CLINICAL STUDY

A prospective study of sex steroids, sex hormone-binding globulin, and non-vertebral fractures in women and men: the Tromsø Study

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Abstract

Objectives: As bone fragility is partly the result of sex hormone deficiency, we sought to determine whether circulating sex steroids or sex hormone-binding globulin (SHBG) predicts non-vertebral fractures.

Methods: Forearm bone mineral density (BMD), total estradiol and testosterone, calculated free levels, and SHBG were measured in 1386 postmenopausal women and 1364 men aged 50–84 years at baseline in the Tromsø Study (1994–1995). Non-vertebral fractures were documented between 1994 and 2005.

Results: During 8.4 years (range 0.01–10.4) of follow-up, 281 women and 105 men suffered non-vertebral fractures. For both sexes, fracture cases had lower BMD and higher SHBG, but sex steroids were not lower. Each standard deviation (s.d.) increase in SHBG increased non-vertebral fracture risk in women (hazards ratio (HR) 1.17; 95% confidence interval (CI) 1.03–1.33) and men (HR 1.26; 95% CI 1.03–1.54). After further adjustment for BMD, the risk was not statistically significant in women (HR 1.09; 95% CI 0.95–1.24) or men (HR 1.22; 95% CI 0.99–1.49). Each s.d. decrease in BMD increased fracture risk in women (HR 1.36; 95% CI 1.19–1.56) and men (HR 1.41; 95% CI 1.15–1.73). Fracture rates were highest in participants with SHBG in the highest tertile and BMD in the lowest tertile and were 37.9 and 17.0 per 1000 person-years in women and men respectively. However, in both sexes the combination of BMD and SHBG was no better predictor of fracture risk than BMD alone. Sex steroids were not associated with fracture risk.

Conclusions: Measurements of sex steroids or SHBG are unlikely to assist in decision making regarding fracture risk susceptibility.

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Introduction

Women and men who sustain fragility fractures do so partly because of low bone mineral density (BMD), which is the result of a reduced peak BMD, bone loss, or both (1, 2). Bone loss occurs before menopause but is accelerated at menopause and is the result of sex hormone deficiency (3–6). Consequently, a low level of circulating sex steroids may be a sensitive and specific predictor of fractures and so may be useful in signaling the need for further investigation or treatment.

Some investigators have reported lower sex steroids and higher sex hormone-binding globulin (SHBG) associated with bone loss (5–14) and increased fracture risk but this is not consistently reported (15–25), perhaps because many factors influence fracture risk, particularly falls (26, 27). However, few prospective data are available examining the contribution of sex steroids and SHBG on fracture risk (15, 18, 20, 22, 24). In another paper, we reported that estradiol and SHBG

predicted bone loss in postmenopausal women and men, but the associations were weak in both (28).

If sex steroids or SHBG (a determinant of the free hormonal fraction) predicts fractures, the combination of BMD and sex steroids or SHBG measurements may improve prediction. We therefore examined the independent and combined contribution of sex steroids, SHBG, and BMD to fracture risk in both sexes. We tested the hypothesis that incident non-vertebral fractures in women and men are predicted by circulating levels of sex steroids or SHBG in a prospective study of 1386 women and 1364 men during 23 034 person-years follow-up.

Subjects and methods

Subjects

The Tromsø Osteoporosis Study (TROST), as part of the Tromsø Study, involved measurement of bone density in

6981 subjects aged 50–84 years (response rate 80%; (29)) and sex steroid levels in a random subgroup of 3017 subjects at baseline in 1994–95 (30, 31). We excluded 267 participants due to hormone medication ($n=185$), pre- or perimenopausal status ($n=48$), or outlying hormone values not believed to be true measurements ($n=34$); 2750 participants are thus included in this study. The participants with invalid scans ($n=45$) were excluded from the analyses including BMD. All participants gave informed written consent. The regional Committee of Research Ethics and the Norwegian Data Inspectorate approved the study.

