The Effects of Serum Testosterone, Estradiol, and Sex Hormone Binding Globulin Levels on Fracture Risk in Older Men

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Context: The relationship between sex steroids and fracture is poorly understood.

Objective: The objective of the study was to examine associations between nonvertebral fracture risk and bioavailable estradiol (bioE2), bioavailable testosterone (bioT), and SHBG.

Design: This was a case-cohort study.

Setting: The Osteoporotic Fractures in Men Study (MrOS) was conducted in a prospective U.S. cohort in 5995 community-dwelling men 65 yr old or older.

Participants: Participants included a subcohort of 1436 randomly chosen white men plus all 446 minorities and all those with incident hip and other nonvertebral fractures.

Main Outcome Measures: Baseline testosterone and estradiol were measured by mass spectrometry (MS) and SHBG by RIA.

Results: Men with the lowest bioE2 (<11.4 pg/ml) or highest SHBG (>59.1 nM) had greater risk of all nonvertebral fractures [adjusted hazard ratio (HR) [95% confidence interval]: 1.5 (1.2–1.9) and 1.4 (1.1–21.8), respectively]. Men with the lowest bioT (<163.5 ng/dl) had no increased fracture risk after adjustment for bioE2 [adjusted HR 1.16 (0.90–1.49)]. A significant interaction between SHBG and bioT ($P = 0.03$) resulted in men with low bioT and high SHBG having higher fracture risk [HR 2.1 (1.4–3.2)]. Men with low bioE2, low bioT, and high SHBG were at highest risk [HR 3.4 (2.2–5.3)].

Conclusions: Older men with low bioE2 or high SHBG levels are at increased risk of nonvertebral fracture. When SHBG levels are high, men with low bioT levels have higher risk. The strongest association occurred when all measures were considered in combination. **(*J Clin Endocrinol Metab* 94: 3337–3346, 2009)**

It has been speculated that sex steroids contribute to fracture risk in older men (1). With aging, sex steroid concentrations decline (2, 3), fracture rate increases (4), and testosterone therapy improves bone density (5). Androgens and estrogens have *in vitro* and *in vivo* bone effects and trophic effects on skeletal development (6). Estradiol has been consistently associated with skeletal characteristics (6–11), but whether testosterone has independent

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Abbreviations: bioE2, Bioavailable estradiol; bioT, bioavailable testosterone; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; HR, hazard ratio; MrOS, Osteoporotic Fractures in Men Study.

effects on bone density, structure, or biochemical indices is uncertain (12). Testosterone may affect various extraskeletal functions relevant to fracture, including muscle strength, physical activity, cognition, and fall rate (13–18).

High SHBG has been independently associated with fracture risk (19–25). By binding to testosterone and estradiol, SHBG reduces circulating sex steroid concentrations and thereby their cellular actions. SHBG may have independent effects via a receptor mediated mechanism or affect sex steroid interaction with cellular receptors (26–28).

Although several publications suggest lower estradiol and/or testosterone or higher SHBG are linked to higher fracture rates (11, 19–21, 29), few studies have adequate power to assess independent and/or interdependent effects of estradiol, testosterone, and SHBG. Most previous studies measured sex steroids using RIA techniques, which are susceptible to artifact, particularly at low concentrations (30, 31).

We report associations between fracture risk and sex steroids in a large cohort of older men. Sex steroid levels were measured using liquid chromatography/mass spectrometry, a method with high accuracy (32, 33). We examined interactions between sex steroids, fracture risk, and other variables including bone mineral density (BMD), age, body composition, physical activity, and physical performance. We assessed the SHBG-fracture association, both independently and in combination with sex steroids.

Subjects and Methods

Study population

The Osteoporotic Fractures in Men Study (MrOS) study enrolled 5995 participants from March 2000 through April 2002 as previously described (34, 35). Community-based recruitment occurred at six U.S. academic medical centers in Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Pittsburgh, PA; Portland, OR; and San Diego, CA. Eligible participants were at least 65 yr old, could walk without assistance, and had not had bilateral hip replacement surgery. The institutional review board at each center approved the study protocol. All participants gave written informed consent. We used a case-cohort design: a random subsample of the original cohort (subcohort) was selected independently of fracture cases, and all cases outside the subsample were selected (Fig. 1). We selected 2048 men for steroid measurements (subcohort). A total of 1436 were randomly chosen plus all 446 minorities were included. They were followed for 4.7 (±.9) yr. Measures were also obtained in men (n = 3865) who experienced an incident nonvertebral fracture between enrollment and July 2006. Therefore, fracture cases could arise from either the subcohort (n = 126) or the remainder of the cohort (n = 299). After exclusions, the final study population included 1738 men in the subcohort and 342 incident fracture cases (102 from subcohort; 240 outside subcohort).

