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Does the use of Testosterone Enanthate as a Form of Doping in Sports Cause Early Closure of Epiphyseal in Bones?

¿El Uso de Enantato de Testosterona como Forma de Dopaje en el Deporte Causa Cierre Epifisiario Temprano de los Huesos?

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LOK, S. Does the Use of Testosterone Enanthate as a Form of Doping in Sports Cause Early Closure of Epiphyseal in Bones? ***Int. J. Morphol., 33(4)***:1201-1204, 2015.

SUMMARY: The purpose of this research is to investigate the morphometric effects of short term usage of testosterone enanthate among the anabolic androgenic steroids used as doping by athletes on humerus bones of male rats. 30 rats (35 days) were utilized in this research. The rats were divided into three equal groups. For the experimental group (n= 10), testosterone enanthate at 10 mg/kg dose in 100 µl peanut oil diluents, for the peanut oil group (n= 10), peanut oil of testosterone enanthate' diluent (100 µl) was executed intraperitoneally 5 days in a week for 3 weeks. The control group (n= 10) was nourished without any practice for 3 weeks. All rats were euthanized end of the research. Humerus bones were exposed by the dissection of rats' front extremite bones and measurements and the averages were taken. When the length of rats' humerus bones were analyzed, the growth of humerus bones of rats testosterone enanthate applied in the experimental group stopped significantly and the difference was significant ($p<0.05$). It was observed that among anabolic androgenic steroids, testosterone enathate's usage on male rats in puberty period caused early epiphyseal closure and stopped the growth of humerus bones significantly.

KEY WORDS: Sport; Doping; Testosterone enanthate; Bone; Humerus; Morphometry.

INTRODUCTION

Anabolic androgenic steroids (AAS) the speed sports in which high performance, power and endurance is needed (Kanayama *et al*., 2009); by athletes, body builders and weightlifters (Al-Ismail *et al*., 2002) to increase muscle mass, strength (Kanayama *et al*.; Attardi *et al*., 2011; Fürstenberger *et al*., 2012; Khan *et al*., 2012; Azzazy *et al*., 2009) and used to be able to achieve high performance (Lood *et al*., 2012; Hildebrandt *et al*., 2011; Di Luigi *et al*., 2012).

AAS damages organs such as liver (Tasgin *et al*., 2010; Pagonis *et al*., 2008), heart (Lok *et al*., 2010; Hassan & Kamal, 2013; Kaushik *et al*., 2010; Baggish *et al*., 2010; Tasgin *et al*., 2011) and kidney (Hassan & Kamal; Neri *et al*., 2011). Immune system (Khan *et al*.), endocrine system, urogenital system (Collins & Basaria, 2012) not only cause the problems like that; but also reveal serious side effects on other systems in the body (Bispo *et al*., 2009) and prompt serious health problems (Di Luigi *et al*.).

One of the most serious side effects of these substances used for doping by athletes especially, the result of usage at an early age, it causes early epiphyseal closure in long bones and prevent bone from draw out (Al-Ismail *et al*.; Soma *et al*., 2008; Lok *et al*., 2011; Zaman *et al*., 2012).

The purpose of this research is to investigate among anabolic androgenic steroids short term usage of testosterone enanthate's, used by athletes for doping, morphometric effects on male rat humerus bones.

MATERIAL AND METHOD

The research was carried on 30 male Wistar rats of 35 days (90–120 g). Rats fed *ad libitum* and were divided into three groups in standard cages. While control group (n= 10) fed without any application, to the peanut oil group (n= 10) the peanut oil (100 µl, Zade peanut oil, Konya-TURKEY), which is used as testosterone enanthate's diluent, executed 5 days intraperitoneally, after 2 days the same application was performed. To the experimental group (n=

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10) testosterone enanthate on (Androne® 250 Enj, Caspian tamin pharmaceitucal Co, Rasht-IRAN) 10 mg/kg dose (Lok and Yalcin, 2010), was applied intraperitoneally 5 days by dilueting in 100 µl peanut oil and after 2 days break, it was applied again. All processes have been resumed 3 weeks.

Before, at the begining and during the study, one day in a week weight of rats in all groups were measured and weekly weight averages were taken. Weekly dose adjustment was done in the weight measures of testosterone enanthate applied group. All rats were euthanized at the end of 3 weeks with pentobarbital (Nembutal sodium, Abfar). Humerus bones of rats were revealed by the dissection of front extremite bones.

