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Vitamin D Status and Sex Hormone Binding Globulin: Determinants of Bone Turnover and Bone Mineral Density in Elderly Women

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ABSTRACT

To examine the relation of the vitamin D status and the remaining estrogen activity with bone turnover and bone mineral density (BMD) in elderly women, BMD was measured at both hips using dual-energy X-ray absorptiometry and at the distal radius using single photon absorptiometry, in 330 healthy women aged 70 and over. Vitamin D metabolites, sex hormone binding globulin (SHBG), PTH(1–84), osteocalcin, alkaline phosphatase, and hydroxyproline and calcium excretion in 2 h fasting urine were measured. Multiple linear regression was used to adjust for potential confounders. In 65% of the women, serum 25(OH)D was below 30 nmol/l. Only values below a threshold for 25(OH)D were negatively related to serum PTH(1–84) ($p = 0.02$, threshold at 25 nmol/l) and to osteocalcin levels ($p = 0.04$, threshold at 30 nmol/l). BMD of the femoral neck and trochanter was positively related to serum 25(OH)D (left neck $p = 0.001$) with thresholds at 30 nmol/l whereas the distal radius was not ($p = 0.32$). Serum PTH was negatively related to BMD at all measurement sites (all $p < 0.001$). Serum SHBG, an inverse measure of estrogen activity, was positively related to osteocalcin levels ($p = 0.004$) and the urinary hydroxyproline/creatinine ratio ($p = 0.002$) and negatively related to the BMD of the trochanter (left trochanter $p = 0.02$) and the distal radius ($p = 0.001$). We conclude that in elderly women, serum 25(OH)D levels below 30 nmol/l are associated with secondary hyperparathyroidism and increased bone turnover. SHBG is positively related to bone turnover. Vitamin D deficiency especially influences BMD of the femoral neck, a cortical area. SHBG mainly influences BMD at the trochanteric region and distal radius, predominantly trabecular areas, which may reflect the effects of remaining estrogen activity. (J Bone Miner Res 1995;10:1177–1184)

INTRODUCTION

OSTEOPOROSIS IS A DISEASE CHARACTERIZED BY DECREASED BONE MASS and structural deterioration resulting in a higher risk of fractures. Hip fractures are an especially important cause of morbidity and mortality in the elderly.[1] The decreasing bone mass[2] and the increasing risk of falls[3] account for the sharp rise in the hip fracture incidence with age. Women as well as men lose bone with age, but in women the onset of menopause aggravates bone loss,[4] while later in life other factors, such as vitamin D deficiency, may play a role.[5]

Vitamin D deficiency is common in the elderly.[5] Patients with a hip fracture usually have a poorer vitamin D status than age-matched controls.[6] The levels of 25-hydroxyvitamin D (25(OH)D) decline with age, due to lesser exposure to sunshine and decreased production in the aging skin.[6,7] Regular nutrition does not compensate

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for this.[1,6,8] Severe, long-standing vitamin D deficiency leads to osteomalacia, but this is uncommon.[1] Nevertheless, vitamin D deficiency may play a role in the pathogenesis of osteoporosis in the elderly. It leads to decreased production of the active metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). It is the main stimulator of the calcium absorption from the gut and indirectly as well as directly inhibits parathyroid hormone (PTH) production.[5,9] The increased secretion of PTH in the case of vitamin D deficiency increases bone turnover and bone loss and will lead to decreased mineralization of newly formed bone.[5] Seasonal variation of serum concentrations of 25(OH)D and $1,25(OH)_2D$ and an inverse variation of serum PTH levels have been found in elderly groups.[8,10,11] Vitamin D supplementation in the case of vitamin D deficiency increases $1,25(OH)_2D$ levels and suppresses PTH secretion in elderly patients.[12] The boundary between a normal and deficient vitamin D status is still a matter of discussion.[13]

In the first years after the onset of menopause, women lose bone at a much faster rate than premenopausal women. The rate of bone loss then gradually decreases,[4] but this may partly depend on the remaining endogenous estrogen production from adrenal androstenedione in adipose tissue.[14,15] Sex hormone binding globulin (SHBG) binds the circulating sex hormones and is the major inverse determinant of the level of free (active) hormone.[16] Indeed, SHBG is a better predictor of BMD and bone loss than the total endogenous estrogen levels, which suggests that it is a suitable measure for the remaining oestrogen activity.[17–19] Higher levels of SHBG have been found in patients with vertebral crush fractures when compared with controls[17] as well as in patients with hip fracture.[20] Recently we found in women over 70 years of age that the relation of the number of years since menopause with BMD of the hip was stronger than its relation with chronological age.[21] These data suggest a relation between the remaining estrogen activity and osteoporosis even long after menopause. However, no studies have been done on the relation of SHBG to bone turnover and BMD in very old people.

