Associations of Serum Sex Hormone-Binding Globulin and Sex Hormone Concentrations with Hip Fracture Risk in Postmenopausal Women

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Context: Endogenous estradiol, testosterone, and SHBG may influence the risk of hip fracture.

Design and Methods: From the Women's Health Initiative Observational Study, 39,793 eligible postmenopausal women did not have a previous hip fracture and were not using estrogen or other bone-active therapies. Of these, 400 who had a first-time nonpathological hip fracture (median follow-up, 7 yr) were matched to 400 controls by age, ethnicity, and baseline blood draw date. Estradiol, testosterone, and SHBG were measured in banked baseline serum.

Results: Compared with women in the lowest tertiles, those with bioavailable testosterone in the highest tertile had a lower risk [odds ratio (OR) = 0.62; 95% confidence interval (CI) = 0.44–0.88]; those with bioavailable estradiol in the highest tertile had a lower risk (OR = 0.44; 95% CI = 0.29–0.66), and those with SHBG in the highest tertile had a higher risk (OR = 1.90; 95% CI = 1.31–2.74) of hip fracture. In models with all three hormones and potential confounders, high SHBG remained a strong independent risk factor (OR = 1.76; 95% CI = 1.12–2.78), high bioavailable testosterone remained protective (OR = 0.64; 95% CI = 0.40–1.00), but estradiol no longer was associated (OR = 0.72; 95% CI = 0.42–1.23).

Conclusions: High serum SHBG is associated with an increased risk of subsequent hip fracture and high endogenous testosterone with a decreased risk, independent of each other, serum estradiol concentration, and other putative risk factors. But endogenous estradiol has no independent association with hip fracture. **(*J Clin Endocrinol Metab* 93: 1796–1803, 2008)**

The number of hip fractures annually is estimated to reach 6 million worldwide by 2050 (1), and Caucasian women have a 17% lifetime risk of hip fracture by age 50 yr (2). Previous studies have reported that endogenous estradiol, testosterone, and SHBG may influence the risk of hip fracture in postmenopausal women. Estrogen deficiency increases bone loss, and previous studies suggest that women with very low estradiol levels have an increased risk of fracture (3–10). For example, the Study of Osteoporotic Fractures (SOF) reported that elderly postmenopausal women who had endogenous estradiol concentrations below 5 pg/ml had a 2.5- to 3-fold greater risk of hip and vertebral fracture than women with higher levels (4). Other prospective studies found associations between higher estradiol levels and a decreased risk of hip and/or vertebral fracture (6, 7, 10, 11).

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Abbreviations: BMD, Bone mineral density; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; OR, odds ratio; SOF, Study of Osteoporotic Fractures; WHI-OS, Women's Health Initiative Observational Study.

Aromatization of testosterone outside the ovary is a major source of endogenous estradiol in postmenopausal women (12). In line with this mechanism of action, SOF found that a protective effect of testosterone on the risk of hip fracture was not independent of estradiol level. But testosterone may also have direct anabolic effects on bone, improve bone mineral density (13, 14), maintain muscle mass, and protect against falls and thus fractures (8, 13, 15, 16).

Several previous studies found high SHBG levels to be associated with higher risks of vertebral and other osteoporotic fractures in postmenopausal women (3, 4, 6, 7, 10, 17, 18). SHBG binds to estradiol and testosterone, thereby reducing the concentrations of these hormones that are available to interact with their receptors on bone and other target tissues. Additionally, SHBG might regulate cellular response to sex hormones at multiple steps by a SHBG-dependent sex hormone signaling mechanism (19, 20) so that higher circulating SHBG might decrease fracture risk by more than one mechanism.

The inter-relations among estradiol, testosterone, SHBG, and the development of hip fracture need to be examined. We used archived baseline specimens and data as well as longitudinal hip fracture information from the Women's Health Initiative Observational Study (WHI-OS) to evaluate the associations between endogenous circulating levels of estradiol, testosterone, and SHBG and the risk of subsequent hip fracture in postmenopausal women.

