Alterations in Bones of Prepubertal Rats with Testicular Torsion
Testiküler Torsiyonlu Prepubertal Ratlarda Kemik Değişimleri

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Summary
Objective: Testicular torsion is one of the leading causes of hypogonadism among children. We aimed to evaluate the effects of testicular torsion in prepubertal rats using densitometry and a histopathological study in adulthood.
Materials and Methods: Thirty-two prepubertal male Wistar albino rats were randomly divided into four equal groups. The rats in the SHAM group underwent scrotal incision only. In the torsion group rats, both testes were torsioned 720° in a clockwise direction. In the torsion/detorsion group, the testes were detorsioned after four days of torsion. The torsion/orchidectomy rats underwent orchidectomy on the fourth day subsequent to bilateral torsion. Three months after surgery, bone mineral density (BMD) and content (BMC) were measured by DXA and bone cortex thickness (BCT) measurements were performed histopathologically.
Results: The lowest values of BMD and BMC were in rats with torsion (mean 0.075 g/cm^2 and 0.290 g), and highest in the SHAM group (mean 0.142 g/cm^2 and 0.488 g). The SHAM group had a mean of 388.52 μm BCT, whereas the torsion group had a mean of only 220.16 μm ($p<0.05$).
Conclusion: Bilateral testicular torsion in prepubertal rats may lead to bone loss in adulthood. Detorsion may prevent the negative effects of torsion on bones in rats. *Turk J Phys Med Rehab 2008;54:1-3.*
Key Words: Bone mineral density, rat, osteoporosis, testicular torsion

Özet
Amaç: Testiküler torsiyon çocuklarda hipogonadizmin nedenlerinden birisidir. Bu çalışmada, prepubertal testis torsiyonunun erişkinlikte kemikler üzerindeki etkisini dansitometre ve histopatolojik yöntemleri kullanarak değerlendirmeyi amaçladık.
Gereç ve Yöntem: Otuz-iki prepubertal erkek Wistar albino rat randomize olarak dört eşit gruba ayrıldı. SHAM grubuna yalnızca skrotal insizyon uygulandı. Torsiyon grubunda her iki testis saat yönünde 720° torsiyone edildi. Torsiyon/detorsiyon grubunda testisler torsiyondan dört gün sonra detorsiyone edildi. Torsiyon/orşiektomi grubunda bilateral torsiyondan dört gün sonra orşiektomi uygulandı. Cerrahiden üç ay sonra, kemik mineral yoğunluğu (KMY) ve kemik mineral içeriği (KMİ) DXA ile, kemik korteks kalınlığı (KKK) da histopatolojik olarak değerlendirildi.
Bulgular: En düşük KMY ve KMİ değerleri torsiyon grubunda (ortalama 0,075 g/cm^2 and 0,290 g), en yüksek ise SHAM grubunda (ortalama 0,142 g/cm^2 and 0,488 g) bulundu. KKK, SHAM grubunda ortalama 388,52 μm, torsiyon grubunda ise ortalama 220,16 μm bulundu ($p<0,05$).
Sonuç: Prepubertal ratlarda bilateral testiküler torsiyon, erişkin dönemde kemik kaybına yol açabilir. Ratlarda tedavide detorsiyon uygulanması, torsiyonun kemik üzerindeki olumsuz etkilerini azaltabilir. *Türk Fiz Tıp Rehab Derg 2008;54:1-3.*
Anahtar Kelimeler: Kemik mineral yoğunluğu, rat, osteoporoz, testiküler torsiyon

Introduction

Osteoporosis (OP) is a disease characterized by low bone mass and deterioration of microarchitecture of bone tissue, which leads to increased bone fragility with an increase in fracture risk (1). Although OP occurs more commonly in women, it affects both men and women over the age of 75 (1, 2). Estrogen deficiency plays a major role in bone loss in postmenaupausal women (1). Loss of gonadal function results in metabolic changes which are associated with a reduction in bone mass by an unknown mechanism (3). In spite of the unknown etiology and mechanism, sex hormones are recognized as important factors in the maintenance of bone mass and architecture both in men and women (13). Clinical and animal studies have demonstrated that hypogonadism is associated with OP and fracture in men (48). It is firmly established that androgen withdrawal induced by orchidectomy (ORX) results in decreased bone mass in experimental studies (7, 9, 10).

Testicular torsion is a common genitourinary tract emergency in childhood (11). Twisting or torsion of a testis causes occlusion of the gonadal blood supply and may lead to necrosis and hypogonadism (11, 12). Since the experimental model of hypogonadism in adult rats has demonstrated that it causes loss of bone mass, ORX is used as

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a model of OP in rats (10). However, there has been no investigation concerning the effects of testicular torsion on the development of OP up to the present. An experimental model of testicular torsion has been described previously. According to this model, at least four hours of 720° unilateral testicular torsion causes sufficient testicular tissue injury in both ipsilateral and contralateral testes in rats (13). No study evaluating the effects of experimental torsion using this model has yet been reported in the literature. Furthermore, the testicular torsion in prepubertal rats has not been investigated for the influence on the bones of adult rats either.

