ORIGINAL ARTICLE

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High Serum SHBG Predicts Incident Vertebral Fractures in Elderly Men

Liesbeth Vandenput,[1] Dan Mellström,[1,2] Andreas Kindmark,[3] Helena Johansson,[1] Mattias Lorentzon,[1,2] Jason Leung,[4] Inga Redlund-Johnell,[5] Björn E Rosengren,[5] Magnus K Karlsson,[5] Yi-Xiang Wang,[6] Timothy Kwok,[4] and Claes Ohlsson[1]

[1]Centre for Bone and Arthritis Research, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[2]Geriatric Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[3]Department of Medical Sciences, University of Uppsala, Uppsala, Sweden
[4]Jockey Club Centre for Osteoporosis Care and Control, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China
[5]Clinical and Molecular Osteoporosis Research Unit, Department of Clinical Sciences, Lund University, and Department of Orthopaedics, Skåne University Hospital, Malmö, Sweden
[6]Department of Imaging and Interventional Radiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China

ABSTRACT

Previous prospective cohort studies have shown that serum levels of sex steroids and sex hormone-binding globulin (SHBG) associate with nonvertebral fracture risk in men. The predictive value of sex hormones and SHBG for vertebral fracture risk specifically is, however, less studied. Elderly men (aged ≥65 years) from Sweden and Hong Kong participating in the Osteoporotic Fractures in Men (MrOS) study had baseline estradiol and testosterone analyzed by gas chromatography–mass spectrometry (GC-MS) and SHBG by immunoradiometric assay (IRMA). Incident clinical vertebral fractures ($n = 242$ cases) were evaluated in 4324 men during an average follow-up of 9.1 years. In a subsample of these men ($n = 2256$), spine X-rays were obtained at baseline and after an average follow-up of 4.3 years to identify incident radiographic vertebral fractures ($n = 157$ cases). The likelihood of incident clinical and radiographic vertebral fractures was estimated by Cox proportional hazards models and logistic regression models, respectively. Neither serum estradiol (hazard ratio [HR] per SD increase = 0.93, 95% confidence interval [CI] 0.80–1.08) nor testosterone (1.05, 0.91–1.21) predicted incident clinical vertebral fractures in age-adjusted models in the combined data set. High serum SHBG, however, associated with increased clinical vertebral fracture risk (1.24, 1.12–1.37). This association remained significant after further adjustment for FRAX with or without bone mineral density (BMD). SHBG also associated with increased incident radiographic vertebral fracture risk (combined data set; odds ratio [OR] per SD increase = 1.23, 95% CI 1.05–1.44). This association remained significant after adjustment for FRAX with or without BMD. In conclusion, high SHBG predicts incident clinical and radiographic vertebral fractures in elderly men and adds moderate information beyond FRAX with BMD for vertebral fracture risk prediction. © 2015 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals, Inc. on behalf of American Society for Bone and Mineral Research.

KEY WORDS: SEX STEROIDS; OSTEOPOROSIS; GENERAL POPULATION STUDIES; FRACTURE RISK ASSESSMENT; MEN

Introduction

Osteoporosis and osteoporosis-related fractures in men account for considerable disease burden and costs for society. Both androgens and estrogens affect bone health in men, and previous studies have shown that serum sex steroid levels associate with nonvertebral fracture risk.[1] In particular, low serum estradiol and testosterone and high sex hormone-binding globulin (SHBG) levels result in increased likelihood of all and nonvertebral fractures.[2–8]

The predictive value of sex steroids and SHBG specifically for vertebral fracture risk in men is, however, less studied. Both total and bioavailable estradiol associated with increased risk of incident radiographic vertebral fractures in the Rancho Bernardo Study, including 352 older men with 28 fracture cases over an 8-year follow-up.[9] Yet, the Rotterdam Study, evaluating 178 older men with 45 radiographic vertebral fractures during an average follow-up of 6.5 years, found no significant associations of sex steroids or SHBG with fracture risk.[10] Regarding incident clinical vertebral fractures, Meier and colleagues reported high

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Address correspondence to: Claes Ohlsson, MD, PhD, Centre for Bone and Arthritis Research, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Vita Stråket 11, SE-413 45 Gothenburg, Sweden. E-mail: claes.ohlsson@medic.gu.se
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Table 1. Characteristics of the Study Subjects

