is associated with certain adverse physical effects and the risk of the development of addiction, owing to the improvement observed within a short time period in the performance and physical appearance of athletes, today, unfortunately, these agents are still frequently used (W.E. Buckley et al, 1988, M.S. Bahrke et al, 1998).

The prohibition of the use of performance-enhancing doping agents is aimed at protecting the health of athletes (A. Dirix et al, 1988). In professional sports, the career and future of athletes depend on their performance in races. The profession of athletes is sports and the pressure of achieving success in the particular sports branch dealt with may impel athletes to consult all kinds of methods to reach this target. Nonetheless, the prohibition of the use of doping agents is also closely related to the maintenance of the self-respect of athletes (C.E. Yesalis, 2000).

Today, anabolic androgenic steroids (AAS), apart from their medical use, are preferred rather for enhancing athletic performance (as doping agents) and to improve physical appearance. Regular reports on the use of these agents were started to be compiled only after the year 1971 (E. Vardar et al, 2002).

Starting from the 1950s and up to the early 1970s, AAS were commonly used by athletes. In Switzerland, 30% of the athletes involved in indoor sports and other sports branches admitted to having used anabolic androgenic steroids (A. Ljungqvist, 1995). In the 1980s, these steroids began to be used commonly in outdoor sports as well (C.E. Yesalis et al, 1989).

Steroid hormones are used particularly by athletes, such as weight lifters, who perform heavy endurance workouts. Furthermore, it has been demonstrated that heavy endurance workouts bring about an increase in the number of steroid receptors in these athletes, thereby, further aggravating the effects of steroids (T.D. Fahey, 1998).

Studies conducted on anabolic steroids have pointed out to the continued and even increased use of these agents by both adult athletes and other persons (J.C. Wagner, 1989, R. Windsor., D. Dumitru, 1989).

Attention has been drawn to the health risks associated with the frequent use of anabolic steroids not only by scientists but also in reports published by many healthcare and sports organisations (C. Maravelias., A. Dona, 2005). In general, it has been clearly indicated in these reports that the use of anabolic steroids, which damage health, is prohibited (E. Marshall, 1988, L.Goldberg et al, 1990). Despite all these efforts, for many years, anabolic steroids have been available on the black market and have been readily accessed in many sports clubs and gymnasiums (D. Duchaine, 1989).

It is observed that, despite their adverse physical effects and the risk of misuse and addiction, androgens are frequently used by adolescent athletes with an aim to enhance performance (M.S.Bahrke et al, 1998, E. Vardar et al, 2004).

The general side effects of anabolic androgenic steroids include, increased water retention, liver

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dysfunction, oedema, jaundice, increased cardiac workload, increased risk of malign and benign liver tumours, increased blood pressure, kidney dysfunction, increased cholesterol levels, induced tumour growth, risk of cardiovascular diseases, increased blood glucose levels, pustulation, epistaxis, muscle cramps and spasms, thyroid dysfunction, tendon damage or rupture, psychological disorders and aggressiveness. The side effects of anabolic androgenic steroids in females include hirsutism, nymphomania, hair loss, menstrual irregularity or menopause, reduced breast size, deepening of voice and clitoral enlargement. The side effects of AAS in preadolescent males include the early ossification of cartilages and short stature. Furthermore, it has been reported that AAS cause hair loss, infertility, decreased production of male sex hormones, gynecomastia, sexual anorexia, reduced testicular size, impotence, decreased production of male sex hormones, enlargement of the prostate gland, prostate gland cancer, reduced sperm production, Wilms' tumour and abnormal sperms in males (K. Livanelioğlu, 2010).

The differences between males and females for muscular growth resulting from workouts, and muscular hypertrophy being more evident in males, as well as the differences of secondary sex traits of males and females have all been attributed to testosterone being produced at higher levels in males. As a matter of fact, testosterone, together with the hormones of the hypophysis and adrenal glands, render the morphological structure of males and females different (N. Akgün, 1993). Testosterone, which is an androgenic hormone, belongs to the group of steroids responsible for the primary and secondary male sex traits (B. Starcevic et al, 2003).

Due to its androgenic and anabolic effects, testosterone has influence on several organs. Its anabolic (myotropic) effects are observed as the development of a larger mass of muscles, increased maturity of skeletal bones and increased mineralization (C.D. Kochakian., J.R. Murlin, 1935).

Anabolic steroids, which are the illegal synthetic derivatives of testosterone (J.A. Potteiger., V.G. Stilger, 1994, M.E. Powers, 2002, N.A. Evans, 2004), have strengthened anabolic efficacy and reduced androgenic effect. Drugs such as oxymesterone or oxandrolone are frequently used by athletes to increase strength and endurance (F. Muscatelli et al, 1994, C.J. Bagatell., W.J. Bremner, 2003). It has been stated that, today, the use of anabolic steroids with an aim to decrease body fat, increase strength and enhance athletic performance, has increased even among high school students (G.L. Gaa et al, 1994, J.A. Potteiger., VG. Stilger, 1994, ME. Powers, 2002).

