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The Morphometric Effects of Testosterone, Used as a Doping Agent, on the Humerus and Femur of Pubescent Male and Female Rats

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ABSTRACT

The aim of this study was to examine the morphometric effects of testosterone on some osteological structures in pubescent rats. For this purpose, 16 male and 16 female rats were allocated equally to control and experimental groups. After calculating the average weight of all rats, testosterone was administered at a dose of 5 mg/kg body weight (bw) by subcutaneous route, 5 days a week for 10 weeks. At the end of the 10th week, all the rats were euthanized; and corpus and height measurements of the femur and humerus, and cortex and cavum medulla morphometric measurements were made. A significant decrease was detected in the corpus femoris and height measurements of the male rats administered with testosterone ($P<0.05$). However, no significant difference was found for female rats ($P>0.05$). A significant decrease was determined in the corpus humeri and height measurements of female and male rats administered with testosterone ($P<0.05$). Statistically significant differences were determined between the femur-humerus and cortex-cavum medulla measurements of the testosterone and control groups ($p<0.05$). In result, testosterone caused some morphometric changes in the extremity bones of pubescent rats included in the experimental group. A parallelism can be drawn between the results of this study and results derived from the use of anabolic-androgenic steroids by young athletes. The results of the present study may contribute to raising awareness among athletes on the adverse effects of anabolic-androgenic steroids.

Key Words: Bone, morphometry, rat, testosterone

INTRODUCTION

Anabolic-androgenic steroids (AASs) are synthetic derivatives of testosterone, known as the male sex hormone (24, 15) When given orally and parenterally, testosterone is quickly metabolized and exhibits no effect. On the other hand, when administered by oral and intramuscular route, testosterone derivatives are effective owing to the ester groups in their structure. By inhibiting gonadotropin release from the hypothalamic-hypophyseal axis via a negative feedback mechanism, AASs decrease blood LH and FSH levels. As a result, endogenic testosterone and estradiol levels decline (18). In males, AASs contribute to muscle and bone growth and sexual performance (8,19).

Depending on the use of anabolic-androgenic steroids; mood disorders including addiction, mania and depression; aggressive behaviour such as psychotic disorders, homicide, psychiatric symptoms and disorders such as reduced sexual interest and insomnia may occur (7,16,17). Despite negative physical effects and the risk of misuse and addiction; it is observed that some adolescent athletes often use androgens to increase their performance (3,25).

The aim of this study was to determine the probable morphometric or structural changes in certain bones (the humerus and femur) and internal organs resulting from the administration of testosterone, an AAS commonly used by athletes as a doping agent, to pubescent rats.

MATERIALS AND METHODS

Thirty-two 50-day-old Sprague Dawley rats, of which 16 were male and 16 female, were obtained from Selcuk University Centre for Experimental Medical Research and Application. The study protocol was approved (2007/022) by the Ethics Committee of Selcuk University, Faculty of Veterinary Medicine. The animals were fed *ad libitum* and kept in standard cages. In the laboratory, temperature and humidity were stabilized at 25^0C and 52.00 % Rh, respectively. The rats (16 males, 16 females) were divided into 2 equal groups (n=16). While the first group was maintained as the control group, the second group was subjected to testosterone administration.

Prior to the conduct of the trial, the weight of all animals, including the control group, was determined on an assay balance (Ohaus CS 200 Compact scale, Mexico) on Monday each week. The experimental groups were left to rest on Saturdays and Sundays throughout the trial, and were injected

daily with 5 mg/kg bw (5) of testosterone (Sustanon® 250 amp., Organon, Istanbul, Turkey) by subcutaneous route, 5 days a week for 10 weeks. At the end of the 10th week, the animals were euthanized with an intraperitoneal injection of 200 mg/kg bw pentobarbital (Pentotal sodium®, Abbott, Istanbul, Turkey). Subsequently, the materials were dissected such that the fore and rear extremities were revealed, and maceration was applied.