	MrOS Sweden ($n = 2847$)	MrOS Hong Kong ($n = 1477$)	Combined ($n = 4324$)
Age (years)	75.4 ± 3.2	72.5 ± 5.0	74.4 ± 4.1
Height (cm)	174.7 ± 6.6	163.0 ± 5.7	170.7 ± 8.4
Weight (kg)	80.6 ± 12.1	62.1 ± 9.4	74.3 ± 14.3
BMI (kg/m^2)	26.4 ± 3.6	23.3 ± 3.1	25.3 ± 3.7
Serum sex steroids			
Estradiol (pg/mL)[a]	21.2 ± 7.5	24.6 ± 14.3	22.4 ± 10.7
Bioavailable E2 (pg/mL)	13.2 ± 5.0	15.1 ± 9.6	13.9 ± 7.1
Testosterone (ng/dL)[a]	457 ± 176	548 ± 204	490 ± 192
Bioavailable testosterone (ng/dL)	180 ± 70	207 ± 59	190 ± 67
SHBG (nmol/L)	47.2 ± 21.8	51.2 ± 20.4	48.5 ± 21.4
Validated vertebral fractures (*n*, %)			
Incident clinical vertebral fractures	221/2847 (7.8)	21/1477 (1.4)	242/4324 (5.6)
Incident radiographic vertebral fractures	91/1108 (8.2)	66/1148 (5.7)	157/2256 (7.0)
Prevalent radiographic vertebral fractures	149/1108 (13.4)	43/1148 (3.7)	192/2256 (8.5)
FRAX without BMD (%)	11.0 ± 4.4	6.9 ± 2.8	9.6 ± 4.4
FRAX with BMD (%)	9.8 ± 5.5	6.7 ± 3.2	8.7 ± 5.1
Lumbar spine BMD (g/cm^2)	1.14 ± 0.20	0.95 ± 0.18	1.08 ± 0.22
Calcium intake (mg/d)	898 ± 433	628 ± 298	805 ± 413
Physical activity (km/d)	4.0 ± 3.2	1.4 ± 1.5	3.1 ± 3.0
Grip strength (kg)	39.9 ± 7.4	31.0 ± 6.6	36.8 ± 8.3
Corticosteroid use (*n*, %)	52 (1.8)	19 (1.3)	71 (1.6)
Smoking (*n*, %)	241 (8.5)	175 (11.8)	416 (9.6)
Alcohol ≥3 units per day (*n*, %)	62 (2.2)	10 (0.7)	72 (1.7)
Falls during past 12 months (*n*, %)	466 (16.4)	224 (15.2)	690 (16.0)
Fractures after the age of 50 years (*n*, %)	186 (6.5)	101 (6.8)	287 (6.6)
Major prevalent diseases (*n*, %)			
Cancer	380 (13.4)	60 (4.1)	440 (10.2)
COPD	238 (8.4)	179 (12.1)	417 (9.7)
Diabetes	269 (9.5)	225 (15.2)	494 (11.4)
Stroke	180 (6.3)	84 (5.7)	264 (6.1)
Rheumatoid arthritis	41 (1.4)	17 (1.2)	58 (1.3)

Data are presented for those subjects with fracture information and serum SHBG levels available, and excluding those with surgical or chemical castration and androgen or anti-androgen treatment. Values are given as mean ± SD or *n* (%). FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture.

COPD = chronic obstructive pulmonary disease.

[a]Serum testosterone levels in healthy adult males vary from 315 to 1000 ng/dL (11 to 35 nmol/L), whereas estradiol concentrations are around 20 to 30 pg/mL (70 to 110 pmol/L).[30]

SHBG to be associated with increased fracture risk in the Dubbo Osteoporosis Epidemiology Study in which 55 clinical vertebral fractures were reported in 609 men during a median follow-up of 5.8 years.[5] Taken together, the available studies investigating the association between sex steroids and future vertebral fracture risk are scarce, not well powered, and include a limited number of incident fractures. We, therefore, investigated the predictive value of serum sex steroids and SHBG for incident vertebral fracture risk in more detail in two large, well-characterized cohorts of older men with high fracture incidence in Sweden and Hong Kong during an average follow-up of 9.1 years.

Materials and Methods

Study sample

The Osteoporotic Fractures in Men (MrOS) study is a multicenter, prospective study including older men in Sweden ($n = 3014$), Hong Kong ($n = 2000$), and the United States ($n = 5994$). In this study, associations between serum sex steroids, SHBG, and incident vertebral fractures were investigated in the cohorts from Sweden and Hong Kong (Table 1). The MrOS Sweden cohort consists of three subcohorts from three different Swedish cities ($n = 1005$ in Malmö, $n = 1010$ in Gothenburg, and $n = 999$ in Uppsala). Study subjects (men aged 69 to 81 years) were randomly selected using national population registers, contacted, and asked to participate. To be eligible for the study, the subjects had to be able to walk without assistance, provide self-reported data, and sign an informed consent.[11] The study was approved by the ethics committees at the Universities of Gothenburg, Lund, and Uppsala. Informed consent was obtained from all study participants. The MrOS Hong Kong cohort includes 2000 Chinese men aged 65 years or older who were recruited by advertisements placed in housing estates and community centers for elderly people. All subjects had to be community dwelling, able to walk without assistance, and not have bilateral hip replacement. Stratified sampling was adopted to achieve approximately 33% of the participants in each of the three age groups: 65–69, 70–74, and ≥75 years.[12] The study protocol was approved by the Chinese University of Hong Kong ethics committee, and written informed consent was obtained from all subjects. Participants with surgical or

chemical castration, and androgen or anti-androgen treatment were excluded from the analyses.

