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Longitudinal Association between Sex Hormone Levels, Bone Loss, and Bone Turnover in Elderly Men

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Male osteoporosis is an increasingly important health problem. It is known that sex steroid hormones play an important role in regulating bone turnover and bone mass in males as well as in females. However, the exact mechanism of bone loss in men remains unknown. In the present study, 200 elderly men (age range, 55–85 yr) were followed for 4 yr to evaluate the relationships between hormone levels, bone turnover markers, bone mineral density, and rates of bone loss. Femoral and lumbar bone mineral density, bone ultrasound parameters at the os calcis, serum testosterone (T), serum estradiol (E_2), SHBG levels, and bone turnover markers (urinary crosslaps and bone alkaline phosphatase) were evaluated for each man at enrollment and 4 yr afterward. The free androgen index (FAI) and free estrogen index (FEI) as well as measures of the bioavailable sex hormones [calculated bioavailable E_2 (c-bioE_2) and T (c-bioT)] were calculated from total hormone levels and SHBG. In the total population, T, c-bioT, c-bioE_2, FAI, and FEI, but not E_2, decreased significantly with age, whereas SHBG increased significantly. Subjects with FEI, c-bioE_2, and E_2 levels below the median showed higher rates of bone loss at the lumbar spine and the femoral neck as well as higher speed-of-sounds decrease at the calcaneus with respect to men with FEI, c-bioE_2, and E_2 levels above the median. Serum bone alkaline phosphatase and urinary crosslaps were significantly higher in men with FEI, c-bioE_2, and E_2 in the lower quartile than in men with FEI, c-bioE_2, and E_2 levels in the higher quartile. No statistically significant differences were observed in relation to T, c-bioT, or FAI levels. Finally, the ratio between E_2 and T, an indirect measure for aromatase activity, increased significantly with age and was higher in normal than in osteoporotic subjects. In conclusion, results from the present study indicate an important role of estrogens, and particularly of the ability to aromatize T to E_2, in the regulation of bone loss and bone metabolism in elderly men. (*J Clin Endocrinol Metab* 88: 5327–5333, 2003)

RECENT EPIDEMIOLOGICAL STUDIES pointed out that male osteoporosis is an increasingly important health problem. A 50-yr-old man has about a 6% risk of hip fracture and a 16–25% risk of any osteoporotic fracture in his remaining life (1). Although the exact incidence and gender ratio varies from country to country, it has been estimated that about one third of all fractures occur in men (2, 3). With the increase in life expectancy, the number of elderly men will increase dramatically in the years to come, and the number of fractures in men is expected to double by 2025 (4). Moreover, the mortality rate after hip fracture is even higher in men than in women (5).

Despite the considerable public health burden attributable to male osteoporotic fractures, the exact mechanism regulating bone mass and bone loss in men remains unknown. Several hormonal and biochemical factors known to affect bone metabolism in women have been shown to change with age in men and have been supposed to influence the age-related decline of bone mineral density (BMD) also in males (6–9). Evidence from cross-sectional studies suggested that declining levels of sex hormones, IGF-I, and 25-OH vitamin D, together with a parallel increase in SHBG and PTH may contribute to age-related bone loss in elderly men (9, 10). In particular, several clinical and experimental observations indicated estrogen as the dominant sex steroid regulating bone metabolism in men (11), and in cross-sectional analysis, estradiol (E_2) levels appeared to correlate better with BMD than circulating testosterone (T) (12–17). However, evidence from longitudinal studies is scarce. In a preliminary observation for an average of 2.1 yr, Slemenda *et al.* (12) described lower E_2 levels in men loosing BMD at more than 1% per year than in those losing BMD at less than 1% per year. In a more recent 4-yr study by Khosla *et al.* (18), elderly men with bioavailable E_2 levels below the median value of 40 pmol/liter showed higher rates of bone loss at the midradius and -ulna than men with bioavailable E_2 levels above the median. Other additional longitudinal studies in larger and ethnically different male populations are needed to further address this issue.

The aim of this study was to longitudinally evaluate the biological interactions among sex hormones, SHBG, and dehydroepiandrosterone sulfate (DHEAS), as well as their role in the regulation of bone turnover and bone loss in a sample of elderly Italian men.

