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Morphometric Effect of Erythropoietin Used as Doping and Swimming Exercise in Female Rats in Puberty on Humerus and Femur Bones

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Abstract

In this study, the effect of human erythropoietin (rhEPO) with aerobic exercise on the femur and humerus bone of female rats in the pubertal period was investigated by morphometric method. The study was performed on 40 female rats of the Spraque – Dawley genus.

The rats were divided into four groups as erythropoietin, swimming exercise with erythropoietin, swimming and sedentary. For 4 weeks, every other day all rats were injected with rhEPO (100 IU / kg, IP) 4 days a week. After the injection, the swimming group with rhEPO and the solo swimming group were swam for 30 minutes. At the end of 4 weeks, the rats were euthanized, and corpus, height and cortex and cavum medulla measurements of humerus and femur bones were done.

Statistical evaluation also indicated that there were no differences ($p>0.05$) between rhEPO group, swimming group and rhEPO + swimming group femur length and femur cavum medulla da sedentary group. Sedentary group was found to be thicker ($p<0.05$) in femoral Corpus than swimming and rhEPO group, while there was no statistical difference in femoral cortex but a numerical difference was found.

While Humerus bone length and Corpus were not significant in our study ($p>0.05$), although the numerical values of rhEPO + swimming group in Cortex and Cavum medullada were different compared to other groups, no statistical difference was found ($p > 0.05$). As a result, it is thought that long-term use of erythropoietin may cause bone development disorders.

Keywords: Doping, Erythropoietin, Exercise, Morphometry

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1. Introduction

It is well known that blood and bone share a unique, regulatory relationship with one another. But the details of the relationship between these two are still unanswered (McGee et al., 2012). Erythropoietin (EPO) is a hematopoietic growth factor that stimulates the formation of red blood cells. EPO is known as a doping agent in high-performance sports, and especially in cycling. In the clinical setting, this erythropoiesis stimulating agent is used to treat anemia, especially if it is caused by a lack of endogenous EPO production due to chronic kidney failure. In recent years, the non-hematopoietic functions of EPO, also known as pleiotropic functions, have been extensively investigated. Of interest for orthopedics and musculoskeletal tissue engineering, EPO's non-hematopoietic capabilities include osteogenic and angiogenic potencies (Rölfing, 2014).

For EPO's bone-forming role, the tissue may contain a protective hetereceptor (Brines et al., 2004). However, Epo receptors (Epo-R's) have been reported to be outside hematopoietic tissues, suggesting that EPO also plays a role in non-hematopoietic tissues (Mennini et al., 2006). The presence of Epo-R mRNAs has been reported in the brain, testes, placenta, heart, lungs, bone marrow and spleen (Tan et al., 1991; Fandrey & Bunn, 1993; Agnello et al., 2002 ; Brines & Cerami, 2006).

The differential role of EPO on different organs indicates tissue-specific functions of EPO (Haroon, et al., 2003). The finding that EPO has pleiotropic roles in non-hematopoietic tissues has led to research into the role of EPO in bone formation and homeostasis (Li & Li, 2006; Wilson & Trumpp, 2006; Yin & Li, 2006). Therefore, combining hematopoiesis with skeletal homeostasis via Epo signaling makes important sense (Foldes et al., 1989 ; Gazit et al., 1990 ; Bab et al., 1992 ; Bab & Einhorn, 1993 ; Bab, 1995; Greenberg et al., 1995). In their studies, Lee et al., (1991) showed that Epo therapy can increase cortical thickness.

Studies have repeatedly shown the morphological effect of EPO on bone, but the mechanisms that regulate the

process remain unclear. One suggestion is that EPO may play an important role in regenerating newly absorbed bone by stimulating JAK-STAT signaling pathways through Epo-R in HSCs (Shiozawa et al., 2010).

Among studies that observed bone formation in response to EPO, there are several notable major differences between those that did not. The first is the doses used. Supraphysiological doses are used in most studies showing bone formation. Those who used supraphysiological doses often used EPO doses that fell within a normal range. There may also be age-related differences in the animals ' response to EPO. We recorded that the reaction of young animals to EPO was more robust than the reaction in older animals (unpublished observations). Also, there are well-known differences in the hormonal response of different bones. For example, Singbrant et al. He studied the effects of EPO on the proximal tibia (Singbrant et al., 2011).

Recent and ongoing studies show that hematopoietic stimulation improves bone formation in the context of both early ossification and fraction recovery and advances in mechanical strength (Ferguson et al., 1999; Brager et al., 2000; Bozler et al., 2006; Holstein et al., 2007).