Assessment of covariates

In both the MrOS Sweden and Hong Kong cohorts, we used a standardized questionnaire to gather information about self-reported previous fractures after age 50 years, falls (yes/no) during the last 12 months preceding the baseline visit, amount of physical activity, nutritional intake, smoking, alcohol use, prevalent major diseases (diabetes, stroke, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer), and use of glucocorticoids. Physical activity was the subject's average total daily walking distance, including both walking as a means of exercise and leisure and as a means of outdoor transportation in activities of daily life. Calcium intake was estimated from dairy product intake. Alcohol use was expressed as three or more glasses of alcohol-containing drinks per day, calculated from the reported frequency and amount of alcohol use. Grip strength was analyzed using a dynamometer; two measurements were taken on each side and the average of right and left was used in the analyses. Standard equipment was used to measure height and weight. Body mass index (BMI) was calculated by dividing the weight in kg by the height in meter squared.

Areal BMD (aBMD, g/cm^2) of the femoral neck and lumbar spine (L_1 to L_4) was assessed at baseline using the Lunar Prodigy dual-energy X-ray absorptiometry (DXA; GE Lunar Corp., Madison, WI, USA) in the Uppsala and Malmö cohorts and the Hologic QDR 4500/ A-Delphi (Hologic, Waltham, MA, USA) in the Gothenburg cohort. The coefficients of variation (CVs) for the aBMD measurements ranged from 0.5% to 3% depending on the application. To be able to use DXA measurements performed with equipment from two different manufacturers, a standardized BMD (sBMD) was calculated, as described previously.[11] In MrOS Hong Kong, aBMD at the femoral neck and lumbar spine was measured using the Hologic QDR 4500/ A-Delphi with CVs $<1\%$.

The country-specific FRAX tool was used to assess a subject's calculated 10-year probability of a major osteoporotic fracture (clinical spine, distal radius, proximal humerus, or hip) (www.shef.ac.uk/FRAX/). It integrates the fracture risks associated with clinical risk factors as well as femoral neck BMD.[13]

Assessment of vertebral fractures

Incident clinical vertebral fractures

In MrOS Sweden, central registers covering all Swedish citizens were used to identify the subjects, and at the time of fracture evaluation, computerized X-ray archives were searched for new vertebral fractures occurring after the baseline visit.[4] In MrOS Hong Kong, fractures were ascertained by the Hong Kong Hospital Authority electronic database, which included computerized X-ray archives. In addition, the subjects were contacted at 4-monthly intervals for fracture incidence during the first 4 years of follow-up.[8] All fractures were verified by X-ray. The mean follow-up time for fractures was 8.6 years in Sweden and 9.9 years in Hong Kong.

Prevalent and incident radiographic vertebral fractures

In Sweden, X-rays of the lateral thoracic and lumbar spine were obtained both at baseline and after an average follow-up of 4.7 years in 1156 subjects. The radiographs were evaluated for vertebral fractures at both time points by an expert radiologist (IR-J) using a modified semiquantitative method developed by Genant and colleagues.[14,15] In Hong Kong, lateral thoracic and lumbar spine radiographs were obtained at baseline and after 4 years in 1566 participants. The films were assessed by two radiologists using Genant's semiquantitative scoring system.[14,16]

Serum analyses

A validated gas chromatography–tandem mass spectrometry system[17–19] was used to analyze serum testosterone (limit of detection 0.05 ng/mL, intra-assay CV 2.9%, interassay CV 3.4%) and estradiol (limit of detection 2.00 pg/mL, intra-assay CV 1.5%, interassay CV 2.7%) in the samples from MrOS Sweden and Hong Kong. Serum was also assayed for SHBG using immunoradiometric assay (IRMA) (Spectria, Orion Diagnostica, Espoo, Finland) with an intra-assay CV of less than 5.5% and an interassay CV of less than 6.9%. More than half of the subjects in Sweden (1928/2847, 67.7%) had morning samples (drawn before 10.00 am); the remaining were drawn around noon. All Hong Kong samples were fasting samples. Bioavailable estradiol and testosterone levels were calculated using law-of-mass-action equations using association constants estimated from a systematic review of published binding studies and an iterative numeric method.[20]

