Are Bioactive and Free Sex Steroids Associated with Bone Mineral Density and Bone Turnover Markers in Middle Aged Men?

Orta Yaşlı Erkeklerde "Biyoaktif ve Serbest Seks Steroidleri" Kemik Mineral Yoğunluğu ve Kemik Döngüsü Belirteçleri ile İlişkili mi?

Melek Sezgin, Burak Çimen*, Handan Çamdeviren Ankaralı**, İsmet As, Neslihan Erçetin*, Özlem Bölgen Çimen, Günşah Şahin

Department of Physical Medicine and Rehabilitation, Mersin University, Mersin
**Department of Biochemistry, Mersin University, Mersin*
***Department of Biostatistics, Karaelmas University, Zonguldak, Turkey*

Summary

Aim: To investigate whether bioactive and free sex steroids are associated with bone mineral density (BMD) and bone turnover markers in middle aged men.
Material and Methods: One hundred and fifteen out of 165 volunteers aged 35-65 years presenting to our outpatient clinic were included in the study. Serum albumin, total testosterone (T), total estradiol (E2), SHBG, osteocalcin (OC) and C-terminal telopeptide (CTx) levels were measured. Free and bioactive sex steroids, free androgen index (FAI) and free estrogen index (FEI) were calculated. BMD in the lumbar spine and the hip was measured in all participants and effects of sex steroids on BMD and bone turnover markers were investigated.
Results: The mean age and the mean body mass index (BMI) in all participants were 52.4±7.8 years and 26.1±3.4 kg/m^2 respectively. There was no significant difference in sex hormone levels and bone turnover markers between the individuals with osteoporosis and osteopenia and the individuals with normal BMD (p>0.05). There was a significant relation between age and FAI (r=-0.23, p=0.01), but there was no significant relation between age and bioactive and free sex steroids, FEI and SHBG. However, there was a positive correlation between BMI and bioactive E2 (r=0.35, p:0001), free E2 (r=0.29, p:0.002) and FEI (r=0.39, p=0.0001). After an adjustment for variables effective on BMD was made; no relation was found between BMD measures from the lumbar spine and the hip and serum bioactive sex steroids, free sex steroids, FAI, FEI and SHBG (p>0.05). However, there was a weak positive relation between serum bioactive T, FEI and OC, CTx levels (p=0.05).
Conclusion: We think that bioactive and free sex steroids are not independent variables effective on BMD in the spine and the hip in middle aged men and that further studies are needed to elucidate the pathophysiology of idiopathic male osteoporosis. *(From the World of Osteoporosis 2009;15:59-65)*
Key words: Male osteoporosis, bioactive sex steroids, free sex steroids, bone mineral density, bone turnover markers

Özet

Amaç: Orta yaşlı erkeklerde biyoaktif ve serbest seks steroidlerinin kemik mineral yoğunluğu (KMY) ve kemik döngüsü belirteçleri ile ilişkili olup olmadığını araştırmak.
Gereç ve Yöntemler: Çalışmaya polikliniğimize başvuran, 35 ile 65 yaşları arasındaki 165 gönüllü arasından 115 erkek alındı. Serum albumin, total testosteron (T), total östradiol (E2), SHBG, osteokalsin (OC), C-terminal telopeptid (CTx) seviyeleri ölçüldü. Serbest ve biyoaktif seks steroidleri, serbest androjen indeksi (SAİ) ve serbest östrojen indeksi (SÖİ) hesaplandı. Çalışma grubunun bel ve kalçalarından KMY'u ölçüldükten sonra seks steroidlerinin, KMY ve kemik döngüsü belirteçleri üzerine etkisi araştırıldı.
Bulgular: Çalışma grubunun yaş ve vucüt kitle indekslerinin ortalamaları sırasıyla 52,4±7,8 yıl ve 26,1±3,4 kg/m^2'di. Osteoporozlu ve osteopenik bireylerin hem seks hormonu düzeyleri hem de kemik döngüsü belirteçleri KMY normal olan bireylerden farklı değildi (p>0,05). Yaş ile, SAİ hariç (r=-0,23, p=0,01), biyoaktif ve serbest seks steroidleri, SÖİ ve SHBG arasında ilişki yoktu. Ancak VKİ ile biyoaktif E2 (r=0,35, p=0001), serbest E2 (r=0,29, p=0,002) ve SÖİ (r=0,39, p=0,0001) arasında pozitif korelasyon vardı. KMY üzerine