The debate about the effects of EPO on bone formation still needs to be resolved. Also, there is much to learn about the molecular mechanisms of EPO and bone formation; is EPO's effects on the skeleton coupled with hematopoiesis or secondary to EPO's effects on hematopoiesis? If there is a direct effect on the skeleton, the HSC or MSC is the target or both, and both activities need to be present simultaneously for bone formation to occur (McGee et al., 2012).text text text text text

2.Material And Method

1- Subject Selection: 40 female Sprague-Dawley type rats from the Selçuk University Experimental Medicine Research and Application Center were used in the study**.**

Rats were housed in polycarbonate cages (Tecniplast, Italy) in a light-dark cycle of 14:10 hours at 21±2 0C, with 1 rat in 250 cm2 area, adlibitum was fed with standard rat feed (Purina, Canada) and water (normal tap water in glass bottles). The study was continued for 4 weeks.

Forming groups: the groups in the study were formed as follows.

1. Group (Drug Trial Group, n=10). RhEPO for four weeks (EPOBEL ® 2000 IU / 0.6 mL LV, Nobel Pharmaceutical Marketing & Industries Ltd. Şti) 100 IU / kg (Nuno et al 2009) dose was applied 4 times per week in peritoneal and 30 min swimming exercise was performed after each drug application.

2. Group (Swimming control group, n=10): rats were fed normally, given peritoneal saline 4 times per week and 30 min swimming exercise after each drug application.

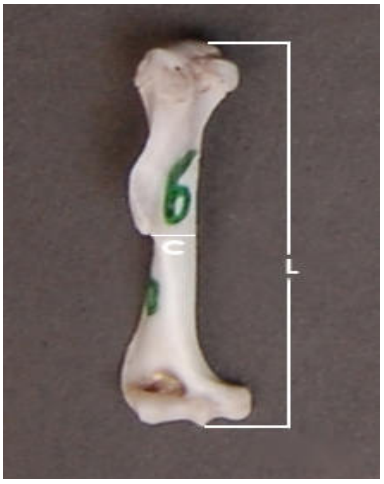
3. Group (Drug Control Group, n=10). RhEPO was administered 4 times peritoneal times per week at a dose of 100 IU/kg for four weeks.

4. Group (healthy control group, n = 10): the rats were fed normally.

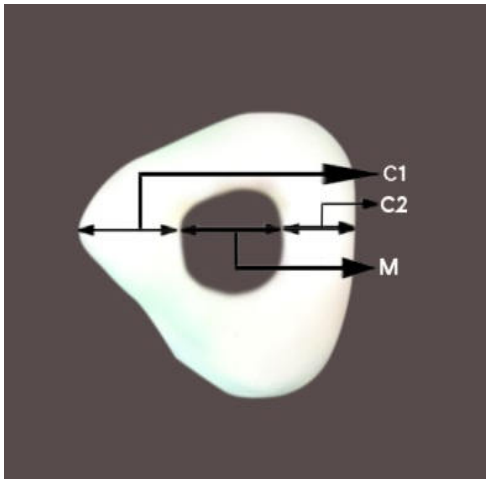
At the end of the fourth week, the rats were euthanized by intraperitoneal injection of 200 mg/kg (Pentotal sodium, Abbott) of pentobarbital. The anterior and posterior extremities of the materials were then uncovered and dissected and subjected to maceration. The uncovered humerus and femur bones were marked and preserved in special plastic bags. Morphometric measurements of height length, Corpus, cortex and cavum medulla of humerus and femur bones, diameter were made from the reference points shown in figures 2.1 and 2.2. Measurements were taken with a stainless hardened digital caliper (China) caliper of 0-100 mm.

The images of the bones were taken with the Nikon DSLR D200. The statistical evaluation of the data was based on the package program SPSS 13.0 (SPSS 13.0 for Windows/ SPSS® Inc, Chicago, USA). The results were presented as mean±Sd. Anova and Duncan test were used to compare the data between groups. $p<0.05$ value was considered statistically significant.

Picture 2.1. Reference points of length (L), Corpus (C), Cortex (C1-C2) and Medullar diameters (M) of humerus

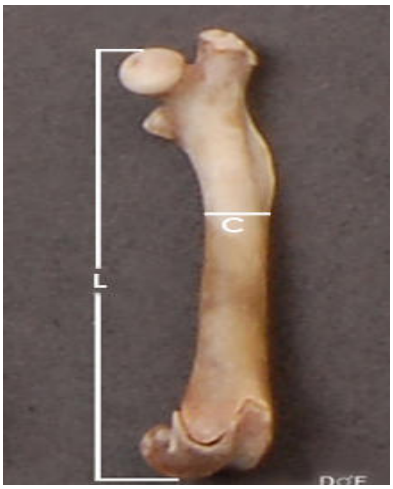


L: distance between endpoints of Caput humer and trochlea humer.C: corpus of humerus thickness (lower border level of Tuberositas deltoidea).

