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Morphometric Effect of Nandrolone on Humerus of the Pubertal Term Rats

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Abstract: The purpose of this research was to evaluate the morphometric effects on nandrolone used as doping in sports on humerus of rats in the pubertal term, 30 days, 60 pieces Sprague Dawley rats were used in the study. Male (n:30) and female (n:30) rats were divided into three equal groups as nandrolone, peanut oil and control groups. Nandrolone was injected 10 mg kg^{-1} intraperitoneally within peanut oil diluents of the commercial drug during 4 weeks as 5 days and left for 2 days of rest by the break (Nandrolone group). Peanut oil group was applied intraperitoneally like nandrolone group. The control group was no any treat. Rats were euthanatized at the end of the 4 weeks. Front extremities of the rats were dissected. Morphometric measurements were made with calipers to every one of the anatomical reference points determined of the humerus bones. Difference between humerus height averages of female peanut group, female control groups and female nandrolone group was found statistically significant ($p<0.05$). There was no statistically significance determined between the height of humerus of the nandrolone group ($p>0.05$) compared to sex difference. Cortex thickness and medullar diameter of nandrolone female humerus were statistically significant than other groups ($p<0.05$). As a result, it can be concluded that nandrolone used mainly athletes in the puberty period and in all athletes, stops elongation of the bones such as humerus.

Key words: Humerus, morphometry, nandrolone, rats, peanut group, doping, Turkey

INTRODUCTION

Now a days, the usage rate of ergogenic is rising with the intention of increasing competition and physical performance (Jenkins, 2002). Doping is a kind of absorption which enables sportsman to increase his performance in an artificial and illegal way during the game (Gunay, 1998). Stimulants, narcotic analgesics, peptide hormone analogues, diuretics, masking agents and anabolic agents are materials used to dope (Schanzer and Thevis, 2007). It is possible to identify anabolic agents in two groups as beta 2 agonists and Anabolic Androgenic Steroids (AAS) (Suzer, 2004). It is well known that sportsmen use AAS to increase their physical performance since 1950 (Lombardo, 1996).

ASS are frequently used by sportsman who engaged in athleticism, weight-lifting and bodybuilding (Vardar *et al*., 2002). Using ASS during weight-lifting trainings brings muscle building herewith (Akgun, 1993). Testicle, adrenal cortex and ovarium secrete androgenic steroids (Dokmeci, 2000). In order to gain more efficient results AAS testosterone's countless derivations synthesized to extend the half life of main molecule (Maravelias *et al*., 2005). Nandrolone, exogenic and analogue of testosterone is anabolic androgenic steroid. Nandrolone is derived after C-19 methyl group split up from testosterone (Ozer, 1994). Metabolism and metabolites of nandrolone is similar with testosterone. Anabolic effect of nandrolone decanoate is strong and it is also one of the most influential medicine used in muscle and body building. It is also known that androgenic effect is less (Baysaling, 2000). Nandrolone is parent drug in treatment of osteoporosis after menopause, anemia, chronic kidney failure etc. Nandrolone commercially is produced between the doses 25 and 200 mg mL^{-1} and selling in many countries (Engel *et al*., 1957).

In addition to common adverse effects in gynecomastic changes in lipid profile, hepatotoxicity, barrenness, gonadal hypertrophy, depression of immune system and psychological corruptions, ASS have important cardiovascular effects. They also have thrombotic, atherogenic and vasospasmic effects (Maravelias *et al*., 2005; Akalin, 2006). Main adverse effects of AAS on skeleton are closure of epiphysis both

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in adults and children. It is also observed that sportsman using androgen for a long term becomes shorter. Frequent trainings and increased steroid use may result in unexpected lengthening of muscles and tendons. In some of the published studies states that it can also increase the risk of tendon rupture (Maravelias *et al*., 2005). Another notification related to nandrolone is that it has positive effects on increment in bone mass, hypertrophy in skeleton muscles (Taylor *et al*., 1999) more brawn and reparation of wounding in skeleton muscles (Vignaud *et al*., 2004).

Although, many source surveys done in this category defines ASS many adverse effects, it is still unknown whether nandrolone has any effect on bone development or not. The point of this survey is to determine morphometric effect of nandrolone which is used to dope in sport on rat's humerus in puberty.

