

Older Men With Low Serum Estradiol and High Serum SHBG Have an Increased Risk of Fractures

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ABSTRACT: Osteoporosis-related fractures constitute a major health concern not only in women but also in men. To study the predictive role of serum sex steroids for fracture risk in men, serum sex steroids were analyzed by the specific gas chromatography-mass spectrometry technique at baseline in older men ($n = 2639$; mean, 75 yr of age) of the prospective population-based MrOS Sweden cohort. Fractures occurring after baseline were validated (average follow-up of 3.3 yr). The incidence for having at least one validated fracture after baseline was 20.9/1000 person-years. Estradiol (E2; hazard ratio [HR] per SD decrease, 1.34; 95% CI, 1.22–1.49), free estradiol (fE2; HR per SD decrease, 1.41; 95% CI, 1.28–1.55), testosterone (T; HR per SD decrease, 1.27; 95% CI, 1.16–1.39), and free testosterone (fT; HR per SD decrease, 1.32; 95% CI, 1.21–1.44) were all inversely, whereas sex hormone-binding globulin (SHBG; HR per SD increase, 1.41; 95% CI, 1.22–1.63) was directly related to fracture risk. Multivariable proportional hazards regression models, adjusted for age, suggested that fE2 and SHBG ($p < 0.001$), but not fT, were independently associated with fracture risk. Further subanalyses of fracture type showed that fE2 was inversely associated with clinical vertebral fractures (HR per SD decrease, 1.57; 95% CI, 1.36–1.80), nonvertebral osteoporosis fractures (HR per SD decrease, 1.42; 95% CI, 1.23–1.65), and hip fractures (HR per SD decrease, 1.44; 95% CI, 1.18–1.76). The inverse relation between serum E2 and fracture risk was nonlinear with a strong relation <16 pg/ml for E2 and 0.3 pg/ml for fE2. In conclusion, older Swedish men with low serum E2 and high SHBG levels have an increased risk of fractures.

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INTRODUCTION

OSTEOPOROSIS-RELATED FRACTURES constitute a major health concern not only in women but also in men, in whom the lifetime fracture risk at age 45 has been estimated to be 24%.⁽¹⁾ Apart from major social and economic costs associated with fractures, mortality increases after both hip and vertebral fractures. This mortality is even higher in men than in women.⁽²⁾ Thus, it is important to clarify the pathogenesis of osteoporosis and fractures in men, who have been less widely studied than women, to aid in prevention and treatment.

Sex steroids are important for the skeletal growth and the maintenance of both the female and the male skeleton.^(3–5)

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However, the relative contribution of androgens versus estrogens in the regulation of the male skeleton is unclear. The effects of testosterone (T) can be exerted either directly through the androgen receptor or indirectly through aromatization to estradiol (E2) and further through estrogen receptor- α and/or - β .^(6,7) All three of these sex steroid receptors are expressed in bone,^(8–11) and experimental animal studies have indicated that each of these three receptors mediates site-specific skeletal effects of sex steroids.^(12–15)

Both human cross-sectional observational and prospective studies in men have, in general, shown that serum E2 is a stronger predictor of BMD than serum T.^(16–27) Furthermore, in an interventional study by Falahati-Nini et al.,⁽²⁸⁾ using E2 or T treatment in aging men with eliminated endogenous E2 and T, it was shown that E2 is the dominant sex steroid inhibiting bone resorption, whereas both E2 and T are important in maintaining bone formation. This finding might explain why BMD is more strongly associated with serum E2 than with serum T in men. However, Leder

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et al.⁽²⁹⁾ found that T had a clear impact on both bone formation and bone resorption, as studied in young men with eliminated endogenous E2 and T, and experimental animal studies have clearly established an important role of androgens for the male skeleton.⁽⁷⁾ Fracture risk is not only dependent on BMD. In cross-sectional studies, inverse associations between both serum E2^(18,30) and T⁽¹⁸⁾ and prevalent fractures were shown. However, the roles of serum E2 and T as predictors of fracture risk in men analyzed in prospective studies remain unclear. Neither serum E2 nor serum T predicted risk of fractures in the Rotterdam study, including 45 men with incident vertebral fractures, or in the Tromsø study, including 105 men with nonvertebral fractures.^(31,32) In the former study, the absence of a significant association between serum E2 and future fracture risk could be caused by insufficient power, because only a limited sample of males from the Rotterdam study was analyzed. Nevertheless, serum E2 levels were associated with BMD in the Rotterdam study. In the Framingham study, including 39 men with fractures, it was shown that E2, but not T, was a significant predictor of fracture risk, whereas it was recently reported in the Dubbo study, including 113 men with fractures, that T but not E2 predicted risk of fractures.^(33,34) These conflicting results might be because of the fact that these previous prospective studies, investigating the role of serum sex steroids for fracture risk, have been underpowered, including few incident fractures, and most of them^(31–33) have analyzed the baseline sex steroid levels using immunoassay-based techniques with a questionable specificity at lower concentrations.