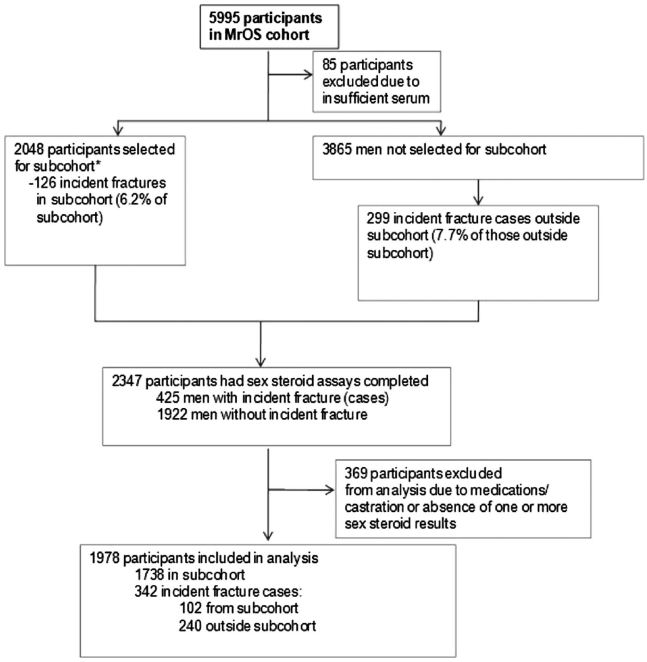

FIG. 1. Case-cohort design for the MrOS sex steroids and fracture study. *, Subcohort consisted of 1436 randomly selected non-Hispanic white men and all 446 minority men. Weighting was used in analyses to account for stratified sampling by race.

Baseline characteristics

Race/ethnicity, education level, smoking and alcohol consumption, occurrence of fracture after age 50 yr, medical history, and previous 12-month fall occurrence were determined by questionnaire at baseline. Current medications were recorded. Physical activity was assessed with the Physical Activity Score for the Elderly (36). Height (centimeters) and weight (kilograms) were measured using standard protocols. Grip strength (kilograms), lower extremity power, time to complete a narrow walk (6 m × 20 cm), and ability to rise from a chair without arms were assessed (34).

Sex steroid measurements

Baseline fasting morning blood was collected. Serum was prepared immediately after phlebotomy and stored at −70 C. Total serum testosterone and estradiol were measured using a combined gas chromatographic-negative ionization tandem mass spectrometry and liquid chromatographic electrospray tandem mass spectrometry bioanalytical method (Taylor Technology, Princeton, NJ). A $1/(\text{concentration})^2$ weighted least squares regression procedure was used to fit a linear function to the calibration data. The lower limit of detection for estradiol is 0.625 pg/ml (2.29 pmol/liter), and for testosterone is 25.0 pg/ml (0.09 nmol/liter). Duplicate aliquots from each participant's serum were assayed and results averaged. Testosterone intraassay coefficient of variation (CV) was 2.5% and interassay CV, 6.0%; the estradiol intraassay CV was 6.4% and interassay CV, 10.1%. Serum SHBG concentrations were measured using an Immulite analyzer with chemiluminescent substrate (Diagnostic Products Corp., Los Angeles, CA). The standard curve ranged from 0.2 to 180 nm/liter. The SHBG intraassay CV was 4.4% and interassay

CV, 6.0%. Albumin values for free hormone calculations were obtained from baseline serum using routine colorimetric methods (interassay CV 2.0%). Calculation of bioavailable fractions of testosterone and estradiol was by the method of Sodergard *et al.* (37). Using this method, the Spearman correlation coefficient for bioavailable testosterone and free testosterone was 0.98 and for bioavailable estradiol and free estradiol was 0.98, both $P < 0.0001$.

BMD

Areal proximal femur BMD was measured using dual-energy x-ray absorptiometry (QDR 4500W; Hologic Inc., Bedford, MA). Participants were scanned according to standardized procedures and scanners were calibrated at baseline. Whole body, spine, hip, and linearity phantoms were measured at all sites at baseline, and spine and hip phantoms were scanned throughout the study to monitor longitudinal changes. Daily quality control scans showed no shifts in scanner performance at any site during enrollment.

Ascertainment of incident fractures

We contacted 99% of participants every 4 months by mail or telephone to ask about recent fractures. All reported nonspine fractures were adjudicated by physician review of radiology reports or x-rays if radiology reports were unavailable. Fracture follow-up was 99%. Using a group of investigators, fractures were adjudicated as traumatic if circumstances leading to the fracture would likely have resulted in a fracture in a normal individual.

Statistical analyses

Cox proportional hazards models, with weighting to accommodate the stratified sampling and case-cohort design, were used to evaluate associations between sex steroids and time to incident fracture.

Three methods were used to evaluate associations between sex steroids and time to first fracture. We first created quartiles of sex steroid variables based on distributions in the subcohort. Because men in second, third, and fourth quartiles had similar risks of fracture, we created dichotomous variables; for testosterone and estradiol, the lowest quartile was compared with the other three quartiles; for SHBG, the highest quartile was compared with the lowest three quartiles. Second, we used restricted cubic spline Cox proportional hazard models to examine sex steroid variables as continuous and to test whether associations with incident fracture were nonlinear (38). Third, we performed exploratory cut point analysis. We dichotomized sex steroids at various quantiles using log likelihoods of Cox proportional hazard models. The cut point at which the sex steroid variable was dichotomized to produce the highest profile log likelihood was considered the best value for further dichotomizing (39). The cubic spline and cut point analyses supported use of the first quartile as a cut point.

