Morphometric effect of nandrolone decanoate used as doping in sport on femur of rats in puberty period

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Manuscript Preparation
E Funds Collection

Sefa Lök[1ABCDE], **Hakan Yalcin**[2ABCDE]

[1] Physical Education and Sports Academy, Konya, Turkey
[2] Department of Anatomy, Veterinary Faculty, Selcuk University, Konya, Turkey

Source of support: Departmental sources

Received: 11 December 2010; **Accepted:** 15 December 2010; **Published online:** 31 December 2010

Abstract

Background and Study Aim: The aim of this research is to show the morphometric effect of nandrolone that is used as doping in sport on femur of rats in puberty period.

Material/Methods: A total of 60 Sprague-Dawley rats (30 days old) were divided into three equal groups. The control group (male n=10, female n=10) was nourished for 4 weeks without any substance being administered. For the peanut oil group (male n=10, female n=10), peanut oil was used as diluent of nandrolone decanoate (500 mcl). It was administered for 5 days via intraperitoneal injection, with a pause of 2 days and the same application was done for 4 weeks.. Nandrolone decanoate was applied for 5 days to the Nandrolone group (male n=10, female n=10) in 10 mg/kg dose by being diluted in 500 mcl peanut oil via intraperitoneal injection and the same was applied for 4 weeks having taken a 2-day long pause. Rats were euthanatized at the end of fourth week. Back extremity bones of materials were revealed by being dissected and put to maceration operation.

Results: Morphometric measures were taken by determining the anatomic reference points (length, corpus, cortex-cortical bone thickness-substantiate compact, medullar wide-cavity medullar) that would be measured of femur bones that are on right side. It was determined that nandrolone caused shortening in femur length ($p<0.05$) in male and female rats when compared to peanut oil and control groups.

Conclusions: Nandrolone may delay the bone formation in the puberty period.

Key words: nandrolone • morphometric • femur

Author's address: Sefa Lok, Physical Education and Sports Academy, Selcuk University, 42075, Konya, Turkey; e-mail: sefalok@selcuk.edu.tr

Background

Humankind has never accepted the physical limitations since it began to exist and has always tried to overcome them. Hence, they began the certain substances [1]. Nowadays, rate of use of ergogenic substances as doping has increased in order to increase physical performance in sportsmen [2]. As for doping, it is taking a substance foreign to the organism in any way in order to increase the performance of sportsmen during competition in an artificial and unethical way [3].

Nandrolone (19-nortestosterone) is produced after leaving the C-19 methyl group from testosterone [4,5]. Nandrolone is metabolised to 19-norandrosterone and 19-noretiocolanolone [6]. Nandrolone is present as endogenous in mouse kidney, horse and pig testis, monkey placenta and pig follicular liquid [7].

Nandrolone has higher anabolic effect via influence muscle cells. It is one of the most effective medicals for muscle power and development. It has been reported that its androgenic effect is too low. In research conducted by experts on the Deca-Durabolin, it has been confirmed that it specially intensified the tendons. Anabolic androgenic steroids (AASs) are frequently used for women during treatment of osteoporosis in postmenopausal period and in the protein deficiency conditions [1].

General side effects are androgenouses are hirsutism, hoarseness, acne, clitoral hypertrophy, amenorrhea, spermatogenesis inhibition, early epiphyseal closure, virilisation of woman fetus [5].

It has been reported that AASs increased the metacarpal bone length and weight of ram [8]. Effect of nandrolone on bone mineral density after ovariectomy in female monkeys has been examined, it has been reported that nandrolone increases the bone mineral density after ovariectomy [9].

Although many side effects of androgenic steroids had been generally described in literatures, it has been determined that an effect of nandrolone on bone development in puberty period had not been researched. When AASs' general side effects are evaluated, it may be set forth that nandrolone also has an effect on bone development.

The aim of this study is to determine whether the nandrolone that is used as doping in sport has a morphometric effect on femur of rats that are in puberty period.

Material and Methods

The research was conducted on 60 Sprague-Dawley rats 30 days old (female n=30, 107±8.62 g, male n=30, 121±13.3 g, Selcuk University Experimental Medicine Research and Application Centre). The research was approved by Selcuk University Veterinary Faculty Ethical Committee. Rats were nourished ad libitum and put in the standardized cages, males and females separately (five for each cage). The temperature of laboratory, in which the study was done, was kept at approximately 25°C, moisture gradient was approximately 52.00% Rh. Male and female rats were divided into three groups. The control group (male n=10, female n=10) was nourished for 4 weeks without any substance being administered. For the peanut oil group (male n=10, female n=10) peanut oil was used as diluent of nandrolone decanoate (500 mcl, Zade peanut oil, Konya). It was administered for 5 days via intraperitoneal injection, with a pause of 2 days and the same application was done for 4 weeks. Nandrolone decanoate (Nandrolone Decanoate® Inc., Norma Hellas SA, Menandrou, Greece) was administered to the Namdrolone group (male n=10, female n=10) for five days via intraperiotaneal injection by being diluted in 500 mcl arachis oil with 10 mg/kg dose [10], and the same was applied for 4 days with a 2-day long pause. All rats were euthanized at the end of fourth week with intraperitoneal injection of pentobarbital (Nembutal sodium Flk, Abfar, Istanbul) medicine. Back extremity bones of materials were revealed by being dissected and put to maceration operation. Then, revealed femur bones were dried.