We herein studied the predictive role of sex steroids, as measured by the specific gas chromatography-mass spectrometry (GC-MS) technique, for fracture risk in a large, prospective population-based cohort of Swedish men.

MATERIALS AND METHODS

Study subjects

The MrOS study is a multicenter, prospective study including older men in Sweden, Hong Kong, and the United States. The Swedish MrOS cohort ($n = 3014$) consists of three subcohorts from three different Swedish cities ($n = 1005$ in Malmö, $n = 1010$ in Göteborg, and $n = 999$ in Uppsala; Table 1), and the study subjects (men 69–80 yr of age) were randomly identified using national population registers. A total of 45% of the subjects who were contacted participated in the study. In this study, associations between serum sex steroids and fractures, which occurred after the baseline visit, were studied in MrOS Sweden. All covariates were measured at baseline. To be eligible for the study, the subjects had to be able to walk without aids. There were no other exclusion criteria.⁽¹⁸⁾ The study was approved by the ethics committee at University of Gothenburg. Informed consent was obtained from all study participants.

Assessment of covariates

Height was measured using a Harpenden stadiometer, and weight was measured by an electric scale. Two consecutive measurements of height were performed in the same

TABLE 1. CHARACTERISTICS OF THE STUDY SUBJECTS IN MrOS SWEDEN

Variables	MrOS Sweden ($n = 3014$)
Age (yr)	75.4 ± 3.2
Height (cm)	174.7 ± 6.5
Weight (kg)	80.7 ± 12.1
Grip strength (kg)	39.9 ± 7.5
SHBG (nM)	43.2 ± 21.9
DXA analyses	
sBMD femur neck (g/cm ²)	0.83 ± 0.13
Serum by GC-MS ($n = 2639$)	
Testosterone (ng/ml)	4.50 ± 1.87
Free testosterone (pg/ml)	80.1 ± 34.1
Dihydrotestosterone (pg/ml)	357 ± 195
Estradiol (pg/ml)	20.9 ± 7.9
Free estradiol (pg/ml)	0.36 ± 0.15
Estrone (pg/ml)	34.3 ± 15.0
Subjects with validated incident fractures	
All fractures	209 (20.9)
Nonvertebral osteoporosis fractures	
Hip	83 (8.2)
Distal radius	38 (3.7)
Proximal humerus	28 (2.7)
Pelvis	17 (1.6)
Clinical vertebral fractures	10 (1.0)
Other fractures	67 (6.5)
	77 (7.6)

Values are given as mean ± SD. For fractures, the numbers of subjects with first fractures are given with the incidence/1000 person-years shown within parentheses. Some subjects, included in the group of “all fractures,” had more than one type of first fracture, and therefore, these subjects were included in more than one of the different subtypes of fractures. Nonvertebral osteoporosis fractures are defined as fractures in hip, distal radius, proximal humerus, and pelvis. Other fractures include all validated fractures minus nonvertebral osteoporosis fractures and clinical vertebral fractures.

sBMD, standardized BMD.

session, and the average of these measurements was calculated. If there was a difference of ≥ 5 mm between the first two measurements, a third measurement was performed, and the average of the two values with the least mutual discrepancy was calculated. Grip strength was analyzed using the Baseline equipment (Baseline, Chattanooga, TN, USA), and the average of the measurements of the dominant and nondominant arm was used in the analyses.

Assessment of fractures

Participants were followed for 3.32 yr on average after the baseline examination. The follow-up time was recorded from the date of the baseline visit to the date of the first fracture or the date of death. When a subject sustained a first fracture at different sites during the follow-up, the various fractures and the follow-up time for each respective first fracture type were included in the analyses. Central registers covering all Swedish citizens were used to identify the subjects and the time of death for all subjects who died during the study, and these analyses were performed after the time of fracture validation. At the time of fracture evaluation, the computerized X-ray archives in Malmö, Göteborg, and Uppsala were searched for new fractures

TABLE 2. ANDROGENS AND ESTROGENS AND RISK OF FIRST FRACTURE IN THE WHOLE AND IN THE THREE SUBCOHORTS OF MROS SWEDEN

	<i>All subjects</i> (<i>n</i> = 2639)	<i>Malmö</i> (<i>n</i> = 956)	<i>Göteborg</i> (<i>n</i> = 998)	<i>Uppsala</i> (<i>n</i> = 685)
Testosterone (per SD decrease)	1.27 (1.16–1.39)	1.27 (1.11–1.46)	1.29 (1.12–1.48)	1.13 (0.84–1.50)
Free testosterone (per SD decrease)	1.32 (1.21–1.44)	1.30 (1.12–1.49)	1.34 (1.18–1.52)	1.24 (0.99–1.57)
Dihydrotestosterone (per SD decrease)	1.31 (1.17–1.46)	1.33 (1.14–1.56)	1.27 (1.07–1.50)	1.24 (0.92–1.66)
Estradiol (per SD decrease)	1.34 (1.22–1.49)	1.36 (1.16–1.60)	1.32 (1.14–1.54)	1.24 (0.93–1.66)
Free estradiol (per SD decrease)	1.41 (1.28–1.55)	1.40 (1.19–1.64)	1.39 (1.21–1.61)	1.38 (1.08–1.79)
Estrone (per SD decrease)	1.30 (1.15–1.47)	1.39 (1.19–1.63)	1.20 (0.92–1.58)	1.06 (0.74–1.52)
SHBG (per SD increase)	1.41 (1.22–1.63)	1.41 (1.09–1.82)	1.41 (1.12–1.77)	1.29 (0.96–1.85)

Age-adjusted hazard ratios are given with 95% CIs within parentheses.