Subjects and Methods

Study population

The WHI-OS is a multicenter prospective study of 93,676 women who were ages 50–79 yr when they enrolled in 1993 through 1998 at 40 clinical centers in the United States. Women were ineligible for the study if they participated in a clinical trial or had less than 3 yr predicted survival, alcohol or drug dependency, mental illness, dementia, or other inability to participate in the study. A description of the study design and rationale has been reported elsewhere (21). This study was approved by the investigators' institutional review boards. Each of the participants signed written informed consent to participate in the study.

Selection of women with incident hip fracture and controls

As of August 31, 2004, 39,793 women met the following eligibility criteria to select women who experienced a first-ever (incident) nonpathological hip fracture during follow-up. We excluded women with a baseline history of hip fracture and women with hip fractures from a known pathological cause. We excluded women who reported using estrogen, androgen, selective estrogen receptor modulators, antiestrogens, or other anti-osteoporotic medications, including bisphosphonates and PTH within 1 yr of baseline.

From among 39,793 eligible women, a total of 404 women suffered their first nonpathological hip fracture during a median follow-up of 7.0 yr. We selected randomly 400 of these women to comprise the incident hip fracture case group. For each case, a control was selected from the 39,793 eligible women who had not had a hip fracture, were not lost to follow-up, and were not deceased at the time of the respective case's hip fracture event. The control was matched to their case by age within 1 yr, race/ethnicity, and baseline blood draw within 120 days. Cases could have been a potential control for other cases whose fracture event occurred previously, but none of the 400 controls had a hip fracture during the study period.

Blood samples and measurements of sex hormones and SHBG

For each study participant, blood was collected at the baseline visit after at least a 12-h fast and then stored at −70 C (22). Samples used for hormone measurements were taken from these baseline specimens. Samples were shipped on dry ice to the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles, CA). Laboratory personnel were blinded to case-control status, and samples were analyzed in random order.

Estradiol and testosterone concentrations were quantified using RIAs after organic solvent extraction and Celite column partition chromatography (23). For the estradiol RIA, the intraassay coefficient of variation (CV) was 7.9% at 34 pg/ml (124 pmol/liter), and interassay CV were 8.0 and 12.0% at 16 pg/ml (58.7 pmol/liter) and 27 pg/ml (99.1 pmol/liter), respectively. For the testosterone RIA, the interassay CV were 12.0% at 4.9 ng/dl (0.17 nmol/liter), 11.0% at 14.3 ng/dl (0.50 nmol/liter), and 10.0% at 47.9 ng/dl (1.66 nmol/liter); the intraassay CV was 6% at 14.3 ng/dl (0.50 nmol/liter). Bioavailable (non-SHBG-bound) and free (non-albumin- and non-SHBG-bound) estradiol (or testosterone) concentrations were calculated using the measured total estradiol (or total testosterone) and SHBG concentrations, an assumed constant for albumin, and affinity constants of SHBG and albumin for estradiol (or testosterone) (24, 25). These calculated values are highly correlated ($r \geq 0.85$) with direct measurement of bioavailable estradiol and testosterone (25, 26). The sensitivities of the estradiol and testosterone assays were, respectively, 3 pg/ml (11.0 pmol/liter) and 1.5 ng/dl (0.052 pmol/liter), and concentrations below these values were deemed undetectable.

SHBG was quantified by a solid-phase, two-site chemiluminescent immunoassay using the Immulite Analyzer (Diagnostic Products Corp., Los Angeles, CA). The solid phase is a polystyrene bead with a monoclonal antibody specific for SHBG. The intraassay CV ranged from 4.1–7.7%, and the interassay CV ranged from 5.8–13%. The assay had a sensitivity of 0.2 nmol/liter, and concentrations below these values were deemed undetectable.