Subjects and Methods

Subjects

Patients eligible for the study were 500 elderly men living in the area of Siena, Italy, contacted by direct mailing, with an age greater than 55

Abbreviations: aBMD, Areal bone mineral density; ANCOVA, analysis of covariance; BALP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; BUA, broadband ultrasound attenuation; c-bioE_2, calculated bioavailable estradiol; c-bioT, calculated bioavailable testosterone; c-fE_2, calculated free estradiol; c-fT, calculated free testosterone; CTX, crosslaps; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; DXA, dual-energy x-ray absorptiometry; E_2, estradiol; FAI, free androgen index; FEI, free estrogen index; QUS, quantitative ultrasound; T, testosterone; vBMAD, volumetric bone mineral apparent density.

* We dedicate this work to the memory of C.G.

yr. Among them, 364 men (age range, between 55 and 85 yr) agreed to participate. From this total cohort of 364 men, 64 men were excluded from the study because of malignancy (*i.e.* prostate cancer), Paget's disease of bone, clinical hypogonadism, malabsorption due to gastrointestinal disorders, or their use of a drug potentially affecting bone metabolism. At the end of the enrollment, 300 men were included in the study, and 200 completed the 4-yr follow-up. Informed written consent was obtained from all participants, and the study was approved by the Institutional Review Board of Siena Medical Center. The age range of the studied men was 55–88 yr, with a mean (±SEM) age of 64.8 ± 0.8 yr. For all subjects, a detailed medical history was obtained, and dietary calcium intake was assessed by a sequential self-questionnaire including foods that account for the majority of calcium in the diet. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. General baseline characteristics of the population are presented in Table 1.

Bone mass measurement

Areal BMD (aBMD; grams per square centimeter) was determined for lumbar spine (L2–L4) and proximal femur by dual-energy x-ray absorptiometry (DXA) using the Hologic 4500 instrument (Hologic, Waltham, MA) with coefficients of variation (CVs) of 0.9 and 1.9%, respectively. Broadband ultrasound attenuation (BUA), speed of sounds (SOS), and stiffness index at the os calcis were also evaluated (Achilles, Lunar, Madison, WI). The CVs were 2.5% for BUA and 0.5% for SOS. DXA and calcaneal measurements were repeated after 4 yr. Volumetric bone mineral apparent density (vBMAD; grams per cubic centimeter) was calculated from densitometric data as previously described (19), using the following formulae: femoral neck vBMAD = BMC/A^2, and lumbar spine vBMAD = $BMC/A^{3/2}$, where BMC is the bone mineral content and A is the projected bone area. Although the World Health Organization's definition of osteoporosis has been established only for women, for the purpose of this study, we defined osteoporosis by either a lumbar spine or femoral neck aBMD value of at least 2.5 SDs below the mean for young normal white males.

Radiographs of the lumbar spine were evaluated for the presence of osteophytosis and facet joint osteoarthritis according to the methods of Orwoll *et al.* (20) and Masud *et al.* (21), respectively.

Laboratory analysis

A 24-h urine collection and fasting serum samples obtained between 0800 and 0900 h were stored at −70 C until assayed. Serum concentrations of calcium, phosphate, bone alkaline phosphatase (BALP; Tandem-R Ostase, Beckman Coulter, Inc., Fullerton, CA; interassay CV < 8.1%) and urinary type I collagen C-telopeptides [urinary crosslaps (CTX); α-CrossLaps RIA, Osteometer Biotech A/S, Copenhagen, Denmark; interassay CV < 6.5%] were evaluated at baseline and after 4 yr. In addition to the above-mentioned markers of bone turnover, the following baseline hormones were evaluated: total E_2 (RIA, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 11 pmol/liter; interassay CV < 7.9%), total T (RIA, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 0.18 nmol/liter; interassay CV < 10.3%); SHBG (SHBG RIA, BIOCODE S.A., Liege, Belgium; sensitivity, 0.8 nmol/liter; interassay CV < 8.0%), DHEAS (R-DHEA-S CTK RIA, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 0.08 μmol/liter; interassay CV < 8.5%), LH (LH-CTK-4 RIA, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 0.2 IU/liter; interassay CV < 3.7%), FSH (FSH-CTK-4 RIA, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 0.2 IU/liter; interassay CV < 4.5%), IGF-I (IGF-I IRMA, Immunotech, France; sensitivity, 3 mg/ml; interassay CV < 7.4%), and 25-OH vitamin D (25-Hydroxyvitamin D ^{125}I RIA kit, Diasorin Diagnostics, Saluggia, Italy; sensitivity, 1.5 ng/ml; interassay CV < 11%). Measurements of circulating E_2, T, and SHBG levels were repeated after 4 yr. The free androgen index (FAI) and free estrogen index (FEI) were calculated as the ratios between total hormone levels and SHBG. As adjunctive measures of biologically active sex hormone values, calculated free T (c-fT), non-SHBG-bound T [calculated bioavailable T (c-bioT)], calculated free E_2 (c-fE_2), and non-SHBG-bound E_2 [calculated bioavailable E_2 (c-bioE_2)] were calculated according to the method described by Vermeulen *et al.* (22) and Van den Beld *et al.* (23), taking the concentration of T, E_2, and SHBG into account, and assuming a fixed albumin concentration of 45 g/liter.