In the present paper, we present data on the influence of the remaining oestrogen activity and the vitamin D status on PTH secretion, parameters of bone turnover, and BMD in a group of healthy elderly women.

MATERIALS AND METHODS

Baseline measurements were taken in women aged 70 and over participating in a clinical trial on the effect of vitamin D supplementation on the incidence of hip fractures. Women who were residents of homes or apartments for the elderly were asked to participate in an additional study involving BMD measurements. The protocol was approved by the Free University Hospital Ethical Committee. All participants gave informed consent. In order to be able to visit the hospital for these measurements, the women had to be reasonably mobile. Exclusion criteria for entry in the trial were: hip fracture in the past, total hip prosthesis, and recent history of urolithiasis, hypercalcaemia, or sarcoidosis. Women taking vitamin D supplements at baseline or suffering from primary hyperparathyroidism were excluded. The date of the measurement was classified by season. Exposure to sunshine was determined by an outdoor frequency score (0: <1 time/week outdoors, 1: 1–2 times/week outdoors, 2: ≥3 times/week outdoors) and a sunshine preference score (0: prefers shade, 1: prefers some sunshine, 2: prefers sunshine). Height and body weight were measured while the participants wore indoor clothes and no shoes.

BMD measurements

BMD was measured by single photon absorptiometry (SPA; Norland OsteoAnalyser) at the distal radius of the dominant forearm. The long-term coefficient of variation in a group of 27 volunteers and osteoporotic patients was 3.1% (local unpublished results). The BMD of both hips was measured using dual-energy X-ray absorptiometry (DXA; Norland XR-26, Norland Corp., Fort Atkinson, WI) at the femoral neck and the trochanter. All DXA scans were reanalyzed by one observer to increase precision, with the most recent Norland software version 2.3.0 which enables rotation of the regions of interest in one plane. The long-term coefficient of variation in a group of 50 volunteers and osteoporotic patients was 2.1% for the femoral neck and 2.4% for the femoral trochanter (local unpublished results with earlier software version).

Biochemical measurements

Blood samples and 2 h morning urine samples[22] were obtained in fasting subjects. Osteocalcin was measured using a radioimmunoassay kit from the Incstar Corporation (Stillwater, MN). Serum intact PTH (PTH(1–84)) was measured in plasma using a two-step immunochemical method involving amino-terminal immunoextraction followed by a midregion immunoassay. The interassay coefficient of variation (CV) of this method is 10.2%.[23] Measurements of the vitamin D metabolites were performed with competitive protein binding assays after isolation by gradient HPLC. The intra- and interassay CVs are 5% and 6%, respectively, for 25(OH)D and 6% and 15% for $1,25(OH)_2D$.[24] Measurement of SHBG was performed using an IRMA kit from Farmos Diagnostica with an interassay CV of 5.3% (Oulunsalo, Finland). Hydroxyproline was measured by HPLC with an interassay CV below 3.2%[25] and expressed as a hydroxyproline/creatinine ratio (Hp/Cr). Serum alkaline phosphatase, calcium, phosphate, albumin, and creatinine were measured using standard laboratory methods. Serum calcium was corrected for serum albumin using the following equation: corrected calcium = serum calcium + [40 − albumin (g/l)] × 0.02 mmol/l.

Statistical analysis

Statistical analysis was performed using SPSS-PC. All relationships were analyzed with multiple linear regression analysis (MLR) with correction made for possible confounding. Regression equations were checked for linearity of the relation, normal distribution, and stability of variance. Nominal variables (e.g., season) and ordinal variables

TABLE 1. MEAN (SD) FOR AGE, YEARS SINCE MENOPAUSE, ANTHROPOMETRIC DATA, AND BMD (g/cm^2) IN 330 PARTICIPATING WOMEN

Age (years)	80.3 (5.6)
Years since menopause	32.5 (7.1)
Body weight (kg)	71.2 (11.4)
BMI* (kg/cm^2)	28.3 (4.0)
BMD† left femoral neck (g/cm^2)	0.687 (0.106)
BMD right femoral neck	0.680 (0.105)
BMD left trochanter	0.604 (0.111)
BMD right trochanter	0.595 (0.109)
BMD distal radius	0.318 (0.081)

* Body mass index.
† Bone mineral density.