Baseline questionnaire and clinical data

At baseline, questionnaire data included demographic information, medical history, medication use, family history, personal habits, physical activity, alcohol use, and dietary habits. Participants were asked to bring all medications and supplements to the clinic for verification of current use. Total calcium intake was derived from the sum of dietary and supplemental sources using a modification of the Block food frequency questionnaire (27) and an interviewer-administered medication inventory. Physical function was measured using the 10-item Rand-36 physical function scale, by which a score of 90 or greater approximates the upper 10th percentile in the WHI-OS (28).

Weight was measured on a balance beam scale while wearing indoor clothing to the nearest 0.1 kg, and height was measured with a fixed stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Ascertainment and validation of hip fracture cases

The incidence of hip fractures was assessed annually by mailed questionnaires, and those who reported a fracture were contacted by phone to obtain medical records and radiology reports. Possible or confirmed pathological hip fractures were excluded from the selection of cases.

Statistical analyses

Baseline characteristics were compared between women with an incident hip fracture and controls, with reported P values from χ^2 tests for categorical variables and t tests for continuous variables. Undetectable hormone concentration readings (four readings for estradiol and no readings for testosterone and SHBG) were set to missing. Generalized

additive modeling (29) was used to determine whether the sex hormone measurements were linearly related to fracture risk, and Pearson correlation coefficients were calculated to assess the degree of linear correlation between sex hormone measures and BMI. Conditional logistic regression models were conducted to assess the associations between levels of sex hormones and the risk of subsequent hip fracture. Odds ratios (OR) with 95% confidence intervals (CI) were estimated per SD change in sex hormone concentration and according to tertile categories (defined by the distribution in controls), with associated *P* values for tests of linear trend. Associations were initially examined with adjustment of the matched factors, age and draw date, to account for any residual confounding. Because ethnicity was matched perfectly in cases and controls, it was not included in the models.

Models were adjusted for factors that were associated with both incident hip fracture and sex hormone or SHBG levels in the initial univariate analyses: total daily calcium intake, current cigarette smoking, corticosteroid use, nulliparity, and diabetes. Because estradiol, testosterone, and SHBG are interrelated, we hypothesized *a priori* that interactions between the sex hormones might influence the risk of hip fracture. BMI, current smoking status, and alcohol use were suspected to be associated with the risk of hip fracture by mediating sex hormone levels. Likelihood ratio tests were used to evaluate whether interactions between these factors and the hormones, and between the hormones themselves, were significant. OR also were estimated between sex hormone levels and hip fracture after stratifying by median BMI and current smoking status. Results are presented for the raw measurements for each hormone, because logarithmic transformation of the concentrations of each hormone did not alter the findings. The level of significance used was P value < 0.05.

Results

Subjects in this study had a mean age of 70.8 yr, and 95% were Caucasian, reflecting the higher risk of hip fracture in this racial group. Among the 400 women with an incident hip fracture, 268 (67%) were aged 70–79 yr, 107 (27%) were aged 60–69 yr, and 25 (6%) were aged 50–59 yr. Taking walks for exercise, alcohol use, prior sex hormone use, thiazide or thyroid medication use, and diabetes status did not differ between cases and controls. Cases were more likely to be nulliparous, currently smoke cigarettes, and use corticosteroids. Cases tended to have a parent who fractured a hip after age 40 yr and also had lower BMI and weight (mean ± SD = 68.3 ± 15.0 kg) than controls (70.4 ± 12.9 kg; $P = 0.03$) (Table 1). Cases also reported lower calcium in-