We evaluated interactions among bioavailable testosterone (bioT), bioavailable estradiol (bioE2), and SHBG. We stratified each dichotomous sex steroid variable (dichotomized at lowest quartile for bioE2 and bioT and highest quartile for SHBG) and evaluated adjusted hazard ratios (HRs) for remaining sex steroid variables in each stratum. For example, we tested the association between bioT and fracture in each stratum of SHBG. Additive interactions were tested in Cox proportional hazards models (40) and were considered statistically significant if $P < 0.10$. We then categorized men into eight mutually exclusive categories. The reference category (lowest risk) contained men with bioT and bioE2 in the highest three quartiles and SHBG in the lowest three quartiles. The eighth category (highest risk) contained men with bioT and bioE2 in the lowest quartile and SHBG in the highest quartile. Each intermediate category contained men who were in one or more high-risk quartiles of bioT, bioE2, or SHBG.

All Cox proportional hazard models were fit using the weighting method of Barlow *et al.* (41) for case-cohort analysis. Age, race, and body mass index (BMI) were included as covariates in all models. Additional potential confounders were added, and if addition changed the HR for the sex steroid variable by more than 10%, it was retained in the model. Primary analyses were of each sex steroid individually. Subsequently models were adjusted for other sex steroids. For example, the model evaluating bioE2 was also adjusted for the dichotomous bioT and SHBG variables to determine whether this altered the HR for bioE2.

To estimate the proportion of fracture cases that would be attributable to low bioE2, low bioT, and high SHBG, we conducted an exploratory attributable fraction analysis. The average attributable fraction method (42) was used to obtain attributable fraction estimates for each sex steroid and SHBG and adjust for the other sex steroid/SHBG measures and for age, BMI, and BMD. To conduct this exploration with readily available statistical code (43), we assumed a simple case-control design and estimated odds ratios using multivariable logistic regression.

To determine the robustness of our findings, we performed sensitivity analyses. To evaluate whether models were robust to potentially influential observations, we calculated Dfβ for each of the sex steroid variables in the final models, with and without interaction terms. Using a cutoff of the absolute value of $2/\sqrt{n}$, no points were considered influential. However, plots of each Dfβ by identification number allowed us to identify those observations with relatively more influence than others. When these were excluded (n = 3 for full model without interaction term, n = 15 for full model with interaction term), there were no changes in tests of the null hypothesis (*i.e.* no term gained or lost statistical significance), and only the adjusted HR for bioE2 was attenuated (by 0.1%). The HRs for other terms were unchanged or strengthened by the exclusion of observations with relatively larger absolute values of Dfβ.

Results

Most nonvertebral fractures were judged as nontraumatic (nontraumatic n = 280, traumatic n = 62). There were few traumatic hip fractures (n = 2), and their exclusion did not affect analyses. The subcohort and fracture case characteristics are shown in Table 1. Correlations between serum levels of sex steroids and SHBG [bioE2 and SHBG: r = −0.13 ($P < 0.0001$), bioT and SHBG: r = 0.27 ($P < 0.0001$)] and between bioE2 and bioT [r = 0.37 ($P < 0.0001$)] were moderate. Age was negatively associated with bioT and bioE2 (r = −0.19 to −0.09) and positively associated with SHBG (r = 0.24) ($P < 0.0001$). BMI was negatively associated with

TABLE 1. Selected characteristics of men in the MrOS sex steroid case-cohort study

	Subcohort (n = 1738)[a] Mean ± SD or %	Subcohort, excluding fracture cases (n = 1636) Mean ± SD or %	Nonvertebral fracture cases (n = 342)[b] Mean ± SD or %
Age (yr)	73.3 ± 5.8	73.2 ± 5.8	75.2 ± 6.4
Race/ethnicity			
White	71.4	70.7	94.7
Black	12.1[a]	12.7	0.9
Asian	7.1	7.3	1.5
Hispanic	5.9	5.8	2.6
Other	3.4	3.6	0.3
Self-reported health			
Excellent	32.3	32.5	30.1
Good	51.8	51.5	55.3
Fair/poor/very poor	15.9	16.0	14.6
Cigarette smoking			
Ever smoked	63.6	63.5	62.6
Current alcohol consumption			
None	35.2	35.2	39.2
Greater than zero and less than seven drinks per week	38.5	38.6	36.8
Seven or more drinks per week	26.2	26.0	24.0
BMI (kg/m^2)	27.4 ± 3.8	27.5 ± 3.7	27.3 ± 4.1
Physical performance			
Narrow walk (m/sec)	1.00 ± 0.5	1.01 ± 0.4	0.92 ± 0.5
Leg power (100 W)	2.0 ± 0.7	2.1 ± 0.7	1.9 ± 0.7
History of falls reported at baseline	19.3	18.3	30.7
Previous nontrauma fracture after age 50 yr	15.7	14.9	29.0
Total testosterone (ng/dl)	404.6 ± 158.6	403.7 ± 159.0	403.6 ± 165.0
Total estradiol (pg/ml)	22.7 ± 7.7	22.7 ± 7.7	22.1 ± 7.6
BioT (ng/dl)[c]	204.6 ± 64.7	204.9 ± 64.9	195.0 ± 65.2
BioE2 (pg/ml)[c]	14.5 ± 4.8	14.6 ± 4.8	13.8 ± 4.8
SHBG (nM)	49.0 ± 19.3	48.6 ± 19.0	53.7 ± 21.6

[a] Subcohort consisted of 1436 randomly selected non-Hispanic white men and all 446 minority men. It includes 102 incident fracture cases (Figure 1). Minorities were oversampled in the subcohort; [b] fracture cases include 102 incident fracture cases inside the subcohort and 240 incident fracture cases outside the cohort (Figure 1). Of the nonvertebral fractures, 74 (21.6%) were hip fractures; [c] to convert bioE2 to picomoles per liter, the conversion factor is 3.671; to convert bioT to nanomoles per liter, the conversion factor is 0.0347.

bioT and SHBG (r = −0.31 to −0.30) and positively associated with bioE2 (r = 0.17) ($P < 0.0001$). Weight decreased by 0.36% per year during follow-up. Correlations between bioT, bioE2, and SHBG and BMD were between −0.05 and −0.2 ($P < 0.0001$).