At baseline, two self-administered questionnaires were filled in. A physical activity score was made by adding the hours per week of moderate and hard physical activity, giving the hours with hard activity double weight: score = moderate + 2hard. Our baseline definition of postmenopausal status was based on self-reported menopause ($n=955$), and age ≥ 54 years if data were missing ($n=431$; (30)). Exclusion of the women with missing data did not change the results. Ninety-one of the included women reported to be within the first 4 years after menopause. We included information on chronic diseases such as diabetes, cardiovascular diseases, asthma and cancer, and use of any medication. None of the participants reported the use of bisphosphonates at baseline. Follow-up data regarding the appearance of diseases or use of medication were not available for all the participants.

All non-vertebral fractures were registered from the X-ray archives of the University Hospital in Tromsø between 1 January 1994 and 12 February 2005. All fractures are registered here, as this is the only X-ray service in the city or within 250 km. The only exception would be fractures occurring while traveling with no control X-ray after returning home. The validation of the fracture registration is previously reported (32). Follow-up time was assigned from baseline to the first non-vertebral fracture to death ($n=506$), when the participant moved ($n=177$), or to the end of follow-up. The mean follow-up time was 8.4 years (range 0.01–10.4) and the number of person-years were 23 034.

Measurements

Height and weight were measured in light clothing without shoes, and body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). Bone density was measured on the non-dominant distal forearm, with single X-ray absorptiometric devices (DTX-100 Osteometer Medi Tech Inc., Hawthorne, CA, USA). The coefficient of variation (CV) was 0.8%, and details of the measurement methods and the strict quality control procedures for densitometry are previously published (29, 33). Briefly, all scans were reviewed and reanalyzed and only the scans free of artifacts were included. The long-term performance of

the densitometers was assessed by phantom measurements twice daily.

Non-fasting blood samples were taken between 0800 h and 1600 h and serum was stored at -70°C for 6–7 years, until first thawed in 2001. All steroids and SHBG were measured on Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA). Estradiol, testosterone, and DHEA sulfate (DHEAS) measurements were based on competitive immunoassays, whereas SHBG measurements were based on immunometric assays. The lower limits of detection were 10 pmol/l for estradiol, 0.1 nmol/l for testosterone, 1.0 $\mu\text{mol}/\text{l}$ for DHEAS, and 1.0 nmol/l for SHBG. The intra- and inter-assay CV for estradiol and DHEAS were between 4 and 15%. The intra- and inter-assay CV for testosterone >1 nmol/l were 3.5 and 5%, while in the range 0.1–1.0 nmol/l it was 12 and 20% respectively. The intra- and inter-assay CV for SHBG were 3 and 7% respectively. Samples with values below the limits of detection were given a value midway between zero and limit of detection: estradiol (327 women, 50 men), testosterone (547 women, 7 men), and DHEAS (354 women, 86 men). All assays were run within a period of a few weeks using the same lot of reagents and assay kits. We used Vermeulen *et al.* method to calculate free estradiol and free testosterone from total estradiol, total testosterone, and SHBG levels (30, 34). A recent validation by Rinaldi *et al.* (35) found that method simple and reliable.

Statistical analysis

Women and men were analyzed separately. We used a two-sided *t*-test and χ^2 to test for differences in means in baseline characteristics and sex steroids by fracture status. To test for a possible linear trend or threshold effect of sex steroids and SHBG on fracture risk, we analyzed fracture probability by quartiles, quintiles, and deciles of sex steroids and SHBG. We estimated the incidence of fractures in tertiles of risk factors, and tested whether combinations of them predicted fracture risk better than either trait alone.

Cox's proportional hazards model was used to determine whether baseline total and free estradiol, total and free testosterone, DHEAS and SHBG predict fractures, and univariate and multivariable measures of associations are presented. We tested the independent and combined contribution of sex steroids, SHBG, and BMD to fracture risk, by combining them in Cox's proportional hazards model. We controlled for age (years), weight (kg), height (cm), current smoking status (no/yes), physical activity score, and BMD (g/cm^2), known to be associated with sex steroids and fracture risk (30, 31, 36, 37). A history of previous fractures, chronic diseases, alcohol consumption, and supplementation of calcium were excluded as covariates, because they had no confounding effects. Exclusion of participants with high-energy trauma fractures ($n=55$)

did not change the results, so all fractures were included. We tested interaction terms between age, BMD, sex steroids, and SHBG as continuous variables in the models and none were significant. The proportionality assumptions of the models were verified. Log transformation corrected for the skewed distribution of the sex steroids, but changed no results.