TABLE 3. SERUM SEX STEROIDS AS INDEPENDENT PREDICTORS OF FRACTURE

	<i>Age-adjusted</i>	<i>Adjusted for age, height and weight</i>	<i>Adjusted for age, height, weight, and BMD</i>	<i>Adjusted for age, height, weight, BMD, and grip strength</i>
Free estradiol (per SD decrease)	1.36 (1.23–1.50)	1.36 (1.23–1.51)	1.31 (1.18–1.46)	1.28 (1.15–1.43)
SHBG (per SD increase)	1.32 (1.14–1.53)	1.26 (1.08–1.47)	1.26 (1.08–1.46)	1.22 (1.05–1.43)

Multivariable proportional hazards regression model including age, free estradiol, and SHBG with or without adjustment for height, weight, femoral neck BMD, and grip strength as risk factors for all validated incident fractures. Age-adjusted hazard ratios are given with 95% CIs within parentheses.

occurring after the baseline visit, using the unique personal registration number, which all Swedish citizens have. All fractures reported by the study subject after the baseline visit were confirmed by physician review of radiology reports. Fractures reported by the study subject, but not possible to confirm by X-ray analyses report, were not included in this study. All validated fractures were included in the main analyses, followed by exploratory subanalyses of fracture type. In the latter, we studied the associations between sex steroids and validated fractures, divided into three main groups: (1) X-ray-verified clinical vertebral fractures, (2) nonvertebral osteoporosis fractures at the major osteoporosis-related locations (defined as hip, distal radius, proximal humerus, and pelvis), and (3) other fractures (radius/ulna, hand, fingers, humerus, elbow, skull, cervical vertebrae, clavicle, scapula, rib, femoral shaft, patella, upper tibia, ankle, foot, toes; Table 1). In addition, subanalyses of the associations between serum sex steroids and hip fracture risk were performed. Fracture rates were expressed as the number of subjects with first fractures per 1000 person-years (Table 1).

Assessment of BMD (DXA)

Areal BMD (aBMD, g/cm²) of the femoral neck was assessed using the Lunar Prodigy DXA (*n* = 2004 from the Uppsala and Malmö cohorts; GE Lunar Corp., Madison, WI, USA) or Hologic QDR 4500/A-Delphi (*n* = 1010 from the Göteborg cohort; Hologic, Waltham, MA, USA). The CVs for the aBMD measurements ranged from 0.5% to 3%. To be able to use DXA measurements performed with equipment from two different manufacturers, a standardized BMD (sBMD) was calculated, as previously described.⁽¹⁸⁾

Serum analyses

The validated GC-MS system^(35,36) was used for the analysis of T (limit of detection, 0.05 ng/ml; intra-assay CV,

2.9%; interassay CV, 3.4%), dihydrotestosterone (DHT) (limit of detection, 0.02 ng/ml; intra-assay CV, 3.1%; interassay CV, 4.1%), E2 (limit of detection, 2.00 pg/ml; intra-assay CV, 1.5%; interassay CV, 2.7%), and estrone (limit of detection, 8.00 pg/ml; intra-assay CV, 1.8%; interassay CV, 1.7%). Analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. Serum samples for sex steroid levels were available for 99% of the subjects in the Göteborg cohort and 96% of the subjects in the Malmö cohort, whereas the 1 ml required for the GC-MS analyses was only available for 68% of the subjects in the Uppsala cohort. Fasting blood samples were collected between 8:00 and 8:30 a.m. Serum sex hormone-binding globulin (SHBG) was measured using IRMA (Orion Diagnostics, Espoo, Finland; limit of detection, 1.3 nM; intra-assay CV, 3%; interassay CV, 7%). Free T (fT) and free E2 (fE2) were calculated according to the method described by Vermeulen et al.⁽³⁷⁾ and van den Beld et al.⁽²⁵⁾ taking the concentrations of total T, total E2, and SHBG into account and assuming a fixed albumin concentration of 43 g/liter. All samples were analyzed in one laboratory.