Address for Correspondence/Yazışma Adresi: Melek Sezgin MD, Department of Physical Medicine and Rehabilitation, Mersin University, Mersin, Turkey
Phone: +90 324 337 43 00/1113-1604 Gsm: +90 542 528 78 00 E-mail: msezgin@mersin.edu.tr **Received/Geliş Tarihi** 27-03.2009 **Accepted/Kabul Tarihi:** 22.06.2009

etkili değişkenler için düzeltme yapıldıktan sonra, serum biyoaktif seks steroidleri, serbest seks steroidleri, SAİ, SÖİ ve SHBG seviyelerinin ne bel ne de kalça KMY ölçümleri ile ilişkisi tesbit edilmedi (p>0,05). Ancak serum biyoaktif T, SÖİ ile OC ve CTx seviyeleri arasında sınırda ilişki vardı (p=0,05).
Sonuç: Biz, orta yaşlı erkeklerde biyoaktif ve serbest seks steroidlerinin bel ve kalça KMY'na etkili bağımsız değişkenler olmadıkları ve idiopatik erkek osteoporozunun patofizyolojisini açıklayacak yeni çalışmalara ihtiyaç olduğu kanısındayız. *(Osteoporoz Dünyasından 2009;15:59-65)*
Anahtar kelimeler: Erkek osteoporozu, biyoaktif seks steroidleri, serbest seks steroidleri, kemik mineral yoğunluğu, kemik döngüsü belirteçleri

Introduction

Osteoporosis is a disease characterized by low bone mineral density (BMD) and increased risk of fracture (1). Male osteoporosis has been the subject of growing interest over the past few years on account of its frequency and cost. In the last few years, there have been several studies suggesting that about 30% of all hip fractures occur in men and that the incidence of vertebral fractures in men is approximately one-half of that in women (2-4). Moreover, the mortality rate after hip fracture is even higher in men than in women (5) and is estimated at 10% to 14% (6).

A major cause or etiological factor of male osteoporosis has been evidenced in only 30% to 50% of the cases. In the remainder of the patients, the etiology is unclear and is termed "primary" or "idiopathic osteoporosis" (7). In recent years several factors, particularly insulin-like growth factor-1 (IGF-1) and sex hormones have been claimed to play important roles in the pathogenesis of idiopathic bone loss in men. In humans, studies have showed age-related declines in circulating IGF-1 as well as IGF-1 stored in the cortical and trabecular bone, and significant correlations between serum IGF-1 levels and BMD in men with idiopathic osteoporosis (8-13). However, other studies have showed no correlation between IGF-1 and BMD after adjusting for potential confounding variables (14,15). Likewise, in our previous study, we found a significant relation between serum IGF-1 levels and BMD only in the lumbar spine after adjusting for potential confounding variables in a Turkish male population (16).

Sex steroids play an important role in the skeletal growth and maintenance both in females and in males. Estrogen (E) deficiency is a major risk factor for postmenopausal osteoporosis in women (17,18). Despite the fact that men do not have the equivalent of menopause and that serum total testosterone (T) and total E levels decline only marginally with age, there are substantial age-related bone losses in men (19,20). The previous studies assessing the relationship of serum total and/or free T and E levels with BMD have generally revealed conflicting results (21-25). Thus, it has been difficult to attribute bone loss in aging men to either T or/and E deficiency. However, measurements of total T or total E levels do not accurately reflect the actual levels of these steroids available to tissues because the fraction bound to sex hormone-binding globulin (SHBG) does not freely pass to target tissues. Besides, free T and E levels constitute only 1-3% of the total sex steroids and the proportions available to target tissues are underestimated (26,27).

Bioavailable sex steroids comprise the fractions that are free or bound to albumin in the circulation, and in contrast to the fraction bound to SHBG, it is these fractions that have rapid access to target tissues (26,27). Ferrini et al. were the first to show that bioavailable T and E levels decline significantly principally in response to marked age-related increases in serum SHBG levels in men (28). Thereafter, Khosla et al. found that bioavailable T and E levels were positively correlated with BMD and negatively correlated with bone resorption marker in men (29).

To our knowledge, there have not been any studies on the effects of bioavailable sex steroids on BMD in Turkish males. For this reason, we attempted to determine the roles of bioavailable and free sex steroids in BMD at various skeletal sites in middle aged Turkish men. In addition, we investigated the relationship between sex steroids and bone turnover markers.

Material and Methods

Study Population

The study included 115 men aged 35 years to 65 years and selected from 165 volunteers referred to the outpatient clinic of Physical Therapy and Rehabilitation at Mersin University Hospital. Data were collected with a questionnaire composed of questions on smoking habits, alcohol consumption, exercise habits, dietary calcium intake, history of chronic diseases and previous and present medication.