Fracture risk and sex steroids

Men with lower levels of bioE2 were at higher risk of nonvertebral fracture. After adjustment for age, race, and BMI, the HR for all nonspine fracture in those in the lowest bioE2 quartile *vs.* the highest three quartiles was 1.48 [95% confidence interval (CI) 1.18–1.86; Table 2 and Fig. 2A]. The association was similar after adjustment for bioT and SHBG but was somewhat attenuated after adjustment for total hip BMD (HR 1.29; 95% CI 1.01–1.64). A similar association was present between bioE2 and hip fracture risk (HR 1.57; 95% CI 0.95–2.59; Table 3). Total estradiol was not significantly associated with nonverte-

TABLE 2. Hazard ratios (95% CI) for association between nonvertebral fractures and sex steroids

	bioE2[a]	bioT[a]	SHBG[b]
Unadjusted	1.49 (1.19–1.87)	1.39 (1.10–1.75)	1.63 (1.30–2.04)
Adjusted for age, race, BMI	1.48 (1.18–1.86)	1.28 (1.00–1.64)	1.44 (1.14–1.82)
Adjusted for bioE2[c]		1.16 (0.90–1.49)	1.42 (1.12–1.80)
Adjusted for bioT[c]	1.42 (1.12–1.80)		1.48 (1.17–1.88)
Adjusted for SHBG[c]	1.46 (1.16–1.83)	1.33 (1.04–1.70)	
Full model including bioE2, bioT, and SHBG[c]	1.39 (1.09–1.76)	1.20 (0.93–1.56)	1.45 (1.14–1.84)
Full model additionally adjusted for BMD[c]	1.29 (1.01–1.64)	1.24 (0.96–1.59)	1.36 (1.07–1.72)

[a] HR is for lowest quartile *vs.* highest three; for bioE2 lowest quartile was less than 11.4 pg/ml (<41.8 pmol/liter); for bioT lowest quartile was less than 163.5 ng/dl (<5.67 nmol/liter); [b] HR is for highest quartile (SHBG ≥59.1 nM) *vs.* lowest three; [c] also adjusted for age, race, and BMI; BMD refers to total hip BMD.

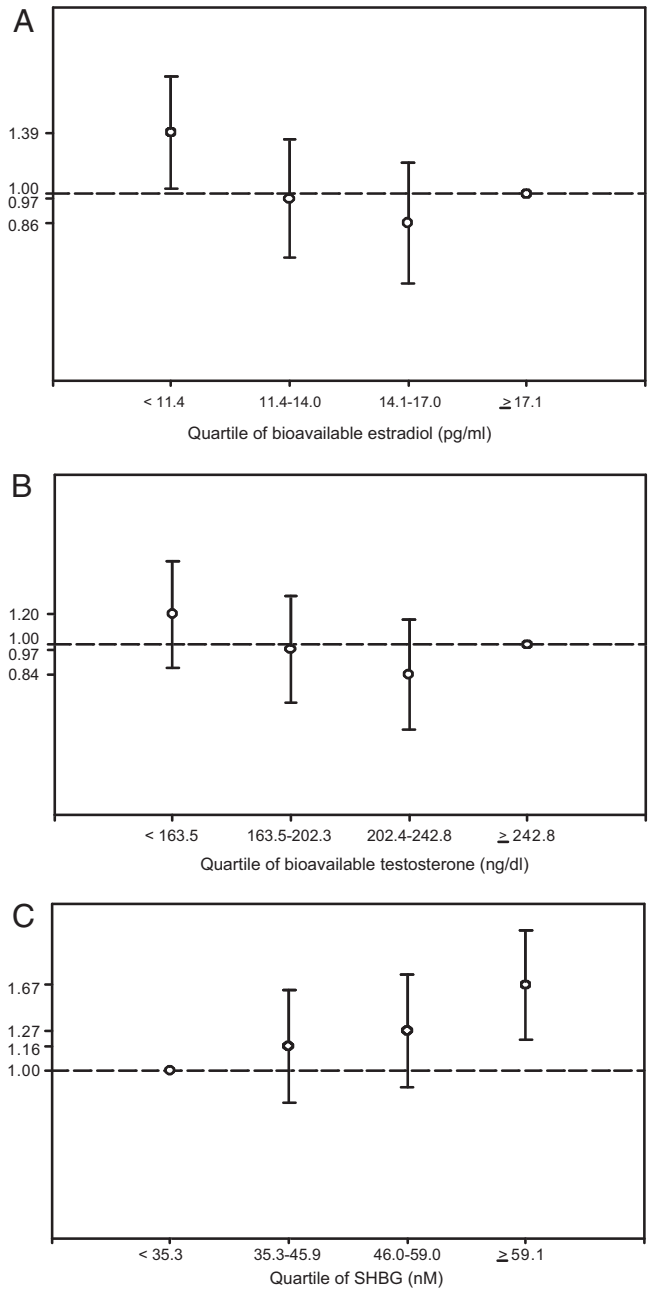

FIG. 2. HRs and 95% CIs for risk of nonvertebral fractures by quartiles of sex steroids (adjusted for age, race, BMI). A, Bioavailable estradiol. B, Bioavailable testosterone. C, SHBG. To convert bioavailable estradiol to picomoles per liter, the conversion factor is 3.671; to convert bioavailable testosterone to nanomoles per liter, the conversion factor is 0.0347.

