Morphometric evaluation of the effect of methenolone enanthate on humeral development in adolescent rats

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The effect of methenolone enanthate (ME), one of the anabolic-androgenic steroids (AAS), used as a muscle amplifier and for doping by athletes, on the humerus bone development of adolescent male and female rats has been researched in this study. Three different groups for each male and female rat, including 8 rats of each, (ME, V, C) were formed and a total of 48 rats were used. The subject groups were given 0.5 mg/kg methenolone enanthate (Primobolan® Depot, Bayer) diluted in peanut oil, 0.5 ml; all animals received five intraperitoneal injections weekly for four weeks. The rats were euthanized at the end of the four weeks and both humerus dissected and macerated. The length, corpus thickness, compact bone thickness and medulla canal diameter were measured with calipers and the average of each was noted. The effect of ME administration on humerus length and corpus femoris thickness was negative in male animals but positive in females and was determined to be statistically significant (p<0.05) in both cases. There was no treatment-related difference in compact bone thickness and medulla diameter (p>0.05), while a significant difference (p<0.05) had been seen between the sexes in medulla diameter in the control groups. Humerus compact bone density in male rats and medulla canal diameter of female rats are significant in terms of the difference between groups (p<0.05), and gender effect on the change arising in medulla canal diameter as a result of ME administration has been revealed to be insignificant (p>0.05). It was concluded that the ME effect on rat humerus, similar to that of other AAS, consists in suppressing bone growth in male animals while enhancing it in females during puberty. Rat models show negative middle-term and long-term effects of this agent, which is still widely in use as a performance enhancer, as opposed to its positive results in the short term.

Key words: Athletes, adolescent rats, bone, methenolone enanthate, morphometric.

INTRODUCTION

The urge to win against competitors and the psychological need of winning have brought about a constant increase in the frequency and intensity of physical training. Even though the incidence of anabolic steroid use is not exactly quantified, widespread use of these substances is observed along with the intensification of training (Anderson and McKeag, 1985). In addition to medical purposes, the AAS are used also to enhance sporting performance (Vardar et al., 2002) and modify body appearance (Buckley et al., 1988; Bahrke et al., 1998; Pope and Brower, 2000). These drugs are generally taken by athletes, weight lifters and body builders (Pope and Brower, 2000). As in the case of many other drugs, anabolic steroids are generally produced naturally in the body (Kaya and Bilgili, 1998).

Recently, not only body builders and athletes but also...
adults who do not practice sports and young adults also often use steroids (Durant et al., 1993; Taner et al., 1995; Scott et al., 1996; Lambert et al., 1998). A primary effect of the anabolic action of testosterone and its derivatives is their myotrophic effect, resulting in greater muscle mass and better endurance. The excitatory effect of these androgens on the brain, which often results in hyperexcitation and aggressivity, has motivated the widespread use of AAS by athletes at any level.

Studies on anabolic steroids have been shown that the ongoing increase of their use is similarly frequent among both practitioners of sports and other people (Wagner, 1989; Windsor and Dumitru, 1989). Health problems related to their frequent use have attracted the attention of many health and athletic organisations along with scientists: urging reports to different preventative measures have been published (Maravelias et al., 2005). The use of AAS has been attributed to psychiatric symptoms and disease like addiction, substance abuse, mood disturbances including mania and depression, psychosis, aggressive behavior including homicidal, loss of libido, and insomnia (Pope and Katz, 1988; Breuer, 1993; Pope and Katz, 1994). Anabolic androgenic steroids were reported to cause early cartilagial ossification and short height among males in their prepubertal period. In the adult males, they cause hair loss, sterility, gynecomastia, loss of libido, testicular atrophy, impotence, low levels of endogenous androgens, prostatic hypertrophy and cancer, oligospermy, Wilms’ tumor and abnormal sperm morphology; however, in the adult females, they cause hirsutism, alopecia, breast shrinkage, thickening of the voice, excessive increase in libido and menstrual irregularity (Livanelioglu, 2010). Vanderschueren et al. (2004) reported that androgens cause changes in the length of bone, cortical diameter and bone density of rat humeruses.

The AAS can yield results that negatively affect the growth such as early closure of the growth plaques (in the growth plate in epiphyseal cartilages) of the young during adolescent period. It has been reported that these effects could be seen more often among young (Maravelias et al., 2005). Thus, orthopedic problems can arise in children as a result of overloading onto bones caused by AAS (Maravelias et al., 2005; Marqueti et al., 2006). ME, like other AAS, is used with the objective of improving patients’ general condition by supporting healing processes during disease situations that require high levels of protein synthesis and preventing damage due to catabolic processes. Examples of conditions in which it is used are convalescence, post-operative care, wasting diseases, cachexia, radiation or cytostatic therapy, progressed breast cancer or genital cancer in women, hematopoietic disease, long-term corticoid treatment, osteoporosis, protein deficiencies of the elderly patient, and chronic liver disease. The use of this drug to enhance muscle and bone growth in healthy individuals has been recorded as abuse (Anonymous, 2010). The objective in this study is to determine probable morphometric or structural changes of ME, an ASS, preferred much and widely used as a performance enhancer by the athletes, in the humerus of adolescent rats.