TABLE 1. General baseline characteristics (mean ± SD and ±95% CI) of the study population

	Mean ± SD	±95% CI
Age (yr)	64.8 ± 0.8	63.5–65.8
Weight (kg)	74.5 ± 0.7	73.2–76.1
Height (cm)	170.7 ± 0.3	169.8–171.6
BMI (kg/m^2)	25.6 ± 0.2	25.2–26.1
Calcium intake (mg/d)	824 ± 28	766–876
Alcohol intake (mg/d)	24 ± 0.5	23–25
Current smokers (%)	41	

CI, Confidence interval.

Statistical analysis

Correlations between the variables were evaluated using Pearson's simple and partial correlation coefficients. ANOVA and analysis of covariance (ANCOVA) were used to analyze the effects of sex hormone quartiles on BMD at different sites, quantitative ultrasound (QUS) parameters, rates of bone loss, and levels of bone biochemical markers. The following covariates were considered for the ANCOVA analysis: age, BMI, calcium intake, smoking status, and alcohol use. The Bonferroni pairwise multiple-comparisons test was used as *post hoc* test to evaluate the difference in bone parameters between a single quartile and each of the other quartiles. A value of $P < 0.05$ was accepted as the value of significance. All statistical tests were two-sided and were performed by using Statistica 5.1 (Statsoft, Inc., Tulsa, OK) and SPSS software for Windows, version 10.0 (SPSS Ltd., Chicago, IL).

Results

Correlations of hormones with age and BMI

The correlation coefficients between baseline sex hormones and age or BMI are reported in Table 2. In the total population, T and DHEAS levels decreased with age (r = −0.23, $P < 0.001$; r = −0.24, $P < 0.001$, respectively), whereas SHBG (r = 0.45; $P < 0.0001$), LH (r = 0.35; $P < 0.0001$), and FSH (r = 0.28; $P < 0.0001$) increased significantly. E_2 and IGF-I levels did not significantly vary by age. By contrast, the FAI and the FEI significantly decreased with age (r = −0.41, $P < 0.0001$; r = −0.23, $P < 0.001$, respectively). A similar age-related decrease in c-fT (r = −0.40; $P < 0.0001$), c-bioT (r = −0.43; $P < 0.0001$), c-f E_2 (r = −0.23; $P < 0.001$), and c-bioE_2 (r = −0.24; $P < 0.001$) concentrations was observed. Circulating E_2 positively correlated with BMI (r = 0.20; $P <$

TABLE 2. Simple correlation coefficients between baseline hormones and age or BMI

Hormone	Age	BMI
Total T	−0.23[a]	−0.12
Total E_2	0.08	0.20[b]
DHEAS	−0.24[a]	0.10
SHBG	0.45[c]	−0.17[d]
IGF-I	−0.02	0.06
LH	0.35[c]	−0.16[d]
FSH	0.28[c]	−0.15[d]
FAI	−0.41[c]	−0.07
FEI	−0.23[a]	0.22[b]
c-fT	−0.40[c]	−0.10
c-fE_2	−0.23[a]	0.20[b]
c-bioT	−0.43[c]	−0.09
c-bioE_2	−0.24[a]	0.22[b]

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.0001$.
[d] $P < 0.05$.

0.01) and total T (r = 0.32; $P < 0.0001$). The correlation between age and BMI was weak (r = 0.09) and not statistically significant.

Results from the 4-yr longitudinal analysis confirmed the age-related decrease in serum T levels (−2.7% per year) and the increase in SHBG levels (+3% per year), whereas E_2 concentrations after 4 yr did not significantly differ from those at baseline.