Humerus bones were measured in the right anatomical references (A) length, corpus thickness [B1 (craniocaudal) + B2 (mediolateral) / 2], cortex-cortical bone thickness [C1 (caudal) + C2 (cranial) + C3 (medial) + C4 (lateral) / 4] and medullar diameter-cavum medullare [D1 (craniocaudal) + D2 (mediolateral) / 2] (Lok *et al*., 2011) each of these points were found and necessary morphometric measures were done (Fig. 1).

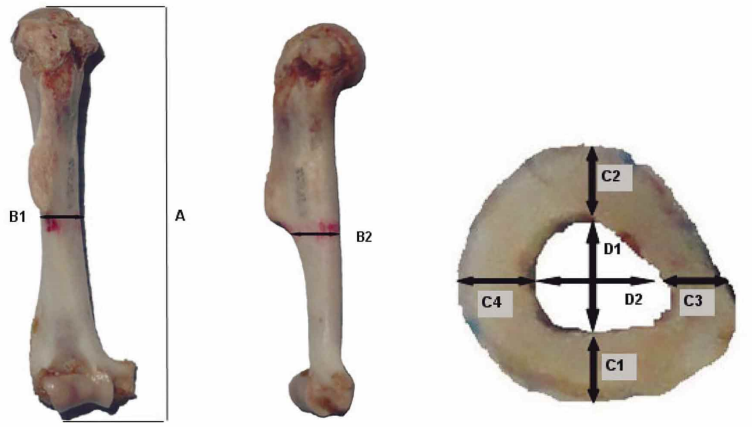

Fig. 1. Reference points (Right crainal and medial face) of humerus length (A), corpus (B1+B2/2), cortex (C1+C2+C3+C4/4) and medullar calibers (D1+D2/2).
A= Pitch between terminal points of caput humeri and trochlea humeri; B1+B2/2= Corpus thickness of humerus;
C1+C2+C3+C4/4= Cortex thickness (cortical bone- substantiate compacta) of humerus level; D1+D2/2= Caliber of cavum medullar of humerus corpus level.

Table I. Comparison of length and thickness of corpus, cortex and medullar diameters of humerus bones (Mean±SD).

	Experimental n= 10	Control n= 10	Peanut oil n= 10
Length	23.63±0.52 [a]	24.90±0.48 [b]	25.38±0.81 [b]
Corpus	2.63±0.09 [a]	2.66±0.08 [a]	2.66±0.12 [a]
Cortex	0.52±0.02 [a]	0.52±0.00 [a]	0.53±0.02 [a]
Medullar	1.55±0.09 [a]	1.63±0.10 [a]	1.59±0.10 [a]

a, b= Different letters in the same line are statistically significant (Duncan test, $p<0.05$).

"Nomina Anatomica Veterinaria" (ICVGAN, 2005) was used in the writing of anatomical terms. It was benefited from SPSS 13.0 package program in data statistical evaluation. Results were presented as Mean ± SD. In the comparison of data, ANOVA and Duncan test was applied ($p<0.05$).

RESULTS

The length, thickness of corpus, cortex and medullar diameters of humerus bones of experimental, control and peanut oil groups were compared (Table I).

When the length of humerus bone of rats was examined; in the testosterone enanthate applied group (experiment) growth of the humerus bones stopped and difference found significant ($p<0.05$). It was found that there is no significant difference at the thickness of corpus and cortex, diameters of medullar of humerus bones between experiment, control and peanut oil groups ($p>0.05$).

DISCUSSION

Short term use of testosterone enanthate's morphometric effects on male rats, in puberty period, and length, corpus thickness, cortex thickness and medullar diameter of humerus bone were analyzed.

Rats' length of humerus (testosterone enanthate applied) in experiment group was found shorter than other groups ($p<0.05$). In the other studies (Lok *et al*., 2011; Li *et al*., 2000) of AAS', it was reported that anabolic androgenic steroids causes early epiphyseal closure and avoid bone from growth. Literature findings show parallelism with this study findings.

Among three groups (experiment, control and peanut oil) in this study, no difference was found in medullar diameter, corpus and cortex thickness of humerus bone. In other words; testosterone enanthate had no significant effect on medullar diameter, corpus and cortex thickness of humerus ($p>0.05$). In the studies done with

different research groups, it was reported that AAS' did not have any impact on bone corpus thickness (Lok & Yalcin, 2010), cortex thickness (Windahl *et al*., 1999) and medullar diameter (Lok *et al*., 2012; Kim *et al*., 2003). Literature diagnosis shows parallelism with labour findings.