Statistical analyses

Associations among variables were examined with Pearson's correlation. Cox proportional hazards models were used to study the associations between serum sex steroids and fracture outcomes. All validated fractures were included in the main analyses, followed by exploratory subanalyses of fracture type. In general, age-adjusted hazard ratios (HRs) per SD change (Tables 2–4) or versus the subjects in the lowest quartile of the respective hormones (Table 5) are given with 95% CIs within parentheses. To further explore the apparent nonlinear relation between E2 and fracture risk, Poisson regression models were used.⁽³⁸⁾ In the Poisson models, spline functions were applied to

TABLE 4. SUBANALYSES OF FREE ESTRADIOL, FREE TESTOSTERONE, AND SHBG AS PREDICTORS OF DIFFERENT TYPES OF FIRST FRACTURE

	<i>Nonvertebral osteoporosis fractures</i>	<i>Hip fractures</i>	<i>Clinical vertebral fractures</i>	<i>Other fractures</i>
Age-adjusted HR				
Free estradiol (per SD decrease)	1.42 (1.23–1.65)	1.44 (1.18–1.76)	1.57 (1.36–1.80)	1.18 (0.95–1.45)
Free testosterone (per SD decrease)	1.31 (1.15–1.50)	1.33 (1.11–1.60)	1.45 (1.28–1.64)	1.13 (0.93–1.38)
SHBG (per SD increase)	1.35 (1.08–1.69)	1.76 (1.27–2.43)	1.48 (1.14–1.91)	1.43 (1.13–1.82)
Age- and BMD-adjusted HR				
Free estradiol (per SD decrease)	1.36 (1.16–1.58)	1.31 (1.05–1.63)	1.47 (1.26–1.71)	1.08 (0.85–1.37)
Free testosterone (per SD decrease)	1.28 (1.12–1.47)	1.27 (1.06–1.53)	1.41 (1.24–1.60)	1.08 (0.87–1.34)
SHBG (per SD increase)	1.21 (0.96–1.52)	1.54 (1.11–2.14)	1.28 (0.98–1.67)	1.34 (1.05–1.73)

Hazard ratios are given with 95% CIs within parentheses. Nonvertebral osteoporosis fractures are defined as fractures in hip, distal radius, proximal humerus, and pelvis. Other fractures include all validated fractures minus nonvertebral osteoporosis fractures and clinical vertebral fractures.

TABLE 5. SERUM SEX STEROIDS IN QUANTILES AND RISK OF FIRST FRACTURE

	<i>All fractures</i>	<i>Nonvertebral osteoporosis fractures</i>	<i>Clinical vertebral fractures</i>
Estradiol			
Q1 (≤ 16.0 pg/ml)	1	1	1
Q2 (>16.0 and ≤ 20.3 pg/ml)	0.40 (0.26–0.61)	0.44 (0.24–0.84)	0.45 (0.23–0.89)
Q3 (>20.3 and ≤ 25.3 pg/ml)	0.53 (0.36–0.77)	0.50 (0.27–0.93)	0.43 (0.22–0.85)
Q4 (>25.3 pg/ml)	0.60 (0.41–0.86)	0.49 (0.26–0.90)	0.40 (0.20–0.82)
Free estradiol			
Q1 (≤ 265 fg/ml)	1	1	1
Q2 (>265 and ≤ 349 fg/ml)	0.49 (0.33–0.73)	0.59 (0.32–1.09)	0.39 (0.20–0.77)
Q3 (>349 and ≤ 437 fg/ml)	0.54 (0.37–0.79)	0.63 (0.35–1.14)	0.32 (0.15–0.65)
Q4 (>437 fg/ml)	0.47 (0.32–0.70)	0.39 (0.19–0.78)	0.27 (0.13–0.58)
Testosterone			
Q1 (≤ 3.36 ng/ml)	1	1	1
Q2 (>3.36 and ≤ 4.38 ng/ml)	0.67 (0.45–1.00)	0.64 (0.35–1.18)	0.71 (0.37–1.38)
Q3 (>4.38 and ≤ 5.54 ng/ml)	0.63 (0.42–0.96)	0.72 (0.40–1.30)	0.41 (0.19–0.90)
Q4 (>5.54 ng/ml)	0.89 (0.61–1.29)	0.51 (0.26–0.98)	0.76 (0.40–1.46)
Free testosterone			
Q1 (≤ 61 pg/ml)	1	1	1
Q2 (>61 and ≤ 79 pg/ml)	0.73 (0.51–1.07)	0.68 (0.39–1.21)	0.70 (0.37–1.31)
Q3 (>79 and ≤ 99 pg/ml)	0.58 (0.39–0.86)	0.59 (0.33–1.07)	0.48 (0.24–0.96)
Q4 (>99 pg/ml)	0.47 (0.31–0.71)	0.26 (0.12–0.57)	0.31 (0.14–0.69)
SHBG			
Q1 (≤ 28.8 nM)	1	1	1
Q2 (>28.8 and ≤ 38.7 nM)	0.76 (0.48–1.20)	0.47 (0.22–0.99)	0.94 (0.40–2.22)
Q3 (>38.7 and ≤ 52.5 nM)	1.30 (0.87–1.95)	1.00 (0.54–1.83)	1.96 (0.94–3.07)
Q4 (>52.5 nM)	1.79 (1.22–2.62)	1.48 (0.84–2.60)	2.07 (1.00–4.29)

Quartile 1 (Q1) is the reference in the analyses. Age-adjusted hazard ratios are given with 95% CIs within parentheses. Nonvertebral osteoporosis fractures are defined as fractures in hip, distal radius, proximal humerus, and pelvis.

study the relationship between serum levels of E2 and the risk of getting a fracture, with age and time included as independent variables. The Poisson model was made up of linear pieces at the ends and quadratic functions in the intermediate intervals, and the knots were chosen at the 25th, 50th, and 75th percentile.