Exclusion criteria were consumption of medications affecting the bone metabolism and hypothalamic-pituitary axis, history of systemic diseases (endocrine, renal, hepatic, gastrointestinal and rheumatic) and presence of hypogonadism (loss of libido and erectile dysfunction and/or decreased total testosterone levels and increased gonadotropin levels).

Weight was measured with a beam balance and height with a stadiometer in all subjects. Body mass index (BMI) was calculated as in the following: weight (kilograms)/height^2 (meters^2).

The study was conducted in accordance with the Declaration of Helsinki. All subjects gave informed consent to participate in the study.

BMD and Biochemical Measurements

BMD was measured at the lumbar vertebrae (L2-4) and the hip (the femoral neck, Ward's triangle and trochanter) with dual energy x-ray absorptiometry and a Norland XR-46 scanner (Ford Atkinson, WI. USA). The coefficient of long-term variations in BMD measurements with the scanner is 2% at the lumbar spine and 2.4% at the neck of the femur.

After an overnight fast, blood samples were taken at 8:00-10:00 am and a 24-hour urine collection was completed. As soon as serum samples were obtained, serum total E_2 (analytic sensitivity: 0.5 ng/ml, within-run precision CV:3.3%), total T (analytic sensitivity: 0.02 ng/ml, within-run precision CV:2.7%), follicle-stimulating hormone (FSH) (analytic sensitivity: 0.1mIU/ml, within-run precision CV:2.6%) and luteinizing hormone (LH) (analytic sensitivity: 0.1 mIU/ml, within-run precision CV:1.2%) levels were determined with electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostic, Manheim, Germany). SHBG (analytic sensitivity: 0.2 nmol/L, within-run precision CV:6.9%) levels were determined with solid phase chemiluminescent immunometric assay (Siemens, Immulite 1000).

As for the bone turnover markers, intact osteocalcin (OC), indicator of bone formation, and serum levels of C-terminal telopeptide of type I collagen (CTx), indicator of bone resorption, were measured. Serum levels of OC (analytic sensitivity: 0.5 ng/ml, within-run precision CV:0.7%) and CTx (analytic sensitivity: 0.01 ng/ml, within-run precision CV:5.5%) were determined with electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostic, Manheim, Germany).

The bioavailable (non-SHBG-bound) fraction of testosterone and estradiol and the free fraction (non-SHBG and non-albumin-bound) of testosterone were calculated in accordance with the method described by Södergard et al. and Vermeulen et al. (26,30). The free androgen index (FAI) was calculated as in the following: FAI=total testosterone/SHBGx100. Likewise, the free estrogen index was calculated as in the following: FEI= total estradiol/SHBGx100.

Statistical Analyses

Statistical Package program for the Social Sciences (SPSS) version 11.5 for Windows was used for statistical analyses. $p<0.05$ was considered significant. Descriptive statistics were expressed in mean±standard deviation (SD) and frequencies (counts and percentages). Kolmogorov-Smirnov test was used for normality test of continuous variables. Univariate analyses were used for simple comparisons of all variables between the osteoporotic, osteopenic and normal subjects. Pearson correlation and simple variance analyses were used to determine the relations between demographic features and BMD measures and sex hormones. Multiple linear regression model which included age and BMI as covariate variables were used to evaluate effects of sex hormones on BMD measures and bone turnover markers. Thus, the effects of age and BMI were eliminated. This model was also used to evaluate effects of bone turnover markers on BMD measures.

Results

Of 165 volunteering men, fifty volunteers did not meet the study criteria and were excluded from the study after physical and laboratory examinations. The mean age and BMI of the subjects included in the study were 52.4±7.8 years and 26.1±3.4 kg/m^2 respectively. The measurements of sex steroids, bone turnover markers and BMD of the study population are shown in Table 1.

Twenty-two subjects (19.2%) were found to have BMD T scores of ≤-2.5 SD (osteoporosis) in at least one skeletal site tested. Fifty-one subjects (44.3%) were found to have BMD T scores of <-1SD or >-2.5 SD (osteopenia), while the rest had bone density T scores of ≥-1 SD (normal). The definition of low BMD used in this study was based on the WHO diagnostic criteria (31). There was no significant difference in serum levels of sex hormones and bone turnover markers between the subjects with osteoporosis or osteopenia and the subjects with normal BMD ($p>0.05$, Table 2, Figure 1 a and b).

