0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 7
Printed in U.S.A.

Relationship of Serum Sex Steroid Levels and Bone Turnover Markers with Bone Mineral Density in Men and Women: A Key Role for Bioavailable Estrogen*

SUNDEEP KHOSLA, L. JOSEPH MELTON, III, ELIZABETH J. ATKINSON, W. M. O'FALLON, GEORGE G. KLEE, AND B. LAWRENCE RIGGS

Endocrine Research Unit (S.K., B.L.R.); Division of Endocrinology, Metabolism, and Nutrition; Departments of Internal Medicine, Health Sciences Research (L.J.M., E.J.A., W.M.O.), and Laboratory Medicine and Pathology (G.G.K.), Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

ABSTRACT

Estrogen (E) deficiency associated with the menopause is the major cause of bone loss in aging women. However, men also lose significant amounts of bone with age, but they do not have the equivalent of menopause, and serum total testosterone (T) and E levels decline only marginally with age in men. Thus, it has been difficult to attribute bone loss in aging men to either T or E deficiency. Here, we show in a population-based, age-stratified sample of 346 men, aged 23–90 yr, that serum total T and E (estradiol plus estrone) levels decreased over the life span by 30% and 12%, respectively, but bioavailable (or nonsex hormone-binding globulin-bound) T and E levels decreased by 64% and 47%, respectively. In these men and in a parallel cohort of 304 women, aged 21–94 yr, serum PTH increased 84% and 64% over the life span, and urinary *N*-telopeptide of type I collagen (NTx) excretion, a bone resorption marker, increased 77% and 80% between age 50–85 yr in the men and women, respectively. By univariate analyses, serum bioavailable T and E levels correlated positively with bone mineral density (BMD) at the total body, spine, proximal femur, and distal radius and negatively with urinary NTx excretion in men and women. Urinary NTx excretion was also negatively associated with BMD in both sexes. By multivariate analyses, however, serum bioavailable E level was the consistent independent predictor of BMD in both men and postmenopausal women. Thus, bioavailable E levels decline significantly with age and are important predictors of BMD in men as well as women. These studies suggest that in contrast to traditional belief, age-related bone loss may be the result of E deficiency not just in postmenopausal women, but also in men. (*J Clin Endocrinol Metab* **83:** 2266–2274, 1998)

ALTHOUGH osteoporosis is more common in women, men also incur substantial bone loss with aging (1, 2), and elderly men have age-specific hip fracture and vertebral fracture rates that are at least half those in women (3). Indeed, recent estimates are that of the $13.8 billion cost to the U.S. health care system attributed to osteoporosis each year, approximately $2.7 billion is related to fractures in men (4). Thus, osteoporosis in men is a significant problem both clinically and economically and is likely to increase in scope as the population ages.

Estrogen (E) deficiency has clearly been identified as a major risk factor for osteoporosis in women, and the effects of E on the skeleton have been the subject of intensive investigation. Recent evidence indicates that E deficiency may be responsible not only for the early postmenopausal phase of rapid bone loss, but also for the late slow phase of bone loss associated with aging. The latter had been attributed at least in part to an age-related increase in bone turnover, due largely to increases in serum PTH levels with aging (5–7). E therapy of elderly postmenopausal women, however, appears to prevent the secondary hyperparathyroidism and increased bone turnover associated with aging (7, 8). This has led to the hypothesis that the extraskeletal effects of E, namely on intestinal calcium absorption (9, 10), renal calcium handling (11, 12), and perhaps direct effects on PTH secretion (13), may be responsible for preventing the age-related increase in serum PTH and in bone turnover in late postmenopausal women treated with E (14).

Despite the fact that men do not have the equivalent of menopause and that serum total testosterone (T) levels decrease only marginally with age (15–17), men have substantial age-related decreases in bone mineral density (BMD) in both cross-sectional (18) and longitudinal (19) studies. Moreover, previous epidemiological studies assessing the relationship between serum total T levels and BMD have generally found either no relationship (17, 20) or even a negative association between total T levels and BMD in aging men (21). Thus, the absence of substantial decreases in serum total T levels in aging men has led to the belief that T does not play a major role in bone loss in aging men.

Recent studies have suggested instead the possibility that, as in women, E may play a key role in regulating bone turnover in men. Smith *et al.* (22) described a male with homozygous mutations in the E receptor gene who, even in the presence of normal T and free T levels, had unfused epiphyses and marked osteopenia, along with elevated indexes of bone turnover. Subsequently, Morishima *et al.* (23) and Carani *et al.* (24) reported clinical findings in two males with homozygous mutations of the gene that codes for the enzyme, aromatase, which is responsible for the conversion of androgens to E. In both instances, BMD was significantly

Received January 13, 1998. Revision received March 16, 1998. Accepted March 24, 1998.

Address all correspondence and requests for reprints to: Sundeep Khosla, M.D., Mayo Clinic, 200 First Street SW, 5–164 West Joseph, Rochester, Minnesota 55905. E-mail: khosla.sundeep@mayo.edu.

* This work was supported by Research Grant AR27065 from the NIAMSD, USPHS.

reduced, and bone turnover markers were markedly elevated despite normal T levels. Treatment with T did not significantly affect bone metabolism in one patient (24), whereas treatment with E markedly increased BMD in both patients (24, 25). Finally, a recent epidemiological study by Slemenda *et al.* (21) found that serum estradiol (E_2), but not T, levels were positively associated with BMD in men over age 65 yr.

Despite these findings, a major conceptual problem with attributing age-related bone loss in men to E deficiency is that, as in the case of total T levels, serum total E levels decline only marginally with age in men (15). However, there are several important limitations of previous studies attempting to define the relationship of T or E to BMD in men. First, most have studied subjects in a narrow age range, such as elderly men (20, 21, 26). Second, they have failed to measure levels of circulating bioavailable sex steroids. The bioavailable sex steroids comprise the fractions that are free or associated with albumin in the circulation (27–30), and it is these fractions that have rapid access to target tissues (31, 32). In contrast, the fraction bound to sex hormone-binding globulin (SHBG) does not have free access to target tissues. As SHBG levels increase with age in men (33, 34), measurement of total T or E levels does not accurately reflect the actual levels of these steroids available to tissues. Moreover, although several studies have measured circulating free T and E levels (21, 26), the free fraction constitutes only 1–3% of the total circulating sex steroids (30), and failure to account for the 35–55% of the circulating steroids bound to albumin vastly underestimates the proportion available to target tissues.