In his recent studies, N.A. Evans (2004) suggested that the short-term use of the physiological doses of anabolic steroids did not induce significant side effects, whilst long-term use was associated with grave harm. This researcher has underlined that still some athletes insist on the illegal use of steroids for the sake of either maintaining or enhancing their performance.

The present study was aimed at the investigation of the possible adverse structural effects of testosterone, an AAS frequently used by athletes, on the internal organs of pubescent rats.

Materials And Methods

Thirty-two 50-day-old laboratory rats of the Sprague Dawley breed constituted the material of the study. The rats were obtained from the Experimental Medicine Research and Practice Centre of Selçuk University (SÜDAM), a legal experimental animal breeder, accompanied by the approval (No. 2007/022) issued by the Ethics Board of Selçuk University, Faculty of Veterinary Medicine. The animals were provided with *ad libitum* feed and were kept in standard cages. Each cage housed four rats. The room temperature was adjusted to an average of 25 C° throughout the trial. The relative humidity of the laboratory was adjusted to an average rate of 52.00 %. The 32 rats (16 males and 16 females) were allocated to two equal groups (n:16). The first group was maintained as the control group, whilst the second group was administered with testosterone.

All of the animals, including the control rats, were weighed on an assay balance (Ohaus CS 200 Compact Scale, Mexico) prior to the start of the trial and on Monday every week throughout the trial. The experimental animals were administered with 5 mg/kg/day (C.R. Blystone et al, 2007) of testosterone (Sustanon 250 ampoule) by subcutaneous route 5 days a week for a period of 10 weeks. The experimental animals were left to rest on Saturdays and Sundays. At the end of the 10^{th} week, the animals were euthanized with an intraperitoneal injection of 200 mg/kg of pentobarbital (Pentotal sodium, Abbott).

Prior to euthanasia, the average body weight of each animal and after euthanasia, the weight of the dissected testes, liver, spleen, heart and kidneys were measured on an assay balance (Kern q Jehy. GmbH, Germany US 9v). The measurements of the group, which was injected with testosterone, were compared with those of the control group. The data obtained from the experimental and control groups were statistically analysed. The comparison of the two groups was performed using the independent t-test. Anatomic terminology conforms to that prescribed by Nomina Anatomica Veterinaria (N.A.V. 2005).

Result

Table 1. The t-test results for some organ weights (g) in male rats included in the control and testosterone-administered groups

		Groups	N	Mean	Std. Deviation	t	P
Male Rats	Heart	Testosterone	8	0.2962	0.01847	-0.653	0.525
		Control	8	0.3025	0.01982		
	Liver	Testosterone	8	3.6388	0.14327	-3.714	0.002*
		Control	8	3.8700	0.10240		
	Right kidney	Testosterone	8	0.3638	0.01302	0.432	0.672
		Control	8	0.3612	0.00991		
	Left kidney	Testosterone	8	0.3688	0.01458	0.522	0.610
		Control	8	0.3650	0.01414		
	Spleen	Testosterone	8	0.1475	0.01488	-2.084	0.056
		Control	8	0.1625	0.01389		
	Right testis	Testosterone	8	0.3325	0.02053	-15.950	0.000*
		Control	8	0.5050	0.02268		
	Left testis	Testosterone	8	0.3350	0.02330	-11.705	0.000*
		Control	8	0.5062	0.03420		

In male rats, the weight of the heart, right kidney, left kidney and spleen did not differ statistically between the testosterone-administered and control groups ($P>0.05$). However, it was determined that testosterone administration led to a statistically significant decrease in the weight of the right and left testes and the liver ($p<0.05$).

Table 2. The t-test results for some organ weights (g) in female rats included in the control and testosterone-administered groups

		Groups	N	Mean	Std. Deviation	t	P
Female Rats	Heart	Testosterone	8	0.3112	0.01885	-1.986	0.067
		Control	8	0.3363	0.03021		
	Liver	Testosterone	8	3.7050	0.13082	-1.706	0.110
		Control	8	3.8612	0.22351		
	Right kidney	Testosterone	8	0.3750	0.03162	2.792	0.014*
		Control	8	0.3362	0.02326		
	Left kidney	Testosterone	8	0.3750	0.02330	1.938	0.073
		Control	8	0.3425	0.04132		
	Spleen	Testosterone	8	0.1688	0.03227	-1.946	0.072
		Control	8	0.2025	0.03694		

It was detected that, in female rats, testosterone administration significantly increased the weight of the right kidney ($p<0.05$), whilst it decreased the weight of the left kidney, heart, liver and spleen ($P>0.05$).

Table 3. The t-test results for the body weights (g) of male rats included in the testosterone-administered and control groups throughout the 10-week-trial period.

