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MORPHOMETRIC EFFECT OF METHANOLONE ENANTHATE ON SCAPULA IN ADOLESCENT RATS

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ABSTRACT

This study was carried out to determine the effect of Methenolone enanthate (ME) on the doping list on male and female rat scapula during puberty. In this study, three groups (ME, FY, K) were created for males and females, each containing 8 rats, and a total of 48 rats were used. Methenolone enanthate (Primobolan Depot ® Bayer) was administered to the experimental groups at a dose of 0.5 mg / kg (0.5 ml, diluted in peanut oil) 5 times a week intraperitoneally for 4 weeks. All rats were euthanized under thiopental (40 mg / kg) anesthesia after 4 weeks. After necropsy, both scapula bones were dissected and macerated. At the end of this process, the bones were dried in a 37 C oven for 24 hours. Morphometric measurements of right and left scapula bones of male and female rats in each group were opened in the semi-automatic image analysis program imageJ (Ver. 1.44, National Institutes of Health). After calibrating the images, the height of the scapula and then the largest distance perpendicular to the height axis were measured by using the freehand line feature of the program (Fig 1). Area measurements of the medial surfaces of the bones were made using the point counting method (Başoglu et al, 2007, Akosman and Özdemir, 2010; Bolat et al, 2011). For this purpose, by using grid function of imagej program (plugins-analyze-grid), grids with 2 mm between two points and 4 mm2 of area represented by each point were created. These grids were superimposed on the bone images and the points falling on the bone surface were counted (Fig 2). The number of points obtained was multiplied by the area represented by each point and the bone surface areas of the animals in the groups were calculated separately in mm2.vAs a result, it was found that the effect of ME on rat scapula height, width and surface area was similar to other AAS's suppressed bone development in male pubers and encouraged bone development in females. The negative effects of the commonly used doping agents on bones in males in the medium and long term have been tried to be demonstrated with rat models.

Key Words: morphometric, rats, Adolescents rats

INTRODUCTION

It is clear that the clinical-therapeutic trials of AAS that doctors usually use are not free of side effects. Side effects are related to the type and dosage of steroids and have effects such as liver enzymes level, cholestatic jaundice, peliosis hepatis (Maravelias et al, 2005) and short stature in the skeletal system and thickening of the bone crust (Özdemir and Yalçın, 2011; Bozkurt et al, 2011ab).

AAS's may have consequences that may adversely affect growth, such as the early closure of the growth centers of long bones (epiphyseal cartilage growth plates) during puberty. It is reported that these effects can be seen more in young people and orthopedic problems arise due to overload caused by AAS especially in children (Peters et al 2002; Maravelias et al, 2005; Marqueti et al, 2006).

Another side effect that has been emphasized is the decrease in length growth in adults taking androgens for a long time and early epiphyseal closure in children or young people (Al-Ismail, 2002). Anabolic steroids suppress estrogen and testosterone production (Kasikcioglu, 2009; Baggish et al, 2010), while decreasing bone production and increasing destruction (Rathi, 2008). Lane (2006) states that steroids mostly affect trabecular bone and cause hyperparathyroidism. Mitchell (2005) and Kasıkcıoğlu (2009) reported that bone loss occurred within a few months of steroid use and that these losses were much more common in the trabecular bone in the vertebral and femoral head than in the cortical bone. In a morphometric study conducted on rats and investigating the effect of AAS on humerus and femur, it is reported that this drug causes a decrease in cortical bone density in males (Özdemir and Yalçın 2011, Bozkurt et al 2011ab). Mitchell (2005) and Kasıkcıoğlu (2009) reported that bone loss due to AAS use can be reduced with load-bearing exercises.

Studies have been reported to have growth-promoting effects of AAS. However, there are no studies in the literature on the effect of AAS on scapula. The aim of this study was to determine the effect of Methanolone Enenthate, one of the most widely used AASs used for doping by athletes, on the scapula height, width and surface area of puberta rats.