While age had significantly negative effects on femoral neck and Ward's triangle BMD ($r=-0.26$, $p=0.007$ and $r=-0.29$, $p:0.004$ respectively), BMI had significantly positive effects on BMD of the lumbar spine ($r=0.32$, $p=0.002$), the femoral neck ($r=0.29$, $p=0.004$), the trochanter ($r=0.35$, $p=0.001$), and the Ward's triangle ($r=0.21$, $p=0.03$). Age was positively correlated with FSH ($r=0.27$, $p=0.003$), LH ($r=0.33$, $p=0.0001$) and total E_2 ($r=0.21$, $p=0.02$), but negatively correlated with FAI ($r=-0.23$, $p=0.01$). There was no significant relation between age and SHBG, bioavailable T, free T, total T, bioavailable E_2, free E_2, FEI ($p>0.05$, Figure 2 a and b). BMI was negatively correlated with total T ($r=-0.30$, $p=0.001$) and SHBG ($r=-0.21$, $p=0.02$), but positively correlated with bioavailable E_2 ($r=0.35$, $p=0.0001$), free E_2 ($r=0.29$, $p=0.002$), FEI ($r=0.39$, $p=0.0001$) and total E_2 ($r=0.18$, $p=0.04$, Table 3).

Table 1. The measures of sex steroids, bone turnover markers and BMD of the study population

	mean±SD	min-max
Total T (nmol/ L)	16.1±5.0	7.5-29.2
Total E_2 (pmol/ L)	94.9±25.3	38.8-158.0
FreeT (nmol/ L)	0.29±0.25	0.08-2.7
FreeE_2 (pmol/ L)	1.45±0.47	0.49-2.99
BioT (nmol/ L)	6.7±2.1	2.26-12.9
BioE_2 (pmol/ L)	59.7±18.6	20.0-117.0
FAI (nmol/nmol)	39.5±13.6	8.9-94.6
FEI (pmol/nmol)	245.3±114.2	38.9-594.0
SHBG (nmol/ L)	48.0±32.9	16.2-258.0
OC (ng/mL)	24.5±7.7	9.9- 52.1
CTx (ng/mL)	0.41±0.22	0.08-1.19
LSTS	-1.25±1.3	-3.80-2.02
FNTS	-0.7±0.9	-2.70-2.17
LSBMD values (g/cm^2)	0.966±0.16	0.663-1.362
FNBMD values (g/cm^2)	0.859±0.14	0.364-1.242
TBMD values (g/cm^2)	0.732±0.11	0.478-0.989
WBMD values (g/cm^2)	0.638±0.15	0.392-1.140

Total T: Total testosterone, Total E_2: Total estradiol, Free T: Free testosterone, FreeE_2: Free estradiol, BioT: Bioavailable testosterone, BioE_2: Bioavailable estradiol, FAI: Free androgen index, FEI: Free estrogen index, SHBG: Sex hormone binding globulin, OC: Osteocalcin, CTx: C-terminal telopeptide, LSTS: Lumbar spine T score, FNTS: Femoral neck T score, LSBMD: Lumbar spine bone mineral density, FNBMD: Femoral neck bone mineral density, TBMD: Trochanter bone mineral density, WBMD: Ward's triangle bone mineral density

After age and BMI were adjusted, the relations between sex hormones and BMD were determined with multiple linear regression analysis. There was no significant relation between all BMD measurements and levels of serum bioavailable T, free T, bioavailable E_2, free E_2, and SHBG ($p>0.05$, Table 4). Also, there was no significant relation between all BMD measurements and FAI, FEI, total T, and total E_2 ($p>0.05$).

When the relation between sex hormones levels and bone turnover markers was investigated, there were weak positive relations between serum OC levels and FEI ($r=0.92$, $p=0.05$) and between serum CTx levels and FEI and bioavailable T ($r=0.86$, $p=0.05$ and $r=0.72$, $p=0.05$ respectively).

Discussion

In the present study, we found that age was positively correlated with FSH, LH and total E_2, and negatively correlated with FAI. However, we did not find any correlations between age and bioavailable T, free T, bioavailable E_2, free E_2, FEI and SHBG levels. First Ferrini et al. and then Szulc et al. reported that free T, bioavailable T, and FAI as well as bioavailable E_2 concentrations decreased and SHBG levels increased with age (28,32). The results of this study are not consistent with their results. It may be that this study only included middle aged men, that is, the men aged 35-65 years, but that prior studies included the men over 65.