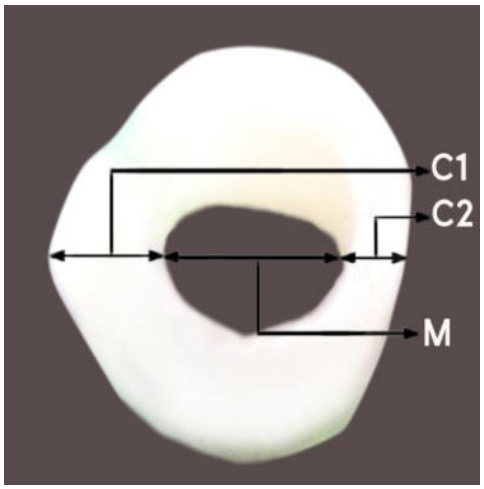


C1 - C2: mean cortex thickness (cortical bonesubstantia) at the corpus level of the humerus compacta).
M: diameter of cavum medullare at the corpus level of humerus.

Pictures 2.2. Reference points of femur length (L), Corpus (C), Cortex (C1-C2) and Medullar diameters (M)



L: distance between caput ossis femoris and endpoints of trochlea ossis femoris.
C: thickness of the femur's corpus (lower bound level of the Trochanter Tertius).



C1-C2: the mean cortex thickness at the corpus level of the femur (cortical bone substantia compacta).
M: diameter of cavum medullare at corpus level of femur.

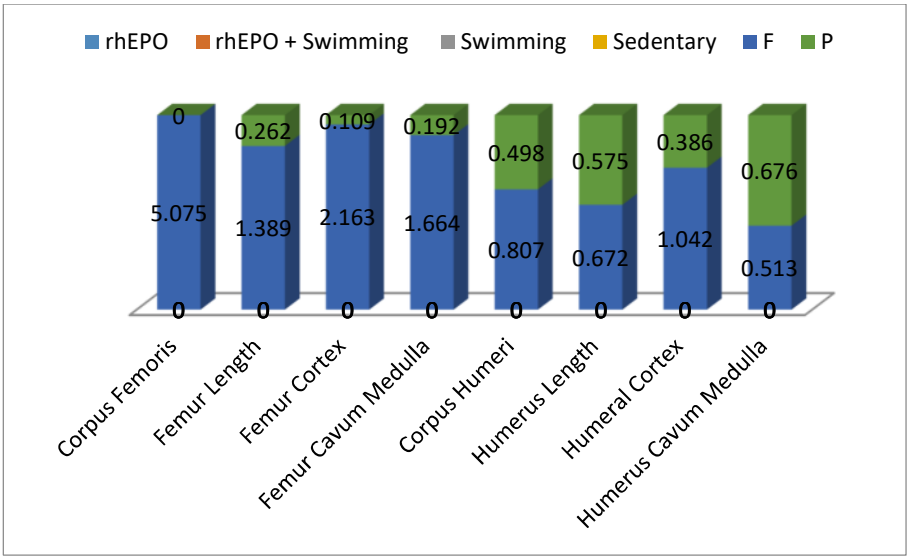
3. Results

Tab. 1.

Morphometric Effect of Erythropoietin Used as Swimming Exercise and Doping on Humerus and Femur Bones in Female Puberty Period (mm) (Mean ± Sd).

	rhEPO	rhEPO + Swimming	Swimming	Sedentary	F	P
Corpus Femoris	3.93 ± .14[b]	4.02 ± .18[ab]	3.90 ± .19[b]	4.16 ± .15[a]	5.075	.005*
Femur Length	30.63 ± .39	31.02 ± .99	30.36 ± 1.03	30.84 ± .39	1.389	.262
Femur Cortex	.83 ± .11	.88 ± .12	.93 ± .14	.95 ± .09	2.163	.109
Femur Cavum Medulla	2.62 ± .21	2.28 ± .21	2.05 ± .38	2.24 ± .18	1.664	.192
Corpus Humeri	2.94 ± .13	2.95 ± .08	2.86 ± .11	2.93 ± .23	.807	.498
Humerus Length	24.17 ± .40	24.32 ± .66	24.02 ± .46	24.27 ± .52	.672	.575
Humeral Cortex	.77 ± .11	.82 ± .09	.75 ± .11	.76 ± .08	1.042	.386
Humerus Cavum Medulla	1.37 ± .26	1.33 ± .18	1.37 ± .20	1.45 ± .24	.513	.676

* P <0.05. a, b, c, There are significant differences between values carrying different letters in the same line (p <0.05)



Grafic1. Morphometric effect of erythropoietin on humerus and Femur Bones (mm) (Mean±Sd) used as swimming exercise and Doping in female rats during Puberta period.

it is stated that there are no differences (p>0.05) between rhEPO group, swimming group and rhEPO + swimming group femur length, femur cavum medulla da sedentary group. Sedentary group was found to be thicker (p<0.05) in femoral Corpus than swimming and rhEPO Grun, but no statistical difference was found in femoral cortex.