bral (HR 1.09; 95% CI 0.86–1.39) or hip fracture risk (HR 1.52; 95% CI 0.91–2.52). These associations were essentially unchanged when only nontraumatic fractures were considered.

After adjustment for age, race, and BMI, men with bioT in the lowest quartile had a higher risk of nonvertebral fracture than those in the highest three quartiles (HR 1.28; 95% CI 1.00–1.64; Table 2 and Fig. 2B). The association was slightly stronger after adjustment for SHBG but was no longer significant after adjustment for bioE2 (HR 1.16; 95% CI 0.90–1.49). When only nontraumatic fractures were considered, the association between bioT and fracture risk was stronger (HR 1.45; 95% CI 1.12–1.89) and remained significant after adjustment for bioE2 (HR 1.31; 95% CI 1.00–1.72). Inclusion of BMD in the model did not significantly affect the association, regardless of trauma status. The HRs for the relationship between bioT and hip fracture risk were similar (Table 3). Total testosterone levels were not associated with nonvertebral (HR 1.02; 95% CI 0.79–1.32) or hip fracture risk (HR 0.93; 95% CI 0.51–1.71).

Fracture risk and SHBG

After adjustment for age, race, and BMI, men with the highest quartile of SHBG were at increased risk of nonvertebral fracture compared with those in the lowest three quartiles (HR 1.44; 95% CI 1.14–1.82; Table 2 and Fig. 2C). The association remained consistent after adjustment for sex steroids but was slightly attenuated after adjustment for BMD. Associations between SHBG level and fracture risk were slightly stronger but not substantively altered when only nontraumatic fractures were considered (HR 1.57; 95% CI 1.22–2.03). Hip fracture risk was approximately doubled in men with high SHBG (HR 2.17; 95% CI 1.31–3.59; Table 3) and was not influenced by further adjustment for sex steroids or BMD.

Consideration of covariates

The associations between fracture risk and sex steroids and SHBG were not substantively altered by sequential adjustment for other potential confounders, including physical activity, physical performance, and previous falls. Limiting analyses to non-Hispanic white participants and excluding hip fractures did not alter the findings.

Threshold analyses

Spline analyses showed a nonlinear association between serum bioE2 and nonvertebral fracture (P for nonlinearity = 0.045; Fig. 3A). Log likelihood cut point analysis showed that dichotomizing bioE2 at 12.5 pg/ml (45.9 pmol/liter) maximized model fit for nonvertebral fractures. This threshold concentration was similar to that associated with increased fracture risk in the lowest quartile of bioE2 [<11.4 pg/ml (41.8 pmol/liter)]. Spline analysis did not reveal nonlinearity in the associations between fracture risk and bioT or SHBG (Fig. 3, B and C).

Interaction between bioT, SHBG, and fracture risk

We observed a significant additive interaction between bioT and SHBG ($P = 0.03$). Nonvertebral fracture risk for

TABLE 3. Hazard ratios (95% CI) for association between hip fractures and sex steroids

	bioE2	bioT[a]	SHBG[b]
Unadjusted	1.56 (0.96–2.54)	1.74 (1.05–2.86)	3.53 (2.20–5.68)
Adjusted for age, race, BMI	1.57 (0.95–2.59)	1.33 (0.79–2.25)	2.17 (1.31–3.59)
Adjusted for bioE2[c]		1.18 (0.68–2.04)	2.14 (1.30–3.54)
Adjusted for bioT[c]	1.50 (0.89–2.54)		2.23 (1.35–3.69)
Adjusted for SHBG[c]	1.54 (0.93–2.53)	1.42 (0.84–2.40)	
Full model including bioE2, bioT, and SHBG[c]	1.43 (0.84–2.43)	1.26 (0.73–2.20)	2.18 (1.31–3.61)
Full model additionally adjusted for BMD[c]	1.00 (0.56–1.77)	1.59 (0.90–2.81)	2.09 (1.23–3.56)

[a] HR is for lowest quartile *vs.* highest three; for bioE2 lowest quartile was less than 11.4 pg/ml (<41.8 pmol/liter); for bioT lowest quartile was less than 163.5 ng/dl (<5.67 nmol/liter); [b] HR is for highest quartile (SHBG ≥59.1 nM) *vs.* lowest three; [c] also adjusted for age, race, and BMI; BMD refers to total hip BMD.