In the present study, we addressed these limitations in several ways. First, we defined the age-related changes in circulating bioavailable T and E levels in a population-based, age-stratified sample of men, aged 23–90 yr (n = 346). Next, we assessed age-related changes in serum PTH and in markers of bone turnover in these men and related these to BMD and the sex steroids. We also assessed the relative importance of bioavailable T *vs.* E levels in determining BMD in men by multivariate analyses. Finally, we performed similar studies in a parallel cohort of 304 women so that these relationships in aging men *vs.* women could be compared.

Subjects and Methods

Study subjects

Subjects were recruited from an age-stratified random sample of Rochester, MN, men and women that were selected using the medical records linkage system of the Rochester Epidemiology Project (35). Over half of the Rochester population is identified annually in this system, and the majority are seen in any 3-yr period. Thus, the enumerated population approximates the underlying population of the community, including both free-living and institutionalized individuals. Altogether 1138 men and 938 women aged 20 yr and over were approached, but 239 men and 126 women were ineligible (among the men, 109 were demented and could not give informed consent, 13 were radiation workers, 91 died before contact, 25 were debilitated due to terminal cancer, and 1 was unable to participate due to pending legal action; among the women, 89 were demented, 11 were pregnant, 9 were radiation workers, 8 were participants in an ongoing clinical trial of osteoporosis prophylaxis, and 9 died before they could be contacted). Of the 899 eligible men, 348 participated and provided full study data, although 2 were excluded from analysis because 1 was receiving T therapy and 1 had inexplicably high (into the range of premenopausal women) E_2 and estrone (E_1) levels. Of the 812 eligible women, 351 participated and provided full study data, although 47 of the 213 postmenopausal women were receiving E replacement therapy and were excluded from this analysis. Thus, the total number of subjects included in this analysis was 650 (346 men and 304 women). All but 13 men and 3 women were Caucasian, reflecting the ethnic composition of the population (96% white in 1990). The men ranged in age from 23–90 yr, and the women ranged in age from 21–94 yr.

As this sample of subjects was population based, the overall results are applicable to the general population of men and women in the community. However, we also performed subset analyses in normal subjects, excluding those with rheumatoid arthritis (4 men and 5 women), Paget's disease (5 men), gastrointestinal resection (11 men and 1 woman), significant renal insufficiency (defined as serum creatinine >2 mg/dL, 6 men), chronic obstructive pulmonary disease (14 men and 3 women), prostate cancer or bilateral orchidectomies (12 men), premature menopause (at <35 yr of age; 1 woman), or current therapy with corticosteroids (7 men and 7 women), thiazide diuretics (21 men and 33 women), anticonvulsants (2 men and 2 women), or oral contraceptives (28 premenopausal women). After all of these exclusions, the subset of normal men and women consisted of 280 and 231 subjects, respectively.

Study protocol

BMD (grams per cm^2) was determined for the total body, spine (L2–L4), proximal femur (total), and middistal radius using dual energy x-ray absorptiometry with the Hologic QDR2000 instrument (Hologic, Waltham, MA) using software version 5.40. As we did not specifically exclude subjects with spinal osteoarthritis or aortic calcification, which can confound the BMD measurement (36), we assessed the midlateral instead of the antero-posterior spine, which largely excludes these confounders from the scanning field. The coefficients of variation (CVs) for the total body, lateral spine, femur, and radius were 0.8%, 2.1%, 1.8%, and 1.7%, respectively.

Fasting state serum samples were obtained between 0800–0900 h, and a 24-h urine collection was completed. All samples were stored at −70 C until analyzed.

Laboratory methods

Fasting serum samples were assayed by RIA for total T (Diagnostic Products Corp., Los Angeles, CA; interassay CV, 11%), E_2 (Diagnostic Systems Laboratories, Webster, TX; interassay CV, 11%), E_1 (Diagnostic Systems Laboratories; interassay CV, 9%), and SHBG (Wien Laboratories, Succasunna, NJ; interassay CV, 7%). In addition, the non-SHBG-bound (bioavailable) fractions of total T, E_2, and E_1 were measured using a modification of the techniques of O'Connor *et al.* (27) and Tremblay *et al.* (28). Briefly, tracer amounts of ^{3}H-labeled T, E_2, or E_1 were added to serum aliquots. An equal volume of saturated solution of ammonium sulfate (final concentration, 50%) was added to precipitate SHBG with its bound steroid. Separation of the SHBG fraction was performed by centrifugation at 1100 × *g* for 30 min at 4 C. The percentage of labeled steroid remaining in the supernatant (the free and albumin-bound fractions) was then calculated. The bioavailable steroid concentration was obtained by multiplying the total steroid concentration, as determined by RIA, by the fraction that was non-SHBG bound. Free T was measured by RIA using a T analog with low affinity for SHBG and albumin (Diagnostic Systems Laboratories; interassay CV, 10%). Serum dehydroepiandrosterone sulfate (DHEAS) was measured by RIA (Diagnostic Products Corp.; interassay CV, <8%). Serum LH and FSH were measured by immunoradiometric assays (Diagnostic Products Corp.; interassay CV, 14% for LH and 11% for FSH).

Serum intact PTH was measured by immunochemiluminometric assay (37) (interassay CV, 14%). Bone formation was assessed by serum osteocalcin, measured by RIA using antibody G12 (interassay CV, <6%) (5), as well as by serum bone alkaline phosphatase (BAP), measured by enzyme-linked immunosorbent assay (5) (ELISA; interassay CV, <11%), and serum carboxyl-terminal propeptide of type I collagen (PICP), also measured by ELISA (Prolagen-C, Metra Biosystems, Mountain View, CA; interassay CV, <7%). Bone resorption was evaluated by measurement of 24-h urinary levels of the *N*-telopeptide of type I collagen (NTx) and free deoxypyridinoline (f-Dpd), both assessed as nanomoles per L glomerular filtrate. NTx and f-Dpd were measured by ELISA kits (Os-

teomark, Ostex, Seattle, WA; interassay CV, 10%; and Pyrilinks-D, Metra Biosystems, Mountain View, CA; interassay CV, 14%). The glomerular filtration rate was assessed by creatinine clearance.

Statistical analysis

Pearson correlations were used to summarize relationships between the various continuous variables. Log transformations were used on highly skewed variables. When appropriate, Spearman correlations were used. The percent change from age 25–85 yr was estimated from predictions using a loess model in S-plus (38), using the following formula: percent change = [(value at age 85 yr − value at age 25 yr)/value at age 25 yr] × 100. The smoother function was also used as a means to visually explore the data in the various plots. Stepwise model selection was used to determine the relationship of sex steroid variables and bone turnover markers with BMD. Higher ordered terms and interactions were checked, but were only included if they made a meaningful change to the model. Finally, model assumptions were checked by examination of the model residuals.