Statistical analyses

In predefined analyses, the associations between serum levels of sex steroids and SHBG and incident clinical vertebral fractures were analyzed using Cox proportional hazards models. Logistic regression models were used to examine the associations between sex steroids and SHBG and incident radiographic vertebral fractures. We show the effect estimates with the 95% confidence intervals (CIs) within parentheses for a 1-SD increase (*Z*-score) in serum sex steroid or SHBG levels. All estimates were adjusted for time of serum sampling, cohort, and MrOS Sweden site. Further adjustments were made for body mass index (BMI), a multivariate model (calcium intake, smoking, alcohol use, falls, grip strength, physical activity, fractures after the age of 50 years/prevalent radiographic vertebral fractures, prevalent diseases [diabetes, cancer, stroke, COPD, rheumatoid arthritis], and glucocorticoid use), and lumber spine BMD to assess the independent effects of serum SHBG on vertebral fracture outcome. The models used to estimate the associations between serum testosterone, estradiol, and SHBG, entered as continuous variables, and vertebral fracture incidence were performed in both cohorts separately as well as in the combined cohort, for which the analyses were adjusted for the individual cohorts. SHBG was also examined as quartiles and further as a dichotomous variable comparing quartile 4 to quartiles 1 to 3. The association between serum levels of SHBG and testosterone was examined using partial correlation, controlling for time of serum sampling, cohort, and MrOS Sweden site.

Fracture discrimination was determined using C-statistics, where the statistical significance of change in the area under the ROC curve (AUC) between models was tested with the roc.test function in *R* using settings according to DeLong and colleagues.[21] Fracture reclassification analyses were performed by calculating the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) according to Pencina and colleagues.[22] The improvements in risk prediction by SHBG as a continuous parameter were evaluated over baseline models, including age or FRAX estimates with or without BMD.

Table 2. Serum Sex Steroids and the Risk of Incident Clinical Vertebral Fractures

	MrOS Sweden	MrOS Hong Kong	Combined
Estradiol (per SD increase)	0.96 (0.82–1.12)	0.45 (0.19–1.04)	0.93 (0.80–1.08)
Bioavailable estradiol (per SD increase)	0.87 (0.74–1.03)	0.22 (0.09–0.55)	0.84 (0.71–0.98)
Testosterone (per SD increase)	1.03 (0.89–1.20)	1.19 (0.80–1.77)	1.05 (0.91–1.21)
Bioavailable testosterone (per SD increase)	0.88 (0.74–1.04)	0.71 (0.46–1.10)	0.85 (0.73–1.00)
SHBG (per SD increase)	1.21 (1.09–1.34)	1.68 (1.21–2.35)	1.24 (1.12–1.37)

Age-adjusted hazard ratios are given with 95% CIs within parentheses. All models are also adjusted for time of serum sampling, cohort, and MrOS Sweden site when applicable.

To identify the proportion of subjects correctly reclassified by adding SHBG levels as extra predictors, the incremental discriminative ability of the SHBG levels compared with age or FRAX estimates (both with and without BMD) was assessed by using category-free NRI and IDI.

All statistical analyses were performed using SPSS software (version 21.0; Chicago, IL, USA).

Results

Characteristics of the study subjects

The baseline characteristics of the study subjects in the MrOS Sweden and Hong Kong cohorts as well as in the combined cohort are shown in Table 1. Data are presented for those subjects with fracture information and serum SHBG levels available, and excluding those with surgical or chemical castration and androgen or antiandrogen treatment. At baseline, the mean age of the men in the combined cohort ($n = 4324$) was 74.4 years. During an average follow-up time of 9.1 years, a total of 242 men had at least one validated incident clinical vertebral fracture (rate of 6.2 per 1000 person-years).

In a combined subsample of 2256 men who had a spine X-ray both at baseline and after an average follow-up of 4.3 years, 8.5% had at least one prevalent radiographic vertebral fracture, whereas 157 incident radiographic vertebral fractures were identified (Table 1).

Serum SHBG as a predictor of incident clinical vertebral fracture risk

Age-adjusted Cox proportional hazards models demonstrated that high serum SHBG associated with increased clinical vertebral fracture risk in the combined data set when analyzed as a continuous variable (24% increased risk per SD increase in SHBG, Table 2). Significant associations between SHBG and clinical vertebral fracture risk were also observed in the individual cohorts. In contrast, neither serum estradiol nor testosterone predicted fracture risk in corresponding analyses (Table 2). Low levels of bioavailable estradiol, but not bioavailable testosterone, associated significantly with increased clinical vertebral fracture risk. However, when both serum SHBG and bioavailable estradiol were included in the same model, only SHBG independently predicted the risk of incident clinical vertebral fractures (hazard ratio [HR] per SD increase = 1.24, 95% confidence interval [CI] 1.12–1.37). Further exploratory analyses showed that when assigning men in quartile 1 of SHBG levels as a referent, clinical vertebral fracture risk was increased in men within the highest quartile of SHBG levels, whereas the fracture risk in the individual quartiles 2 and 3 was similar to that of quartile 1 (Supplemental Table S1). Compared with men within the pooled quartiles 1 to 3, men with SHBG levels within quartile 4 were at significantly greater risk (52%) of a clinical vertebral fracture.