All E_2 measurements were positively correlated with BMI, but total T and SHBG were negatively correlated with BMI. We determined that age had a significantly negative effect on hip BMD, whereas BMI had a significantly positive effect on all BMD measures. After age and BMI were adjusted, neither serum bioavailable sex steroids levels nor serum free sex steroids levels were associated with BMD at the hip and the lumbar spine.

Previously, Khosla et al. in their study on 346 men aged 23-90 yr showed that serum bioavailable T and total and bioavailable E_2 levels were significantly correlated with BMD at various sites. The correlation was considerably stronger for bioavailable E_2 as opposed to total E (29). Likewise, in 534 community-dwelling men aged 50-89 yrs, Greendale et al. reported that bioavailable E_2 and T were significantly associated with BMD at the forearm, the spine and the hip, whereas total T was not associated with BMD measurements (22). Van den Beld et al. found that bioavailable and free T were more strongly related to hip BMD than total T in elderly men (33). Recently, in a longitudinal study, Khosla et al. showed significant associations between bioavailable sex steroid levels and rates of changes in BMD only at the forearm sites, but not at the total hip or the mid lateral spine in subjects over the age of 60. On the other hand, in middle-aged subjects, only ulnar BMD was significantly correlated with bioavailable E_2 (34). Unlike the previous studies, we did not measure BMD at the forearm. This is a limitation of this study, which may explain the lack of association between bioavailable sex hormones and BMD.

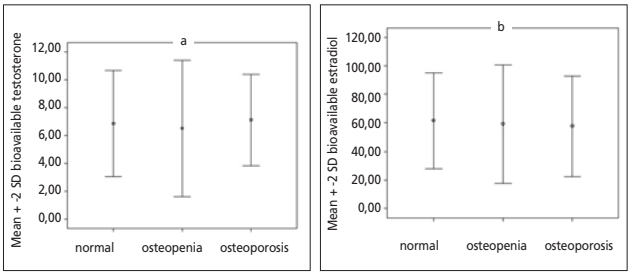

Figure 1. The comparison of bioavailable testosterone levels (a) and bioavailable estradiol levels (b) between the groups

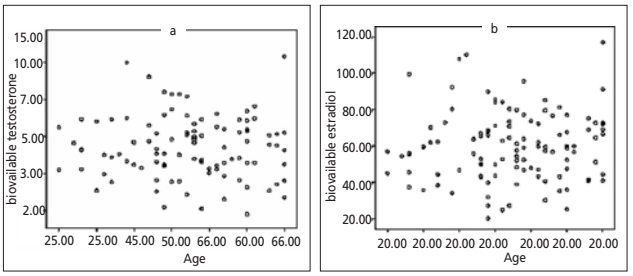

Figure 2. a) Correlation between age and bioavailable testosterone b) and bioavailable estradiol

Table 2. The comparison of sex steroids and bone markers between the groups

Variables	Osteoporosis (n:22) Mean±SD	Osteopenia (n:51) Mean±SD	Normal (n:42) Mean±SD	P values
OC (ng/mL)	23.05±7.4	25.7±8.1	23.9±7.4	0.335
CTx (ng/mL)	0.339±0.15	0.440±0.2	0.339±0.22	0.206
T Tes (nmol/L)	15.8±5.2	15.6±5.0	16.8±5.1	0.503
T E_2 (pmol/L)	88.2±24.4	93.9±26.0	99.5±24.5	0.226
Free Tes (nmol/L)	0.290±0.69	0.261±0.1	0.336±0.40	0.425
FreeE_2 (pmol/L)	1.42±0.4	1.44±0.5	1.48±0.4	0.866
Bio Tes (nmol/L)	7.12±1.64	6.52±2.4	6.86±1.90	0.538
BioE_2 (pmol/L)	57.6±17.5	59.2±20.7	61.5±16.7	0.707
FAI (nmol/nmol)	41.9±7.5	38.4±15.9	39.6±13.0	0.627
FEI (pmol/nmol)	250.4±110.8	246.6±128.7	241.1±99.1	0.949
SHBG (nmol/L)	39.7±13.7	49.4±34.1	50.6±38.1	0.424

OC: Osteocalcin, CTx: C-terminal telopeptide, Total T: Total testosterone, Total E_2: Total estradiol, FreeT: Free testosterone, FreeE_2: Free estradiol, BioT: Bioavailable testosterone, BioE_2: Bioavailable estradiol, FAI: Free androgen index, FEI: Free estrogen index, SHBG: Sex hormone binding globulin