Results

Age-related changes in BMD and serum sex steroids

Table 1 shows the correlation coefficients between BMD and age in the men and women. In this cross-sectional analysis, BMD declined significantly with age in both sexes at all sites, and Table 1 also indicates the percent decrease from age 25–85 yr in BMD at the various sites. Thus, at all sites, the percent decrease in BMD was approximately twice as great in the women as it was in the men.

Table 1 also shows the age-related changes in serum sex steroid levels in the men and women. Between age 25–85 yr, the decline in bioavailable T was almost twice that in total T in both sexes, and bioavailable T decreased by approximately 64% and 28% in the men and women, respectively (Table 1 and Fig. 1, A and B). In the men, total E (E_2 plus E_1) declined only marginally with age, whereas bioavailable E decreased by 47% (Table 1 and Fig. 2, A and B). In contrast, total and bioavailable E levels decreased similarly in the women. For simplicity, we have presented the data for total and bioavailable E_2 plus E_1, although E_2 and E_1 individually showed similar changes. As expected, there was a precipitous drop in serum total and bioavailable E levels in the women around the time of the menopause, whereas there was a gradual, age-related decrease in bioavailable E levels in the men (Fig.

TABLE 1. Correlation coefficients for association between age and BMD, serum sex steroid and SHBG levels, and serum LH and FSH levels among age-stratified samples of Rochester men and women

	Men		Women	
	r	% Change from age 25 to 85 yr	r	% Change from age 25 to 85 yr
BMD				
Total body	−0.29[a]	−7.7	−0.62[a]	−17.6
Lateral spine	−0.44[a]	−26.8	−0.70[a]	−45.2
Proximal femur	−0.36[a]	−15.5	−0.62[a]	−29.5
Radius	−0.51[a]	−17.8	−0.70[a]	−28.4
Sex steroids				
Total T	−0.25[a]	−29.5	−0.13[b]	−15.8
Bioavailable T	−0.62[a]	−64.1	−0.16[b]	−28.4
Total E	−0.09	−11.5	−0.49[a]	−81.2
Bioavailable E	−0.43[a]	−47.1	−0.52[a]	−83.4
T/SHBG (log)	−0.55[a]	−83.2	−0.15[c]	−32.3
E/SHBG (log)	−0.46[a]	−69.3	−0.53[a]	−81.1
Free T (log)	−0.62[a]	−92.4	−0.16[c]	−33.4
DHEAS	−0.68[a]	−83.3	−0.61[a]	−74.5
SHBG	0.50[a]	124.3	−0.03	−1.4
LH	0.36[a]	285.5	0.47[a]	731.4
FSH	0.49[a]	505.2	0.84[a]	1804.9

[a] $P < 0.001$.
[b] $P < 0.05$.
[c] $P < 0.01$.

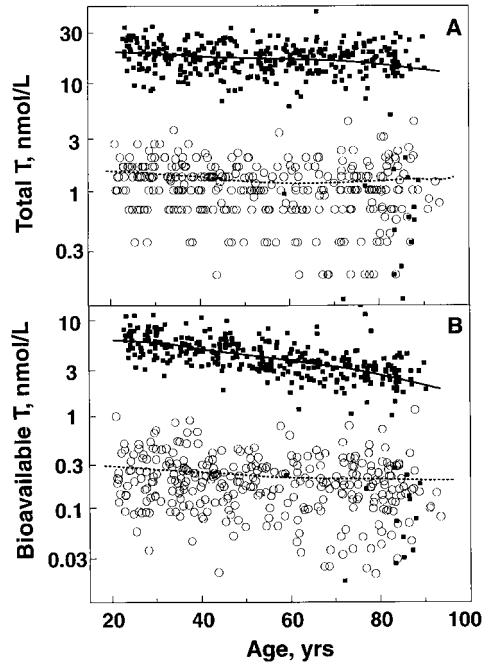

FIG. 1. Serum total T (A) and bioavailable T (B) levels as a function of age among an age-stratified sample of Rochester men (*solid lines, squares*) and women (*dashed lines, circles*). See Table 1 for correlation coefficients with age.

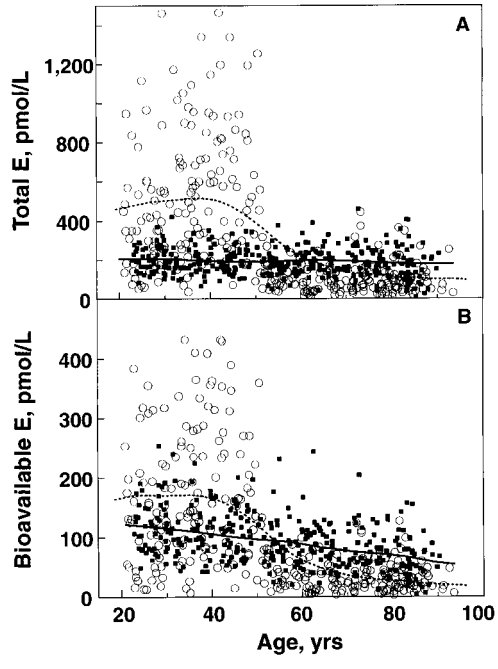

FIG. 2. Serum total estrogen (A) and bioavailable estrogen (B) levels as a function of age among an age-stratified sample of Rochester men (*solid lines, squares*) and women (*dashed lines, circles*). See Table 1 for correlation coefficients with age.

2, A and B). There was also a marked difference between sexes in changes in SHBG with age; SHBG increased by more than 2-fold over the life span in the men, whereas it was virtually unchanged in the women (Table 1 and Fig. 3). Thus, the greater decrease in bioavailable T and E in the men was explained in large part by the age-related increase in SHBG levels. Finally, serum DHEAS levels also decreased significantly with age in both sexes (Table 1).

We also assessed whether in addition to the bioavailable sex steroid measures, other indexes of bioavailable or free sex steroids decreased similarly with age in the men and women (Table 1). Thus, the age-related decreases in the molar ratios of T to SHBG and E to SHBG were comparable to the decreases in directly measured bioavailable T and E, respectively. In addition, the free T level, which represents the T fraction not bound to either SHBG or albumin, decreased with age in a manner similar to bioavailable T in both sexes.