RESULTS

Characteristics of the study subjects

The baseline characteristics and the number and incidence/1000 person-years of validated fractures, which have occurred after the baseline visit of the older men in the

MrOS Sweden cohort, are shown in Table 1. In total, 209 subjects had at least one validated incident fracture, and the average follow-up time of the 3014 subjects was 3.32 yr (Table 1). All sex steroids measured in serum using the specific GC-MS technique, including T ($r = -0.07$, $p < 0.001$), fT, ($r = -0.11$, $p < 0.001$), DHT ($r = -0.07$, $p < 0.001$), E2 ($r = -0.07$, $p < 0.001$), fE2 ($r = -0.11$, $p < 0.001$), and estrone ($r = -0.07$, $p < 0.001$) were negatively associated with age, whereas SHBG ($r = 0.10$, $p < 0.001$) was positively associated with age. Strong correlations between T and E2 ($r = 0.79$, $p < 0.0001$) and between fT and fE2 ($r = 0.81$, $p < 0.0001$) were found.

Androgens and estrogens as risk factors for all validated incident fractures

The impact of serum sex steroids on risk of having any fracture was evaluated in the main analyses. In age-adjusted hazards regression analyses, baseline estrone, E2, fE2, DHT, T, and fT, included as continuous parameters, were all inversely, whereas SHBG was directly, related to fracture risk, when analyzed in the whole MrOS Sweden cohort (Table 2). Similar relations between sex hormones and fracture risk were seen when the three subcohorts (Malmö, Göteborg, Uppsala) were studied separately, although only fE2 was a significant predictor of fractures in all three cities studied (Table 2). The HR per SD decrease of fE2 for fractures was not only significant in each of the three subcohorts but also of very similar magnitude (HR = 1.38–1.40; Table 2). Furthermore, no significant interaction effect was seen between any of the hormones tested and city.

Inclusion of the two highly correlated estrogens, estrone and E2 ($r = 0.64$, $p < 0.0001$), in the same age-adjusted multivariable proportional hazards regression model showed that E2 (HR per SD decrease, 1.34; 95% CI, 1.22–1.49), but not estrone, was an independent significant predictor of fractures. In all three cohorts, fE2 was a stronger predictor of fractures than E2 (Table 2).

Inclusion of the two highly correlated androgens, DHT and T ($r = 0.80$, $p < 0.0001$), in the same age-adjusted multivariable proportional hazards regression model showed that T (HR per SD decrease, 1.27; 95% CI, 1.16–1.39), but not DHT, was an independent predictor of fractures. In all three cohorts, fT was a stronger predictor of fractures than T (Table 2).

Free estradiol and SHBG are independent predictors of all validated fractures

Because fT and fE2 were highly correlated, we included these two parameters in the same age-adjusted multivariable proportional hazards regression model, showing that fE2 (HR per SD decrease, 1.37; 95% CI, 1.10–1.71) but not fT (HR per SD decrease, 1.03; 95% CI, 0.84–1.27) was independently inversely related to all fractures. We next included fE2 and SHBG in the same multivariable proportional hazards regression model, showing that serum fE2 was independently inversely and SHBG was independently directly related to all fractures (Table 3). To determine whether the associations between fE2/SHBG and risk of fractures were mediated by body composition, BMD, or muscle strength, further adjustments were performed (Table 3). The independent associations between fE2/SHBG and all validated fractures were slightly attenuated but remained significant after adjustment for height, weight, femoral neck BMD, and grip strength (Table 3).

Sex steroids as predictors of different types of validated incident fractures

We next performed exploratory subanalyses of the predictive value of fE2, fT, and SHBG for different fracture types. Fractures were divided into three major types of fractures: (1) nonvertebral osteoporosis-related fractures (defined as hip, proximal humerus, distal radius, and pelvis),

(2) clinical vertebral fractures, and (3) other fractures (Table 1). Low fE2, Low fT, and high SHBG were predictors of nonvertebral major osteoporosis-related fractures and clinical vertebral fractures, whereas SHBG, but not fE2 or fT, was a predictor of other fractures (Table 4). Interestingly, when hip fractures were analyzed separately, it was found that fE2, fT, and SHBG were all significantly associated with hip fractures (Table 4). After adjustment for femoral neck BMD, the associations between fE2/fT and fractures were slightly attenuated. However, they both remained significant predictors of nonvertebral major osteoporosis-related fractures, clinical vertebral fractures, and hip fractures (Table 4). The impact of SHBG as a predictor of nonvertebral osteoporosis fractures and clinical vertebral fractures was partly lost and was not significant any more after adjustment for femoral neck BMD.