In addition, we found no significant relation between FEI, FAI, total E_2, total T, SHBG and BMD at the lumbar spine and the hip. However, in a prior study on 14 elderly Turkish men, Gürlek et al. showed that total T levels were associated with forearm and hip BMD and that total E_2 levels were associated with forearm BMD (35). Thereafter, Keleş et al. showed that neither total T nor total E_2 was associated with any BMD measurements in 174 healthy Turkish men (36). They found a significant relation between free T and only distal radius BMD even though many early studies had revealed correlations between hip and forearm BMD and free T and FAI (25,37-39). Drinka et al. failed to find any correlation between free T and BMD measured at the lumbar spine, the hip and the radius (24). Likewise, Rapado et al. in their study on a group of elderly healthy men did not find any significant relation between androgens or SHBG values and BMD measured at any sites (40).

Table 3. Correlation of sex steroids, bone turnover markers and BMD with age and BMI

Variables	Age		BMI	
	r	p	r	p
T Tes	0.14	0.1	-0.30	0.001*
T E_2	0.21	0.02*	0.18	0.04*
FSH	0.27	0.003*	-0.005	0.9
LH	0.33	0.0001*	-0.03	0.6
Free T	0.12	0.2	-0.05	0.6
FreeE_2	0.07	0.4	0.29	0.002*
Bio Tes	-0.05	0.5	-0.06	0.5
BioE_2	0.06	0.4	0.35	0.0001*
FAI	-0.23	0.01*	0.12	0.1
FEI	-0.12	0.1	0.39	0.0001*
SHBG	0.14	0.1	-0.21	0.02*
OC	0.03	0.7	-0.16	0.1
CTx	-0,06	0.5	0.06	0.6
LSBMD	0.02	0.7	0.32	0.002*
FNBMD	-0.26	0.007*	0.29	0.004*
TBMD	-0.14	0.1	0.35	0.001*
WBMD	-0.29	0.004*	0.21	0.03*

Total T: Total testosterone, Total E_2: Total estradiol, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, FreeT: Free testosterone, FreeE_2: Free estradiol, Bio T: Bioavailable testosterone, BioE_2: Bioavailable estradiol, FAI: Free androgen index, FEI: Free estrogen index, SHBG: Sex hormone binding globulin, OC: Osteocalcin, CTx: C-terminal telopeptide, LSBMD: Lumbar spine bone mineral density, FNBMD: Femoral neck bone mineral density, TBMD: Trochanter bone mineral density, WBMD: Ward's triangle bone mineral density

In this study, the osteoporotic and the osteopenic subjects regarding the levels of total, free and bioavailable sex steroids, SHBG and bone turnover markers were not different from the subjects with normal BMD. There have been conflicting results in the literature regarding levels of sex steroids, SHBG and bone turnover markers between osteoporotic patients and normal individuals. Gillberg et al. reported that the men with idiopathic osteoporosis had significantly lower estradiol levels, FEI and FAI and higher SHBG levels than the normal healthy men (41). However, others did not find any difference in the levels of testosterone and estradiol, bone remodelling markers between patients with osteoporosis and controls, but they showed higher SHBG plasma levels in the osteoporotic patients than in the controls. Moreover, this carrier protein was negatively correlated with BMD at the femoral neck and at the lumbar spine (42,43). Likewise, Rucker et al. suggested that SHBG was a significant predictor for BMD at the total hip and the femoral trochanter (44).

Our results showed positive correlations between bioavailable T and FEI with bone resorption (serum CTx) and/or bone formation (serum OC) markers. Therefore, we thought that both E_2 and T may contribute to the bone turnover in middle aged men. Likewise, Lormeau et al. showed significant positive correlations between E_2 and FEI and bone formation (bone alkaline phosphatase) and resorption (serum CTx) markers (44). In addition, several studies revealed that levels of bone turnover markers were significantly higher in men with the lowest concentration of bioavailable E_2 and FEI or with bioavailable E_2 levels below 40 pmol/L (11pg/ml). They suggested that E_2 played a dominant role in the regulation of bone resorption in elderly men although T may have smaller contributions (32,33,42).