Correlations of sex hormones with BMD and QUS

Baseline aBMD at the femoral neck and lumbar spine were positively correlated with E_2 (r = 0.16; $P < 0.05$), FEI (r = 0.20; $P < 0.01$), c-fE_2 (r = 0.16; $P < 0.01$), and c-bioE_2 (r = 0.20; $P < 0.01$) levels, but not with T, FAI, or the calculated measures of the biologically active T levels (c-fT and c-bioT). Because at all sites BMD decreased with age and increased with BMI, the correlation between baseline bone mass measurements and sex hormones was adjusted for age and BMI. Adjusted aBMD at femoral sites was significantly correlated with the concentrations of E_2, FEI, c-fE_2, and c-bioE_2, but not with total T, FAI, c-fT, or c-bioT levels (Table 3). A similar positive association between estrogen measurements and lumbar aBMD was observed in men without severe osteoarthritis (Table 3). Among QUS parameters, only SOS was positively correlated with FEI, c-fE_2, c-bioE_2, and E_2 levels (Table 3).

To further evaluate the association between estrogen and bone mass, we compared average aBMD and QUS parameters in men grouped according to FEI quartiles (median, 2.4 pmol/nmol; lower quartile, <0.98 pmol/nmol; upper quartile, >3.9 pmol/nmol). At all BMD sites, men in the low FEI quartile had the lowest mean aBMD or QUS values, and those in the highest quartile had the highest mean aBMD and QUS values. This trend was statistically significant at the lumbar spine and the femoral neck (Fig. 1). Although the same trend was seen at the calcaneus, it was not statistically significant. Compared with the low FEI quartile, the mean aBMD in the highest FEI quartile were 11, 6, and 12% higher at the lumbar spine, femoral neck, and ward's triangle, respectively. The corresponding differences in vBMAD were 10 and 8% at the spine and the hip, respectively (Fig. 2). When the analyses were repeated, grouping the men according to either c-fE_2 or c-bio E_2 levels, the results were comparable with those obtained using the FEI.

Association of sex hormones with bone loss and bone turnover

The correlation coefficients between sex hormones and rates of change in aBMD or QUS at the calcaneus are shown on Table 4. As is evident, the decline in spinal aBMD after exclusion of subjects with severe lumbar osteoarthritis was positively correlated to FEI, c-fE_2, c-bioE_2, and E_2 levels, but not to T, FAI, c-fT, and c-bioT. Bone loss at the femoral sites was significantly correlated to FEI, c-fE_2, and c-bioE_2 levels, but not to E_2, T, FAI, c-fT, and c-bioT. The strongest correlations were seen with FEI and c-bioE_2 levels. A similar positive association was observed between FEI and SOS loss at the calcaneus, whereas the correlation between FEI and BUA or stiffness loss did not reach statistical significance. No correlations were found between rates of change in calcaneal ultrasound parameters and, respectively, E_2, c-f E_2, c-bio E_2, T, c-fT, c-bioT, or FAI levels. Serum levels of IGF-I and DHEAS did not correlate with QUS or aBMD loss at any site.

Because FEI appeared to be the most consistent predictor of rates of BMD and QUS loss, we further examined its relationship to these variables by grouping the recruited subjects according to FEI quartiles. Interestingly, men in the lower FEI quartile showed higher rates of bone loss at the lumbar spine and the femoral neck with respect to those in the higher quartiles (Fig. 3). A similar, although not significant, trend was observed for SOS but not BUA measured at

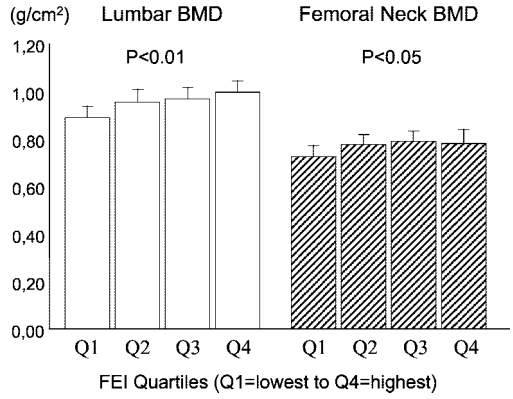

FIG. 1. Average lumbar and femoral aBMD values in elderly men by quartiles of FEI.