Serum sex steroids in quartiles and risk of fractures

Further exploratory analyses of the associations between E2 and fE2 with all fractures, nonvertebral osteoporosis fractures, and clinical vertebral fractures showed that subjects in the lowest quartile of E2 and fE2 had the highest risk of fractures, whereas the risk of fractures was rather similar for subjects in quartiles 2–4 (Table 5). For hip fractures, the statistical power was too low for analyzing the four quartiles separately. However, the age-adjusted HR for hip fracture of the pooled subjects in the three highest quartiles of E2 and fE2 versus those in the lowest quartiles were 0.38 (95% CI, 0.20–0.74) and 0.43 (95% CI, 0.22–0.85), respectively. For both T and fT, subjects in the lowest quartile of T and fT had the highest risk of fractures, although all comparisons versus the other quartiles were not statistically significant (Table 5).

For SHBG, mainly subjects in the highest quartile of SHBG had an increased risk of fractures (Table 5). The age-adjusted HR for hip fractures in the highest quartile versus the pooled subjects in the three lowest quartiles of SHBG was 1.78 (95% CI, 1.33–2.37). These findings indicate that the associations between sex steroids/SHBG and fractures were of a nonlinear nature.

Impact of having low E2 and/or low T on risk of fractures

To show the impact of having low E2 and/or low T, studied as categorical variables, on risk of fractures, subjects were divided into four groups according to both their E2 and T status: group 1 (= referent) was medium or high E2 and medium or high T, group 2 was low E2 and medium or high T, group 3 was medium or high E2 and low T, and group 4 was low E2 and low T (Table 6). Subjects with low E2 had an increased risk of fractures, independent of T status (Table 6). In contrast, subjects with low T but medium or high E2 did not have an increased risk of fractures, suggesting, in a similar manner as when E2 and T were evaluated earlier as continuous parameters, that low E2 but not low T independently predicted risk of fractures (Table 6). The greatest risk of fractures was seen in group 4 with both low E2 and low T, but no significant interaction effect was seen for E2 and T (Table 6).

TABLE 6. IMPACT OF HAVING LOW E2 AND LOW T OR HIGH SHBG ON RISK OF FIRST FRACTURE

	Age-adjusted hazard ratio (95% CI)
Impact of having low E2 and/or low T	
Group 1: medium or high E2 and medium or high T (n = 1667)	1 (referent)
Group 2: low E2 and medium or high T (n = 307)	1.72 (1.14–2.58)
Group 3: medium or high E2 and low T (n = 312)	0.87 (0.51–1.48)
Group 4: low E2 and low T (n = 353)	2.12 (1.48–3.04)
Impact of having low E2 and/or high SHBG	
Group 1: medium or high E2 and medium or low SHBG (n = 1434)	1 (referent)
Group 2: low E2 and medium or low SHBG (n = 505)	2.10 (1.45–3.02)
Group 3: medium or high E2 and high SHBG (n = 526)	1.92 (1.32–2.78)
Group 4: low E2 and high SHBG (n = 152)	3.56 (2.20–5.76)

Age-adjusted hazard ratios are given with 95% CIs within parentheses. Low E2, subjects within the lowest quartile of E2 (Q1 ≤ 16.0 pg/ml); medium or high E2, subjects within quartiles 2–4 of E2 (Q2–Q4, > 16.0 pg/ml); low T, subjects within the lowest quartile of T (Q1 ≤ 3.36 ng/ml); medium or high T, subjects within quartiles 2–4 of T (Q2–Q4, > 3.36 ng/ml); high SHBG, subjects within the highest quartile of SHBG (Q4 > 52.5 nM); medium or low SHBG, subjects within quartiles 1–3 of SHBG (Q1–Q3, ≤ 52.5 nM).

Impact of having low E2 and/or high SHBG on risk of fractures

To show the impact of having low E2 and/or high SHBG, studied as categorical variables, on risk of fractures, subjects were divided into four groups according to both their E2 and SHBG status: group 1 (=referent) was medium or high E2 and medium or low SHBG, group 2 was low E2 and medium or low SHBG, group 3 was medium or high E2 and high SHBG, and group 4 was low E2 and high SHBG (Table 6). Subjects with low E2 had an increased risk of fractures independent of SHBG status, and subjects with high SHBG had an increased risk of fracture independent of E2 status (Table 6). Thus, both low E2 and high SHBG were independently predicting risk of fractures. The greatest risk of fractures was seen in the group with both low E2 and high SHBG, but no significant interaction effect was seen for E2 and SHBG (Table 6).

Poisson regression models of the nonlinear relations between E2/fE2 and fracture risk

As proposed earlier that a threshold E2 level exists, below which E2 is directly associated with BMD,^(16,39) the apparent nonlinear relations between serum E2 and fracture risk were further evaluated using Poisson regression models (adjusted for age and time to event), showing that the yearly incidence of fractures was clearly inversely associated with serum levels less than, but not more than, 16 pg/ml for E2 (669 subjects with E2 < 16 pg/ml) and 0.27 pg/ml for fE2 (701 subjects with fE2 < 0.27 pg/ml; Fig. 1).