CONCLUSIONS

As a result, short term use of testosterone enanthate's morphometric effects on male rats' humerus bone, in puberty period, causes early epiphyseal closure and shuts down the growth of bone as seen in anabolic androgenic steroids.

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RESUMEN: El objetivo de esta investigación fue estudiar en el húmero de ratas macho, los efectos morfométricos del uso a corto plazo de Enantato de testosterona entre los esteroides anabólicos usados como dopaje por atletas. En esta investigación se utilizaron 30 ratas divididas en tres grupos iguales. Un grupo experimental (n= 10), que se le administró Enantato de testosterona en dosis de 10 mg/kg en 100 µl diluido en aceite de maní, a otro grupo sólo se le administró aceite de maní (n= 10). La administración fue realizada por vía intraperitoneal 5 días a la semana durante 3 semanas. El grupo control (n= 10) fue alimentado durante 3 semanas. Todas las ratas fueron sacrificadas al término de la investigación. Los húmeros fueron expuestos por disección, se realizaron las mediciones y se tomaron los promedios. Cuando se analizó la longitud de los húmeros se observó que su crecimiento en las ratas del grupo experimental que recibieron Enantato de testosterona, se detuvo de manera significativa ($p<0,05$). Se observó que entre los esteroides anabólicos androgénicos, el uso de testosterona en ratas macho en el periodo de la pubertad causó el cierre epifisario temprano y detuvo el crecimiento del húmero de manera significativa.

PALABRAS CLAVE: Deporte; Doping; Enantato de testoterona; Hueso; Húmero; Morfometría.

REFERENCES

Al-Ismail, K.; Torreggiani, W. C.; Munk, P. L. & Nicolaou S. Gluteal mass in a bodybuilder: radiological depiction of a complication of anabolic steroid use. *Eur. Radiol., 12(6)*:1366-9, 2002.

Attardi, B. J.; Marck, B. T.; Matsumoto, A. M.; Koduri, S. & Hild, S. A. Long-term effects of dimethandrolone 17ß-undecanoate and 11ß-methyl-19-nortestosterone 17ß-dodecylcarbonate on body composition, bone mineral density, serum gonadotropins, and androgenic/anabolic activity in castrated male rats. *J. Androl., 32(2)*:183-92, 2011.

Azzazy, H. M.; Mansour, M. M. & Christenson, R. H. Gene doping: of mice and men. *Clin. Biochem., 42(6)*:435-41, 2009.

Baggish, A. L.; Weiner, R. B.; Kanayama, G.; Hudson, J. I.; Picard, M. H.; Hutter, A. M. Jr. & Pope, H. G. Jr. Long-term anabolic-androgenic steroid use is associated with left ventricular dysfunction. *Circ. Heart Fail., 3(4)*:472-6, 2010.

Bispo, M.; Valente, A.; Maldonado, R.; Palma, R.; Glória, H.; Nóbrega, J. & Alexandrino, P. Anabolic steroid-induced cardiomyopathy underlying acute liver failure in a young bodybuilder. *World J. Gastroenterol., 15(23)*:2920-2, 2009.

Collins, L. & Basaria, S. Adverse effects of androgen deprivation therapy in men with prostate cancer: a focus on metabolic and cardiovascular complications. *Asian J. Androl., 14(2)*:222-5, 2012.

Di Luigi, L.; Sgrò, P.; Aversa, A.; Migliaccio, S.; Bianchini, S.; Botrè, F.; Romanelli, F. & Lenzi, A. Concerns about serum androgens monitoring during testosterone replacement treatments in hypogonadal male athletes: a pilot study. *J. Sex Med., 9(3)*:873-86, 2012.

Fürstenberger, C.; Vuorinen, A.; Da Cunha, T.; Kratschmar, D. V.; Saugy, M.; Schuster, D. & Odermatt, A. The anabolic androgenic steroid fluoxymesterone inhibits 11ß-hydroxysteroid dehydrogenase 2-dependent glucocorticoid inactivation. *Toxicol. Sci., 126(2)*:353-61, 2012.

Hassan, A. F. & Kamal, M. M. Effect of exercise training and anabolic androgenic steroids on hemodynamics, glycogen content, angiogenesis and apoptosis of cardiac muscle in adult male rats. *Int. J. Health Sci. (Qassim), 7(1)*:47-60, 2013.

Hildebrandt, T.; Yehuda, R. & Alfano, L. What can allostasis tell us about anabolic-androgenic steroid addiction? *Dev. Psychopathol., 23(3)*:907-19, 2011.