TABLE 3. Age and BMI adjusted partial correlation coefficients between bone mass measurements and sex steroid hormones

	Total T	FAI	c-fT	c-bioT	Total E_2	FEI	c-fE_2	c-bioE_2
Spine without OA	−0.13	−0.06	0.04	0.03	0.17[a]	0.19[b]	0.18[b]	0.19[b]
Hip								
Femoral neck	−0.09	−0.03	0.09	0.10	0.16[a]	0.20[b]	0.18[b]	0.19[b]
Trochanter	−0.12	0.02	−0.03	0.04	0.15[a]	0.24[c]	0.22[b]	0.23[c]
Total hip	−0.13	0.02	0.09	0.11	0.15[a]	0.27[d]	0.24[c]	0.25[c]
Ward's triangle	−0.07	0.04	0.03	0.05	0.09	0.22[b]	0.19[b]	0.21[b]
Calcaneus								
BUA	−0.04	0.06	0.03	0.04	0.05	0.06	0.04	0.07
SOS	0.02	−0.01	0.06	0.08	0.14[a]	0.15[a]	0.14[a]	0.14[a]
Stiffness	−0.01	0.08	0.04	0.05	0.10	0.10	0.09	0.10

OA, Osteoarthritis.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.0001$.

the calcaneus. As shown in Fig. 3, no significant differences in aBMD loss were observed between subjects grouped in the two lower quartiles of FEI (Q1 and Q2, below the median) as well as between those men in the upper two quartiles (Q3 and Q4, above the median), suggesting the possibility of a threshold level of FEI corresponding approximately to the median value. Similar but less significant results were obtained when total E_2, c-fE_2, and c-bioE_2 levels were considered. By contrast, no statistically significant differences were observed when subjects were ranked according to T, FAI, c-fT, or c-bioT quartiles.

Consistent with the results on rates of bone loss, we observed a negative correlation between FEI levels and urinary CTX or serum BALP ($r = -0.17$, $P < 0.05$; $r = -0.19$, $P < 0.01$, respectively). Moreover, men with FEI in the lower quartile showed increased urinary CTX levels compared with those of men with FEI in the upper two quartiles (Fig. 4A). Similarly, BALP levels were significantly higher and increased significantly after 4 yr in men with FEI in the lower quartile with respect to men with the FEI in the higher quartile (Fig. 4B). Comparable results were obtained when analyses were performed considering total E_2, c-fE_2, and c-bioE_2 levels.

Sex hormone levels in osteoporotic vs. nonosteoporotic males

Of the 200 men, 66 (33%) were osteoporotic according to the −2.5 SD cutoff value at either the lumbar spine and/or the femoral neck. Clinical characteristics of osteoporotic and nonosteoporotic subjects are shown on Table 5. Age and height did not significantly differ between the two groups, whereas the weight and the average daily intake of calcium were higher in nonosteoporotic compared with osteoporotic subjects. Of interest, the FEI and the c-bioE_2 were significantly lower in osteoporotic subjects with respect to nonosteoporotic subjects. A similar, although not significant, trend was observed for E_2 and c-fE_2 levels, whereas T, c-fT, c-bioT, and SHBG were slightly but not significantly higher in the osteoporotic group. Moreover, the ratio between E_2 and T, an indirect measure for aromatase activity was significantly lower in osteoporotic than in normal subjects. Notably, a significant age-related increase in this ratio was also observed ($r = 0.19$; $P < 0.01$), suggesting that the ability to aromatize androgens into estrogen may be enhanced with aging. The difference in the E_2/T ratio between osteoporotic and nonosteoporotic men remained statistically significant after adjusting for BMI or body weight ($P < 0.05$, ANCOVA).

Discussion

Sex steroid hormones are important regulators of bone physiology and marked alteration in their levels represent a major factor in the pathogenesis of osteoporosis. Although the importance of estrogen in maintaining bone mass in women is firmly established (24), the relative contributions of estrogen *vs.* androgens in regulating male skeletal homeostasis remain unclear. Androgens are the dominant sex steroids secreted in males and have long been assumed to be critical for skeletal maintenance in men (25). However, in the past few years, several clinical and experimental observations indicated a major and likely dominant role of estrogens in the regulation of bone mineralization in the male, sug-

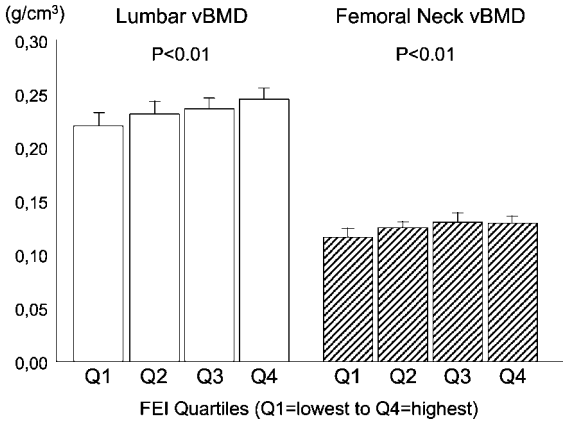

FIG. 2. Average lumbar and femoral vBMAD values in elderly men by quartiles of FEI.