The age-related decreases in sex steroids were accompanied by parallel increases in serum LH and FSH levels (Table 1). Of note, serum LH in the men did not correlate with serum total T or E levels (r = 0.03 and −0.04, respectively), but was inversely correlated with serum bioavailable T and E levels (r = −0.35 and r = −0.29, respectively; both $P < 0.001$). Similarly, serum FSH in the men was only weakly inversely correlated with serum total T and E levels (r = −0.11; $P = 0.05$; and r = −0.15; $P = 0.01$, respectively), but showed a strong negative association with serum bioavailable T and E levels (r = −0.46 and r = −0.35, respectively; both $P < 0.001$). In contrast, in the women, serum LH and FSH were both inversely associated with serum total and bioavailable E levels (r = −0.19 and r = −0.21, respectively; both $P < 0.001$ for total and bioavailable E *vs.* LH; and r = −0.54 and −0.55, respectively; both $P < 0.001$ for total and bioavailable E *vs.* FSH). However, neither serum LH nor FSH was correlated with T levels in the women (data not shown).

Age-related changes in serum PTH and bone turnover markers

Figure 4, A–C, shows the changes in serum PTH, serum osteocalcin, and urinary NTx excretion as a function of age in the men and women. In these cross-sectional data, serum PTH increased similarly with age in both sexes; over the life span, the percent increase in serum PTH was 84% in the men and 64% in the women. Serum osteocalcin and urinary NTx excretion decreased with age in the men up to approximately age 50 yr and increased with age thereafter. Similarly, serum osteocalcin and urinary NTx decreased with age in the premenopausal women and increased with age in the postmenopausal women. Table 2 shows the correlation coefficients between all of the measured bone turnover markers and age in the men and women. Serum BAP had an age-related pattern similar to that of serum osteocalcin, although none of the individual correlation coefficients was statistically significant. Serum PICP decreased with age in the men up to age 50 yr and in the premenopausal women, and then showed little or no change with age in the men over age 50 yr or in the postmenopausal women. Urinary f-Dpd excretion showed age-related changes similar to those for urinary NTx excretion.

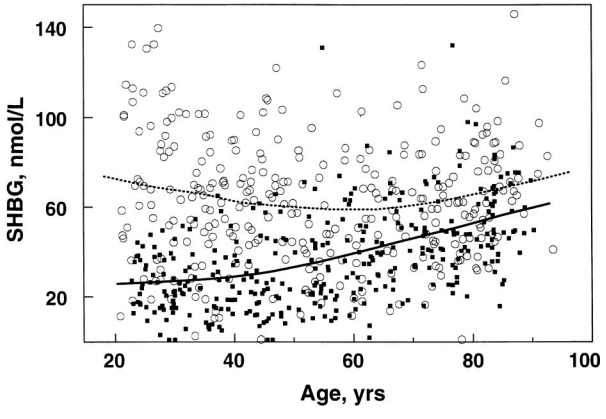

FIG. 3. Serum SHBG levels as a function of age among an age-stratified sample of Rochester men (*solid lines, squares*) and women (*dashed lines, circles*). See Table 1 for correlation coefficients with age.

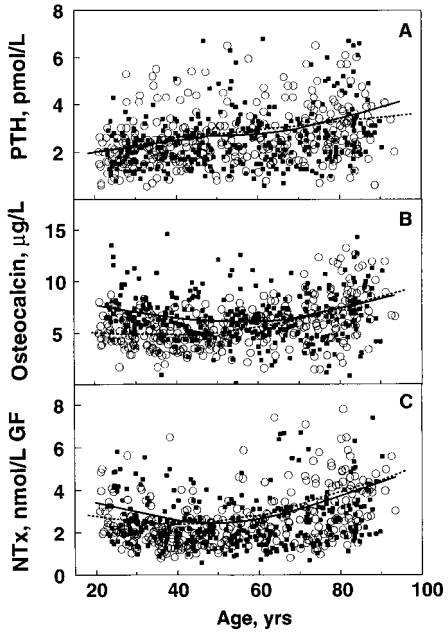

FIG. 4. Serum PTH (A), serum osteocalcin (B), and urinary NTx (C) levels as a function of age among an age-stratified sample of Rochester men (*solid lines, squares*) and women (*dashed lines, circles*). Serum PTH increased significantly with age (r = 0.30; $P < 0.001$ for the men and women). The curves for serum osteocalcin and urinary NTx were biphasic (see text for details and Table 2 for correlation coefficients).

Relationship among BMD, sex steroids, and bone turnover

Table 3 shows the results of the univariate analyses relating BMD at the various skeletal sites to the sex steroid and DHEAS levels in the men and women. In the men, serum total T levels did not correlate with BMD at any site, except weakly at the radius. In contrast, serum bioavailable T levels were significantly correlated with BMD at all sites in the men. In the women, serum total and bioavailable T levels were equally correlated with BMD at the various sites. Serum total and bioavailable E levels were correlated with BMD at the various sites in the men, although, as for T, the correlations in the men were considerably stronger for bioavailable as opposed to total E. In contrast, serum total and bioavailable E levels were equally correlated with BMD in the women.

TABLE 2. Correlation coefficients for association between age and bone formation and resorption markers among age-stratified samples of Rochester men and women

	Men		Women	
	<50 yr (n = 148)	≥50 yr (n = 198)	Premenopausal (n = 138)	Postmenopausal (n = 166)
PTH	0.18[a]	0.19[b]	0.28[c]	0.15
Bone formation markers				
Osteocalcin	−0.31[c]	0.25[c]	−0.14	0.36[c]
BAP	−0.14	0.13	−0.10	0.13
PICP (log)	−0.30[c]	−0.04	−0.20[a]	0.12
Bone resorption markers				
NTx	−0.34[c]	0.28[c]	−0.27[b]	0.37[c]
f-Dpd	−0.13	0.45[c]	−0.21[a]	0.45[c]

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

TABLE 3. Correlation coefficients for association between BMD and sex steroid levels among age-stratified samples of Rochester men and women

BMD	Total T	Bioavailable T	Total E	Bioavailable E	DHEAS
Total body					
Men	−0.01	0.22[a]	0.16[b]	0.31[a]	0.24[a]
Women	0.22[a]	0.22[a]	0.42[a]	0.42[a]	0.39[a]
Lateral spine					
Men	0.09	0.33[a]	0.20[a]	0.38[a]	0.34[a]
Women	0.27[a]	0.29[a]	0.43[a]	0.45[a]	0.45[a]
Proximal femur					
Men	0.00	0.28[a]	0.19[a]	0.38[a]	0.31[a]
Women	0.25[a]	0.30[a]	0.33[a]	0.38[a]	0.44[a]
Radius					
Men	0.15[b]	0.38[a]	0.15[b]	0.34[a]	0.33[a]
Women	0.27[a]	0.28[a]	0.43[a]	0.45[a]	0.47[a]

[a] $P < 0.001$.
[b] $P < 0.01$.