TABLE 1. Baseline characteristics and endogenous serum hormone measures

Characteristic	No fracture (n = 400)	Fracture (n = 400)	*P* value
Age (yr)	70.8 ± 6.2	70.8 ± 6.2	
Caucasian (n)	380 (95%)	380 (95%)	
BMI (kg/m^2)	27.4 ± 5.1	26.0 ± 5.2	<0.001
Current cigarette smoking (n)	10 (2.5%)	36 (9.1%)	<0.001
Walks for exercise (n)	273 (70%)	258 (66%)	0.3
Current alcohol use (n)	247 (63%)	251 (63%)	0.6
Total hip BMD T-score ≤ −2.5[a] (n)	5 (16%)[a]	12 (35%)	0.05
Total calcium intake ≥ 1 g/d (n)	214 (54%)	177 (45%)	0.009
Total vitamin D intake (μg)	9.3 ± 6.9	9.5 ± 9.9	0.7
Age at menopause (yr)	48.9 ± 6.4	48.5 ± 6.2	0.3
Bilateral oophorectomy before age 50 (n)	31 (7.8%)	32 (8.0%)	0.9
Live birth ever (n)	349 (87%)	318 (80%)	0.006
History of parent with hip fracture (n)	64 (16%)	80 (20%)	0.14
Prior estrogen or testosterone use (n)	98 (25%)	95 (24%)	0.8
Thiazide use (n)	23 (6%)	20 (5%)	0.6
Corticosteroid use (n)	4 (1%)	16 (4%)	0.007
Thyroid medication use (n)	58 (15%)	61 (15%)	0.8
Treated diabetes (n)	19 (5%)	24 (6%)	0.1
Excellent to very good health (self-reported) (n)	220 (56%)	194 (49%)	0.05
RAND 36 physical functioning score > 90 (n)	117 (30.1%)	84 (21.8%)	0.009
Sex hormones[b]			
Total estradiol (pg/ml)[c]	11.8 ± 6.3	10.8 ± 6.3	0.039
Bioavailable estradiol (pg/ml)[d]	7.5 ± 4.5	6.6 ± 4.3	0.002
Free estradiol (pg/ml)[d]	0.28 ± 0.17	0.25 ± 0.16	0.002
Total testosterone (ng/dl)	27.4 ± 15.1	25.5 ± 13.4	0.067
Bioavailable testosterone (ng/dl)[d]	12.6 ± 7.0	10.9 ± 6.3	<0.001
Free testosterone (ng/dl)[d]	4.9 ± 2.8	4.3 ± 2.5	<0.001
SHBG (μg/dl)	1.6 ± 0.8	1.8 ± 0.9	<0.001

Unless indicated as number of subjects (n), values are mean ± SD; test of association was from χ^2 test (categorical variables) or *t* test (continuous variables).

[a] By World Health Organization criteria, 34 women with fracture and 32 controls had BMD measurements.

[b] Outlier cutoffs were 50 pg/ml for total estradiol, 100 ng/dl for testosterone, and 200 μg/dl for SHBG; comparison was from *t* test using log-transformed values. For conversion of estradiol to pmol/liter, multiply by 3.671. For conversion of testosterone to nmol/liter, multiply by 0.0347. For conversion of SHBG to nmol/liter, multiply by 40.

[c] Four participants (one with incident fracture) had undetectable total estradiol values, and these were set to missing; three participants had quantity insufficient for one or two hormones, and these were set to missing.

[d] Bioavailable and free sex hormone concentrations were calculated using the measured total sex hormone and SHBG concentrations, an assumed constant for albumin, and affinity constants of SHBG and albumin for the sex hormone (24, 25).

take, poorer general health, and poorer physical functioning. Vitamin D intake did not differ between the two groups. Both age at menopause and early surgical menopause also did not differ. In the sample of 40 cases and 38 controls that had measurements of bone mineral density (BMD) at baseline, 12 (35%) of the cases compared with five (16%) of the controls had total hip BMD T-scores of −2.5 or lower ($P = 0.05$) (Table 1). The controls had a mean hip T-score of −1.2 compared with the cases who had a mean of −2.1 ($P = 0.0006$).

Findings are reported for the bioavailable sex hormones which also represent those for the free forms because they were nearly perfectly correlated in controls. SHBG level was inversely correlated with bioavailable (or free) testosterone (r = −0.20) and bioavailable (or free) estradiol (r = −0.41) concentrations (Table 2). BMI was correlated with total (r = 0.45) and bioavailable (r = 0.51) forms of estradiol and inversely with SHBG levels (r = −0.31). BMI was weakly correlated with total testosterone (r = 0.14) and bioavailable testosterone (r = 0.29). Correlations between total, bioavailable, and free sex hormone concentrations and BMI were no different in fracture cases than in controls ($P > 0.05$).