The revealed humerus and femur bones were marked and kept in special plastic bags. Morphometric measurements of the height and corpus of the humerus and femur were performed on the left side. Measurements were taken with a 0-100 millimetre calliper (stainless hardened digital calliper, China). The anatomic reference points used for the measurement of the humerus and femur are shown in Figures 1 and 2, and were specified for the x and y axes using a Nikon v12 profile projector with 0.001 precision (14).

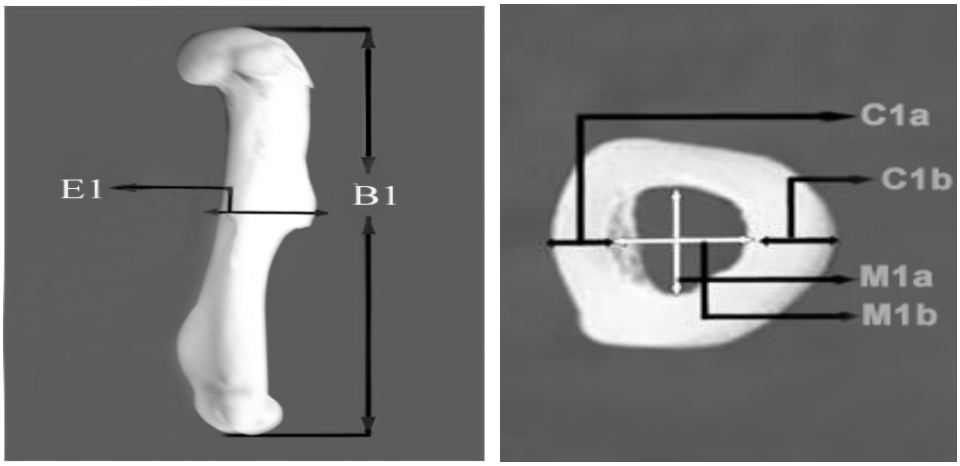

Figure 1. Anterior-Directional Reference Points of the Left Humerus in Male Rats Administered with Testosterone

E1=thickness of the corpus humeri at the lower limit level of the tuberositas deltoidea
B1= distance between the end points of the trochlea humeri and caput humeri of the humerus
M1a, M1b = average thickness of medullar diameter (cavum medullare) of corpus humeri (ventral of the trochanter tertius)
C1a and C1b= average cortex thickness at corpus humeri level [(Substantia compacta (lower limit of the tubersitas deltoidea)]

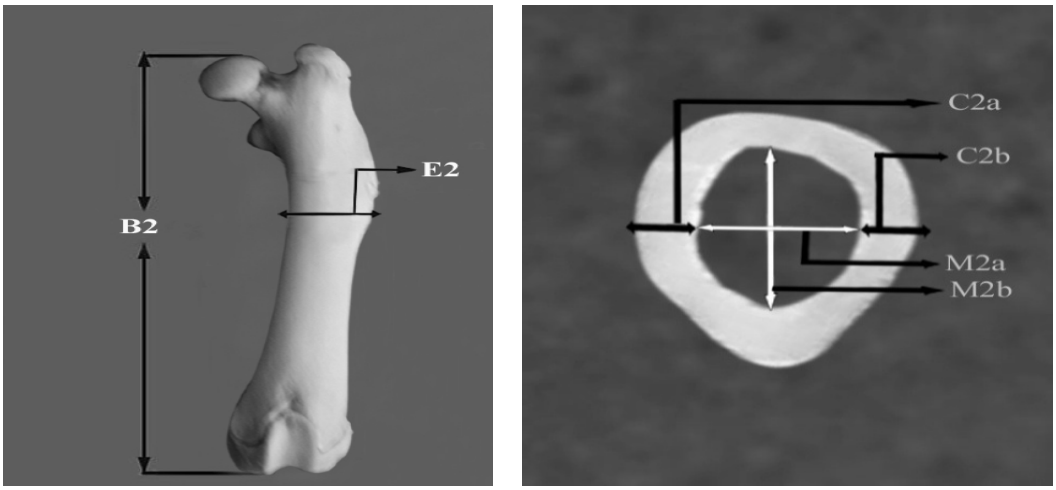

Figure 2. Anterior-Directional Reference Points Of The Left Femur In Male Rats Administered With Testosterone