the lowest quartile of bioT was greater among men with SHBG in the highest quartile (HR 2.10; 95% CI 1.39–3.17; Fig. 4A) than in the lowest three SHBG quartiles (HR 0.99; 95% CI 0.73–1.35; Fig. 4A). These associations remained after adjustment for bioE2 and did not appear to be from a shift in the SHBG distribution; median SHBG levels did not differ between low and high bioT groups (69.0 *vs.* 70.4 nM, respectively, $P = 0.7$), and adjustment of the models with an SHBG^2 term did not affect the interaction. We evaluated whether the stronger association of bioT with fracture risk in the highest SHBG quartile could have been due to particularly low levels of bioT in the high SHBG group. The median bioT levels within the lowest bioT quartile were slightly lower in the highest SHBG quartile compared with the lower quartiles [127.7 *vs.* 138.7 ng/dl (4.43 *vs.* 4.81 nmol/liter, respectively,) $P = 0.03$]. However, in age-, race-, and BMI-adjusted models, even very low levels of bioT were not associated with increases in fracture risk (Fig. 2B). These results indicate that low concentrations of bioT impart particular risk in the presence of high SHBG.

Combinatorial effects of estradiol, testosterone, and SHBG on fracture risk

When the combined effects of sex steroid or SHBG levels were examined, the associations with fracture risk were strengthened. The highest nonvertebral fracture risk was in men (n = 74, 3.7%) in the lowest quartiles of bioT and bioE2 and highest quartile of SHBG (HR 3.39; 95% CI 2.19–5.27; Fig. 4B). Risk estimates were similar or stronger when only nontraumatic fractures were included in the analyses; in the lowest quartiles of bioT and bioE2 and highest quartile of SHBG, the HR was 4.02 (95% CI 2.54–6.37). The effects of combining high-risk categories were also evident for hip fracture; men with low bioE2 and bioT and high SHBG levels had a 3.8-fold higher risk of hip fracture (95% CI 1.48–9.92).

Attributable risk

The fraction of nonspine fracture risk attributable to low bioE2 was 5.7%, 1.5% to low bioT, and 7.7% to high SHBG. For hip fracture risk, the fraction attributed to low bioE2 was 0.1%, to low bioT was 2.7%, and to high SHBG was 14.6%.

Discussion

In this large prospective study of older men, those with the lowest bioE2 or the highest SHBG had higher risks of nonvertebral fracture. BioT had a weak association with nonvertebral fracture that disappeared after adjustment for bioE2. The association between bioT and nontraumatic fracture risk was stronger and remained after adjustment for bioE. When high SHBG levels are present, low bioT was associated with a substantially increased fracture risk even with bioE2 adjustment. The associations were similar, perhaps slightly stronger, for hip fracture. Total sex steroids were not associated with fracture. These results have important implications for understanding how sex steroids and SHBG affect fracture risk and for determining the clinical role of these measurements.

Our finding that low bioE2 was independently associated with increased fracture risk extends earlier reports of estrogen's importance for men's skeletal health (11, 19, 29). Previous studies evaluating the sex steroid-fracture association have been inconsistent and limited by cross-sectional design, low participant and fracture numbers, and/or RIA-based sex steroid measurements (11, 20, 22, 23, 25, 29, 44). Two recent studies used mass spectrometry to more accurately measure testosterone and estradiol. In the Dubbo cohort, total testosterone had a strong and estradiol a weak association with osteoporotic fracture risk, (21), but independent effects were not assessed. Another large, prospective study (MrOS Sweden) (19) found that lower free and total estradiol were associated with nonvertebral and vertebral fracture risk. Their results are very similar to ours and together provide compelling evidence for estradiol's effects on fracture risk. Attenuation of bioE2's association with fracture by adjustment for BMD suggests that estradiol's positive effects on fracture risk may be due, in part, to an effect on bone density (7, 9,