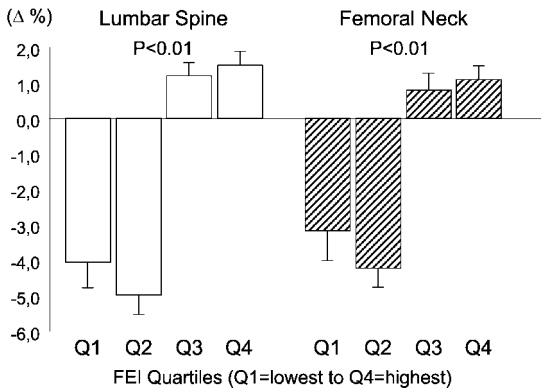

FIG. 3. Rates of aBMD loss (percentage per 4 yr) in elderly men grouped according to FEI quartiles.

TABLE 4. Simple correlation coefficients between baseline hormones and rates of bone loss

	Total T	FAI	c-fT	c-bioT	Total E_2	FEI	c-fE_2	c-bioE_2
LS-BMD loss[a]	0.11	0.10	0.11	0.12	0.20[b]	0.24[c]	0.19[b]	0.24[c]
FN-BMD loss	−0.09	−0.05	0.02	0.07	0.13	0.16[d]	0.14[d]	0.17[d]
WT-BMD loss	−0.13	−0.05	−0.08	−0.06	0.13	0.14[d]	0.12	0.14[d]
SOS loss	−0.04	−0.02	−0.03	−0.02	0.10	0.16[d]	0.12	0.13
BUA loss	−0.06	−0.10	−0.06	−0.04	0.06	0.12	0.09	0.10
Stiffness loss	−0.05	−0.08	−0.04	−0.03	0.10	0.13	0.10	0.12

LS, Lumbar spine; FN, femoral neck; WT, Ward's triangle.
[a] Subjects with severe lumbar osteoarthritis were excluded from analysis.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.05$.

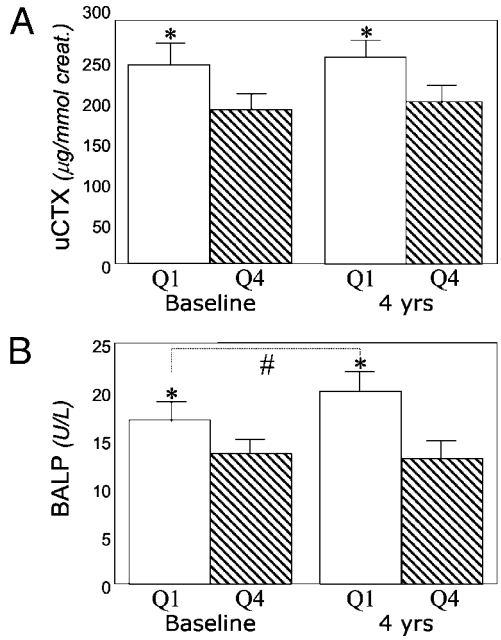

FIG. 4. Bone turnover markers at baseline and after 4 yr in subjects in the low (Q1) *vs.* high (Q4) FEI quartiles. A, uCTX, urinary α-CTX (*, $P < 0.05$, Q1 *vs.* Q4). B, BALP (*, $P < 0.05$, Q1 *vs.* Q4; #, $P < 0.05$, baseline BALP *vs.* 4-yr BALP).

TABLE 5. Clinical characteristics and hormone levels (mean ± SEM) in osteoporotic and nonosteoporotic men

Characteristic	Osteoporotics (n = 66)	Nonosteoporotics (n = 134)
Age (yr)	63.4 ± 1.3	65.5 ± 0.7
Weight (kg)	70.7 ± 1.1	76.5 ± 0.9[a]
Height (cm)	170.4 ± 0.9	170.9 ± 0.5
BMI (kg/m^2)	24.4 ± 0.4	26.2 ± 0.3[a]
E_2 (pmol/liter)	96.5 ± 5.5	108.9 ± 4.0
T (nmol/liter)	15.0 ± 0.9	14.0 ± 0.5
DHEAS (μg/ml)	0.467 ± 0.05	0.470 ± 0.04
SHBG (nmol/liter)	42.4 ± 2.9	39.5 ± 1.8
IGF-I (ng/ml)	123.4 ± 16	126.6 ± 6
25-OH Vitamin D (ng/ml)	25.07 ± 1.2	26.5 ± 0.9
FEI (pmol/nmol)	2.71 ± 0.2	3.10 ± 0.1[b]
FAI (nmol/nmol)	0.450 ± 0.05	0.431 ± 0.02
c-fT (nmol/liter)	0.234 ± 0.02	0.226 ± 0.02
c-fE_2 (pmol/liter)	2.41 ± 0.13	2.5 ± 0.09
c-bioT (nmol/liter)	6.2 ± 0.5	6.0 ± 0.3
c-bioE_2 (nmol/liter)	0.072 ± 0.004	0.088 ± 0.003[b]
E_2/T ratio (pmol/nmol)	7.05 ± 0.5	8.50 ± 0.3[a]