To determine the anatomic reference that would be measured [A (height), B (corpus), C1-C2 (cortex-cortical bone thickness-substantiate compact) and D points (medullar wide-cavity medullar)] of femur bones that are on right side, necessary morphometric measures were done to each of these points with 0–100 mm stick (Stainless hardened digital calliper, China) (Figure 1).

Photos of the bones were taken with digital camera (Nikon D200, China) (Figure 1). Besides, average body weight of all samples before euthanasia was measured for the last time with microbalances.

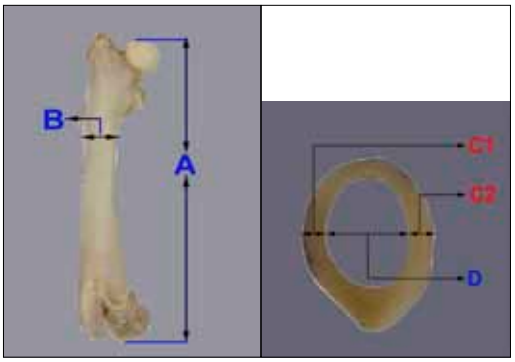

Figure 1. Reference points (Right medial face, ♂) of Femur Height (**A**), Corpus (**B**), Cortex (**C1-C2**) and Medullar calibres (**D**). **A** – Pitch between terminal points of rag ossis femoris and trochlea ossis femoris. **B** – Corpus thickness of femur (sublimit level of Trochanter tertius). **C1-C2** – Average cortex thickness (cortical bone-substantiate compacta) of femur in corpus level. **D** – Calibre of covum medullar of femur in corpus level.

In writing, the anatomic terms from"Nomina Anatomica Veterinaria, 2005" was used [11].

In statistical evaluation, ANOVA and Duncan tests were applied (SPSS 13.0 for Windows/ SPSS® Inc., Chicago, USA). Results were presented as mean ±SE ($p<0.05$ was accepted statistically significance).

Results

It was determined that nandrolone application caused shortening ($p<0.05$) in length of femur when compared to oil groups in both sexes (Table 1). It was determined that nandrolone application had no effect on corpus thickness, cortex thickness and medullar calibre ($p>0.05$).

Discussion

The anabolic androgenic steroid (AAS) used for medical purposes, appear to have a lot of side effects [1,5]. It has been frequently stated that AASs are used by sportsmen

Table 1. Effect of nandrolone on morphometric lengths (mm) of femur in pubertal term rats (mean ±SD).

	Control		Peanut oil		Nandrolone	
	Male (n=10)	Female (n=10)	Male (n=10)	Female (n=10)	Male (n=10)	Female (n=10)
Femur length	30.1±1.06*	29.5±0.39*,**	30.1±1.22*	29.5±0.68*,**	28.8±0.94**,***	28.5±0.77***
Corpus thickness	2.74±0.15*	2.69±0.12*	2.76±0.16*	2.71±0.21*	2.79±0.13*	2.68±0.13*
Cortex thickness	0.54±0.04*	0.57±0.02*	0.55±0.05*	0.58±0.01*	0.56±0.04*	0.53±0.02*
Medullar caliber	1.63±0.12*	1.55±0.11*	1.65±0.12*	1.55±0.10*	1.66±0.09*	1.63±0.10*

*, **, *** – different letters in the same line are statistically significant (Duncan test, P<0.05).

unrestrainedly in order to increase muscle power and muscle mass [12]. In the presented research, it has been determined that nandrolone application causes shortening in femur height ($p<0.05$) of male and female rats in puberty period (Table 1). Xiaodong et al [13], set up an experiment and a control group and injected the experiment group with nandrolone for six weeks. Their study was conducted in order to determine the effects of nandrolone application on bone mass and metabolism in adult rats. They have observed that stature of humerus bone of male and female rats that are in the group which was administered the medication is smaller than the control group. Prakasam et al. [14] examined the effect of testosterone and growth hormone on cortical bone formation and bone growth in rats. They found femur stature to be shorter in both male and female compared to the other group. It was stated that AASs administration may result in short stature in adults and sportsmen using AASs [15]. Probable reason of this effect is early closure of condyle (epiphyseal of growth plates) in adolescents [16].

There was no determined statistically significance in male and female control and peanut oil group animals, when measured parameters of femur were evaluated ($p>0.05$, Table 1). In addition, it was no statistically significance determined that effect of nandrolone on femur height in both male and female rats. Similarly, Weismann et al. [17] stated that testosterone had no differentiate effect on stature of humerus bone in both male and female rats.

In the present study, nandrolone did not affect on the measurement values of femur except its length (Table 1). Weismann et al. [17] examined effect of testosterone on bone cell in male and female rats. They separated experiment and control groups with respective sexes in their study. They stated that nandrolone had no effect on average cortex thickness of humerus bone in both male and female rats. Qu et al. [18] stated that there was no statistically meaningful difference between them in terms of medullar length calibre averages of femur and humerus bones of estrogen female rats. Vidal et al. [19] examined the effects of estrogen receptors on bone growth and maturation in male mice. They confirmed that the difference between femur bones of male experiment and control groups in terms of cortex thickness was meaningful. The probable reason for differences between this research and the existing research may be difference in the active substance applied age group.

Conclusions

Consequently, it can be assumed that nandrolone can stop bone growth and cause short stature especially when used by sportsmen in puberty period.

Acknowledgements

This study is a portion of Ph thesis and is supported by Selcuk University Scientific Research Projects Coordinator (SUBAPK, 09-202021).

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