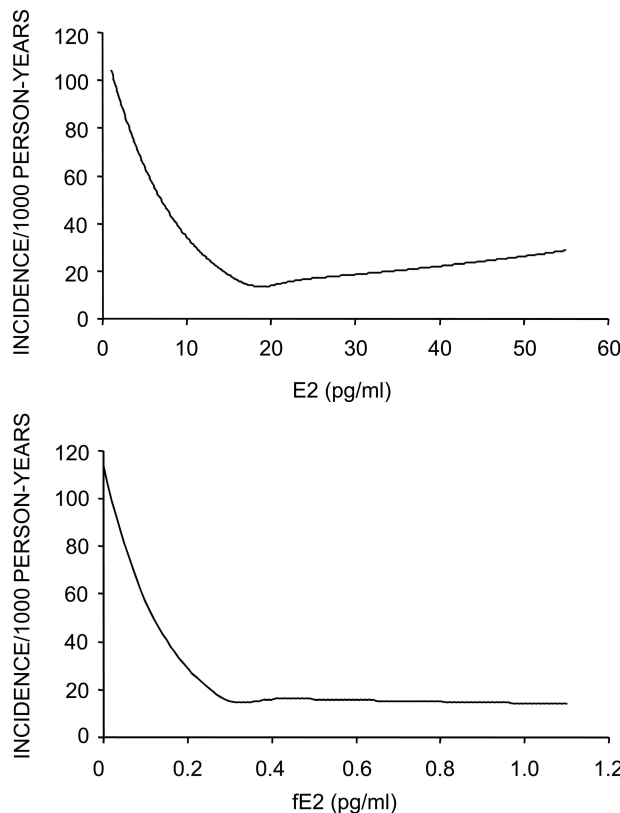


FIG. 1. Yearly incidence of fractures in relation to total E2 and fE2. Poisson regression models were used to determine the relation between serum hormone levels and fracture risk (all validated fractures).

DISCUSSION

This large, prospective population-based study, with serum sex steroids analyzed by the specific GC-MS technique, showed that older men with low serum E2 and high serum SHBG have an increased risk of fractures.

In this study, both low fE2 and low fT were, when studied separately, related to elevated fracture risk in older Swedish men. However, fT was strongly correlated with fE2 (r = 0.81), and multivariable proportional hazards regression models, with fE2 and fT included as continuous parameters, showed that fE2, but not fT, was independently associated with fracture risk. Thus, these calculations indicate that serum E2, and not T, is the major sex steroid with an impact on fracture risk in this cohort of older Swedish men. However, because T and E2 are strongly correlated, one should be cautious in the interpretation of these results. However, in this study, an independent impact of low E2 (lowest quartile), but not of low T (lowest quartile), was further supported by the fact that subjects with low E2 had an increased risk of fractures, independent of T status, whereas subjects with low T, but not low E2, did not have an increased risk of fractures. The greatest risk of fractures was seen in the group with both low E2 and low T, but no significant interaction term was found for E2 and T. Thus, both low E2 and low T clearly predicted risk of fractures,

but in this large cohort of older Swedish men, serum T analyses did not add much information to the serum E2 analyses. We have previously reported that both E2 and T were associated with prevalent fractures in the Swedish MrOS cohort.⁽¹⁸⁾ However, the morbidity associated with the previously occurred fractures might have confounded the serum sex steroid levels in this cross-sectional study using the same cohort.

A major strength of this study is that the serum sex steroids of the older men were analyzed using the specific GC-MS technique, which is not associated with a questionable specificity at lower concentrations as described for previously used immunoassay-based techniques.^(40,41) Serum levels of all sex steroids analyzed, including T, fT, DHT, E2, fE2, and estrone, were, in this study, all negatively associated with age, indicating that their levels decrease with age in older men. The rather strong correlation between fT and fE2 seen in this study is in contrast to the previously described weak ($r = 0.20$)⁽⁴²⁾ and moderate ($r = 0.59$)⁽¹⁸⁾ associations found in two large cohorts of older men when using immunoassay-based analyses of sex steroids. We, therefore, suggest that the more specific GC-MS technique is preferable for future analyses of E2 and T in older men. Especially, we believe that immunoassay-based analyses of low serum levels of E2 might be confounded by lack of specificity. However, a recent comparative study in men showed that serum levels of E2 and T measured with immunoassays showed a similar correlation with BMD as found for levels analyzed by MS.⁽⁴³⁾ Taken together, it is clear that fE2 and fT were strongly correlated in older men, supported by the notion that, in this study, 66% of the variance in serum fT is explained by serum fE2, and this covariance between fE2 and fT explains why fT was a predictor of validated fractures in separate analyses but not in multivariable models including fE2 as a covariate.