While Humerus bone length and Corpus were not significant in our study (p>0.05), although the numerical values of rhEPO + swimming group in Cortex and Cavum medullada were different compared to other

groups, no statistical difference was found ($p > 0.05$).

4.Discussion

The bone that forms the basis of skeletal mobility is a dynamic organ that plays a variety of roles in the body (Anjos-Afonso & Bonnet, 2007). EPO bone creation may also include a hetereceptor that protects tissue (Brines, et al., 2004). EPO supplementation may increase cortical thickness in bone (Lee et al., 1991). EPO can improve bone formation (Ferguson et al., 1999; Brager et al., 2000; Bozler et al., 2006; Holstein et al., 2007). In their study, Sakunya et al., (2019) states that EPO therapy makes thickening in the corpus by regulating low bone mass in long bones. In our study, it was determined that the sedentary group was thicker ($p<0.05$) in femoral Corpus than in swimming and rhEPO group, while there was no statistical difference in femoral cortex but a numerical difference was found. Özdemir, (2013) reports that the widest corpus femoris ($p<0.05$) measurement was determined in the sedentary group.

In another study, Röfling, (2014) in his study on bone fracture recovery in mice, states that EPO increases bone volume and decreases bone marrow cavity. After methanolone enanthate application to adolescent rats, Bozkurt et al., (2011) found that there was a significant ($p<0.05$) thinning of the corpus femorist while finding that there was a significant ($p < 0.05$) shortening of the femur length.

In our study, the length and Corpus of humerus bone were not significant ($p>0.05$), although the numerical values of rhEPO + swimming group in Cortex and Cavum medullada were different compared to other groups, no statistical difference was found ($p>0.05$). In their study, Lavoie et al., (1998) examined the effect of anaerobic exercise on rat metabolism by using rhEPO supplementation and found that rhEPO contributes to bone tissue in a low proportion. Mohammadian et al., (2003) noted that the increase in iron load made growth retardation in bone particularly apparent in the age of puberta. Similarly, Low., (1997) stated that it inhibits growth in bone in his study looking at growth functions in beta-thalassemia patients.

Low bone mineral density in some elite athletes suggests that intense exercise may have negative effects (Hatun, 2000). There are studies that show that high intensity exercises performed before and during puberta have positive or negative effects on growth and skeletal development. Studies on this subject show that girls with rhythmic gymnastics are shorter and weaker than their peers in other sports and non-sports (Benardot & Czerwinski 1991, Damsgaard et al., 2000). Akin et al., (2004) reported that in their study with rhythmic gymnasts before puberta, intense exercise is shortening in the neck of gymnasts and thinning in bone thickness.

5.Conclusion

As a result, while rhEPO, commonly used as a doping agent in recent years, not only improves performance but provides positive effects in bone forty repairs in the long term, its negative effects on healthy bone tissue have been tried to be explained by rat models. Although the effects of rhEPO and intense exercise on bone development have not been demonstrated, further studies are needed to reveal their effects on other organs.

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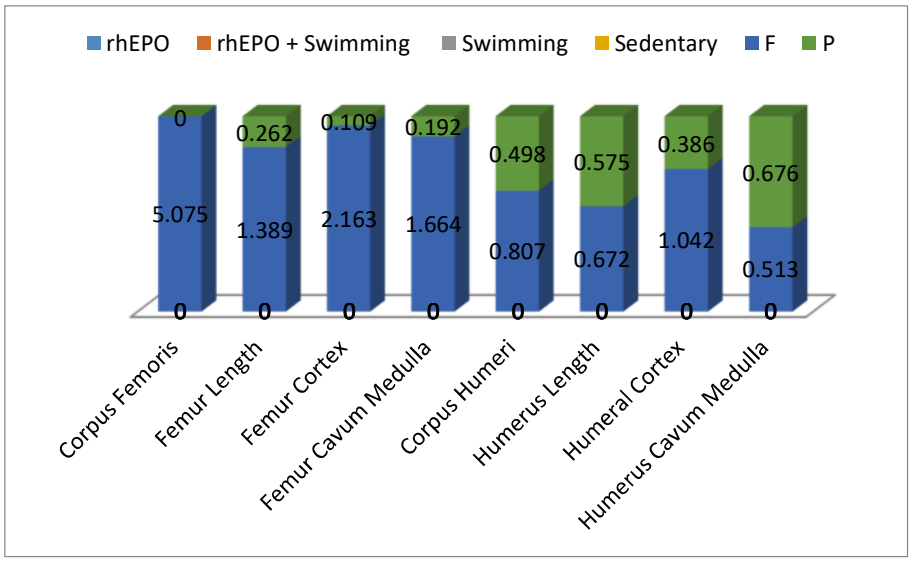

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