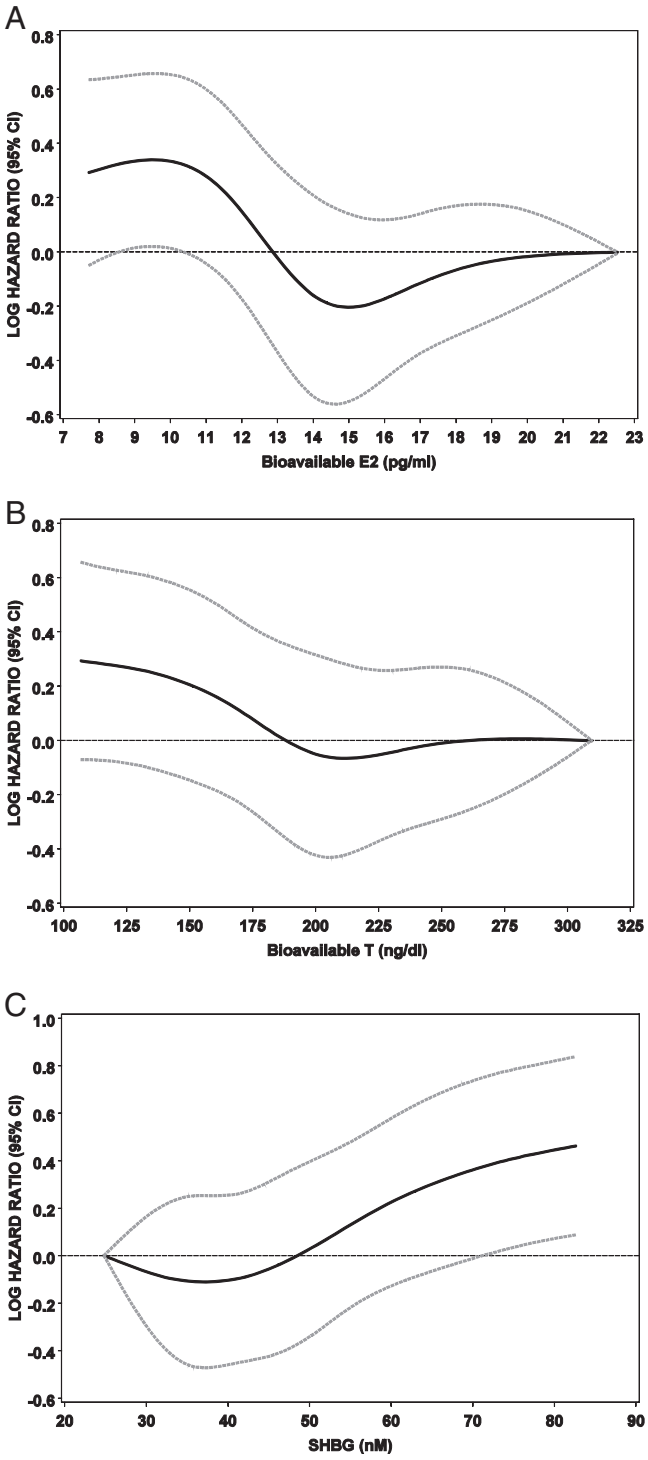

FIG. 3. Spline models for the detection of any nonlinear relationships between sex steroids or SHBG and nonvertebral fracture risk. A, bioE2. B, bioT. C, SHBG. To convert bioavailable estradiol to picomoles per liter, the conversion factor is 3.671; to convert bioavailable testosterone to nanomoles per liter, the conversion factor is 0.0347.

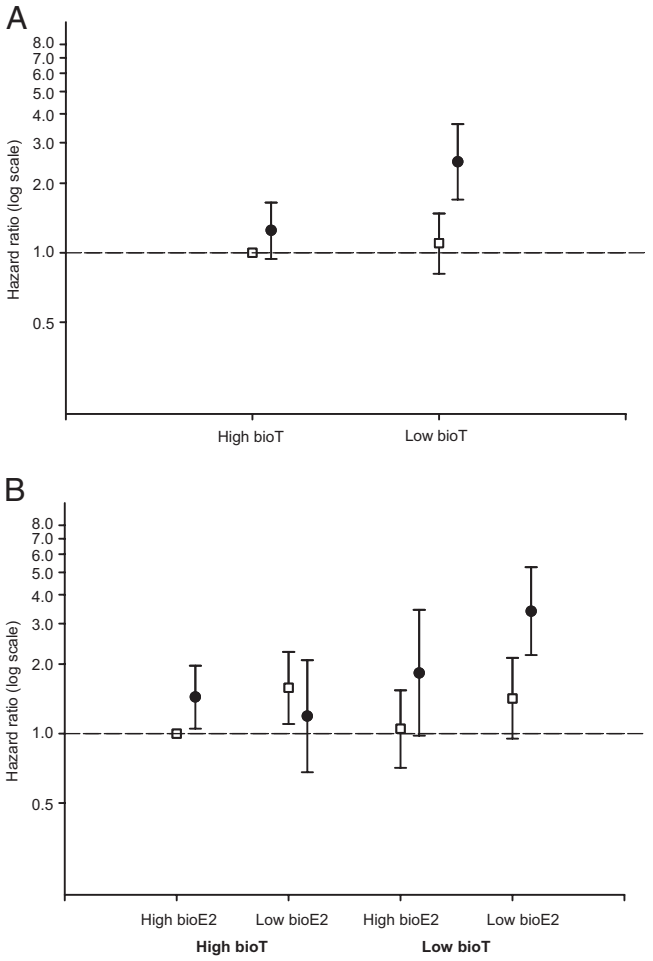

FIG. 4. Combinations of sex steroids and SHBG and risk of nonvertebral fracture. A, bioT and SHBG. B, bioT, bioE2, and SHBG. There were 1079 men in the high bioT, low SHBG category; 397 men in the high bioT, high SHBG category; 392 men in the low bioT, low SHBG category; and 110 men in the low bioT, high SHBG category. □, Low SHBG; ●, high SHBG.

11). However, the association remained significant after BMD adjustment, suggesting additional effects.

We found a nonlinear association between estradiol and fracture risk. Evaluations using quartile analysis, spline analysis, and log likelihood cut point analysis identified similar thresholds of bioE2 below which fracture risk was increased [11.4–12.5 pg/ml (41.8–45.9 pmol/liter); free estradiol: 0.4–0.5 pg/ml (1.47–1.84 pmol/liter)]. MrOS Sweden found a similar fracture risk threshold level [free estradiol: 0.3 pg/ml (1.10 pmol/liter)] (19). Together these results support the hypothesis that a threshold range of bioE2 is necessary for skeletal health (45).

