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Full Length Research Paper

Morphometric evaluation of the effect of methenolone enanthate on femoral 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 femur 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) dilued in peanut oil, 0.5 ml; all animals received five intraperitonal injections weekly for four weeks. The rats were euthanized at the end of the four weeks and both femurs 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 femur 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. It was concluded that, the ME effect on rat femurs, similar to that of other AAS, consists in supressing bone growth in male animals while enhancing in females during pubertas. 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: AAS, Methenolone enanthate, athletes, rat, bone, 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 McKean, 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 et al., 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

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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; Brower, 1993; Pope and Katz, 1994). Anabolic Androgenic Steroids were reported to cause early cartilagal ossification and short height among males in their pre-pubertal period.

In 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 (Livanelioğlu, 2010). Vanderschueren et al. (2004) reported that androgens cause changes in the length of bone, cortical diameter and bone density of rat femurs. The AASs can yield results that negatively effect growth such as early closure of the growth plaques (in the growth plate in epiphyseal cartilages) of young during adolescent period. It has been reported that these effects could be seen more often among the 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 catabolical 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 of this study is to determine possible morphometric or structural changes of ME, an ASS, preferred much and widely used as a performance amplifier by athletes, on the femur among adolescent rats.

MATERIALS AND METHODS

In the study, 48 Sprague-Dawley rats aged 40 days and weighing 150 to 250 g were used. During the study, the animals were kept in polycarbonate cages, one animal per 250 cm^2 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 were divided into the groups. The treatment group received ME, 5 mg/kg (Özdemir and Yalçin, 2010; Blystone et al., 2007) dilued 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 a week during 4 weeks.

All rats were euthanized with thiopental anesthesia, 40 mg/kg, at the end of the fourth week. Both femurs were dissected during necropsy and macerated. The femurs 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 was 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 femur length and corpus diameters are shown in Table 1; compact bone thicknesses and medulla space diameters are summarized in Table 2. When comparing the values for the ME, peanut oil and control groups shown in Table 1, the differences in femur length were found to be statistically significant ($p<0.05$), indicating that ME represses growth in the male animal while it enhances it in the female when compared to the controls. The analysis of femur corpus thickness showed that, the males in the ME group had a significantly thinner bone when compared to V and C, while the corpus thickness in the female rats was significantly higher in the ME group relative to that of V and C ($p<0.05$ for both comparisons). When gender differences are compared, it has seen that the corpus femoris of both control and peanut oil groups male rats are significantly thicker than those of female rats ($p <0.05$). It has been found that the Methanolone Enanthate administration does not make statistically significant difference on the corpus femoris thickness of male and female rats ($p>0.05$).

The comparison of femoral cortex thickness by groups revealed that, it was significantly thicker in female rats when compared to both V and C ($p<0.05$), while there was no significant difference among the groups for the male animals ($p>0.05$). Comparison for cortical thickness according to gender did not pose any significant results ($p<0.05$). The medulla canal diameter of male rats given ME was perceived as being smaller than the two other groups while it was not statistically so ($p>0.05$), and all three groups had similar values when considering only the females ($p>0.05$).

The femoral medulla canal diameters showed a significant difference between male and female animals