A threshold E2 level has been described by Khosla et al.^(16,39) (bioavailable E2 [bE2] of 40 pM [11 pg/ml] and total E2 of 114 pM [31 pg/ml]), below which E2 is directly associated with BMD and inversely associated with markers of bone resorption.⁽²⁰⁾ A threshold E2 level was also reported by Szulc et al.⁽²⁶⁾ in the MINOS study, describing that men in the lowest quartile of bE2 (<53 pM = 14 pg/ml) had significantly lower BMD and elevated bone turnover markers than men in the upper three quartiles. Moreover, a longitudinal study by Gennari et al.⁽⁴⁴⁾ showed that subjects with E2 levels <98 pM had increased rates of bone loss at the femoral neck and lumbar spine. Similarly as previously described for BMD and bone resorption markers, we showed in this study that an E2 threshold level also exists for fracture risk in older men. Levels of serum fE2 less than but not greater than 0.3 pg/ml (= 1 pM), which correspond to an E2 level of 16 pg/ml (= 59 pM) and bE2 level of 6.2 pg/ml (= 23 pM; data not shown), were clearly inversely associated with fracture risk. The threshold E2 level for fracture risk in this study is slightly lower than those previously described for BMD and markers of bone resorption.^(20,26,39,44) This difference could be because of the fact that, in the previous studies, serum E2 was analyzed using immunoassay-based techniques, whereas it was analyzed by GC-MS in this study. Alternatively, the mechanisms for the

impact of E2 on BMD and bone resorption may differ from these explaining the impact of E2 on fracture risk. A BMD-independent impact of E2 on fracture risk is supported by this finding that the association between fE2 and fractures was only slightly attenuated by adjustment for BMD. In a similar manner, the association between E2 and fracture risk was independent of BMD in older postmenopausal women included in the Study of Osteoporosis Fractures (SOF) cohort.⁽⁴⁵⁾ One might therefore speculate that the threshold of E2 deficiency for fractures is not only caused by direct skeletal effects but also to relative E2 deficiency in other tissues of importance for neuromuscular function and/or fall risk.

Serum levels of SHBG have previously been shown to be directly related to prevalent fractures in men.^(46,47) Previous prospective studies have shown conflicting data regarding the predictive role of SHBG for fracture risk. Serum SHBG predicted fracture risk in the Tromsö and Dubbo studies but not in the Rotterdam study.^(31,32,34) This large prospective study, showing that men with high serum SHBG have an increased risk of fractures, clearly supports the data from the Tromsö and Dubbo studies.^(31,34) Interestingly, in this study, both low E2 and high SHBG were independently associated with elevated risk of fractures, and the highest risk of fractures was seen in subjects with both low E2 and high SHBG. Serum E2 is decreased and serum SHBG is increased by age in both older men and women.⁽⁴⁸⁾ Cummings et al.⁽⁴⁵⁾ have previously described, using the SOF cohort, that postmenopausal women with undetectable serum E2 concentrations and high serum concentrations of SHBG have an increased risk of hip and vertebral fractures. Thus, these findings, together with the previous findings from the SOF study, indicate that low serum E2 and high SHBG are clear predictors of fracture risk in both postmenopausal women and older men.

Our study has several limitations. The results are based on single measurements of sex steroids and may underestimate the true associations between the markers we studied and the risk of fractures. Also, the relation between serum sex steroid levels and asymptomatic vertebral fractures was not explored. In addition, although this study has the advantage of being population-based, this could imply the inclusion of subjects treated with antiresorptive agents and/or with compounds affecting bone metabolism and fracture risk (i.e., glucocorticoids) that could affect the interpretation of our results. Furthermore, sex steroid levels at baseline in this population-based cohort may be confounded by comorbidity.⁽⁴⁸⁾ Therefore, low serum sex steroids might in part be more generally a marker of poorer health and frailty, which would fit the fact that the associations with fracture remained significant after adjustment for BMD. Another limitation could be that only the serum samples from the Göteborg cohort were fasting morning samples, whereas the Uppsala and Malmö cohorts also included a few nonfasting samples taken throughout the day. However, the described associations between serum sex steroids and fracture risk were very similar in all three subcohorts, indicating that time of serum sampling did not substantially alter the associations between serum sex steroids and fracture risk. Whereas serum samples were available

for 99% of the subjects in the Göteborg cohort and 96% of the subjects in the Malmö cohort, the 1 ml required for the GC-MS analyses for sex steroid analyses was only available for 68% of the subjects in the Uppsala cohort. However, as can be seen in Table 2, the strength of the associations, as indicated by the hazard ratios, was very similar in the three different subcohorts when studied separately, and no significant interaction effect was seen between any of the hormones evaluated and city, suggesting that the 32% missing samples in the Uppsala cohort did not have a major impact on the described associations.

In conclusion, serum E2 and T were inversely, whereas serum SHBG was directly, related to fracture risk in older Swedish men. Subanalyses established that low serum E2, low serum T, and high serum SHBG predicted clinical vertebral fractures, nonvertebral osteoporosis fractures, and hip fractures. Multivariable proportional hazards regression models suggested that E2 and SHBG, but not T, were independently associated with fracture risk. In addition, we showed that a threshold E2 level exists, below which E2 is related to fracture risk. Taken together, our data show that older Swedish men with low serum E2 and/or high serum SHBG have an increased risk of fractures.

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