SHBG, sex hormones, and risk of hip fracture

Mean concentrations of endogenous bioavailable estradiol and testosterone were, respectively, 14 and 16% lower in cases, and mean SHBG levels were 11% higher in cases than controls (Table 1). Four participants (one with hip fracture) had undetectable estradiol levels. There was no significant evidence of nonlinear or threshold relations between these measurements and the risk of hip fracture ($P > 0.1$).

Expressed as continuous values, each SD [SD = 4.5 pg/ml (16.5 pmol/liter)] increase in bioavailable estradiol was associated with a 23% lower risk of hip fracture (OR = 0.77; CI = 0.65–0.91). Each SD [6.67 ng/dl (0.23 pmol/liter)] increase in bioavailable testosterone level was similarly associated with a 24% lower risk (OR = 0.76; CI = 0.65–0.89), whereas each SD [0.83 μg/dl (33.2 nmol/liter)] increase in SHBG was associated with a 37% higher risk (OR = 1.37; CI = 1.18–1.59).

Bioavailable estradiol was no longer associated with hip fracture after adjustment for either bioavailable testosterone (OR = 0.86 per SD; CI = 0.72–1.04) or SHBG (OR = 0.90 per SD; CI = 0.75–1.08). In contrast, higher testosterone was associated with a 20% lower risk (OR = 0.80 per SD; CI = 0.67–0.96) after adjustment for estradiol and a 17% lower risk (OR = 0.83 per SD; CI = 0.71–0.98) after adjustment for SHBG. Also, higher SHBG was associated with a 30% higher risk (OR = 1.30 per SD; CI = 1.11–1.52) after adjustment for testosterone and a 39% higher risk (OR = 1.39 per SD; CI = 1.17–1.66) after adjustment for estradiol.

With all three hormones in the same model, higher bioavailable testosterone was associated with an 18% (OR = 0.82 per SD; CI = 0.69–0.99) lower risk, and higher SHBG level was associated with a 36% (OR = 1.36 per SD; CI = 1.14–1.63) higher risk. But bioavailable estradiol was not associated with the risk of hip fracture (OR = 0.99 per SD increase; CI = 0.81–1.21). For more direct clinical relevance, the results were expressed by tertiles of bioavailable sex hormone and SHBG levels. High SHBG remained an independent risk factor in models with more than one hormone or all three hormones (Table 3).

Of the 400 hip fractures, 246 (62%) were classified as femoral neck and 147 (37%) as intertrochanteric fractures. The patterns of associations among bioavailable estradiol, bioavailable testosterone, SHBG, and risk of these subtypes of hip fracture did not differ by type of hip fracture (data not shown). The screening discriminatory ability of SHBG, testosterone, and estradiol concentrations for hip fracture of any type was weak (c-statistic = 0.57–0.59).

Risk factors of hip fracture, endogenous sex hormones, and risk of hip fracture

Current smoking, parity, corticosteroid use, physical functioning, and BMI each were associated with hip fracture (Table 4). These variables remained associated with hip fracture in multivariable models that included sex hormone and SHBG levels. After adjustment for potential confounding factors, high SHBG level remained an independent risk factor (OR = 1.76; CI = 1.12–2.78), high bioavailable testosterone remained associated with protection against hip fracture (OR = 0.64; CI = 0.40–1.00), but bioavailable estradiol was not associated (OR = 0.72; CI = 0.42–1.23) (Table 4). Additional adjustment for BMI, which correlated with estradiol and somewhat with SHBG levels, attenuated the risk estimates for bioavailable estradiol and somewhat attenuated those for SHBG but not those for bioavailable testosterone (Table 4). Further adjustment for diabetes, calcium

TABLE 2. Correlations between baseline endogenous serum hormone concentrations in the women without incident hip fracture