MATERIALS AND METHODS

Experimental group is consist of 30 days old 60 Sprague Dawley rat (female n:30, 108±8.62 g, male n:30, 121±13.3 g) provided from SUDAM. Experiment is approved by Selcuk University faculty of veterinary science's ethics committee. Rats are fed on *ad libitum* and placed to standard cages in the way that 5 female and 5 male for each cage. Temperature is kept at 25°C and humidity is kept at 52.00% Rh. Male and female rats divided into three groups; Control group (male n:10, female n:10) had been fed without any application for 4 weeks. Peanut oil used as the diluents of nandrolone decanoate (500 mcl, Zade peanut oil, Konya, Turkey) was implemented to the peanut oil group as intraperitoneal for 5 days, then 2 days break and the same application carried out for 4 weeks. The drug was applied for (male n:10, female n:10) 5 days as intraperitional by diluting the nandrolone decanoate (Nandrolone Decanoate® Inj, Norma Hellas SA, Menandrou, Greece) in the 10 mg kg^{-1} (Andrade *et al*., 2008) peanut oil to the experimental group and the same procedure was applied for 4 weeks after again 5 days pause. At the end of the 4th weeks all rats were euthanatized by injection of pentobarbital (Nembul Sodium, Abfar Medicine Industry, Istanbul, Turkey). Front extremite bones of materials were uncovered by making diseke and were subjected to maceration operation. Then humerus bones which were uncovered were withered. By determining the anatomic reference points of right side humerus bones which will be measured [A (height), B (corpus), C1-C2 (cortex-cortical bone thickness-substantia compacta) and D (medular scale-cavum medullare)] required morphometric measurements for each points by 0-100 mm, caliper

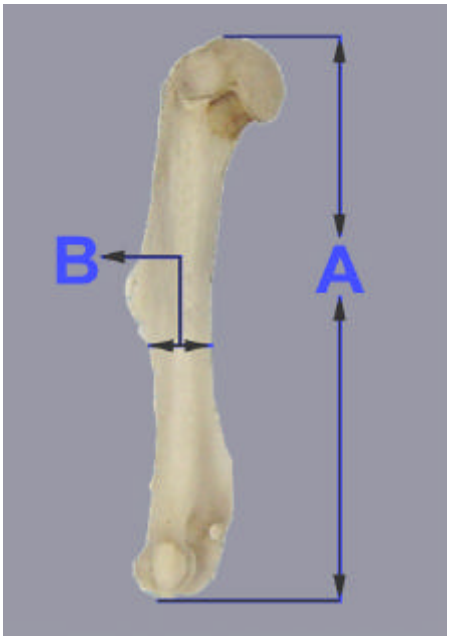

Fig. 1: Reference points of humerus height (A), Corpus (B) (Right Medical Face, ♂), A: Distance between peak points of caput humer and trochlea humer, B: Corpus thickness of humerus (bottom border level of tuberositas deltoidea)

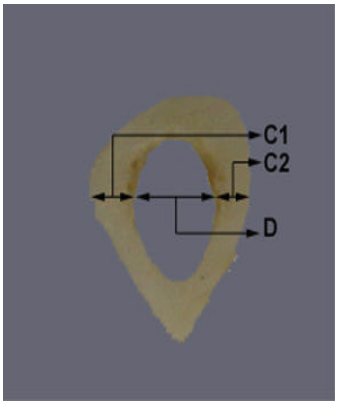

Fig. 2: Reference points of humerus cortex (C1-C2) and medullar scales (D) (right medical face, ♂), C1-C2: Average cortex thickness of humerus on the corpus level (cortical bone-substantia compacta), D: Scale of cavum medullare of humerus on the corpus level

(stainless hardened digital caliper, China) were done (Fig. 1 and 2). For writing of anatomic term Nomina Anatomica Veterinaria (NAV, 2005) was used. For evaluation of statistical data SPSS 13.00 package programme was used. Results were come out as mean±SE.

To compare the data between groups, ANOVA and Duncan test were carried out. To compare the data between genders, independent t-test was used. On the statistical side $p<0.05$ value was regarded as important.

RESULTS AND DISCUSSION

It is determined that when Humerus height is assessed ND application cause curtailment on female rats ($p<0.05$). Both male and female rats humerus bones are at the same scale in all test groups (Table 1). Both male and female rats averages between corpus thickness of humerus bones are not found important on the statistical aspect ($p>0.05$). When comparing the corpus thickness of Humerus bones according to genders, differences between corpus thickness of male and female nandrolone group rats humerus bones are not found important on the statistical aspect ($p>0.05$). However, differences between corpus thickness of male and female peanut oil and control group rats humerus bones are found important on the statistical aspect ($p<0.05$). Cortex thickness of female rats of nandrolone application is measured shorter than male rats ($p<0.05$). Medullar scale of nandrolone's female rats is found longer than peanut oil and control group ($p<0.05$). When comparing according to gender difference between averages of medullar scales of male and female rats in peanut oil and control group is found important ($p<0.05$). It is expressed so frequently that anabolic and androgenic steroids are used for developing muscle strength and muscle mass by athletes in uncontrolled way (Kurling *et al*., 2005).