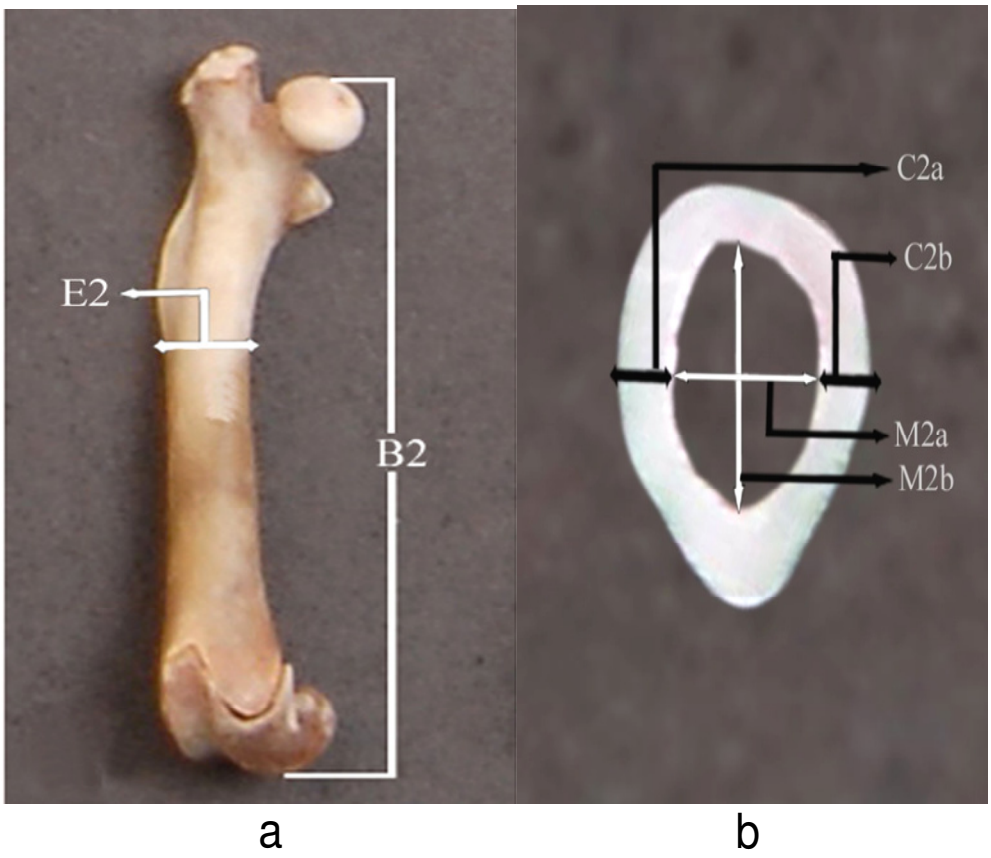

Figure 1. Anterior oriented reference points of the femur (Ozdemir and Yalçin, 2010). (a) (B2) Distance between the extreme points of the head of femur and of the trochlea ossis femoris; (E2) Thickness of the femur shaft on the ventral face of the third trochanter. (b). (C2a+C2b/2) Mean cortical (compact bone) thickness of the femur shaft; (M2a+ M2b/2) mean diameter of the femur shaft medullary canal (Cavum medullare).

Table 1. Femur length and corpus diameter in the ME, V and C groups (Unit: mm, mean±SD).

Group	Femur length		Corpus diameter	
	Male (n:8)	Female (n:8)	Male (n:8)	Female (n:8)
ME	31.46±0.50^{b,B}	32.22±0.30^{a,A}	3.74±0.06^{b,A}	3.75±0.10^{a,A}
V	32.09±0.49^{a,A}	31.12±0.23^{b,A}	3.82±0.05^{a,A}	3.63±0.17^{b,B}
C	32.54±0.38^{a,A}	31.10±0.66^{b,B}	3.87±0.10^{a,A}	3.55±0.11^{b,B}

a,b: Lowercase letters in the same column indicate a statistically significant difference between the numbers marked by *a* vs. those marked *b* ($p<0.05$). A,B: Uppercase *A* versus *B* in the same row indicate a statistically significant difference between the numbers marked by *A* vs. those marked *B* ($p<0.05$).

in the C group but not in the two others ($p>0.05$).

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; Gumuşel and Kandilci, 2005; Ozdemir 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 (Peters et

Table 2. Femur cortex and medullary canal diameter measurements for the ME, V and C groups (Unit: mm, Mean±SD).

Group	Femur cortex		Femoral medulla canal	
	Male (n:8)	Female (n:8)	Male (n:8)	Female (n:8)
ME	$0.80\pm0.06^{a,A}$	$0.82\pm0.03^{a,A}$	$2.08\pm0.11^{a,A}$	$2.06\pm0.10^{a,A}$
V	$0.83\pm0.14^{a,A}$	$0.75\pm0.05^{b,A}$	$2.14\pm0.10^{a,A}$	$2.01\pm0.14^{a,A}$
C	$0.82\pm0.16^{a,A}$	$0.73\pm0.10^{b,A}$	$2.21\pm0.12^{a,A}$	$1.99\pm0.09^{a,B}$

a,b: Lowercase letters in the same column indicate a statistically significant difference between the numbers marked by *a* vs. those marked *b* ($p<0.05$). A,B: Uppercase *A* versus *B* in the same row indicate a statistically significant difference between the numbers marked by *A* vs. those marked *B* ($p<0.05$).

al., 2002; Vardar et al., 2002; Gumuşel and Kandilci, 2005; Maravelias et al., 2005; Sevin et al., 2005; Özdemir and Gültürk, 2008). The fact that the femurs of male rats given ME in this study were shorter than the controls is showing similarity with known data.

While the present study comparing the ME, V and C groups determined that femur 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 femur length shorter than the controls in the male animals and longer in the females (Ozdemir and Yalçin, 2010). 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 Yalçin (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, Onoe 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 Ozdemir and Yalçın (2010), while they confirm that testosterone loading causes a reduction of femoral corpus diameter in male rats they also reported that, this effect is not seen in the female. The reduction in the femoral corpus 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 show similarity with them.

It has been reported that, androgens stimulate bone growth through osteoblastical 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 effects on bone in male and female rats. Windahl et al. (1999) also report 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). Qu et al. (1998) stated that estrogene addition does not have a significant effect on femur cavum medulla thickness of male rats. It was realized that, statistical difference between groups are also insignificant, though, femoral cavum medulla diameters of ME-treated male rats were measured as being smaller than peanut oil and control groups ($p>0.05$). It was thought that this situation is due to the suppression of bone development in male rats by mentioned active substance.

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 in the male animals. The present study indicates that, the differences among the different treatment groups are insignificant both for males and females; this agrees with the reported results for males. The females in the study do not show the difference that would be expected based on the existing reports even though their values were higher than control. This result was evaluated as possibly related to the sensitivity of the measurement methods. Sims et al. (2002) on the other hand, indicated that testosterone treatment has no significant effect on the size of the medulla canal. In this sense, the results seem to agree with existing literature.

CONCLUSION

It can be said that the effect of ME on adolescent rats' femur 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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