MATERYAL AND METOT

In this study, the average weight of 115-250 gr in 48 females of 40 days, 110-250 g in males, Sprague-Dawley rats obtained from the experimental animals unit of Selcuk University Faculty of Medicine were used. The research procedure was approved by the Ethics Committee of the Faculty of Veterinary Medicine of Selcuk University. The rats were housed in polycarbonate cages

(Tecniplast®, Italy) during the study at 21 ± 2 0C, 14:10 hours in a light-dark cycle with 1 rat on an area of 250 cm2, and ad libitum fed with standard rat feed (Purina®, Canada) and water.

After the rats were divided into male and female, 8 rats were formed in each group experimental (Methanolone enanthate-NE) group, vehicle group (peanut oil-FY) and control (K) were formed into 6 groups. ME (Primobolan Depot ® Bayer) groups received 5 mg / kg dose (Blystone, 2007; Özdemir, 2010) (0.5 ml, diluted in peanut oil) 5 times per week intraperitoneally. Vehicle groups received peanut oil 5 times per week (0.5 ml) intraperitoneally and control groups received the same amount of physiological saline intraperitoneally. The study was continued for 4 weeks.

All rats were euthanized by thiopental (40 mg / kg) anesthesia after 4 weeks. After necropsy, both scapula bones were dissected and macerated. At the end of this process, the bones were dried in a 37 C oven for 24 hours.

Right and left scapula bones of male and female rats in each group were placed on play dough parallel to the ground and medial faces on top for morphometric measurements. Millimetric paper was placed in the same plane as the bones on the play dough and the bones were taken in JPG format using a camera (Sony DSC-H55) placed perpendicular to this plane. After the images were uploaded to the computer, the semi-automatic image analysis program imageJ (Ver. 1.44, National Institutes of Health) was opened. After calibrating the images, the width of the scapula was measured at the widest distance perpendicular to the height axis using the freehand line feature of the program (Fig 1). Area measurements of the medial surfaces of the bones were made using point counting method (Basoglu et al, 2007, Akosman and Ozdemir 2010; Bolat et al, 2011). For this purpose, by using grid function of imagej program (plugins-analyze-grid), grids with 2 mm between two points and 4 mm2 of area represented by each point were created. These grids were superimposed on bone images and counted points falling on the bone surface (Fig 2). The number of points obtained was multiplied by the area represented by each point and the bone surface areas of the animals in the groups were calculated separately in mm2.