Table 4. Correlation between sex steroids and BMD

	LSBMD		FNBMD		TBMD		WBMD	
	r	p	r	p	r	p	r	p
T Tes	0.12	0.7	-0.14	0.6	0.06	0.8	0.20	0.5
T E2	0.14	0.7	-0.14	0.8	-0.006	0.9	-0.32	0.5
Free T	0.10	0.3	0.02	0.8	0.01	0.8	-0.01	0.9
FreeE_2	-0.40	0.4	-0.25	0.6	-0.45	0.3	-0.03	0.9
Bio Tes	-0.34	0.3	0.20	0.5	-0.29	0.4	0.04	0.9
BioE_2	0.57	0.4	0.62	0.4	0.76	0.3	0.35	0.6
FAI	0.24	0.5	-0.15	0.6	0.33	0.3	-0.21	0.5
FEI	-0.33	0.4	-0.30	0.5	-0.59	0.2	0.02	0.9
SHBG	-0.007	0.9	0.12	0.6	-0.07	0.8	-0.03	0.9

Total T: Total testosterone, Total E_2: Total estradiol, FreeT: Free testosterone, FreeE_2: Free estradiol, BioT: Bioavailable testosterone, BioE_2: Bioavailable estradiol, FAI: Free androgen index, FEI: Free estrogen index, SHBG: Sex hormone binding globulin

This study has some limitations. First, 115 adult men referring to our outpatient clinic were included in the present study. This type of recruitment cannot exclude the possibility of bias in the results. Second, the sample size was small and BMD in the forearm was not measured. Third, this study had a cross-sectional design. Despite these limitations, this is the first study in the literature performed to determine whether bioavailable T, bioavailable E_2 and SHBG levels were associated with BMD and bone turnover markers in a Turkish male population. Most of the prior studies included elderly subjects as well. Unlike those studies, we included only middle aged men into this study. All above limitations might have caused failure to determine a relation between BMD measures and sex hormones and SHBG levels.

In conclusion, we found that bioavailable and free sex steroids did not have any impact on hip and lumbar spine BMD measures in a middle aged Turkish male population. We think that further studies are needed to elucidate the etiology of idiopathic osteoporosis in middle aged males.

References

1. Braga V, Sangalli A, Malerba G, Mottes M, Mirandola S, Gatti D, et al. Relationship among VDR (BsmI and FokI), COLIA1, and CTR polymorphisms with bone mass, bone turnover markers, and sex hormones in men. Calcif Tissue Int 2002;70:457-62.
2. Ray NF, Chan JK, Thamer M, Melton LJ 3rd. Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res 1997;12:24-35.
3. Cooper C, Campion G, Melton LJ. Hip fractures in the elderly: a world-wide projection. Osteoporos Int 1992;2:285-9.
4. Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ 3rd. Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985-1989. J Bone Miner Res 1992;7:221-7.
5. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA. Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet 1999;353:878-82.
6. Kellie SE, Brody JA. Sex-specific and race-specific hip fracture rates. Am J Public Health 1990;80:326-8.
7. Legroux-Gerot I, Blanckaert F, Solau-Gervais E, Negahban M, Duquesnoy B, Delcambre B, et al. Causes of osteoporosis in males. A review of 160 cases. Rev Rhum Engl Ed 1999;66:404-9.
8. Leifke E, Gorenoi V, Wichers C, Von Zur Mühlen A, Von Büren E, Brabant G. Age-related changes of serum sex hormones, insulin-like growth factor-1 and sex-hormone binding globulin levels in men: cross-sectional data from a healthy male cohort. Clin Endocrinol 2000;53:689-95.
9. Landin-Wilhelmsen K, Wilhelmsen L, Lappas G, Rosén T, Lindstedt G, Lundberg PA, et al. Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin Endocrinol 1994;41:351-7.
10. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol 1997;145:970-6.
11. Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C. Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. J Clin Endocrinol and Metab 1998;83:135-9.
12. Ljunghall S, Johansson AG, Burman P, Kämpe O, Lindh E, Karlsson FA. Low plasma levels of insulin-like growth factor 1 (IGF-1) in male patients with idiopathic osteoporosis. J Intern Med 1992;232:59-64.
13. Kurland ES, Rosen CJ, Cosman F, McMahon D, Chan F, Shane E, et al. Insulin-like growth factor-I in men with idiopathic osteoporosis. J Clin Endocrinol Metab 1997;82:2799-805.
14. Langlois JA, Rosen CJ, Visser M, Hannan MT, Harris T, Wilson PW, et al. Association between insulin-like growth factor-I and bone mineral density in older women and men: the Framingham Heart Study. J Clin Endocrinol Metab 1998;83:4257-62.
15. Barret-Connor E, Goodman-Gruen D. Gender differences in insulin-like growth factor and bone mineral density association in old age: the Rancho Bernardo Study. J Bone Mineral Res 1998;13:1343-9.
16. Sezgin M, Cimen B, Kanık A, As I, Ercetin N, Arinci Incel N, et al. Serum IGF-1 and IGFBP-3 levels in middle aged Turkish males: Relationship with bone mineral density and markers of bone turnover. J From the World of Osteoporosis 2007;13:37-43.
17. Riggs BL, Khosla S, Melton LJ 3rd. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998;13:763-73.
18. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev 2004;25:389-425.
19. Harman SM, Tsitouras PD. Reproductive hormones in aging men. I. Measurement of sex steroids, basal luteinizing hormone, and Leydig cell response to human chorionic gonadotropin. J Clin Endocrinol Metab 1980;51:35-40.
20. Foresta C, Ruzza G, Mioni R, Guarneri G, Gribaldo R, Meneghello A, et al. Osteoporosis and decline of gonadal function in the elderly male. Horm Res 1984;19:18-22.
21. Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC. Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest 1997;100:1755-9.
22. Greendale GA, Edelstein S, Barrett-Connor E. Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. J Bone Miner Res 1997;12:1833-43.
23. Taaffe DR, Cooper CS, Holloway L, Duret C, Marcus R. Lack of association of anabolic hormone status and muscle strength with regional and whole body bone mineral density in healthy men aged 60-79 years. Aging 1999;11:4-11.
24. Drinka PJ, Olson J, Bauwens S, Voeks SK, Carlson I, Wilson M. Lack of association between free testosterone and bone density separate from age in elderly males. Calcif Tissue Int 1993;52:67-9.
25. Murphy S, Khaw KT, Cassidy A, Compston JE. Sex hormones and bone mineral density in elderly men. Bone Miner 1993:20:133-40.
26. Södergård R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801-10.
27. Manni A, Pardridge WM, Cefalu W, Nisula BC, Bardin CW, Santner SJ, et al. Bioavailability of albumin-bound testosterone. J Clin Endocrinol Metab 1985;61:705-10.
28. Ferrini RL, Barrett-Connor E. Sex hormones and age: a cross-sectional study of testosterone and estradiol and their bioavailable fractions in community-dwelling men. Am J Epidemiol 1998;147:750-4.
29. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 1998;83:2266-74.
30. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-72.