[a] $P < 0.01$, ANOVA nonosteoporotics *vs.* osteoporotics.
[b] $P < 0.05$, ANOVA nonosteoporotics *vs.* osteoporotics.

gesting that the skeletal effects of T are, at least in part, mediated by its aromatization into E_2 (11). Unfused epiphyses, higher rates of bone loss, and severe osteoporosis were described in a young men with an inactivating mutation in the estrogen receptor-α gene (26) as well as in males with homozygous mutations in the aromatase gene (27–30). A similar, albeit less striking, osteopenic phenotype was noted in mouse knockout models of either the estrogen receptor-α and aromatase genes (31–34). Taken together, these observations clearly demonstrated that estrogen is necessary for the acquisition of peak BMD and the consolidation of growth cartilages in young men, but its role in regulating bone loss in elderly men is not as clear. Although cross-sectional studies in aged males suggested a correlation of circulating estrogens with bone mass and bone turnover (12–17), evidence from longitudinal studies is confined to a single observation showing a positive association between bioavailable E_2 levels and rates of bone loss at the forearm (18). Indeed, in a previous study, Slemenda *et al.* (12) examined the role of sex steroids on bone mass in a group of elderly men followed longitudinally for an average of 2.1 yr. However, as pointed out by the authors, the study addressed bone loss only in a preliminary manner because of the relatively short period of follow-up (12). Due to the greater noise/signal ratio in rates of bone loss in that relatively short period, the longitudinal analysis was limited to the comparison of men losing bone density at more than 1% per year *vs.* those losing BMD at less than 1% per year and suggested a significant role of estrogen in maintaining bone mass. The data presented here confirm and extend these findings, indicating estrogen as the main steroid regulating bone loss at multiple skeletal sites in elderly men.

Consistent with previous observations (6, 7, 13), in our cohort, the concentrations of total T, DHEAS, FEI, and FAI, as well as all of the calculated measures of the biologically active sex hormone levels decreased significantly with age, whereas those of total E_2 remained stable. The decrease of FEI and c-bioE_2, as index of the biologically active fraction of E_2, may result from the increase in SHBG and the decrease in bioavailable androgens, which are a substrate for peripheral aromatization. Interestingly, FEI, c-f E_2, and c-bioE_2 concentrations were higher among men losing bone mass at slower rates. Subjects with bioavailable estrogen measures in the lowest quartile showed lower baseline BMD and QUS values and higher rates of bone loss at the lumbar spine and the femoral neck compared with those men in the highest quartile. Importantly, weak differences in bone loss were observed between subjects grouped in the two lower quartiles as well as between those men in the upper two quartiles, suggesting the possibility of a threshold level of bioavailable estrogen to suppress bone resorption, corresponding approximately to the median value (FEI, 2.4 pmol/nmol). This observation is consistent with the results of the previous longitudinal study by Khosla *et al.* (18) showing that elderly men with a measured bioavailable E_2 level of less than 40 pmol/liter (which corresponds to a total E_2 level of 114 pmol/liter) are at greatest risk for increases in bone resorption and bone loss, whereas men with bioavailable E_2 levels above that value are relatively protected against bone loss. On the basis of the average SHBG levels in our population, the estimated total E_2 level required to achieve a FEI level of 2.4 pmol/nmol was 98 pmol/liter, which is quite similar to the threshold value reported by Khosla *et al.*, given the different assay methods used. Together with the findings of a recent cross-sectional study (13) showing significantly lower BMD at multiple sites in men in the lowest quartile for bioavailable E_2 levels (<53 pmol/liter) compared with men in the upper three quartiles, these results are consistent with the hypothesis of a threshold level of non-SHBG-bound E_2 for skeletal sufficiency in the elderly male.