MATERIALS AND METHODS

In the study, 48 live Sprague-Dawley rats aged 40 days and weighing 110 to 250 g were used. During the study, the animals were kept in polycarbonate cages, one animal per 250 cm² surface area, at 21±2°C with a 4:10 h light/dark cycle, fed with standard rat diet (Purina, Canada) and water ad libitum. The study was authorized by the ethical committee. A total of six groups, ME (Primobolan Depot®, Bayer), vehicle (V, peanut oil), and control (C), were formed including 8 female and male rats, each after rats was distributed to the groups. The treatment group received ME, 5 mg/kg (Ozdemir and Yalcin, 2010; Blaystone, 2007) diluted in 0.5 ml peanut oil, while the V group was given 0.5 ml peanut oil and the C group the same volume of normal Physiol (Physiological Water with NaCl) intraperitoneally, five times weekly during 4 weeks. All rats were euthanized with thiopental anesthesia, 40 mg/kg, at the end of the fourth week. Both humeruses were dissected during necropsy and macerated.

The humeruses were later dried and kept in specially marked plastic bags. The length, shaft thickness, corpus thickness, compact bone thickness and medullary canal diameter were measured at the points indicated in Figure 1a-b using a 0 to 100 mm caliper and the mean measurements noted. The SPSS 13.0 software package (SPSS® 13.0 for Windows, SPSS Inc, Chicago, USA) was used for the statistical evaluation of the data. Group values were presented as Mean ±SD. ANOVA and Duncan’s tests were used for the comparison of the different treatments performed by ANOVA and Duncan’s tests, while that between the genders was done by the t-test for independent samples. A p value of <0.05 was accepted as statistically significant.

RESULTS

The group humerus length and corpus diameters are shown in Table 1 while compact bone (cortex) thicknesses and medulla space (cavum medulla) diameters are summarized in Table 2. The length of humerus is longer than FY and K groups in methanolone-treated female rats, while it is smaller in male rats and this difference in ME groups was determined to be statistically significant (p<0.05) according to healthy controls. The evaluation of gender comparison has revealed that statistical difference is significant for each of the three groups (p<0.05). This situation is defined as longitudinal contraction of humerus in males and lengthening of humerus in females and bone development for both males and females was observed to approximate to each other. However, this increase in female rats is remarkable, in that it can never reach the length of the humerus of healthy male rats. (Table 1). When corpus humeri thickness is taken into consideration, it was revealed that changes in male rats are parallel to changes in humerus length and thickness of corpus humeri significantly decreases in ME group.
Each of the three groups is different in female rats (p<0.05), besides ME administration causes thickening in corpus humeri. Methanolone enanthate administration causes a considerable amount of reduction in the thickness of humerus cortex in male rats (p<0.05), while it causes thickening in female rats and this was determined to be statistically insignificant for female rats (p>0.05). The research revealed a gender difference; ME and V male humerus cortex is relatively thinner than that of female rats (p<0.05). In the analysis of humerus cavum...
medullary canal diameter, although the statistical difference is insignificant, ME administration was observed to cause a decrease in the width of cavum medullare in male rats. However, width of the cavum medullare in female rats considerably increases following the control (p<0.05). When gender difference is taken into consideration, the difference in V and C groups has been found significant, whereas it is insignificant in ME group. This fact has been evaluated as follows; ME administration, by having counter effect on bone development in female and male rats, has removed initial gender difference.

**DISCUSSION**

AAS are used basically for doping and to modify the body configuration than for medical purposes. These kind of drugs were first used by weight lifters and other athletes including body builders (Vardar et al., 2002). A majority of coaches and athletes believe that AAS doses that are 10 to 200 higher than the endogenous level of these substances increase power and motivation, thus enhancing athletic performance (Sevin et al., 2005). The AAS have been used in male children with delayed onset of puberty, aiming at enhancing growth (Vardar et al., 2002; Sevin et al., 2005; Gümüşel and Kendilci, 2005; Özdemir and Gulturk, 2008). During high-dose or long term therapy, however, while growth occurred rapidly normally it was reported that the expected height could not be reached due to early closing of the epiphyseal plaques (Vardar et al., 2002; Gümüşel and Kendilci, 2005; Maravelias et al., 2005; Sevin et al., 2005; Özdemir and Gulturk, 2008). The fact that the humeruses of male rats given ME in this study were shorter than the controls is compatible with known data. While the present study comparing the ME, V and C groups determined that humerus length was shorter in males who were given ME than in the V and C groups (p<0.05) and longer in the females receiving ME (p<0.05) (Table 1), a study of testosterone administration to rats in puberty also showed a humerus length shorter than the controls in the male animals and longer in the females (Özdemir and Yalcın, 2010).