Serum SHBG as an independent predictor of incident clinical vertebral fracture risk

Multivariate adjustment for traditional risk factors for fracture (BMI, calcium intake, smoking, alcohol use, falls, grip strength, physical activity, fractures after the age of 50 years, prevalent diseases [diabetes, cancer, stroke, COPD, rheumatoid arthritis], and glucocorticoid use) did not substantially change the association between serum SHBG and clinical vertebral fractures in the combined cohort or the individual cohorts (Table 3). Moreover, the association remained significant after adjustment for FRAX without BMD and FRAX with BMD (Table 3). To address whether the association between serum SHBG and clinical vertebral fracture risk is mediated by BMD, further adjustments were made. After inclusion of lumber spine BMD in the model, the association between serum SHBG and long-term risk of clinical vertebral fracture was slightly attenuated but remained significant (HR per SD increase = 1.14, 95% CI 1.03–1.27).

Table 3. Serum SHBG as an Independent Predictor of Incident Clinical Vertebral Fracture Risk

	MrOS Sweden	MrOS Hong Kong	Combined
SHBG (per SD increase)			
A. Base model	1.21 (1.09–1.34)	1.75 (1.27–2.43)	1.21 (1.09–1.34)
B. Multivariate model	1.20 (1.07–1.34)	1.77 (1.29–2.44)	1.22 (1.10–1.35)
C. FRAX without BMD	1.23 (1.11–1.37)	1.60 (1.14–2.23)	1.26 (1.14–1.39)
D. FRAX with BMD	1.22 (1.09–1.35)	1.59 (1.13–2.25)	1.25 (1.13–1.38)

Cox proportional hazards regression models for SHBG in (A) a base model (adjusted for age and BMI); (B) a multivariate model (age, BMI, calcium intake, smoking, alcohol use, falls, grip strength, physical activity, fractures after the age of 50 years, prevalent diseases [diabetes, cancer, stroke, COPD, rheumatoid arthritis], and glucocorticoid use); (C) a model including FRAX without BMD; and (D) a model including FRAX with BMD. All models are adjusted for time of serum sampling, cohort, and MrOS Sweden site when applicable. Hazard ratios are given with 95% CIs within parentheses. FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture.

Table 4. Serum SHBG and Incident Clinical Vertebral Fracture Risk Discrimination and Reclassification in MrOS Sweden

	Discrimination	Reclassification IDI			Reclassification NRI		
	C index	IDI	Event	Nonevent	NRI	Event	Nonevent
Age	0.56						
+ SHBG	**0.59**	**0.005**	**0.005**	0.000	**0.200**	−0.078	**0.278**
FRAX without BMD	0.57						
+ SHBG	0.59	**0.005**	**0.005**	0.000	**0.194**	−0.088	**0.282**
FRAX with BMD	0.61						
+ SHBG	0.62	**0.004**	**0.004**	0.000	**0.178**	−0.094	**0.272**

IDI = integrated discriminative improvement; NRI = net reclassification improvement.

Incident clinical vertebral fracture risk discrimination and reclassification for different models (age, FRAX without BMD, and FRAX with BMD) after addition of serum SHBG. FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture. Bold indicates $p < 0.05$.

Serum SHBG improves clinical vertebral fracture risk reclassification

Serum SHBG slightly improved clinical vertebral fracture risk discrimination (AUC) from a model adjusted for age ($p < 0.05$) when evaluated in the MrOS Sweden cohort. No significant improvements in discrimination with SHBG were observed from models adjusted for FRAX without BMD or FRAX with BMD (Table 4). When evaluated with the more sensitive IDI and NRI, SHBG significantly but modestly improved clinical vertebral fracture reclassification models of age, of FRAX without BMD, and of FRAX with BMD.

Serum SHBG as a predictor of incident radiographic vertebral fracture risk

Age-adjusted logistic regression models in the MrOS Hong Kong subsample showed that high serum SHBG associated with an increased risk of radiographic vertebral fractures. The same tendency was present in the MrOS Sweden subsample, but this association was not significant (Supplemental Table S2). In the combined subsample, high serum SHBG significantly associated with increased radiographic vertebral fracture risk (23% increased risk per SD increase in SHBG, Table 5). Serum levels of estradiol, bioavailable estradiol, and bioavailable testosterone were not significantly associated with radiographic vertebral fracture risk, but (somewhat surprisingly) subjects with high testosterone were at increased risk of fracture. However, when both SHBG and testosterone were tested in the same model, only SHBG independently predicted the risk of incident radiographic vertebral fractures (odds ratio [OR] per SD increase = 1.22, 95% CI 1.04–1.43).