We also found an inverse association between bioavailable

estrogen levels and urinary CTX, in keeping with evidence from some recent interventional studies that directly tested the relative contributions of estrogen *vs.* androgens in preventing the increase in bone resorption after the induction of hypogonadism and aromatase inhibition (35–37). Subjects in the lower FEI quartiles showed increased urinary CTX levels with respect to those in the upper quartile. Thus, estrogen appears to play a dominant role in regulating bone resorption in elderly men. The observed parallel increase in BALP among subjects in the lower FEI quartile may reflect the increase in bone turnover.

Of interest, our finding of lower FEI and c-bioE_2 concentrations levels in the group of subjects with osteoporosis, compared with those with normal BMD values, provides additional evidence for the importance of estrogen in regulating bone metabolism in elderly males. Notably, we also observed a lower E_2/T ratio in osteoporotic than in nonosteoporotic men, suggesting an impairment of aromatase activity as a possible etiopathogenetic factor for osteoporosis in elderly men. Indeed, a likely explanation for the higher E_2/T ratio observed in nonosteoporotic subjects could be linked to the higher BMI of these subjects and thus to an increased aromatization of androgens into estrogen by adipose tissue. However, the difference in E_2/T ratio between osteoporotic and nonosteoporotic men remained statistically significant after adjusting for BMI or body weight, indicating an additive influence on peripheral aromatase activity, independent from the amount of body fat. Of interest, both aromatase activity and amounts of aromatase mRNA in bone have been shown to vary widely among subjects (38, 39), and this variation could indicate a genetic component. These findings are consistent with our preliminary observation of genetically determined differences in aromatase activity due to a TTTA repeat polymorphism in the *CYP19* aromatase gene (40). Male subjects with a high *CYP19* TTTA repeat genotype showed a more efficient aromatase activity both *in vivo* and *in vitro*, with higher estrogen production that was protective for bone loss, particularly at trabecular sites (40, 41). These individual differences in aromatase efficiency may be a more important determinant than the circulating androgen level of whether a man will become osteoporotic and may explain the lack of correlation between bone loss and T levels. By contrast, the observed correlation between bone loss and circulating bioavailable and total estrogen levels also suggest that, although local aromatization of androgens to estrogen in bone cells may contribute significantly to skeletal homeostasis (42), a minimum circulating level of E_2 derived from peripheral nonskeletal aromatization is necessary to prevent bone loss.

Our study has some important limitations. First, the calculated bioavailable indexes of either T or E_2 concentrations are indirect measurements for free bioavailable estrogen and androgens, although widely used and validated in previous studies (22, 23). Moreover, results from a previous longitudinal observation showed a similar correlation of FEI and measured bioavailable E_2 with bone turnover and bone loss (18). Thus, for the purpose of the present study, FEI as well as c-bioE_2 concentrations may be considered as a reasonable surrogate for bioavailable E_2. Second, because the results obtained from DXA are a combination of effects on trabecular and cortical bone and express aBMD, but not volumetric BMD, the present study did not allow exploration of the supposed role of androgens in regulating periosteal apposition and bone size (25). Peripheral-quantitative computed tomography or histomorphometric studies should be required to address this issue and to better define the direct role of androgens on bone. Finally, the proportion of men with osteoporosis in our population was higher than expected and could indicate a disease-based selection bias, with the osteoporotic men responding at a higher rate than healthy men (the average ± SD T scores were -1.6 ± 1.0 and -1.4 ± 1.3 at the femoral neck and the lumbar spine, respectively). Moreover, 200 of the 300 recruited subjects completed the 4-yr follow-up. The analysis of the baseline DXA scan of the 100 drop-out subjects showed the presence of osteoporosis only in 3 (3%), with the remaining 97 men being nonosteoporotic. Thus, the men who were osteoporotic at the baseline visit were more interested in continuing the follow-up examinations than those who were nonosteoporotic. When we considered all of the 300 recruited men, the proportion of osteoporotic subjects was lower. An additive explanation for the higher proportion of osteoporotic men is represented by the low physical activity and by the relatively low calcium intake (800 mg/d *vs.* the recommended optimal intake of 1000 mg/d) of the recruited population.

In conclusion, results from the present study show that FEI and c-bioE_2 concentrations, as indirect measures for bioavailable E_2, are the most consistent predictor of bone turnover and bone loss in elderly men and suggest that peripheral aromatization of T into E_2 plays a significant role in the regulation of bone metabolism in elderly males. Further studies are needed on the diagnostic and potentially therapeutic role of estrogen on bone metabolism and fracture incidence in men.

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