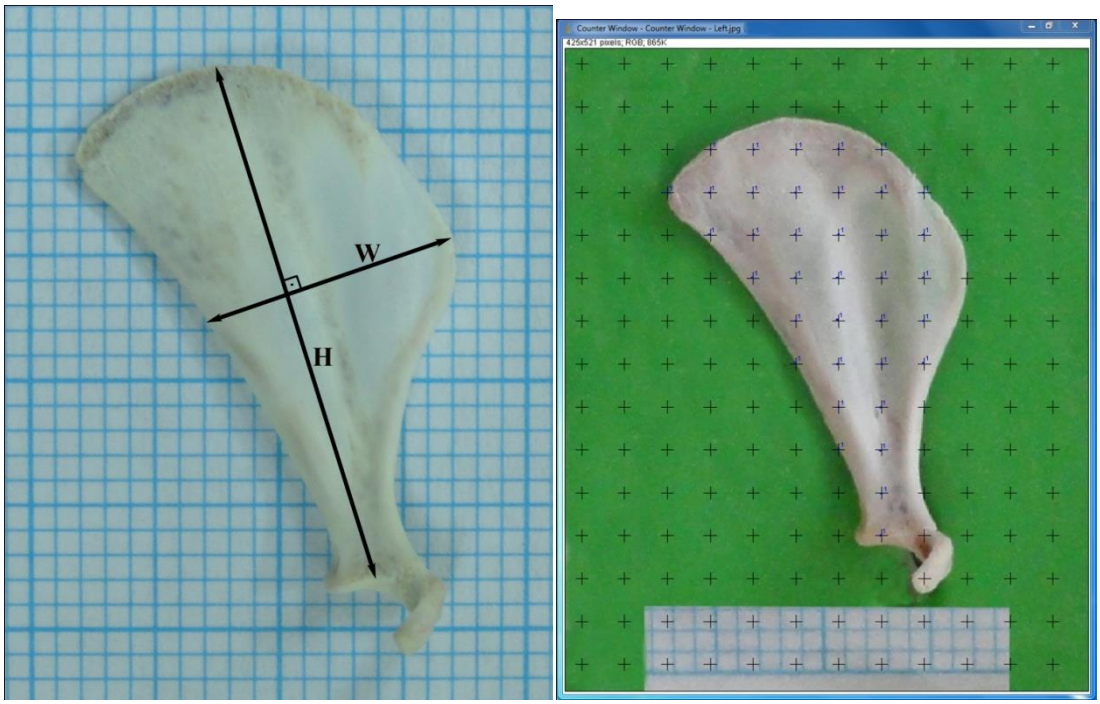

Fig 1. Height (H) and width (W) measurement points on the scapula.

Fig 2. The dotted area measuring scale randomly thrown onto the medial surface image in the scapula.

SPSS 13.0 (SPSS 13.0 for Windows / SPSS® Inc, Chicago, USA) was used for statistical analysis of the data. There was no difference in the statistical evaluation of the morphometric data of the right and left scapula bones ($p > 0.05$). Therefore, the results were presented as mean ± SEM over the total number of bones in the groups. ANOVA and Duncan tests were used to determine the differences between the groups and the sexes. $p<0.05$ was considered statistically significant

FINDINGS

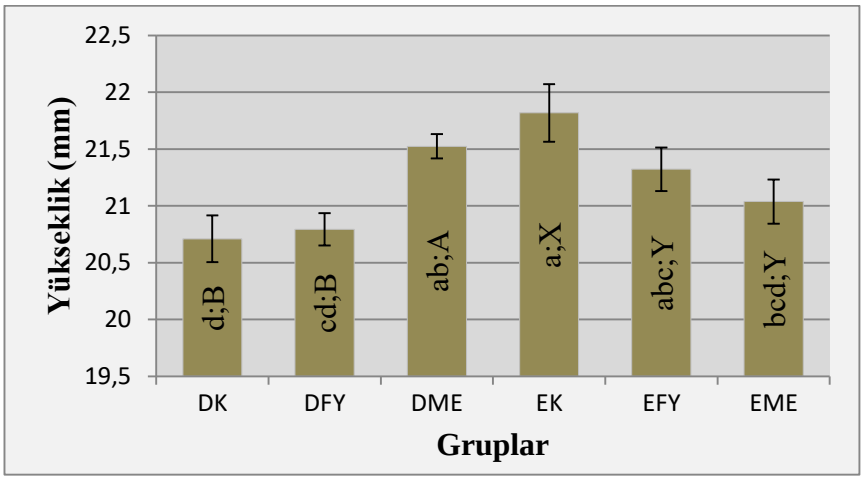

Fig 3. Scapula height, DME, Female methenolone enanthate group, DFY; Female fistic oil group, DK; Control group D, EME; Male methenolone enanthate group. EFY; Male fistic oil group, EK; The male control group, the different letters in the a-d columns indicate the difference between the female and male working groups, the A-B female groups, and the X-Y male groups.

When female scapula height ME (methanolone enanthate), peanut oil and control groups were compared, it was observed that ME (methanolone enanthate) application increased significantly compared to peanut oil and control groups (Fig 3; $P < 0.05$). In male rats, it was determined that ME and vehicle application had a negative effect on the increase in scapula height compared to the control group.