Table 5. Serum Sex Steroids and the Risk of Incident Radiographic Vertebral Fractures

	OR (95% CI)
Estradiol (per SD increase)	1.05 (0.88–1.25)
Bioavailable estradiol (per SD increase)	0.97 (0.79–1.19)
Testosterone (per SD increase)	1.22 (1.03–1.44)
Bioavailable testosterone (per SD increase)	1.06 (0.88–1.27)
SHBG (per SD increase)	1.23 (1.05–1.44)

Age-adjusted odds ratios (ORs) are given with 95% CIs within parentheses for the combined cohort. All models are also adjusted for time of serum sampling, cohort, and MrOS Sweden site.

Serum SHBG as an independent predictor of incident radiographic vertebral fracture risk

As expected, prevalent radiographic vertebral fractures at baseline strongly predicted the risk of incident radiographic vertebral fractures (OR = 5.21, 95% CI 3.62–7.50). Multivariate adjustment for traditional risk factors for fracture including this risk factor (BMI, calcium intake, smoking, alcohol use, falls, grip strength, physical activity, prevalent radiographic vertebral fractures, prevalent diseases [diabetes, cancer, stroke, COPD, rheumatoid arthritis], and glucocorticoid use) did not substantially change the association between serum SHBG and radiographic vertebral fractures (Table 6). Furthermore, this association remained significant after adjustment for FRAX without BMD or FRAX with BMD.

Discussion

In this large, prospective study of Swedish and Chinese men, subjects with the highest SHBG levels had higher risk of incident radiographic and clinical vertebral fractures. The predictive role of SHBG for vertebral fracture risk was partly independent of BMD and largely independent of other known risk factors for fracture.

The association between sex steroids and SHBG and non-vertebral fracture risk in men is well studied (for review see

Table 6. Serum SHBG as an Independent Predictor of Incident Radiographic Vertebral Fracture Risk

	OR (95% CI)
SHBG (per SD increase)	
A. Base model	1.22 (1.05–1.43)
B. Multivariate model	1.22 (1.03–1.45)
C. FRAX without BMD	1.24 (1.06–1.44)
D. FRAX with BMD	1.23 (1.05–1.43)

Binary logistic regression models in the combined cohort for SHBG in (A) a base model (adjusted for age and BMI); (B) a multivariate model (age, BMI, calcium intake, smoking, alcohol use, falls, grip strength, physical activity, prevalent radiographic vertebral fractures, prevalent diseases [diabetes, cancer, stroke, COPD, rheumatoid arthritis], and glucocorticoid use); (C) a model including FRAX without BMD; and (D) a model including FRAX with BMD. All models are adjusted for time of serum sampling, cohort, and MrOS Sweden site. Odds ratios (ORs) are given with 95% CIs within parentheses. FRAX is the country-specific calculated estimate of the 10-year risk of a major osteoporotic fracture.

Vanderschueren and colleagues[1]). Yet, the role of sex steroids and SHBG for vertebral fracture risk particularly is not thoroughly investigated in men, with the few available studies being small and having a limited number of incident fractures. In the present study with the largest number of subjects and highest fracture incidence to date, we show that high levels of SHBG associated with increased risk of clinical vertebral fractures in older men. When analyzed as quartiles, those subjects within the highest quartile of SHBG were at highest risk of fracture. Our study confirms a previous report from the Dubbo Osteoporosis Epidemiology Study showing a significant independent association between serum SHBG levels and an increased risk of symptomatic vertebral fractures. In a multivariate analysis including testosterone, high serum SHBG levels independently predicted a 55% increased risk per SD increase in SHBG.[5] The association in our study was not altered after adjustment for multiple known risk factors for fracture such as smoking, use of alcohol, physical performance, previous fractures, previous falls, and several chronic conditions.

The association of SHBG with incident clinical vertebral fractures was partially attenuated after adjustment for lumbar spine BMD, suggesting that almost half of the impact of SHBG is mediated via effects on bone density. SHBG may affect fracture risk indirectly by binding testosterone and estradiol and hereby reducing the free and bioavailable levels of sex steroids. It has also been speculated that SHBG may directly amplify sex steroid intracellular signaling via a putative membrane receptor and SHBG-sex steroid interactions.[23] Alternatively, SHBG could be a marker for nonskeletal determinants of fracture risk. SHBG levels have been associated with frailty,[24] but in our study, adjustment for grip strength, physical activity, and previous falls did not affect the association between SHBG and vertebral fracture risk.

We also tested the predictive value of serum SHBG levels for fracture risk discrimination. When tested in Cox models, the association between SHBG and clinical vertebral fractures remained unchanged after addition of probability estimates from the risk assessment tool FRAX and that with or without including BMD. Furthermore, SHBG slightly improved fracture risk reclassification when added to base models of FRAX with or without BMD. This suggests that serum SHBG levels are a risk marker for clinical vertebral fractures, by adding information beyond risk estimates from the FRAX tool, with or without BMD.