The discriminatory power of the models was assessed by the mean of the area under the receiver operating characteristics (ROC) curve (c-statistics). The curve is a measure of the discriminating ability of the risk model and is a plot of sensitivity versus 1-specificity. We included ROC graphs to show the relative additional contribution of sex steroids and SHBG to the prediction of fracture risk. The c-index is a rank correlation that compares the predicted probabilities from a model with the observed frequencies of fracture. We calculated the c-index from logistic regression models to compare the ability of risk factors to predict a fracture. The SAS Software package, v9 (SAS Institute Inc., Cary, NC, USA) was used, and significance level was chosen at $P < 0.05$.

Results

During a mean follow-up of 8.4 years (range 0.01–10.4) and 23 034 person-years, 281 (20.3%) of 1386 postmenopausal women and 105 (7.7%) of 1364 men suffered incident non-vertebral fractures (Table 1). Women with fractures were older, taller, and had lower BMI than women without fractures. For both sexes, fracture cases had lower BMD and higher SHBG, but circulating levels of sex steroids were not lower (Table 2).

In women, no significant association between sex steroids and risk of non-vertebral fractures was detected (Table 2). Women with undetectable levels of estradiol (total estradiol < 10 pmol/l, $n = 327$) had no higher risk of fractures than women with detectable levels, HR 0.99 (95% CI 0.75–1.31). In men, there was a lower risk of fractures by higher levels of DHEAS in a univariate model, but not after adjustment for age. No other association between sex steroids and fractures was detected (Table 2).

In both sexes, each s.d. increase in SHBG increased the risk of non-vertebral fractures by about 20% in women (HR 1.17; 95% CI 1.03–1.33, $P = 0.02$) and

men (HR 1.26; 95% CI 1.03–1.54, $P = 0.02$) after adjustment for age, weight, height, smoking, and physical activity (Table 3). After further adjustment for BMD, the increased risk was attenuated and no longer statistically significant in women (HR 1.09; 95% CI 0.95–1.24, $P = 0.22$) nor men (HR 1.22; 95% CI 0.99–1.49, $P = 0.06$). In women, the age-adjusted risk of wrist fractures increased by each s.d. increase in SHBG (HR 1.25; 95% CI 1.06–1.47), but not after adjustment for other covariates (HR 1.18; 95% CI 0.98–1.42). In men, the age-adjusted risk of hip fractures increased for each s.d. increase in SHBG (HR 1.52; 95% CI 1.00–2.31), but not after adjustment for other covariates (HR 1.58; 95% CI 0.98–2.54).

In both sexes, baseline BMD was associated with risk of non-vertebral fractures and each s.d. decrease in BMD increased the risk for fracture by 40% in the adjusted models in women (HR 1.36; 95% CI 1.19–1.56) and men (HR 1.41; 95% CI 1.15–1.73; Table 3). Fracture incidences were highest in women and men with the combination of SHBG in the highest and BMD in the lowest tertile and were 37.9 and 17.0 per 1000 person-years respectively (Fig. 1). There was no significant interaction between BMD and SHBG in women ($P = 0.88$) or in men ($P = 0.53$). However, the combination of BMD and SHBG was no better predictor of non-vertebral fracture risk than BMD alone, as the c-indexes and the area under the ROC curves were similar in both models in either sex (Fig. 2).

In addition, we reran analyses after exclusion of subjects with fractures in fingers, toes, and the face and got similar results. We also reran analyses after exclusion of fracture cases with reported high-energy trauma involved, and the results were similar. Adjustment for sampling hour or season had no effect on the relations between sex steroids and risk for fractures. Finally, neither the combination of free estradiol and free testosterone nor other combinations of sex steroids or SHBG predicted risk for fracture. None of the sex steroids or SHBG showed a threshold effect on risk for fractures.