	Groups	N	Mean	Std. Deviation	t	P
Week 1	Testosterone	8	152.0000	24.28403	-0.682	0.633
	Control	8	159.2500	17.70190		
Week 2	Testosterone	8	181.3750	22.63965	-1.030	0.669
	Control	8	190.8750	12.95528		
Week 3	Testosterone	8	194.2500	23.27322	-0.318	0.854
	Control	8	197.3750	15.15574		
Week 4	Testosterone	8	205.1250	22.05472	-0.851	0.407
	Control	8	212.6250	11.58740		
Week 5	Testosterone	8	217.8750	23.94898	-0.678	0.416

	Control	8	224.3750	12.71599		
Week 6	Testosterone	8	232.5000	24.61707	-0.338	0.401
	Control	8	236.0000	15.87451		
Week 7	Testosterone	8	241.7500	26.27737	0.031	0.735
	Control	8	241.3750	21.95409		
Week 8	Testosterone	8	249.5000	27.66122	-0.125	0.972
	Control	8	251.1250	24.34536		
Week 9	Testosterone	8	256.0000	29.18414	-0.494	0.333
	Control	8	262.1250	19.46746		
Week 10	Testosterone	8	265.2500	28.17167	-0.697	0.404
	Control	8	273.7500	19.89077		
Week 11	Testosterone	8	271.0000	28.20841	-0.503	0.465
	Control	8	277.0000	18.50097		

Based on the data presented in Table 3, it was determined that testosterone administration did not have a significant effect on the body weight of male rats (P>0.05).

Table 4. The t-test results for the body weights (g) of female rats included in the testosterone-administered and control groups throughout the 10-week-trial period.

	Groups	N	Mean	Std. Deviation	t	P
Week 1	Testosterone	8	155.0000	18.49324	5.336	0.096
	Control	8	115.7500	9.52815		
Week 2	Testosterone	8	192.1250	15.04695	10.750	0.234
	Control	8	126.0000	8.73417		
Week 3	Testosterone	8	199.3750	18.37652	7.793	0.023*
	Control	8	144.6250	7.55811		
Week 4	Testosterone	8	215.8750	16.08404	10.480	0.050*
	Control	8	151.5000	6.56832		
Week 5	Testosterone	8	227.2500	16.67119	11.035	0.055
	Control	8	157.3750	6.54517		
Week 6	Testosterone	8	239.7500	19.96962	9.965	0.067
	Control	8	166.0000	6.27922		
Week 7	Testosterone	8	245.2500	26.04803	7.778	0.011*
	Control	8	171.2500	6.75595		
Week 8	Testosterone	8	254.0000	27.74373	7.860	0.001*
	Control	8	174.7500	6.60627		
Week 9	Testosterone	8	266.5000	23.04034	10.638	0.005*
	Control	8	177.5000	5.39841		
Week 10	Testosterone	8	277.0000	23.10844	11.437	0.021*
	Control	8	180.2500	6.20484		
Week 11	Testosterone	8	280.5000	22.45631	10.558	0.067
	Control	8	189.7500	9.31589		

Based on the results presented in Table 4, it was ascertained that, testosterone administration significantly increased the body weight of female rats during weeks 3, 7, 8, 9 and 10 (P<0.05).

Discussion and conclusion

In the present study, it was determined that, in male rats, testosterone administration did not have an effect on the weight of the heart, right kidney and left kidney, but led to an insignificant decrease in the weight of the spleen when compared to the control group. Furthermore, it was observed that the weight of the liver and right and left testes had decreased

significantly in male rats administered with the hormone ($p<0.05$) (Table 1). C.R. Blystone et al (2007) reported that the administration of testosterone to pubescent male rats significantly reduced the weight of certain organs. The results of the present study are in compliance with the report of J.A. Carson et al. (2002) indicating that nandrolone administration significantly reduced testis weight in male rats. M. Balkaya et al. (2002) reported to have observed significant decrease in the weight of certain organs in male rats administered with testosterone. In the present study, it was determined that, compared to the control group, the weight of the heart, liver and spleen had reduced only quantitatively, whilst the weight of the right kidney had increased statistically in the female rats included in the experimental group (Table 2).

Data obtained in the present study demonstrated that the body weight of male control and experimental animals did not differ from each other significantly between weeks 0 and 10 of the trial. However, it was observed that the body weights of the female experimental animals had significantly increased in comparison to the female control rats. In a study on the investigation of the effect of testosterone in adult male rats, J. Iwamoto et al. (2002) reported that no significant difference was determined between the initial and final body weights of the animals. In another study conducted by A. Bisschop et al. (1997), it was determined that in healthy female rats administered with nandrolone decanoate, body weight and muscle weight had increased after treatment compared to pre-treatment values, whilst in male rates no alteration was observed. The results of the present study are in agreement with the data reported by (A. Bisschop et al, 1997).

Side effects observed with the use of AAS in research conducted in experimental animals may provide valuable insight into the use of doping agents by athletes. The data obtained from experimental animals in the present study may contribute to raising awareness among young athletes on the adverse effects of AAS, as well as to protecting their health. It is also considered that the present study may contribute to similar studies to be conducted in the future.

In conclusion, it was determined that testosterone led to certain morphometric changes in the organs of pubescent experimental rats.

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