Hormone	Total E_2 (n = 391)	Bio E_2 (n = 391)	Free E_2 (n = 391)	Total T (n = 391)	Bio T (n = 398)	Free T (n = 398)	BMI (n = 399)
Total E_2							0.45
Bio E_2	0.96						0.51
Free E_2	0.96	1.00					0.51
Total T	0.34	0.24	0.24				0.14
Bio T	0.45	0.45	0.45	0.88			0.29
Free T	0.45	0.45	0.45	0.89	0.99		0.29
SHBG	−0.19	−0.41	−0.41	−0.21	−0.20	−0.20	−0.31

Bioavailable and free sex hormone concentrations were calculated using the measured total sex hormone and SHBG concentrations, an assumed constant for albumin, and affinity constants of SHBG and albumin for the sex hormone (24, 25). Correlations did not differ in cases compared to controls ($P > 0.05$). Bio, Bioavailable; E_2, estradiol; T, testosterone.

TABLE 3. Associations between tertiles of baseline hormone and SHBG levels and risk of hip fracture

Hormones	OR (95% CI)	
	Separate bivariate models[a]	Single multivariate model with all three hormones[b]
Bioavailable estradiol (pg/ml)		
First tertile <5.1	1.00	1.00
Second tertile 5.1 to <8.2	0.83 (0.59–1.16)	1.04 (0.71–1.51)
Third tertile 8.2+	0.44 (0.29–0.66)	0.66 (0.40–1.08)
Bioavailable testosterone (ng/dl)		
First tertile <9	1.00	1.00
Second tertile 9 to <14	0.72 (0.51–1.02)	0.78 (0.54–1.14)
Third tertile 14+	0.62 (0.44–0.88)	0.75 (0.50–1.15)
SHBG (μg/dl)		
First tertile <1.2	1.00	1.00
Second tertile 1.2 to <1.7	1.25 (0.86–1.82)	1.24 (0.84–1.84)
Third tertile 1.7+	1.90 (1.31–2.74)	1.72 (1.14–2.62)

Bioavailable sex hormone concentration was calculated using the measured total sex hormone and SHBG concentrations, an assumed constant for albumin, and affinity constants of SHBG and albumin for the sex hormone (24, 25).

[a] Conditional logistic regression model for each sex hormone individually, with adjustment for matched factors: age and blood draw date.

[b] Single multivariate model with all three hormones in the model and with adjustment for matched factors: age and blood draw date.

intake, and vitamin D intake did not attenuate these estimates (data not shown). We tested for but found no significant interactions ($P_{\text{interaction}} > 0.1$) between SHBG, the bioavailable sex hormones, and possible mediators BMI, current smoking, and alcohol use on the risk of hip fracture. Results did not alter when BMI was replaced by weight in analyses.

Discussion

We found that high SHBG concentration is a risk factor of subsequent hip fracture independent of circulating estradiol and testosterone levels and other risk factors. Also, high endogenous bioavailable testosterone appeared associated with a lower risk