Discussion

The main finding from this population-based prospective study was that measurements of circulating sex steroids did not predict the incident non-vertebral fractures in either sex. A higher SHBG and lower BMD were associated with risk for fracture in both sexes, but the combination of the two traits was no better predictor of fracture risk than BMD alone.

An association between circulating estradiol, bone loss, or risk of vertebral and non-vertebral fractures is reported in some but not all studies in women or men (5–25, 38–41). Circulating estradiol is reported as being associated with prevalent vertebral fractures in women (16), in men but not women (19), whereas others reported no such association in men (17, 21). These

Table 1 Number and incidence of non-vertebral fractures per 1000 person-years by sex from 1994–95 to 2005. The Tromsø Study.

	Women		Men	
	<i>n</i>	Incidence	<i>n</i>	Incidence
All non-vertebral fractures	281	24.5	105	9.1
Hip fractures	42	3.7	27	2.3
Wrist fractures	128	11.2	21	1.8

Table 2 Baseline characteristics by fracture status and the corresponding univariately-associated fracture risk. The Tromsø Study.

Women	Fracture; <i>n</i> =281; mean (S.D.)	No fracture; <i>n</i> =1105; mean (S.D.)	Hazards ratio by s.d. units and (95% CI)
Age (years)	65.6 (6.2)	64.0 (6.2) [‡]	1.31 (1.16–1.47) [‡]
Height (cm)	162.0 (6.2)	160.8 (6.0) [†]	1.20 (1.06–1.35) [†]
Weight (kg)	67.8 (11.5)	68.7 (12.4)	0.93 (0.83–1.05)
Body mass index (kg/m ²)	25.9 (4.3)	26.6 (4.6) [*]	0.87 (0.77–0.97) [*]
Physical activity score ^a	2.6 (2.1)	2.7 (2.1)	0.94 (0.84–1.06)
Forearm BMD (mg/cm ²)	360 (63)	387 (66) [‡]	1.48 (1.31–1.66) [‡]
Current smoker (no/yes %)	28.8	29.5	1.04 (0.80–1.34)
Total estradiol (pmol/l)	28.3 (20.8)	28.7 (21.4)	0.99 (0.88–1.11)
Free estradiol (pmol/l)	0.57 (0.43)	0.61 (0.48)	0.93 (0.82–1.05)
Total testosterone (nmol/l)	0.57 (0.77)	0.50 (0.66)	1.08 (0.97–1.20)
Free testosterone (pmol/l)	6.33 (9.23)	6.16 (9.36)	1.00 (0.90–1.12)
DHEAS (μmol/l)	1.65 (1.19)	1.70 (1.19)	0.95 (0.84–1.08)
SHBG (nmol/l)	80.7 (34.6)	73.4 (32.6) [†]	1.23 (1.10–1.37) [‡]
Men	<i>n</i> = 105	<i>n</i> = 1259	
Age (years)	63.9 (7.0)	62.9 (6.6)	1.25 (1.03–1.51) [*]
Height (cm)	175.8 (6.2)	174.7 (6.9)	1.15 (0.94–1.40)
Weight (kg)	80.6 (11.3)	79.8 (12.2)	1.05 (0.86–1.27)
Body mass index (kg/m ²)	26.1 (3.2)	26.1 (3.5)	0.97 (0.80–1.18)
Physical activity score ^a	3.3 (2.3)	3.7 (2.5)	0.85 (0.69–1.03)
Forearm BMD (mg/cm ²)	511 (70)	536 (69) [‡]	1.48 (1.23–1.77) [‡]
Current smoker (no/yes %)	37.1	34.4	1.19 (0.80–1.77)
Total estradiol (pmol/l)	62.9 (28.8)	60.4 (31.4)	1.09 (0.91–1.30)
Free estradiol (pmol/l)	1.42 (0.68)	1.40 (0.72)	1.04 (0.86–1.26)
Total testosterone (nmol/l)	14.0 (5.6)	13.0 (5.2)	1.18 (0.99–1.41)
Free testosterone (pmol/l)	198 (67)	194 (69)	1.02 (0.85–1.24)
DHEAS (μmol/l)	2.77 (1.54)	3.12 (1.90)	0.80 (0.64–0.99) [*]
SHBG (nmol/l)	59.5 (26.7)	54.1 (24.2) [*]	1.26 (1.06–1.50) [†]

**P*<0.05; [†]*P*<0.01; [‡]*P*<0.001. Differences in means are examined by *t*-tests. BMD, bone mineral density at the distal forearm; DHEAS, DHEA sulfate; SHBG, sex hormone-binding globulin.