TABLE 4. Correlation coefficients for association between BMD and serum PTH levels and bone turnover markers among age-stratified samples of Rochester men and women

BMD	PTH	Osteocalcin	BAP	PICP (log)	NTx	f-Dpd
Total body						
Men	−0.08	−0.13[a]	−0.21[b]	−0.06	−0.23[b]	−0.06
Women	−0.19[b]	−0.49[b]	−0.42[b]	−0.10	−0.47[b]	−0.40[b]
Lateral spine						
Men	−0.18[b]	0.05	0.07	0.14[c]	−0.06	−0.14[a]
Women	−0.17[c]	−0.39[b]	−0.31[b]	0.0	−0.39[b]	−0.32[b]
Proximal femur						
Men	−0.16[c]	−0.15[c]	−0.10	0.05	−0.18[c]	−0.12[a]
Women	−0.21[b]	−0.44[b]	−0.32[b]	−0.11	−0.48[b]	−0.32[b]
Radius						
Men	−0.17[c]	−0.17[c]	−0.14[a]	0.00	−0.32[b]	−0.25[b]
Women	−0.21[b]	−0.42[b]	−0.35[b]	−0.11	−0.51[b]	−0.39[b]

[a] $P < 0.05$.
[b] $P < 0.001$.
[c] $P < 0.01$.

Serum DHEAS levels were also correlated with BMD in both sexes.

Table 4 shows the results of the univariate analyses relating BMD at the various sites to serum PTH and the bone turnover markers. Serum PTH was inversely associated with BMD at all sites in men and women, except for total body BMD in the men. All of the bone turnover markers, except for serum PICP, were generally inversely correlated with BMD at the various sites, although the associations between the turnover markers and BMD were much stronger in the women than in the men. Finally, the sex steroids, particularly the bioavailable fractions, were inversely correlated with bone resorption markers ($r = -0.20$ and $r = -0.19$, respectively; both $P < 0.001$ for bioavailable T *vs.* urinary NTx in the men and women; and $r = -0.12$; $P = 0.02$ and $r = -0.29$; $P < 0.001$ for serum bioavailable E *vs.* urinary NTx in the men and women, respectively). Serum osteocalcin was not correlated with bioavailable T in men or women, but did correlate inversely with bioavailable E in the women ($r = -0.26$; $P < 0.001$).

As the associations between sex steroids, PTH, and bone turnover markers were similar at the various skeletal sites, we selected the proximal femur as a representative site to further explore the relationships between BMD and the sex steroids and bone turnover markers as a function of age and menopausal status. As shown in Table 5, serum total T was negatively correlated with proximal femur BMD in the men under age 50 yr. Serum E, particularly bioavailable E levels, were positively correlated with BMD in the younger and in the older men. Serum total and bioavailable T levels were correlated with proximal femur BMD in the pre- and postmenopausal women. Neither serum total nor bioavailable E levels correlated with proximal femur BMD in the premenopausal women, but were strongly associated with BMD in the postmenopausal women. Proximal femur BMD was inversely correlated with bone turnover markers in the postmenopausal women and in the men over age 50 yr, but was only weakly related or had no relationship to the turnover markers in the premenopausal women or men under age 50 yr.

To assess the relative contributions of androgens *vs.* E in determining BMD, multivariate models were constructed in which BMD was the dependent variable, and serum bioavailable T and E, nonbioavailable (*i.e.* SHBG-bound) T and E, and DHEAS were the independent variables. Table 6 shows the results of the analysis at the proximal femur. The results of the analysis were similar in the men under and over age 50 yr and are therefore presented for the group as a whole. However, the relationships were significantly different in the pre- *vs.* postmenopausal women and are therefore presented separately for both groups. In the men, serum bioavailable E was the most important sex steroid predicting proximal femur BMD. After this had entered the model, neither serum T nor DHEAS was an independent predictor. Of note, once bioavailable E had entered the model, nonbioavailable E was negatively associated with proximal femur BMD. In the postmenopausal women, serum bioavailable E was also the most important sex steroid predicting BMD, although DHEAS also entered the model after bioavailable E. In contrast, in the premenopausal women, serum bioavailable T, rather than E, was an independent predictor of BMD.

TABLE 5. Correlation coefficients for associations between proximal femur BMD and sex steroids, PTH, and bone turnover markers among age-stratified samples of Rochester men and women, grouped by age less than or greater than 50 yr (men) and menopausal status (women)

	Men		Women	
	<50 yr	≥50 yr	Premenopausal	Postmenopausal
Total T	−0.17[a]	0.00	0.26[b]	0.16[a]
Bioavailable T	0.11	0.12	0.28[b]	0.25[b]
Total E	0.15	0.19[b]	−0.07	0.39[c]
Bioavailable E	0.26[b]	0.30[c]	0.01	0.43[c]
DHEAS	0.12	0.16[a]	0.18[a]	0.35[c]
PTH	−0.08	−0.10	−0.11	−0.12
Osteocalcin	−0.12	−0.17[a]	−0.08	−0.43[c]
BAP	−0.10	−0.17[a]	−0.05	−0.25[b]
PICP (log)	−0.02	−0.06	−0.09	−0.21[b]
NTx	−0.05	−0.20[b]	−0.17[a]	−0.49[c]
f-Dpd	−0.01	−0.06	−0.10	−0.28[c]

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

TABLE 6. Multiple regression analysis relating proximal femur BMD to sex steroids among age-stratified samples of Rochester men and women

	Predictors (coefficient ± SE × 10^3)		*P* value
Men	Bioavailable E (1.77 ± 0.19)		<0.001
	Non-bioavailable E (−3.64 ± 0.73)		<0.001
	r^2 (%)	20	
Premenopausal women	Bioavailable T (212.05 ± 62.77)		0.001
	r^2 (%)	8	
Postmenopausal women	Bioavailable E (0.94 ± 0.22)		<0.001
	DHEAS (24.55 ± 10.16)		0.017
	r^2 (%)	21	

As the effects of sex steroids on BMD are probably mediated at least in part by changes in bone turnover, we repeated the above multivariate analysis after adjusting for the effects of bone turnover on BMD. Thus, urinary NTx and serum osteocalcin were either allowed to enter or forced into the models, and residual effects of the sex steroids were then examined. In these analyses, even after adjusting for the relationship between bone turnover markers and BMD, serum bioavailable E remained a significant independent predictor of BMD in the men and the postmenopausal women, and bioavailable T remained significant in the premenopausal women (data not shown). The entire analysis was repeated at the other sites in the men and the women, with similar results at the total body and lateral spine in the men and the women and at the distal radius in the women. At the distal radius site in the men, both bioavailable T and E were approximately equally correlated with BMD (Table 3), and which was picked in the multivariate model depended on whether the bone turnover markers were included in the model; in the absence of the bone turnover markers, bioavailable T was picked as the more important predictor of radius BMD, whereas if the bone turnover markers were included, bioavailable E was selected.