Moreover, Xiaodong and Ark (2000) stated that nandrolone administration in 6-weeks-rat caused reduction in the length of humerus in male rats following the control. It is interesting to see that the results agree with the existing data. The report that anabolic steroids are necessary and play a major role in skeletal development and its continuity (Vanderschueren et al., 2004) is interpreted as the reason for the growth in length of the bone. While Lok and Yalcın (2009), in their 4-week study of nandrolone administration to rats report that no relevant differences are seen in the diameter of the femoral corpus in either male or female animals. Once et al. (1997) found in their experiment on β-estrogen receptors, that β-agonists substantially increase the femoral corpus thickness in both males and females. As for Özdemir and Yalcın (2010), while they confirm that testosterone loading causes a reduction of femoral corpus diameter in male rats. The reduction in the femoral shaft thickness of male rats in the present study and its increase in the female animals is thus similar to the reports in the available literature on other AAS and agrees with them. When the effect of gender on corpus humeral thickness is analyzed, ME administration was observed to remove the important difference between male and female controls. Özdemir and Yalcın (2010) took attention to the fact that although testosterone administration has effect on corpus humeral thickness in rats; the difference between male and female control groups does not exist in those which are testosterone-treated.

It has been reported that androgens stimulate bone growth through osteoblastic proliferation, osseous matrix protein production and the increase of growth hormone synthesis, and that during puberty they increase the cortical bone thickness by enhancing both endosteal and periosteal thickening (Shahidi, 2001). Similarly, it was reported in the study by McDougall et al. (2002) on bone trauma in the rat that anabolic support increases cortical bone thickness. In the present study, such an increase was observed in female rats given ME when compared to males in all groups and to controls; these results agree with the available AAS literature in indicating that while ME stimulates bone growth in the female rat, it represses it in the male of the species. Sato et al. (2002) wrote that androgen loading in rats was followed by thickening of the femoral cortex, while Weismann et al. (1993) indicate that cortical bone was not affected in their investigation of testosterone effect on bone in male and female rats. Windahl et al. (1999) also reported absence of change on the cortical bone thickness of male rats that were given testosterone. The absence of a relevant difference among the different groups as to cortical bone thickness in the study shows the absence of such an effect of ME treatment, a result compatible with those of Weismann et al. (1993) and Windahl et al. (1999). The revelation that ME administration in male rats reduces bone cortex density, as opposed to the findings of Weismann and Ark (1993) and Windahl and Ark (1999), has shown that it affects cortex density. Özdemir and Yalcın (2010) also stated that cortex density reduces in testosterone-treated male rats.

Qu et al. (1998) stated that estrogen addition does not have a significant effect on humerus cavum medullare thickness of male rats. In their study of the effect of hormonal differences between male and female rats on bone growth, Kim et al. (2003) observed that growth hormone increases medullary canal diameter in female rats but not the male animals. The present study indicates that the differences among the different treatment groups are insignificant for males; whereas this
agrees that it has increased at a considerable level as a result of controlling in female rats in ME group (p<0.05). This situation is compatible with the existing literature for both male and female rats. When gender difference in changes in humerus cavum medullary canal diameters is taken into consideration, whereas the differences in FY and K groups are significant, the difference in ME group was found insignificant; this fact has been evaluated as follows; ME administration, by having counter effect on bone development in female and male rats, has removed initial gender difference. Sims et al. (2002) on the other hand, indicate that testosterone treatment has no significant effect on the size of the medullary canal. The fact that the measurements in both male and female rats, in FY group, are between ME and control and since they are statistically close to ME group, have implied that arachis oil may have negative effect on humerus bone development. This fact shows that instrumental substances used in drug injections may have either positive or negative effects on researches. Besides, the necessity to further explore the effect of different doses of arachis oil on bone development has emerged.

Conclusion

It can be said that the effect of ME on adolescent rat’s humerus is similar to that of other AAS, repressing bone growth in the male animal and enhancing it in the female. Rat models have been used to explain the middle and long-term negative effects of ME, widely used today for doping, on bone development, especially in males. This action of the substance is opposed to its short-term benefits. Even though the effects of ME on bone growth have been exposed, more work is needed to investigate the short-term or long-term effects on other organs and tissues.

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