Serum estradiol and testosterone did not associate independently with clinical vertebral fracture risk in our study. Low bioavailable estradiol levels associated with increased risk of clinical vertebral fractures, but this association was lost after adjusting for SHBG. It is plausible that different sex steroid-related fracture risk determinants exist for bone sites containing either predominantly trabecular (vertebrae) or cortical bone (appendicular sites and hip). Our findings indicate that SHBG independently predicts clinical vertebral fracture risk, whereas previous evidence from us and others implicates serum estradiol and to a lesser extent serum testosterone in risk prediction of nonvertebral fractures.[1] The latter is supported by studies showing that the threshold estradiol level for maintaining bone mass is more apparent at cortical than trabecular sites[25] and that estradiol levels are related to cortical porosity in men.[26]

The predictive role of sex steroids and SHBG for incident radiographic vertebral fractures has not yet been studied in well-powered cohorts. In our large cohort of older men, we found that high serum SHBG associated with increased fracture risk, similarly as for incident clinical vertebral fractures. Notably, SHBG associated with incident radiographic vertebral fractures even after adjustment for prevalent radiographic vertebral fractures at baseline. This makes it unlikely that our results are based only on the increased fracture risk in subjects with poor general health status. This association between SHBG and radiographic vertebral fractures also remained significant after addition of probability estimates from the risk assessment tool FRAX and that with or without including BMD. This suggests that serum SHBG levels are a risk marker not only for clinical but also for radiographic vertebral fractures.

Serum estradiol levels (total or bioavailable) did not associate with radiographic vertebral fracture risk in our older men. This is in agreement with data from the Rotterdam Study[10] but contradicts findings from the Rancho Bernardo Study in which men in the lowest quintile of total or bioavailable estradiol had significantly higher odds for vertebral fracture than those in the highest quintile.[9] High levels of testosterone associated with increased risk for incident radiographic vertebral fractures. However, when both SHBG and testosterone were tested in the same model, only SHBG independently predicted the risk of incident radiographic vertebral fractures in our study. The association for testosterone can be explained by the fact that serum levels of testosterone and SHBG are highly correlated ($r = 0.57$, $p < 0.001$), so when serum SHBG levels are high, the total testosterone concentrations are increased as well, which could explain the observed association.

Our study has a number of strengths. It is population based and consists of the largest data set to date with a high fracture incidence and well-characterized fracture phenotypes. Moreover, the findings are largely consistent among older white and Asian men. However, this study also has limitations. The results are based on single serum measurements and may thus underestimate true associations. The inclusion of some non-morning samples might have contributed to increased variability, but this was adjusted for in all the analyses by time of sampling. In addition, the bioavailable sex steroids levels were calculated and not, as preferable, directly measured by equilibrium dialysis-liquid chromatography-tandem mass spectrometry. A limitation of all association studies analyzing circulating sex steroid levels is that a valid estimate of the local levels of testosterone and estradiol in target tissues such as bone is lacking. It is plausible that serum SHBG regulates the local levels of testosterone, estradiol, or the balance between estradiol and testosterone in bone and, thereby, influences sex steroid action in bone. Importantly, these possible effects of circulating SHBG on bioavailable sex steroid levels locally in bone might differ substantially from its effect on circulating total levels of sex steroids. Also, the FRAX tool provides a 10-year probability of hip fracture and major osteoporotic fracture (including vertebral fracture) but no specific clinical vertebral fracture risk calculation, making FRAX less optimal as a covariate for the evaluation of vertebral fracture risk in the present study. Still, although FRAX is not developed for estimating vertebral fracture risk, other studies also used FRAX probability estimates to assess vertebral fracture risk.[27–29] Finally, we have tried to adequately adjust for confounders in all our analyses but cannot rule out residual confounding.

In conclusion, high SHBG associated with increased clinical and radiographic vertebral fracture risk in elderly men and adds moderate information beyond FRAX with BMD for vertebral fracture risk prediction.

Disclosures

All authors state that they have no conflicts of interest.