Finally, as the above analyses were performed in the entire population-based sample of men and women, we repeated the analyses in the strictly defined normal subjects (see *Materials and Methods*) with the same results in terms of predictors of BMD as those shown in Table 6 for the entire cohort.

Discussion

In women, E deficiency is the major cause of early postmenopausal, and perhaps also the subsequent phase of late postmenopausal, slow bone loss (7, 8, 14). This leads to a conceptual problem in defining the cause of age-related bone loss in men because men do not have a menopause and because serum levels of total T or E decline only minimally with age (15–17). Moreover, previous epidemiological studies have found either no association (17, 20) or even a negative association between serum total T levels and BMD in aging men (21). The latter study did note a positive association between serum E_2 levels and BMD in elderly men (21), but as serum total E levels remain relatively constant over the

life span in men (15), it was difficult to attribute bone loss with aging in men to E deficiency. Thus, with the exception of clearly hypogonadal men, age-related bone loss in men had been attributed principally to factors other than sex steroid deficiency (39).

Our data for serum bioavailable E and T levels may help to resolve these issues. By studying subjects over the broad age range of 23–90 yr and by directly measuring bioavailable T and E levels, we were able to demonstrate that elderly men have marked age-related decreases in both serum bioavailable T and E levels. Moreover, although both serum bioavailable T and E levels were correlated with BMD, serum bioavailable E was found to be the consistent independent predictor of BMD by multivariate analysis. Taken together, our data are consistent with the hypothesis that age-related decreases in E availability could at least in part account for the decrease in BMD with age in men. Our findings are also consistent with those of Greendale *et al.* (40), who recently reported that in elderly men and women, serum bioavailable E was more strongly associated with BMD than was bioavailable T. However, as they only studied elderly subjects, the relationship of sex steroids to age-related decreases in BMD were unclear. Despite these findings, however, further longitudinal and direct intervention studies are clearly needed to quantify the relative contributions of T *vs.* E in determining age-related bone loss in men.

In contrast to women, who have a precipitous decrease in serum E levels around the time of the menopause, the age-related decrease in serum bioavailable T and E levels is much more gradual in aging men. This would suggest that the dramatic decrease in serum E levels at the time of the menopause in women triggers a rapid phase of bone loss, which is absent in men. Indeed, this probably accounts for our observation that the percent decrease in BMD over the life span at all sites was approximately twice as great in women as it was in men. However, men made acutely hypogonadal by orchidectomy also have a rapid phase of bone loss (39); as both T and E levels fall to extremely low levels after orchidectomy, these studies do not address the issue of the relative contributions of T *vs.* E in mediating postorchidectomy bone loss. Nonetheless, the similar relationships between bioavailable E and BMD noted in this study in both men and postmenopausal women suggest a fundamentally similar role for E in determining BMD in both sexes. We also found, however, that E levels did not predict BMD in premenopausal women, suggesting that these women all had E levels above some threshold such that variations in E levels were no longer associated with BMD.

These studies also demonstrate that whereas serum total and bioavailable E levels decrease in women principally because of a decrease in ovarian E production, they decrease in men principally because of an age-related increase in SHBG levels. Indeed, although serum SHBG levels increased markedly in the men, they changed little over the life span in the women. Similar gender differences in SHBG levels with aging have been reported by Goodman-Gruen *et al.* (41) in a study of men and women, aged 50–82 yr. The reasons for this difference in changes in serum SHBG with age between men and women are unclear. However, the biological relevance of the decrease in bioavailable sex steroids in the men is supported by the much stronger inverse correlations in these subjects between bioavailable T and E levels and LH and FSH levels compared with the relationship between total T and E levels and gonadotropin levels.

Our findings are also consistent with previous observations in E receptor-negative (22) and aromatase-deficient males (23–25), which had suggested an important role for E in skeletal metabolism in men. Moreover, in a study of aged male rats, Vanderschueren *et al.* (42) found no differences in the effects of orchidectomy or treatment with the aromatase inhibitor, vorazole, on the decrease in bone density, suggesting that the aromatization of androgens to estrogens was playing a major role in skeletal maintenance in the male rats.

We also assessed serum PTH levels and biochemical markers of bone turnover and found, as we (5–7) and others (43) have previously reported, that there is a significant age-related increase in serum PTH levels in both men and women. Our findings also indicate that bone formation and resorption markers decreased in men between 20 and 50 yr of age and in premenopausal women, probably reflecting higher bone turnover in young individuals in the third decade who are completing the phase of skeletal consolidation. After age 50 yr in the men and in postmenopausal women, however, the bone resorption markers (urinary NTx and f-Dpd) increased significantly with age, as we have shown previously for women (44). Of the bone formation markers, only serum osteocalcin showed a consistent increase with age in both sexes. In general, bone turnover markers showed inverse correlations with BMD in the men and the women, although the relationships were stronger in the women. However, even adjusting for effects of bone turnover, serum bioavailable E levels remained significant predictors of BMD in the men and the postmenopausal women. The persistent relationship between BMD and serum bioavailable E (even after adjusting for the effects of bone turnover) noted in our study may be due to less variability in the bioavailable E measurement than in the measurement of the bone turnover markers. Alternatively, these findings could be due to the fact that E may be affecting both bone resorption and formation, whereas markers assessing these processes separately, such as osteocalcin and NTx, may not have as strong a predictive value. Finally, our data show that circulating total and bioavailable E levels are approximately twice as high in men as those in postmenopausal women. In recent studies, we have demonstrated that reduction of the low levels of serum E in a group of late (mean age, 69 yr) postmenopausal women to near undetectable levels by the administration of letrozole, an aromatase inhibitor that blocks the conversion of weak androgens to E in adipose and other peripheral tissues, increased bone resorption by about 15% (45). Thus, it is likely that the circulating levels of E in aging men have significant effects on bone turnover, although similar studies using aromatase inhibitors are needed to establish a causal relationship between E and bone turnover in aging men as well as the relative contributions of E *vs.* T in determining rates of bone turnover in men.