31. Kanis JL, Melton LS, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9:1137-41.
32. Szulc P, Munoz F, Claustrat B, Garnero P, Marchand F, Duboeuf F, et al. Bioavailable estradiol may be an important determinant of osteoporosis in men: the MINOS study. J Clin Endocrinol Metab 2001;86:192-9.
33. Van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 2000;85:3276-82.
34. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM. Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocrinol Metab 2001;86:3555-61.
35. Gürlek A, Gedik O. Endogenous sex steroid, GH and IGF-I levels in normal elderly men: relationships with bone mineral density and markers of bone turnover. J Endocrinol Invest 2001;24:408-14.
36. Keleş I, Aydin G, Başar MM, Hayran M, Atalar E, Orkun S, et al. Endogenous sex steroids and bone mineral density in healthy men. Joint Bone Spine 2006;73:80-5.
37. Mellström D, Johnell O, Ljunggren O, Eriksson AL, Lorentzon M, Mallmin H, et al. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 2006;21:529-35.
38. Kelly PJ, Pocock NA, Sambrook PN, Eisman JA. Dietary calcium, sex hormones, and bone mineral density in men. BMJ 1990;300:1361-4.
39. Ongphiphadhanakul B, Rajatanavin R, Chailurkit L, Piaseu N, Teerarungsikul K, Sirisriro R, et al. Serum testosterone and its relation to bone mineral density and body composition in normal males. Clin Endocrinol 1995;43:727-33.
40. Rapado A, Hawkins F, Sobrinho L, Díaz-Curiel M, Galvao-Telles A, Arver S, et al. Bone mineral density and androgen levels in elderly males. Calcif Tissue Int 1999;65:417-21.
41. Gillberg P, Johansson AG, Ljunghall S. Decreased estradiol levels and free androgen index and elevated sex hormone-binding globulin levels in male idiopathic osteoporosis. Calcif Tissue Int 1999;64:209-13.
42. Legrand E, Hedde C, Gallois Y, Degasne I, Boux de Casson F, Mathieu E, et al. Osteoporosis in men: a potential role for the sex hormone binding globulin. Bone 2001;29:90-5.
43. Lormeau C, Soudan B, d'Herbomez M, Pigny P, Duquesnoy B, Cortet B. Sex hormone-binding globulin, estradiol, and bone turnover markers in male osteoporosis. Bone 2004;34:933-9.
44. Rucker D, Ezzat S, Diamandi A, Khosravi J, Hanley DA. IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clin Endocrinol 2004;60:491-9.