In the current study, we aimed to evaluate the effects of testicular torsion of prepubertal rats on the bone mass in adult rats using Dual energy XRay absorptiometry (DXA), which is a method measuring bone mineral content (BMC) and bone mineral density (BMD) in the bones (14). We measured the BMD and BMC of young adult rats with testicular torsion, as well as bone cortex thickness (BCT) of the vertebrae by histopathology, to determine the loss of bone.

Materials and Methods

Study design

This study was carried out at the Ondokuz Mayis University Medical and Surgical Research Center, Samsun, Turkey. Thirtytwo healthy male Wistar albino rats three months of age weighting approximately 150 g were used in this investigator blinded controlled experimental study. The study was performed with the approval of the animal research committee. All procedures were performed by the same surgeon (UB). The animals were housed separately in plastic cages and kept in a temperaturecontrolled room with a standard 12:12 h lightdark illumination cycle. All rats were fed ad libitum with free access to water and rat cow. All rats were prepubertal and they were divided into four equal groups of eight rats.

Surgical technique

Each rat was anesthized by intraperitoneal 5% ketamine hydrochloride (Warner Lambert, Pfizer Inc., Istanbul, Turkey) injection (50 mg/kg) and then rightsided midscrotal vertical incisions were made. The testes were pulled out of the incision. A 720° torsion and/or orchiectomy were performed.

Group 1: SHAM group (n= 8), rats underwent scrotal incision only.

Group 2: Torsion group (n= 8), both testes were twisted 720° in a clockwise direction and fixed to the scrotum.

Group 3: Torsion/detorsion group (n= 8), initially, both testes were twisted 720° in a clockwise direction and detorsion was performed after 4 days of torsion.

Group 4: Torsion/ORX group (n= 8), initially, both testes were twisted 720° in a clockwise direction and both were removed after four days of torsion.

Three months after surgery, in the young adulthood of the rats, DXA protocols were performed on the vertebral column by BMD and BMC measurements.

Bone status measurement

In all rats, the BMD and BMC were calculated using a rectangular region of interest drawn over the midportion of the lumbar vertebral column by DXA, performed using a pencil beam Xray and Samarium KEdge filtering with two energy peaks at 46.8 and 80 keV. A regular DXA instrument (Norland, USA) was used in high resolution mode. During the measurements, the animals were anesthetized with an intraperitoneal administration of 5% ketamine hydrochloride (Warner Lambert, Pfizer Inc., Istanbul, Turkey) at a dose of 50 mg/kg and were supine, with hindlimbs maintained in external rotation with tape. The hip, knee, and ankle articulations were in 90°, 0°, and 90° flexion, respectively.

Histopathology

Thereafter, all rats were killed and bone cortex thickness measurements of L2L4 segments were performed by histopathological investigation. Bone specimens were extracted from the rats and fixed in buffered neutral formaline solution for 24 hours. After fixation, specimens were decalcified in 10% formic acid (GoodingSteward) for 72 hours. After decalcification, sections were taken longitudinally and transversally from bone. All the tissues were processed overnight (Shandon Citadel 2000, UK). Tissues were embedded in paraffin and sectioned at a thickness of 46 μm (Leica RM 2155 Rotary Microtom, Germany). The sections were stained with hematoxylin and eosin (Leica ST4040, Germany). Three randomly selected areas were measured for bone cortex thickness in Samba, the image analysis system, Morphometry software (Samba Technologies, France).

Statistical Analysis

Analysis of data was performed using computer software SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA). The study was designed with a target number of 8 rats per group for statistical analyses. All data is presented as the mean and standard deviation (Mean±SD) in tables. The normality of the variables was evaluated by the KolmogorovSmirnov test. A one way ANOVA was done for the comparison of mean and standard deviations of BMC, BMD and BCT. The Mann Whitney U test was used for the comparison between measurements according to all groups, when significance was found on ANOVA. The level of significance was set at $p<0.05$.

Results

BMD and BMC measurements

A summary of BMD (g/cm^2, Mean±SD) and BMC (g, Mean±SD) measurements of the groups are shown in Table 1. We found a decrease of BMD and BMC measurements in rats with torsion. The reduction of these measurements in rats with torsion/detorsion was significantly less. The amount of decrease in rats with torsion/ORX was between these two groups. Although there was an obvious reduction of BMD and BMC, we did not detect any significance between the groups ($p>0.05$).

Histopathologic analysis

Table 2 shows the Mean±SD of BCT in experimental groups. We found the best results in SHAM operated animals, while rats with torsion only had the thinnest mean cortex thickness. Removal of both testes after torsion led to greater bone thickness results. Detorsion after torsion also results in thicker bone cortex measurements than other torsioned animals. In spite of all these

Table 1. Mean values of Bone Mineral Density (BMD) and Bone Mineral Content (BMC) of the vertebrae.