International Committee on Veterinary Gross Anatomical Nomenclature (ICVGAN). *Nomina Anatomica Veterinaria (NAV)*. 5th ed. Knoxville, ICVGAN & General Assembly of the World Association of Veterinary Anatomists WAVA, 2005.

Kanayama, G.; Brower, K. J.; Wood, R. I.; Hudson, J. I. & Pope, H. G. Jr. Issues for DSM-V: clarifying the diagnostic criteria for anabolic-androgenic steroid dependence. *Am. J. Psychiatry, 166(6)*:642-5, 2009.

Kaushik, M.; Sontineni, S. P. & Hunter, C. Cardiovascular disease and androgens: a review. *Int. J. Cardiol., 142(1)*:8-14, 2010.

Khan, J. I.; Kennedy, T. J. & Christian, D. R. Jr. *Basic Principles of Forensic Chemistry.* New York, Humana Press/Springer, 2012.

Kim, B. T.; Mosekilde, L.; Duan, Y.; Zhang, X. Z.; Tornvig, L.; Thomsen, J. S. & Seeman, E. The structural and hormonal basis of sex differences in peak appendicular bone strength in rats. *J. Bone Miner. Res., 18(1)*:150-5, 2003.

Li, X.; Takahashi, M.; Kushida, K.; Shimizu, S.; Hoshino, H.; Suzuki, M. & Inoue, T. The effects of nandrolone decanoate on bone mass and metabolism in ovariectomized rats with osteopenia. *J. Bone Miner. Metab., 18(5)*:258-63, 2000.

Lok, S. & Yalcin, H. Morphometric effect of nandrolone decanoate used as doping in sport on femur of rats in puberty period. *Arch. Budo, 6(4)*:217-20, 2010.

Lok, S.; Tasgin, E. & Yalcin, H. The morphometrical response of the combined nandrolone and testosterone usage to the femur. *Sci. Res. Essays, 6*:4867-69, 2011.

Lok, S.; Tasgin, E. & Yalcin, H. Morphometric effects of the combined usage of anabolic androgenic steroids on humerus. *Energy Educ. Sci. Technol. Pt. B-Soc. Educ. Stud., 4(4)*:2625-30, 2012.

Lok, S.; Tasgin, E.; Demir, N. & Ozdemir, M. Long term used testosterone may cause heart and liver damage. *J. Anim. Vet. Adv., 9(18)*:2343-5, 2010.

Lood, Y.; Eklund, A.; Garle, M. & Ahlner, J. Anabolic androgenic steroids in police cases in Sweden 1999-2009. *Forensic Sci. Int., 219(1-3)*:199-204, 2012.

Neri, M.; Bello, S.; Bonsignore, A.; Cantatore, S.; Riezzo, I.; Turillazzi, E. & Fineschi, V. Anabolic androgenic steroids abuse and liver toxicity. *Mini Rev. Med. Chem., 11(5)*:430-7, 2011.

Pagonis, T. A.; Koukoulis, G. N.; Hadjichristodoulou, C. S.; Toli, P. N. & Angelopoulos, N. V. Multivitamins and phospholipids complex protects the hepatic cells from androgenic-anabolic-steroids-induced toxicity. *Clin. Toxicol. (Phila.), 46(1)*:57-66, 2008.

Soma, L. R.; Uboh, C. E.; Guan, F. & McDonnell, S. Plasma concentrations of testosterone and 19-nortestosterone (nandrolone) in the nonracing intact male horse by liquid chromatography-mass spectrometry. *J. Vet. Pharmacol. Therap., 31(6)*:587-90, 2008.

Tasgin, E.; Lok, S. & Demir, N. Combined usage of testosterone and nandrolone may cause heart damage. *Afr. J. Biotechnol., 10(19)*:3766-8, 2011.

Tasgin, E.; Lok, S.; Demir, N. & Ozdemir, M. The effect of testosterone used in sportsmen on routine biochemical parameters. *J. Anim. Vet. Adv. 9(15)*:2038-40, 2010.

Windahl, S. H.; Vidal, O.; Andersson, G.; Gustafsson, J. A. & Ohlsson, C. Increased cortical bone mineral content but unchanged trabecular bone mineral density in female ERbeta(-/-) mice. *J. Clin. Invest., 104(7)*:895-901, 1999.

Zaman, F.; Chrysis, D.; Huntjens, K.; Fadeel, B. & Sävendahl, L. Ablation of the pro-apoptotic protein Bax protects mice from glucocorticoid-induced bone growth impairment. *PLoS One, 7(3)*: e33168, 2012.

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