Our findings may also have practical, clinical implications for the prevention and treatment of osteoporosis in aging men. As noted earlier, we (7, 8) and others (46) have

shown that E treatment of elderly women can prevent or reverse the age-related increase in bone turnover and prevent bone loss (47). As we found that serum bioavailable E levels decreased markedly in aging men and also correlated with BMD, it is plausible that treatment of elderly men with E or selective E receptor modulators, such as raloxifene, may reduce bone turnover and prevent bone loss in aging men. Indeed, a preliminary study by Taxel *et al.* (48) found that E treatment of elderly men (2 mg/day micronized 17β-estradiol) for 9 weeks significantly reduced markers of bone resorption by 11 to 27%. Moreover, as T therapy, in fact, represents combined therapy with T and E (due to the aromatization of T to E), T therapy of aging men may well have significant beneficial effects on BMD and bone turnover, as suggested by several preliminary studies (49–51). Indeed, one recent study of T therapy of eugonadal men with spinal osteoporosis found that the increase in spine BMD after T therapy correlated with the T-induced increase in serum E_2 levels, but not with increases in the serum T levels themselves (51).

Although our data indicate an important role for E in the regulation of skeletal metabolism in men, T clearly also has significant skeletal effects. Osteoblasts contain androgen receptors (52), and T is almost certainly responsible for the sexual dimorphism of the skeleton that develops after puberty and probably also stimulates periosteal growth of cortical bone (53). It is, therefore, of interest that at the distal radius site in the men, bioavailable T and E levels were approximately equally correlated with BMD, and which was picked in the multivariate model depended on whether the bone turnover markers were included in the model. This would suggest that at this predominantly cortical site, bioavailable T may have a greater effect on BMD in the men than at the other sites assessed, although more work is clearly needed to address this issue. Finally, serum bioavailable T was a significant independent predictor of BMD in the premenopausal women, suggesting that when T levels are low, as in this group, variations in these levels are significantly related to BMD. Conversely, when both T and E levels are low (as in the case of postmenopausal women), or when T levels are relatively high but E levels are low (as in the case of men), the variations in E levels are more important predictors of BMD.

In summary, these data indicate that aging men have significant decreases in bioavailable sex steroid levels, which correlate with BMD. Moreover, serum bioavailable E levels predict BMD in men and in postmenopausal women, suggesting fundamentally similar roles for E in skeletal metabolism in both sexes. We also demonstrate very similar changes in serum PTH and bone turnover in aging men and women. Taken together with the recent data from E receptor-negative and aromatase-deficient males (22–25), our findings suggest the need to reevaluate the traditional belief that the effects of sex steroids on the male skeleton are almost entirely due to T. Additional longitudinal studies should be made to compare the effects of T and E on bone and calcium homeostasis in men and to test the hypothesis that E deficiency is a major cause of bone loss in aging men.

Acknowledgments

We thank Ms. Vickie Gathje and Ms. Joan Muhs for their help in recruiting and studying the subjects; Ms. Roberta Soderberg for sample handling; and Ms. Carol A. McAlister for technical assistance.

References

1. **Riggs BL, Melton III LJ.** 1986 Involutional osteoporosis [Review]. N Engl J Med. 314:1676–1686.
2. **Orwoll ES, Klein RF.** 1995 Osteoporosis in men. Endocr Rev. 16:87–116.
3. **Melton III LJ, Riggs BL, eds.** 1995 Osteoporosis. Etiology, diagnosis, and management. In: Epidemiology of fractures, 2nd ed. Philadelphia: Lippincott-Raven; 225–247.
4. **Ray NF, Chan CK, Thamer M, Melton III LJ.** 1997 Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res. 12:24–35.
5. **Delmas PD, Stenner D, Wahner HW, Mann KG, Riggs BL.** 1983 Increase in serum bone gamma-carboxyglutamic acid protein with aging in women. Implications for the mechanism of age-related bone loss. J Clin Invest. 71:1316–1321.
6. **Eastell R, Yergey AL, Vieira NE, Cedel SL, Kumar R, Riggs BL.** 1991 Interrelationship among vitamin D metabolism, true calcium absorption, parathyroid function, and age in women: evidence of an age-related intestinal resistance to 1,25-dihydroxyvitamin D action. J Bone Miner Res. 6:125–132.
7. **Khosla S, Atkinson EJ, Melton III LJ, Riggs BL.** 1997 Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population-based study. J Clin Endocrinol Metab. 82:1522–1527.
8. **McKane WR, Khosla S, Risteli J, Robins SP, Muhs J, Riggs BL.** 1997 Role of estrogen deficiency in pathogenesis of secondary hyperparathyroidism and bone turnover abnormalities in elderly women. Proc Assoc Am Physicians. 109:174–180.
9. **Gallagher JC, Riggs BL, Eisman JA, Hamstra A, Arnaud SB, DeLuca HF.** 1979 Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients: effect of age and dietary calcium. J Clin Invest. 64:729–736.
10. **Gennari C, Agnusdei D, Nardi P, Civitelli R.** 1990 Estrogen preserves a normal intestinal responsiveness to 1,25-dihydroxyvitamin D_3 in oophorectomized women. J Clin Endocrinol Metab. 71:1288–1293.
11. **Nordin BEC, Need AG, Morris HA, Horowitz M, Robertson WG.** 1991 Evidence for a renal calcium leak in postmenopausal women. J Clin Endocrinol Metab. 72:401–407.
12. **McKane WR, Khosla S, Burritt MF, et al.** 1995 Mechanism of renal calcium conservation with estrogen replacement therapy in women in early postmenopause–a clinical research center study. J Clin Endocrinol Metab. 80:3458–3464.
13. **Cosman F, Nieves J, Horton J, Shen V, Lindsay R.** 1994 Effects of estrogen on response to edetic acid infusion in postmenopausal women. J Clin Endocrinol Metab. 78:939–943.
14. **Riggs BL, Khosla S, Melton III LJ.** 1998 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. 13:763–773.
15. **Harman SM, Tsitouras PD.** 1980 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. 51:35–40.
16. **Foresta C, Ruzza G, Mioni R, et al.** 1984 Osteoporosis and decline of gonadal function in the elderly male. Horm Res. 19:18–22.
17. **Meier DE, Orwoll ES, Keenan EJ, Fagerstrom RM.** 1987 Marked decline in trabecular bone mineral content in healthy men with age: lack of association with sex steroid levels. J Am Geriatr Soc. 35:189–197.
18. **Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton III LJ.** 1981 Differential changes in bone mineral density of the appendicular and axial skeleton with aging: relationship to spinal osteoporosis. J Clin Invest. 67:328–335.
19. **Jones G, Nguyen T, Sambrook PN, Kelly PJ, Eisman JA.** 1994 Progressive loss of bone in the femoral neck in elderly people: longitudinal findings from the Dubbo osteoporosis epidemiology study. Br Med J. 309:691–695.
20. **Murphy S, Khaw K-T, Cassidy A, Compston JE.** 1992 Sex steroids and bone mineral density in elderly men. Bone Miner. 20:133–140.
21. **Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC.** 1997 Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest. 100: 1755–1759.
22. **Smith EP, Boyd J, Frank GR, et al.** 1994 Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. N Engl J Med. 331:1056–1061.
23. **Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K.** 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab. 80:3689–3698.
24. **Carani C, Qin K, Simoni M, et al.** 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med. 337:91–95.