Group	BMD (g/cm^2) Mean±SD	BMC (g) Mean±SD
Group 1 (SHAM)	0.142±0.03	0.488±0.34
Group 2 (Torsion)	0.075±0.02	0.290±0.09
Group 3 (Torsion/Detorsion)	0.124±0.22	0.516±0.06
Group 4 (Torsion/ORX)	0.108±0.02	0.331±0.06
ORX: Orchidectomy		

Table 2. Mean values of cortex thickness of bones of the vertebrae and comparison of the groups.

Group	Cortex Thickness (μm) Mean±SD
Group 1 (SHAM)	388.52±40.21*
Group 2 (Torsion)	220.16±29.21*
Group 3 (Torsion/Detorsion)	334.82±28.72
Group 4 (Torsion/ORX)	328.37±36.18
*Statistically significant difference between Group 1 and Group 2 ($p<0.05$) ORX: Orchidectomy	

results, we detected a statistically significant difference only between the sham operated rats (Group 1) and torsion (Group 2) rats ($p<0.05$). The differences between other groups were not significant statistically ($p>0.05$).

Discussion

Osteoporosis has become a public health problem of epidemic proportions, affecting an estimated 75 million people in the US, including one out of three postmenopausal women and a majority of the elderly, including men (2). In women, the clinical features, treatment and mechanism of postmenopausal OP are well known to be related to estrogen deficiency (1, 2). In contrast to women, OP in males has received little attention (15), although, a third of hip fractures occur in men and there are well documented negative effects of aging and hypogonadism on the adult male skeleton (16, 17). One of the important reasons may be that hypogonadism is less common in men. Spermatogenesis prevents the depletion of stock germ cells. Although there are some exceptions such as hormone ablation in prostate cancer, sudden androgen deficiency is rare.

It is still not clear how androgen deficiency causes OP. It has been shown that osteoblasts from male and female subjects have androgen receptors, and androgens can stimulate proliferation of osteoblastic cells. The loss of bone mass in androgen deficiency may be caused by impairment of bone formation (9, 15, 17). The animal studies have shown that hypogonadism in males results in osteopenia (7, 9, 10). Male rats gonadectomized as young adults (age 46 months) soon become relatively as osteopenic as female rats (18). Hypogonadism may stimulate OP in male ORX rats (10). However, we still do not know how the loss and/or torsion of gonads of prepubertal rats affects the bones in adulthood.

Testicular torsion is an important cause of hypogonadism during the childhood period (11). Up to the present, there has been no investigation regarding the effects of prepubertal testicular torsion on the development of OP, BMC and BCT beyond the childhood period. Therefore, we conducted a study involving prepubertal torsion and postpubertal measurement of osteopenia parameters of bone in rats. A common experimental model of testicular torsion was used in the current study (19).

In our study we used DXA, a noninvasive method, and considered it the gold standard for bone density and mass measurement (1,2). DXA was used to determine BMD, BMC of tibia and lumbar vertebrae in osteoporotic rats (8,14,20,21). According to the DXA results, we have shown an obvious decrease of BMC and BMD. However, this reduction of BMD and BMC was either insignificant or not severe.

The rats with torsion had more loss of bone mass compared to rats with torsion/detorsion and torsion/ORX. Interestingly, all of the parameters of the torsion/ORX group have better values than the torsion only group. We speculate that the prolonged presence of the necrotic gonad in the scrotum may have a negative influence on the bone mass. This relationship may be due to an unknown immunologic (i.e. AbAg reactions), hormonal or metabolic mechanism. It seems that detorsion subsequent to torsion may prevent loss of bone mineral content and thinning of bone cortex.

We found a thinning of the bone cortex after three months of torsion. Erben et al. (9) showed that androgen deficiency may lead to loss of cancellous bone in the axial and appendicular skeleton of aged male rats, and osteopenia increases the bone turnover. The rats may have been too young to have had osteoporotic alterations at that time. Therefore, the limitations of the current study are a relative small number of rats in each group and a relative short latent period between the onset of torsion, induced hypogonadism and measurement of bone parameters. Furthermore, we did not detect hormonal alterations either.

In conclusion, prepubertal rats with torsion had a marked decrease of BMD and BMC in the young adult period. Furthermore, the bone thickness was significantly lowered when compared to the shamoperated control rats. Detorsion may prevent the negative effects of the torsion on bones to some extent. Orchidectomy after torsion also gives slightly better results when compared to torsion only. It is clear that testicular torsion in childhood has various negative effects on bones in adult life. Although the current study is one of the first experimental trials focused on male osteoporosis induced by a childhood event, controlled studies evaluating the impact of childhood testicular torsion on this important public health problem in adulthood should also be carried out in human beings.

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