E2= corpus thickness of the trochanter tertius of the femur at ventral level.
B2= distance between the end points of the trochlea ossis femoris and caput ossis femoris of the femur.
M2a, M2b = average thickness of medullar diameter (cavum medullare) of corpus ossis femoris (ventral of the trochanter tertius)
C2a, C2b= average cortex thickness of corpus ossis femoris [(Substantia compacta (lower limit of the tubersitas deltoidea)

The pictures of the bones were taken with a Nikon DSLR D200 digital camera. The groups were compared using the independent t-test. The terminology of "Nomina Anatomica Veterinaria" (13) was followed.

RESULTS

Stature and corpus thickness (mm) of the humerus and femur in testosterone-treated and control groups was presented in table 1. It was determined that, compared to the controls, testosterone administration caused reduced humeral height in males ($P<0.05$), but increased height in females ($P<0.05$). The comparison of the two sexes revealed a difference in the control group ($P<0.05$). The investigation of the effect of testosterone on the thickness of the corpus humeri demonstrated that there was no statistical difference between the two sexes in any of the groups ($P>0.05$). However, testosterone administration reduced the corpus thickness in animals of the same sex ($P<0.05$).

Cortex and cavum medullar diameter measurements (mm) of the humerus and femur in testosterone-treated and control groups were shown in table 2. It was determined that the diameter of the humeral cortex of female rats administered with testosterone was considerably greater than values of the control group ($P<0.05$). The cavum medullar diameter of the humerus of male control animals was larger than the cavum medullar diameter of female control animals ($P<0.05$). It was determined that the femoral height and corpus thickness of male rats administered with testosterone were significantly smaller than the values of the male control animals ($P<0.05$). The femoral height and corpus thickness of female control rats were found to be smaller ($P<0.05$) than the values of the male control rats. Furthermore, the medullar diameter of the femur was found to be greater in the female controls compared to male animals ($P<0.05$), whereas it was determined that the medullar diameter was reduced in female rats administered with testosterone ($P<0.05$).

DISCUSSION

The main indicators of the anabolic efficiency of anabolic steroids are the stimulation of protein synthesis, maintenance of the masculine volume and strength of skeletal muscle mass, and acceleration of somatic growth when administered to humans and experimental animals in the growth period (9). Androgens have been claimed to have a very important role in maintaining skeleton mass and bone density during adulthood and preadolescence, owing to their effect on the development of extremity bones (4).

Table 1. Stature And Corpus Thickness (Mm) Of The Humerus And Femur In Testosterone-Treated And Control Groups (Mean±Sd).

	Length		Corpus thickness	
	Male (n:8)	Female (n:8)	Male (n:8)	Female (n:8)
Humerus				
Testosterone	$26.25\pm1.19^{b,A}$	$25.93\pm0.50^{a,A}$	$2.67\pm0.11^{b,A}$	$2.58\pm0.10^{b,A}$
Control	$27.64\pm0.67^{a,A}$	$24.86\pm0.63^{b,B}$	$2.89\pm0.20^{a,A}$	$2.75\pm0.18^{a,A}$
Femur				
Testosterone	$33.28\pm1.09^{b,A}$	$32.70\pm0.86^{a,A}$	$4.05\pm0.30^{b,A}$	$4.04\pm0.14^{a,A}$
Control	$35.34\pm0.87^{a,A}$	$31.97\pm0.74^{a,B}$	$4.45\pm0.19^{a,A}$	$3.91\pm0.13^{a,B}$

Different superscripts within the same column (a, b) and row (A, B) are statistically significant ($P<0.05$).

Table 2. Cortex and Cavum Medullar Diameter Measurements (Mm) of the Humerus and Femur in Testosterone-Treated and Control Groups (Mean±Sd).