It is said that adverse effects of anabolic and androgenic steroids on skeleton systems resulted in closing growth plaque in tip bone in children and curtailment of height in adults and athletes using so much androgen (Al-Ismail *et al*., 2002; Maravelias *et al*., 2005). Height of humerus bone in male and female rats in nandrolone group is measured shorter than other groups. There is not found any differences in comparison of height of humerus bones (Table 1). Weisman *et al.* (1993) express in their research that height of humerus bone in male rats given testosterone does not change according to genders. This result is the same with the study findings too. Xiaodong *et al.* (2000) form experiment and control group and inject nandrolone to experiment group during 6 weeks for searching effects of using nandrolone on bone mass and metabolism. It is expressed that height of humerus bone of male and female in experiment group given medicine is shorter than control group. While height of humerus bone in nandrolone group is found shorter than other groups numerically, it is not found statistically meaningful (Table 1). These results are valid only humerus bone of female rats in research. However, they are not valid statistically while they are valid for humerus bone of male rats.

In research, it is not found any differences among nandrolone group, peanut oil and control group in comparison of corpus thickness of humerus bone in male and female rats (Table 1). Onoe *et al.* (1997) find that statistically meaningful difference in the corpus thickness of humerus bone of rat-treated beta agonist. This result was not similar the study. However, there was not found any differences among corpus thicknesses of humerus bones in present research. While corpuses of male rats of peanut oil and control group in humerus bone are thicker than female (Table 1) when they are examined, the same is not valid for nandrolone group. It is determined that nandrolone cause corpus thickness of humerus bone of female rats to be closer to corpus thickness of humerus bone of male rats (Table 1). In study, cortex thicknesses of humerus bones of female rats applied nandrolone are measured in a lower value than other groups. Difference among cortex thicknesses of humerus bones of female nandrolone group, peanut oil and control group is found statistically important ($p<0.05$, Table 1).

Besides, in study averages of cortex thickness of humerus bones in male and female nandrolone group is found statistically meaningful in aspect of gender ($p<0.05$, Table 1). Vidal *et al.* (2000) are analyzed effects of estrogen receptor in male rats on bone growth and maturation. They find that difference among cortex thicknesses of femur bone in male experiment and control group is meaningful. In this aspect, this study results are not parallel with present study findings. In this study, cortex thicknesses of humerus bone of male rats in nandrolone group are measured in higher value than female rats in nandrolone group. Difference among cortex thicknesses of humerus bone of male and female rats in

Table 1: Comparing of morphometric measurements of humerus (mean±SD)

	Height (mm)		Corpus (mm)		Cortex (mm)		Medulla (mm)	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female
ND	23.6±0.74^{a,A}	23.1±0.13^{b,A}	2.21±0.07^{a,A}	2.18±0.04^{a,A}	0.51±0.07^{a,A}	0.46±0.05^{b,B}	1.23±0.23^{a,A}	1.28±0.12^{a,A}
PO	24.1±0.81^{a,A}	23.7±0.68^{a,A}	2.26±0.09^{a,A}	2.14±0.07^{a,B}	0.46±0.03^{a,A}	0.56±0.55^{a,B}	1.34±0.13^{a,A}	1.05±0.11^{b,B}
Control	24.3±08.6^{a,A}	23.7±0.60^{a,A}	2.25±0.09^{a,A}	2.13±0.07^{a,B}	0.46±0.09^{a,A}	0.54±0.04^{a,B}	1.31±0.13^{a,A}	1.04±0.09^{b,B}

ND: Nandrolone PO: Peanut Oil, Different letters in the same column a,b and line A,B are important on the statistical aspect ($p<0.05$)

experimental group is found statistically meaningful in the aspect of gender ($p<0.05$, Table 1). Windahl *et al.* (2000) observe difference among humerus bones in the aspect of gender in their research which they analyze effects of androgen receptor on bones in new-born rats. These results are parallel with present study findings. Weisman *et al.* (1993) search effect of testosterone on bone cell of male and female rats and expressed that nandrolone does not affect averages of cortex thickness of humerus bone in male and female rats. In this aspect, the study of Weisman *et al.* (1993) is not parallel with results of present study. In present study, cortex thicknesses of rats in nandrolone group are statistically meaningful in aspect of gender (Table 1). In this study, it was not found differences between medullar scale of male rats humerus bones on peanut oil, nandrolone and control groups (Table 1). In the study of Qu *et al.* (1998), it is determined that there is not found any differences statistically among estrogens in the aspect of average heightness of medullar scale of male rats humerus bones. In present study, medullar scale of female rats humerus bones in nandrolone groups is found bigger than the other groups. These difference between female nandrolone group and peanut oil, control groups is found statistically important ($p<0.05$, Table 1). Lindberg *et al.* (2001) in their study about the effect of androgenic receptor on bone specifity of female rats, cortex thickness of female experimental groups humerus bones is found bigger than control group. Result of the study whose name is mentioned above are same as present study.

CONCLUSION

As a result, it can be concluded that using nandrolone by sportsmen especially who are in younger period, growing of long bone can be stopped and also for women cortex scale of humerus bones can be decreased.

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