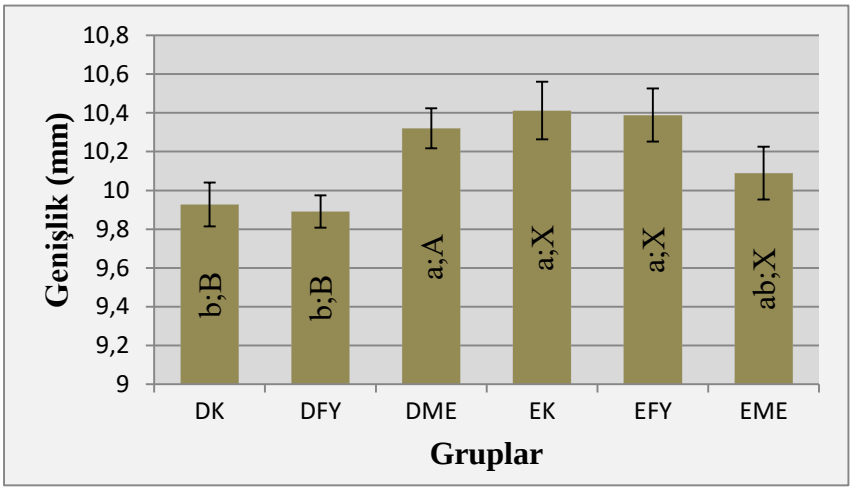

Fig. 4. Scapula width, DME, Female methenolone enanthate group, DFY; Female fistic oil group, DK; Control group D, EME; Male methenolone enanthate group. EFY; Male fistic oil group, EK; The male control group, the different letters in the a-d columns indicate the

difference between the female and male working groups, the A-B female groups, and the X-Y male groups.

When the ME, peanut oil and control groups were compared in female rats, it was observed that this morphometric value increased significantly in the ME group compared to the other two groups (Fig 4; $p < 0.05$). In male rats, no statistically significant difference was observed between groups in terms of scapula width (Fig 4; $p > 0.05$).

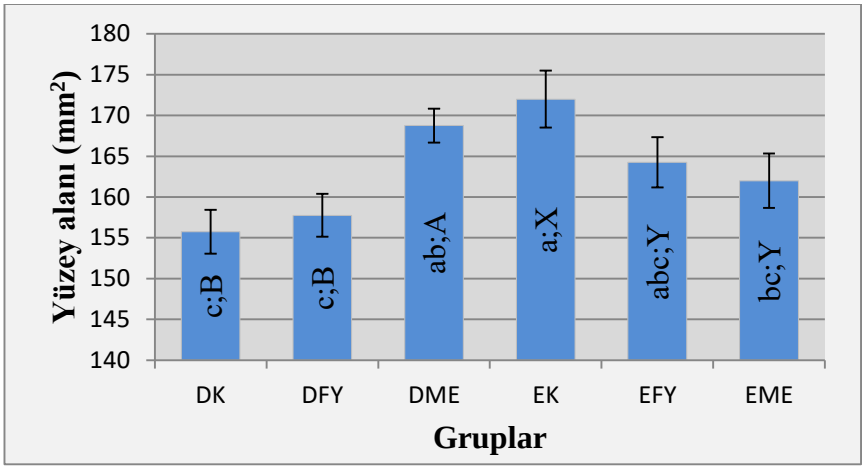

Fig 5. Medial surface area of Scapula, DME, Female methenolone enanthate group, DFY; Female fistic oil group, DK; Control group D, EME; Male methenolone enanthate group. EFY; Male fistic oil group, EK; The male control group, the different letters in the a-d columns indicate the difference between the female and male working groups, the A-B female groups, and the X-Y male groups.

It was observed that ME application in female rats significantly increased the surface area of scapula compared to FY and K groups (Fig 5; $p > 0.05$). In male rats, this effect was found to be negative in vehicle and ME groups compared to the control group (Fig 5; $p > 0.05$).

It is reported that AASs are used for the purpose of accelerating growth in boys with delayed puberty for treatment purposes, but when treatment is continued at high doses and for a long time, it causes premature closure of growth plates in bones and causes short stature (Peters et al, 2002; Vardar et al, 2002; Sevin et al, 2005; Özdemir and Gültürk, 2008). Similarly, it has been reported that long-term AAS applications in puberta rats cause a decrease in humerus and femur length and diameter development in males and increase in height and diameter development in females (Lök, 2009, Özdemir and Yalçın, 2011; Bozkurt et al, 2011ab). However, the effects of these drugs on flat bones are not known. In this study, morphometric changes of the scapula were examined for the first time by using long-term ME application on rats, similar to the effect of ME on long bones in the literature, it was determined that the increase in scapula height, width and surface area in females decreased in males.

In conclusion, this study shows that long-term use of ASS has similar morphometric effects in male and female individuals as well as in flat bones.

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