High SHBG levels were associated with increased nonvertebral fracture risk, independent of sex steroids and BMD. SHBG has been associated with bone density (22, 46), bone turnover markers (22, 46), proximal femur expansion and bending resistance (47), and fracture risk in men (19, 22, 46) and women (24). SHBG may directly influence intracellular signaling via a membrane receptor that requires SHBG-sex steroid interactions (26, 27) or a megalin-mediated endocytic pathway that involves unbound SHBG (26, 28). Through these pathways, SHBG could amplify the effects of sex steroid sufficiency or de-

ficiency (26). However, SHBG could also be a marker for nonskeletal factors affecting fracture risk. Lower insulin or IGF-I levels could increase SHBG, resulting in the SHBG-fracture risk association. SHBG increases with age but decreases with obesity. It is affected by frailty and nutritional status. In our study adjustment for age, leg power, physical activity, BMI, and previous falls did not alter the association between SHBG and fracture risk.

Despite strong cellular and animal data suggesting androgens have positive bone effects, clinical studies offer no clear evidence of an independent androgen effect on bone mass or fracture (11, 20, 22, 23, 25, 44). Consistent with previous reports (19, 21), we found men with low bioT had higher fracture risk, but the association weakened when adjusted for bioavailable estradiol. The association was more robust when only nontraumatic fractures were considered, suggesting a stronger link with osteoporotic fractures. This could be a reflection of low testosterone's effects on fall risk (48), potentially mediated through extraskeletal functions including muscle strength, physical activity, and cognition (13–18). Indeed, the association between low bioT and fracture risk was not attenuated by BMD adjustment, suggesting non-BMD-related factors are important.

We found novel evidence of a bioT-SHBG interaction. Men with low bioT and high SHBG were at substantially higher risk of nonvertebral and hip fracture even after adjustment for bioE2. Men with low bioT and bioE2 and high SHBG had even greater risk of nonvertebral (HR 3.4) and hip fracture (HR 3.8), especially when only nontraumatic fractures were considered. Thus, bioT, bioE2, and SHBG each play a role in fracture determination, but the cumulative effects of sex steroid, and SHBG levels may be most important. Although the findings in MrOS Sweden (19) are similar to ours, combinatorial effects of sex steroids and SHBG have rarely been reported. Given these results, combinatorial effects should be evaluated in additional studies and with other endpoints (*e.g.* bone loss, body composition changes, cardiovascular events, mortality). However, these results should be interpreted with caution because delineating each hormone's independent effect on fracture risk by statistical methods is challenging in the presence of complex interrelationships among bioE2, bioT, and SHBG. This is a particular issue in our study because bioavailable levels were derived from mass action equations that included SHBG. Nevertheless, several analytical approaches (see *Results*) provided consistent evidence of a nonartifactual interaction between SHBG and bioT. Our findings cannot be considered proof of independent molecular effects of bioavailable sex steroids and SHBG, but they are consistent with that hypothesis.

Our results have potential clinical implications. They affirm the robust and independent effects of bioE2 and SHBG in fracture prediction. Moreover, we provide further evidence for a threshold level of bioE2, below which fracture risk is increased. Hence, estradiol and SHBG measurements should be valuable in clinical situations. Although estradiol and SHBG levels are not commonly measured when assessing skeletal health or fracture risk in men, our results and those of MrOS Sweden (19) suggest revision of these practices (49). Second, our results support previous findings that bioavailable or free levels of sex steroids are more robustly associated with fracture risk than are total sex steroid concentrations. Although some investigators argue that total T and total E are biologically more relevant than bioT or bioE2, our results suggest that bioavailable, not total, levels are associated with fracture risk. It remains common to measure total sex steroid levels in clinical situations; however, bioavailable or free levels may be more appropriate as predictive tools. Given the limitations of the analog free testosterone assays, clinical application of these findings would require more accurate and standardized assay methods and development of consensus concerning assay result use in clinical decision making. Third, the associations we observed were most apparent when sex steroids and SHBG were considered in combination. Men with low bioT and bioE2 and high SHBG levels are at highest risk. If validated, approaches that incorporate all three measures into clinical algorithms should be developed.

This study has several limitations. We did not measure changes in sex steroids and SHBG over time so cannot determine how hormonal changes associate with fracture risk. Use of dichotomous cutoffs for sex steroid levels were based on observed associations with fracture and could have overestimated the associations. The cohort was relatively healthy and primarily Caucasian and although similar to more representative populations such as National Health and Nutrition Examination Survey, caution should be used in generalizing our results to other groups of men. The number of hip fractures that occurred during follow-up was relatively small, but nevertheless, the associations between sex steroid and SHBG levels and hip fracture risk were robust. Our findings need to be validated in other cohorts of older men.

This study also has considerable strengths. It is one of the largest to address the association between sex steroids and fracture risk in elderly men. Fractures were carefully ascertained and verified, potentially important confounding variables were evaluated, and sex steroid measurements were performed using gas chromatography/mass spectrometry to avoid inaccuracy at low concentrations (30, 31). Many participants are over age 80 yr, a segment

of the population that is expanding and is at high fracture risk but has not been well studied.

In summary, men with low bioE2 levels and high SHBG levels had increased rates of incident fractures. Low bioT was associated with an increased risk of nontraumatic fractures and there was an interaction between SHBG and bioT; men with low bioT were at higher risk in the presence of high SHBG levels. Men who were in the highest-risk quartiles for bioT, bioE2, and SHBG had a markedly increased fracture risk. Our results suggest that bioavailable sex steroid and SHBG measurements may be useful in the clinical assessment of fracture risk in older men and that the physiological implications of hypogonadism should be considered in light of possible interactions among sex steroids and SHBG.

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