TABLE 4. Hormonal and other predictors of hip fracture in postmenopausal women

Variables	Bivariate separate models[a]	*P*[b]	Hormones and risk factors[c]	*P*[b]	Hormones and risk factors including BMI[d]	*P*[b]
BMI	0.95 (0.92–0.98)	<0.001			0.95 (0.92–0.99)	0.014
Current smoker	5.27 (2.20–12.60)	<0.001	4.75 (1.91–11.81)	<0.001	4.69 (1.88–11.68)	<0.001
Live birth ever	0.58 (0.39–0.85)	0.006	0.56 (0.36–0.87)	0.007	0.52 (0.33–0.81)	0.004
Corticosteroid use	4.38 (1.42–13.54)	0.010	2.84 (0.87–9.26)	0.096	3.00 (0.91–9.94)	0.072
RAND 36 physical functioning >90	0.64 (0.46–0.90)	0.010	0.67 (0.46–0.98)	0.041	0.61 (0.41–0.90)	0.013
Parent broke hip before age 40	1.33 (0.92–1.93)	0.133				
Total calcium intake (per 500 mg)	0.91 (0.82–1.01)	0.074				
Diabetes	1.26 (0.69–2.32)	0.456				
Bioavailable estradiol (pg/ml)		0.002		0.867		0.323
First tertile <5.1	1.00		1.00		1.00	
Second tertile 5.1 to <8.2	0.83 (0.59–1.16)		1.06 (0.70–1.61)		1.20 (0.78–1.85)	
Third tertile 8.2+	0.44 (0.29–0.66)		0.72 (0.42–1.23)		0.97 (0.54–1.73)	
Bioavailable testosterone (ng/dl)		<0.001		0.029		0.023
First tertile <9	1.00		1.00		1.00	
Second tertile 9 to <14	0.72 (0.51–1.02)		0.81 (0.54–1.22)		0.78 (0.51–1.18)	
Third tertile 14+	0.62 (0.44–0.88)		0.64 (0.40–1.00)		0.63 (0.39–1.00)	
SHBG (μg/dl)		<0.001		0.002		0.003
First tertile <1.2	1.00		1.00		1.00	
Second tertile 1.2 to <1.7	1.25 (0.86–1.82)		1.26 (0.83–1.93)		1.13 (0.73–1.74)	
Third tertile 1.7+	1.90 (1.31–2.74)		1.76 (1.12–2.78)		1.63 (1.02–2.59)	

For conversion of estradiol to pmol/liter, multiply by 3.671 pmol/liter. For conversion of testosterone to nmol/liter, multiply by 0.0347. For conversion of SHBG to nmol/liter, multiply by 40. Bioavailable sex hormone concentration was calculated using the measured total sex hormone and SHBG concentrations, an assumed constant for albumin, and affinity constants of SHBG and albumin for the sex hormone (24, 25).

[a] Each variable was modeled separately from the others, with adjustment for matched factors: age and blood draw date.

[b] *P* value for linear trend.

[c] A single multivariable model with adjustment for matched factors: age and date of blood draw.

[d] A single multivariable model with adjustment for matched factors: age and date of blood draw and includes BMI.

of hip fracture independent of the other hormones and risk factors. But endogenous estradiol concentration is not associated with hip fracture independent of either SHBG or testosterone.

Most previous studies have found a significant or suggestive association between higher SHBG and increased osteoporotic fracture risk (3, 4, 6, 7, 10, 17, 18). Two studies observed that women with high SHBG and lower endogenous estradiol levels had a very high risk of vertebral and hip fractures (4, 6, 10). The present study did not observe such an interaction between SHBG and either estradiol or testosterone but suggests that SHBG affects hip fracture risk independent of endogenous estradiol or testosterone.

It has been widely assumed that SHBG exerts its effect on bone indirectly by binding circulating estradiol and testosterone, thereby limiting their bioavailability to bone cells. However, our results complement findings that expand SHBG's role as a mediator of multiple signaling pathways in sex hormone-responsive cells (19, 20, 30, 31). Sex hormone-bound SHBG may bind its own cell membrane receptor (19, 32) and steroid-free SHBG to an endocytic receptor (20) to mediate intracellular sex hormone signaling and cell function. SHBG itself might directly influence BMD; certain mutations in the SHBG gene are associated with circulating SHBG levels and BMD (33). These lines of evidence need to be reconciled and addressed in bone cells to help clarify SHBG's role in fracture.

Although we observed a protective relation between estradiol and risk of hip fracture, the inverse association with SHBG and positive association with testosterone concentrations appeared to account for that relation. Our results differ from SOF, which observed strong and independent associations for estradiol and SHBG (4). SOF and a subsequent study suggested a threshold effect where only low estradiol was associated with a higher risk of fracture (4, 6). However, the present study did not observe such a threshold. Subjects in this study were somewhat younger and heavier, and using a similar assay, had somewhat higher baseline estradiol than in SOF. One third of the women had total estradiol levels less than 8 pg/ml (29 pmol/liter), whereas one third in the SOF study had levels less than 5 pg/ml (18 pmol/liter) (4). Bioavailable estradiol was not measured in SOF, but it is likely that bioavailable levels were also higher in the current study. The relation of endogenous estradiol with hip fracture risk might be somewhat weaker in younger, heavier women who have a lower risk of fracture and have higher baseline estradiol levels than postmenopausal women who weigh less or are older. Measurements of postmenopausal estradiol in our study by RIA after chromatographic extraction appear to be at least as accurate and precise as other estradiol assay methods, but assay standardization across studies of postmenopausal women would make direct comparisons more feasible (34). Our current study is substantially larger than SOF or other studies of this association.