25. **Morishima A, Grumbach MM, Bilezikian JP.** 1997 Estrogen markedly increases bone mass in an estrogen deficient young man with aromatase deficiency. J Bone Miner Res. 12(Suppl 1):S126.
26. **Drinka PJ, Olson J, Bauwens S, Voeks S, Carlson I, Wilson M.** 1993 Lack of association between free testosterone and bone density in elderly men. Calcif Tissue Int. 52:67–69.
27. **O'Connor S, Baker HWG, Dulmanis A, Hudson B.** 1973 The measurement of sex steroid binding globulin by differential ammonium sulphate precipitation. J Steroid Biochem. 4:331–339.
28. **Tremblay RR, Dube JY.** 1974 Plasma concentrations of free and non-TeBG bound testosterone in women on oral contraceptives. Contraception. 10:599–605.
29. **Stumpf PG, Nakamura RM, Mishell DR.** 1981 Changes in physiologically free circulating estradiol and testosterone during exposure to levonorgestrel. J Clin Endocrinol Metab. 52:138–143.
30. **Sodergard R, Backstrom T, Shanbhag V, Carstensen H.** 1982 Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem. 16:801–810.
31. **Manni A, Pardridge WM, Cefalu W, et al.** 1985 Bioavailability of albumin-bound testosterone. J Clin Endocrinol Metab. 61:705–710.
32. **Giorgi EP, Moses TF.** 1975 Dissociation of testosterone from plasma protein during superfusion of slices from human prostate. J Endocrinol. 65:279–280.
33. **Pirke KM, Doerr P.** 1974 Age-related changes and interrelationships between plasma testosterone, oestradiol, and testosterone-binding globulin in normal adult males. Acta Endocrinol (Copenh). 74:792–796.
34. **Vermeulen A.** 1991 Androgens in the aging male. J Clin Endocrinol Metab. 73:221–224.
35. **Kurland LT, Molgaard CA.** 1981 The patient record in epidemiology. Sci Am. 245:54–63.
36. **Reid IR, Evans, Ames R, Wattie DJ.** 1991 The influence of osteophytes and aortic calcification on spinal mineral density in postmenopausal women. J Clin Endocrinol Metab. 72:1372–1374.
37. **Kao PC, van Heerden JA, Grant CS, Klee GG, Khosla S.** 1992 Clinical performance of parathyroid hormone immunometric assays. Mayo Clinic Proc. 67:637–645.
38. **Venables WN, Ripley BD.** 1994 Modern applied statistics with S-plus. New York: Springer-Verlag.
39. **Stepan JJ, Lachman M, Zverina J, Pacovsky V, Baylink DJ.** 1989 Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab. 69:523–527.
40. **Greendale GA, Edelstein S, Barrett-Connor E.** 1997 Endogenous sex steroids and bone mineral density in older women and men: The Rancho Bernardo Study. J Bone Miner Res. 12:1833–1843.
41. **Goodman-Gruen D, Barrett-Connor E.** 1996 A prospective study of sex hormone-binding globulin and fatal cardiovascular disease in Rancho Bernardo men and women. J Clin Endocrinol Metab. 81:2999–3003.
42. **Vanderschueren D, Van Herck E, Nijs J, Ederveen AGH, De Coster R, Bouillon R.** 1997 Aromatase inhibition impairs skeletal modeling and decreases bone mineral density in growing male rats. Endocrinology. 138:2301–2307.
43. **Epstein S, Bryce G, Hinman JW, et al.** 1986 The influence of age on bone mineral regulating hormones. Bone. 7:421–425.
44. **Melton III LJ, Khosla S, Atkinson EJ, O'Fallon WM, Riggs BL.** 1997 Relationship of bone turnover to bone density and fractures. J Bone Miner Res. 12:1083–1091.
45. **Heshmati HM, Khosla S, Robins SP, Geller N, McAlister CA, Riggs BL.** 1997 Endogenous residual estrogen levels determine bone resorption even in late postmenopausal women. J Bone Miner Res. 12(Suppl 1):S121.
46. **Prestwood KM, Pilbeam CC, Burleson JA, et al.** 1994 The short term effects of conjugated estrogen on bone turnover in older women. J Clin Endocrinol Metab. 79:366–371.
47. **Lufkin EG, Wahner HW, O'Fallon WM, et al.** 1992 Treatment of postmenopausal osteoporosis with transdermal estrogen [see comments]. Ann Intern Med. 117:1–9.
48. **Taxel P, Raisz LG.** 1997 The effect of estrogen therapy on older men with low bone mass. J Bone Miner Res. 12(Suppl 1):S353.
49. **Tenover JS.** 1992 Effects of testosterone supplementation in the aging male. J Clin Endocrinol Metab. 75:1092–1098.
50. **Anderson FH, Francis RM, Faulkner K.** 1996 Androgen supplementation in eugonadal men with osteoporosis: effects of six months' treatment on bone mineral density and cardiovascular risk factors. Bone. 18:171–177.
51. **Anderson FH, Francis RM, Peaston RT, Wastell HJ.** 1997 Androgen supplementation in eugonadal men with osteoporosis: effects of six months' treatment on markers of bone formation and resorption. J Bone Miner Res. 12:472–478.
52. **Colvard DS, Eriksen EF, Keeting PE, et al.** 1989 Identification of androgen receptors in normal human osteoblast-like cells. Proc Natl Acad Sci USA. 86:854–857.
53. **Turner RT, Wakley GK, Hannon KS.** 1990 Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res. 8:612–617.