^aA physical activity score was made by adding hours/week of moderate and hard physical activity; score = moderate + 2hard.

results from retrospective data are difficult to interpret, as alterations in estradiol could have occurred after the fractures. Prospective studies including incident vertebral fractures or a combination of vertebral and non-vertebral fractures as the end point have reported lower estradiol associated with fracture risks in women

(15, 20, 22, 23). Prospective studies including incident hip fractures have reported low estradiol associated with a risk of fractures in women (15, 18) and in men (24), but the effect was dependent on weight in one study (18). In a case-cohort study, women with undetectable levels of estradiol (< 5 pg/ml) had an increased fracture risk, and

Table 3 The hazards ratio with 95% confidence interval of non-vertebral fractures by sex-specific s.d. units of predictors in multivariable models in women and men. The Tromsø Study.

Women	Unit (s.d.) of comparison	Hazards ratio in Cox's proportional hazards model	
		Without BMD	With BMD
Age	+ 6.2 years	1.34 (1.18–1.52) [‡]	1.22 (1.06–1.40) [†]
Height	+ 6.0 cm	1.31 (1.15–1.50) [‡]	1.33 (1.16–1.51) [‡]
Weight	+ 12.0 kg	0.91 (0.79–1.04)	0.96 (0.83–1.11)
Physical activity	+ 2.1 score	0.95 (0.84–1.07)	0.97 (0.86–1.09)
Smoking	No/yes	0.98 (0.74–1.29)	0.98 (0.75–1.30)
SHBG	+ 33 nmol/l	1.17 (1.03–1.33) [*]	1.09 (0.95–1.24)
Bone mineral density	– 66 mg/cm ²	–	1.36 (1.19–1.56) [‡]
Men			
Age	+ 6.6 years	1.23 (1.01–1.50) [*]	1.17 (0.94–1.45)
Height	+ 6.9 cm	1.14 (0.91–1.44)	1.12 (0.89–1.42)
Weight	+ 12.0 kg	1.12 (0.88–1.42)	1.22 (0.96–1.57)
Physical activity	+ 2.5 score	0.88 (0.72–1.07)	0.87 (0.71–1.07)
Smoking	No/yes	1.19 (0.79–1.81)	1.17 (0.77–1.79)
SHBG	+ 25 nmol/l	1.26 (1.03–1.54) [*]	1.22 (0.99–1.49)
Bone mineral density	– 69 mg/cm ²	–	1.41 (1.15–1.73) [‡]

**P*<0.05; [†]*P*<0.01; [‡]*P*≤0.001. SHBG, sex hormone-binding globulin.

Figure 1 The incidence of non-vertebral fractures per 1000 person-years in tertiles of sex hormone-binding globulin (SHBG) and bone mineral density (BMD) in women and men. SHBG tertiles in women, <57, 57–84, and >84 nmol/l; men, <43, 43–58, and >58 nmol/l. BMD tertiles in women, <0.362, 0.362–0.423, and >0.423 g/cm², in men: <0.509, 0.509–0.565, and >0.565 g/cm². The Tromsø Study.