	Cortex thickness		Cavum medulla	
	Male (n:8)	Female (n:8)	Male (n:8)	Female (n:8)
Humerus				
Testosterone	$0.68\pm0.05^{a,A}$	$0.73\pm0.04^{a,A}$	$1.21\pm0.07^{a,A}$	$1.29\pm0.39^{a,A}$
Control	$0.70\pm0.07^{a,A}$	$0.67\pm0.06^{b,A}$	$1.29\pm0.08^{a,A}$	$1.14\pm0.05^{a,B}$
Femur				
Testosterone	$0.74\pm0.72^{a,A}$	$0,71\pm0.05^{a,A}$	$2.15\pm0.20^{a,A}$	$2.13\pm0.16^{b,A}$
Control	$0.81\pm0.11^{a,A}$	$0,72\pm0.08^{a,A}$	$2.23\pm0.11^{a,B}$	$2.40\pm0.45^{a,A}$

Different superscripts within the same column (a, b) and row (A, B) are statistically significant ($P<0.05$).

In the present study, the evaluation of the corpus and height measurements of the femur and humerus demonstrated that the measurements of the testosterone group were generally significantly smaller than the values of the control group (Table 1). In a study, in which rats were administered with nandrolone for 4 weeks, Lök (11) determined a statistically significant shortening of the femoral length in males and both the femoral and humeral lengths in females. Similarly, Bonnet et al. (6) and Kitaura et al. (10) reported that metaphyseal growth of the femur was inhibited in rats, which were administered with clenbuterol. In the present study, the humeral length of female rats administered with testosterone was determined to be significantly greater than that of the control group ($P<0.05$), (Table1). As reported by Alim et al. (2), supplying male rats with extra testosterone caused an important increase in femoral length. Tapp (21) reported that testosterone had a significant effect on bone growth in female rats and this finding is in compliance with the humeral length measurements of female rats detected in the present study.

In his study on bone injuries in rats, McDougall et al. (12) reported that extra androgen increased the development of cortical bone diameter. However, in this study, while an insignificant decrease ($P>0.05$) was observed in femoral and humeral cortex and cavum medullar measurements of male rats administered with testosterone (Table 2), compared to the control group, an insignificant increase was observed in the cavum medullar measurements of the femur and cortex and in the cavum medullar diameters of the humerus of female rats (Table 2). In the previously mentioned study, in which nandrolone was administered to rats, Lök (11) found that there was no significant difference between the cavum medullar diameters of the femur amd humerus in male rats included in the nandrolone and arachis oil-administered groups and the control group. Tramontana et al. (22) found that testosterone administration caused a significant decrease in bone cavum medullar diameter. Sims et al. (20) determined that testosterone administration had no important effect on cortical bone diameter in studies conducted in female rats. The report of Windahl et al. (27) indicating that testosterone administration caused no difference in femur cortex and cavum medullar diameters in male rats was important as it is in compliance with this study. The cortex and cavum medullar diameters of the femur and humerus in male rats administered with testosterone did not show any difference in this respect (Table 2).

The comparison of the testosterone-treated groups for sex demonstrated that neither the thickness of the femoral and humeral cortices nor the cavum medullar diameter differed significantly between males and females ($P>0.05$) (Table 2). However, it was ascertained that, in male control rats, while the cavum medullar diameter of the humerus decreased significantly ($P<0.05$), the cavum medullar diameter of the femur was significantly greater ($P<0.05$) (Table 2).

It has been reported that the enlargement of the cavum medullar diameter in males and the increase of cortical thickness is characteristic of puberty, and that while oestrogens reduce bone formation, androgens increase radial growth (1). Sims et al. (20) reported that testosterone administration to rats increased the cavum medullar diameter of the femur. Venken et al. (26) reported a direct stimulating effect of testosterone on bones via androgen receptors. Turner et al. (23) claimed that a lack of androgens during the growth period reduced bone formation in the periosteum.

In the present study, it was determined that, in male rats treated with testosterone, the length and corpus of both the femur and humerus shortened and the cortical and cavum medullar diameters decreased. Based on these results, it can be suggested that the use of anabolic androgenic steroids by young athletes may result in their having a short stature and their skeletal structure being distorted.

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