Other studies have shown that testosterone exerts its effects on bone remodeling and bone loss as a precursor to estradiol (12). Our results support that bioavailable testosterone exerts direct beneficial effects on bone formation independent of endogenous estradiol (35, 36). The observed relation between testosterone and lower fracture risk might at least partly be due to more intracellular production of estradiol by aromatase that is not reflected by circulating estradiol concentrations. Having heavier women in the current study is consistent with this; more aromatase conversion from testosterone to estradiol in adipose cells may occur. Higher testosterone levels might also increase muscle strength and decrease the risk of falling seen in older men (15), although the few studies in women have not shown clear associations (8, 16). Early oophorectomy or early natural menopause in cases more than controls could explain why estradiol was not associated with fracture independent of testosterone because it eliminates ovarian androgen production and decreases androgen substrate for conversion to estrogen, but we did not observe such a difference based on the WHI information collected on menopausal status.

We confirmed that several factors, including lower weight, lower BMI, current smoking, nulliparity, and corticosteroid use are associated with an increased risk of hip fracture. The detrimental effects of, for example, smoking might in part be mediated through lowering estradiol levels (37); however, we showed that these associations are not in large part explained by circulating estradiol, testosterone, or SHBG levels.

This study has several strengths. It is the largest prospective study of endogenous hormones and hip fracture to date. Rates of follow-up for potential hip fractures were high, and fractures were validated by radiographs. Estradiol and testosterone were measured by very sensitive extraction-based RIAs. We measured and analyzed the interrelations among endogenous estradiol, testosterone, and SHBG concentrations in the risk of subsequent hip fracture.

Nevertheless, this study has several limitations. BMD was measured on a small minority, so we could not assess whether this is an intermediary between sex hormone levels and fracture. A limited number of fractures by subtype hindered testing whether associations differ by type of hip fracture. As in previous studies, our subjects were almost all Caucasian, limiting the generalizability of our results to women of other races. Hormone levels were measured at a single time point, and fracture occurred up to 7 yr after baseline measures. However, single measurements of postmenopausal estradiol are moderately correlated ($r \geq 0.7$) with measurements made 2–3 yr later (38). Use of anti-osteoporosis medications during the study period was obtained at only yr 3 (follow-up midpoint), was not verified, and thus could not be assessed. But less than 10% of the eligible study population self-reported taking anti-osteoporosis medications at yr 3, so use is unlikely to have materially altered our results. Low estradiol might act on bone by increasing FSH levels (39). We did not measure FSH, but studies have found that high testosterone in fact may resist FSH-induced bone loss (40). Also, serum SHBG was not measured in these studies. In addition, menopausal exogenous estrogen increases both SHBG and estradiol levels while protecting against fractures; studies of how SHBG and estrogen levels might mediate the effect of exogenous estrogen use on fracture risk are warranted.

In summary, higher circulating levels of SHBG are associated with an increased risk of hip fracture independent of its effects on circulating levels of bioavailable testosterone and estradiol. This suggests that potential direct effects of SHBG on bone cells de-

serve additional study. Moreover, endogenous testosterone appears to reduce the risk of hip fracture in postmenopausal women. This suggests that declines in testosterone production may be an explanation for the increased risk of hip fracture after menopause and with aging, and interventions to maintain or improve bioavailable testosterone levels after menopause might reduce the risk of hip fracture. Finally, our study does not confirm that endogenous estradiol plays an important independent role in the risk of hip fracture in older postmenopausal women.

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