33% of the women selected as controls had undetectable levels of estradiol (15). Women with estradiol in the lowest quartile are reported to have an increased risk of fracture (20), whereas others found neither women with undetectable estradiol levels, estradiol <5 pg/ml or estradiol in the lowest quartile to have an increased risk of hip fracture, but women with estradiol in the upper quartile were protected against hip fractures (18). Devine *et al.* (23) reported free estradiol index, but not estradiol, to be associated with increased fracture risk. The only prospective study of the association between estradiol and risk of non-vertebral fractures in men included only 39 hip fractures (24). Altogether there seems to be a possible weak effect of the circulating levels of estradiol, even very low levels in postmenopausal women, on fracture risk in most of the published studies on this topic. However, whether measurement of estradiol improves the predictive ability above that of

Figure 2 ROC curves for prediction of non-vertebral fractures by sex hormone-binding globulin (SHBG), bone mineral density (BMD), and the combination of both in women and men. The Tromsø Study.

BMD alone has not been shown in ROC curves as far as we know.

The lack of association between circulating sex steroids and risk of non-vertebral fractures in the present study may be the result of several factors. First, non-vertebral fractures are traumatic and the contribution of bone fragility to the fracture event may be relatively less than in vertebral fractures, as these generally involve minimal trauma. Secondly, peak bone mass is an important determinant of bone strength in old age and may make a more important contribution to bone fragility than estrogen deficiency (9, 42). Thirdly, bone loss occurs before menopause and is independent of sex steroids. Lastly, a single measurement of sex steroids has a large variance and so may not adequately reflect long-term exposure to estrogen deficiency.

In the present study, higher SHBG was associated with increased risk of incident non-vertebral fractures, but the risk explained by SHBG was small in both sexes and partly explained by BMD. In women, higher SHBG is reported to be associated with increased risk of incident vertebral, hip, and a combination of vertebral and non-vertebral fractures in prospective studies (15, 18, 20, 22). In men, SHBG is associated with prevalent vertebral fractures and the combination of vertebral

and non-vertebral fractures as the endpoint (17, 21). In men, the effect of SHBG on incident non-vertebral fractures has not been studied in prospective data. Most authors suggest that higher SHBG increases the risk of fractures by binding estradiol and decreasing its bioavailability (15, 20, 21). The independent effect of SHBG on risk of fractures adjusted for total estradiol in the present study is against this explanation. Some investigators report that subjects with both high SHBG and low estradiol had the highest risk of incident vertebral and hip fractures (15, 22). Center *et al.* (17) reported SHBG, but not estradiol, to be associated with prevalent fractures (vertebral and non-vertebral) in men, and the effect was independent of BMD.

As the fracture incidences were highest in participants with the combination of SHBG in the highest and BMD in the lowest tertile in our initial analyses, this combination could identify a subgroup with high risk of fractures. If so, this would support the notion of an independent effect of SHBG other than by affecting the BMD or bioavailability of estradiol. SHBG is more than a transport protein, and may act through specific membrane receptors (43). An anti-estrogenic effect of SHBG is reported in breast cancer cells, but there is no evidence of direct effect of SHBG on bone. However, the fracture risk prediction by the combination of the two traits was no better than BMD alone, as shown in the ROC graphs. We therefore conclude that SHBG is probably a weak predictor of fracture risk and does not improve the predictiveness over and above that of BMD alone. Larger samples might be needed to show a BMD-independent role of SHBG.

This study has limitations and the true strengths of the relationships between sex steroids, SHBG, and fracture risk may have been underestimated. Although assays with low limits of detection were used, estradiol values were below the limits of detection in 24% of postmenopausal women. However, excluding the women with values below the limit of detection did not change the results. The measurement uncertainty could weaken true associations, and the more precisely measured SHBG could therefore appear to be more strongly associated with the outcome. However, we believe that the large sample size in this study offsets some of the uncertainty related to low precision, and reduces the threat on the validity of the results. Variations in the assays used could also explain some of the variability in the findings in the literature.

Responders to study invitations are often more healthy than non-responders. However, previous comparisons between responders and non-responders in TROST, and between the subgroup with hormone measurements and total TROST population, gave no indication of differences between the groups (29, 30). The potential for selection bias is therefore assumed to be small. Since we did not have data regarding the use of medication known to affect bone during follow-up, we were not able to account for this in our analyses.

This could again result in underestimation of true associations.

The lack of association between sex steroids and fracture risk and the weak association between SHBG and fracture risk make these traits unlikely to assist in decision-making regarding fracture risk susceptibility.

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