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References

1. Vanderschueren D, Laurent MR, Claessens F, et al. Sex steroid actions in male bone. Endocr Rev. 2014;35(6):906–60.
2. Amin S, Zhang Y, Felson DT, et al. Estradiol, testosterone, and the risk for hip fractures in elderly men from the Framingham Study. Am J Med. 2006;119(5):426–33.
3. Bjornerem A, Ahmed LA, Joakimsen RM, et al. A prospective study of sex steroids, sex hormone-binding globulin, and non-vertebral fractures in women and men: the Tromso Study. Eur J Endocrinol. 2007;157(1):119–25.
4. Mellström D, Vandenput L, Mallmin H, et al. Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. J Bone Miner Res. 2008;23(10):1552–60.
5. Meier C, Nguyen TV, Handelsman DJ, et al. Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. Arch Intern Med. 2008;168(1): 47–54.
6. LeBlanc ES, Nielson CM, Marshall LM, et al. The effects of serum testosterone, estradiol, and sex hormone binding globulin levels on fracture risk in older men. J Clin Endocrinol Metab. 2009;94(9): 3337–46.
7. Roddam AW, Appleby P, Neale R, et al. Association between endogenous plasma hormone concentrations and fracture risk in men and women: the EPIC-Oxford prospective cohort study. J Bone Miner Metab. 2009;27(4):485–93.
8. Woo J, Kwok T, Leung JC, Ohlsson C, Vandenput L, Leung PC. Sex steroids and bone health in older Chinese men. Osteoporos Int. 2012;23(5):1553–62.
9. Barrett-Connor E, Mueller JE, von Muhlen DG, Laughlin GA, Schneider DL, Sartoris DJ. Low levels of estradiol are associated with vertebral fractures in older men, but not women: the Rancho Bernardo Study. J Clin Endocrinol Metab. 2000;85(1):219–23.
10. Goderie-Plomp HW, van der Klift M, de Ronde W, Hofman A, de Jong FH, Pols HA. Endogenous sex hormones, sex hormone-binding globulin, and the risk of incident vertebral fractures in elderly men and women: the Rotterdam Study. J Clin Endocrinol Metab. 2004; 89(7):3261–9.
11. Mellström D, Johnell O, Ljunggren O, et al. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res. 2006;21(4):529–35.
12. Lau EM, Leung PC, Kwok T, et al. The determinants of bone mineral density in Chinese men—results from Mr. Os (Hong Kong), the first cohort study on osteoporosis in Asian men. Osteoporos Int. 2006;17(2):297–303.
13. Kanis JA, Oden A, Johnell O, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007; 18(8):1033–46.
14. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993;8(9):1137–48.
15. Kherad M, Rosengren BE, Hasserius R, et al. Low clinical relevance of a prevalent vertebral fracture in elderly men—the MrOs Sweden study. Spine J. 2015;15(2):281–9.
16. Kwok AW, Gong JS, Wang YX, et al. Prevalence and risk factors of radiographic vertebral fractures in elderly Chinese men and women: results of Mr. OS (Hong Kong) and Ms. OS (Hong Kong) studies. Osteoporos Int. 2013;24(3):877–85.
17. Labrie F, Belanger A, Belanger P, et al. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. J Steroid Biochem Mol Biol. 2006;99(4–5):182–8.
18. Vandenput L, Labrie F, Mellstrom D, et al. Serum levels of specific glucuronidated androgen metabolites predict BMD and prostate volume in elderly men. J Bone Miner Res. 2007;22(2):220–7.
19. Orwoll ES, Nielson CM, Labrie F, et al. Evidence for geographical and racial variation in serum sex steroid levels in older men. J Clin Endocrinol Metab. 2010;95(10):E151–60.
20. Mazer NA. A novel spreadsheet method for calculating the free serum concentrations of testosterone, dihydrotestosterone, estradiol, estrone and cortisol: with illustrative examples from male and female populations. Steroids. 2009;74(6):512–9.
21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3): 837–45.
22. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008; 27(2):157–72; discussion 207–12.
23. Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA. Androgen and estrogen signaling at the cell membrane via G-proteins and cyclic adenosine monophosphate. Steroids. 1999;64(1–2):100–6.
24. Mohr BA, Bhasin S, Kupelian V, Araujo AB, O'Donnell AB, McKinlay JB. Testosterone, sex hormone-binding globulin, and frailty in older men. J Am Geriatr Soc. 2007;55(4):548–55.
25. Khosla S, Melton LJ 3rd, Robb RA, et al. Relationship of volumetric BMD and structural parameters at different skeletal sites to sex steroid levels in men. J Bone Miner Res. 2005;20(5):730–40.
26. Vandenput L, Lorentzon M, Sundh D, et al. Serum estradiol levels are inversely associated with cortical porosity in older men. J Clin Endocrinol Metab. 2014;99(7):E1322–6.
27. Kanis JA, Johansson H, Oden A, McCloskey EV. A meta-analysis of the efficacy of raloxifene on all clinical and vertebral fractures and its dependency on FRAX. Bone. 2010;47(4):729–35.
28. Melton LJ 3rd, Atkinson EJ, Achenbach SJ, et al. Potential extensions of the US FRAX algorithm. J Osteoporos. 2012;2012:528790.
29. Harvey NC, Kanis JA, Oden A, et al. FRAX and the effect of teriparatide on vertebral and non-vertebral fracture. Osteoporos Int. Epub 2015 Jun 20. 10.1007/s00198-015-3173-3.
30. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev. 2005;26(6):833–76.