(e.g., outdoor frequency and sunshine preference scores) were entered in the regression model as separate indicator variables for each category. When necessary, log transformations of dependent and independent variables were performed. The relationship of 25(OH)D and SHBG with PTH(1–84), parameters of bone turnover, and BMD were examined in a single regression model for each dependent variable. Since the relationship of 25(OH)D with most dependent variables was obviously nonlinear, various transformations were performed. These included logarithmic and inverse transformations as well as thresholds. In case of threshold transformations, serum 25(OH)D levels above certain values, ranging from 15–45 nmol/l with steps of 5 nmol/l, were recoded to the value of the threshold. This transformation implicates that no relation is assumed above the threshold. After each transformation, the fit of the regression model was tested, and the transformation with the best fit was reported. A high fit of a threshold transformation indicates a distinct boundary between a vitamin D–deficient and –replete state. Interaction of the effects of 25(OH)D and SHBG was tested by entering the product term of the transformations of SHBG and 25(OH)D with the best fit in the regression model. All reported p values are two-sided.

RESULTS

Of the total of 348 women who underwent the BMD measurements, one suffered from primary hyperparathyroidism and 17 were taking vitamin D–containing tablets and were therefore excluded from the analysis. Mean values for age, years since menopause (YSM), body weight, BMI, and BMD of the remaining 330 women are shown in Table 1. Table 2 shows the mean, standard deviation, median, 5th, and 95th percentiles for the biochemical parameters in blood and 2 h fasting urine. The percentiles of serum-corrected calcium and phosphate were within the reference values provided by the laboratory.

Determinants of 25(OH)D and SHBG levels

In 65% of all subjects, the serum 25(OH)D level was below 30 nmol/l, and in 34% it was below 20 nmol/l. In winter 83% and in summer 50% of the 25(OH)D levels were below 30 nmol/l. The median levels of serum 25(OH)D were significantly higher for inhabitants of apartments for the elderly than residents of homes for the elderly (29.0 and 22.0 nmol/l, respectively; $p < 0.001$). The vitamin D status was related to age ($p < 0.0001$), outdoor frequency score ($p = 0.004$), sunshine preference score ($p < 0.0001$), and season ($p < 0.0001$). The multiple linear regression (MLR) model that included these four independent variables accounted for 26% of the variance of serum 25(OH)D. Vitamin D values at age 90 were 15.3% lower than at age 70, corrected for season, the sunshine preference, and outdoor frequency scores. Table 3 shows the median 25(OH)D levels by sunshine preference and outdoor frequency scores in the summer. The levels were highest in the summer and lowest in the winter. In stepwise MLR, serum SHBG was best determined by age and body weight, while adding the number of years since menopause, age at menopause, or BMI to the model did not result in a significant improvement. According to this model, serum SHBG was significantly higher in older subjects (+1.41 nmol/l/year; $p = 0.0001$), and lower in heavier subjects (−0.74 nmol/l/kg; $p = 0.0001$).

Relation of 25(OH)D and SHBG with 1,25(OH)$_2$D, PTH(1–84), and parameters of bone turnover

Serum 1,25(OH)$_2$D, corrected for age and serum creatinine, showed a significant variation over the seasons ($p < 0.0001$), parallel to the variation in serum 25(OH)D levels, as shown in Fig. 1. The serum 1,25(OH)$_2$D levels were related to 25(OH)D levels over the whole range ($p < 0.0001$), independent of age and serum creatinine. However, 1,25(OH)$_2$D increased linearly with the logarithm of 25(OH)D, implicating a stronger relation at the lower end of the 25(OH)D range.

To determine the relation of serum 25(OH)D and SHBG with bone turnover, a single MLR model was made for each bone turnover parameter in which we corrected for age, age at menopause, and serum creatinine. The relation of 25(OH)D with the logarithm of PTH(1–84) was best described by a linear model with a threshold for 25(OH)D at 25 nmol/l, which represents a negative relation below and no relation above the threshold. Below this threshold, PTH increased 14.1% for every 10 nmol/l of lower serum 25(OH)D ($p = 0.02$). Likewise serum osteocalcin was 0.3 μg/l higher for every 10 nmol/l of lower serum 25(OH)D ($p = 0.04$) below a threshold at 30 nmol/l. No significant relation of serum 25(OH)D was found with alkaline phosphatase and the fasting urinary Ca/Cr and Hp/Cr ratio (all $p > 0.15$). Higher serum SHBG levels were significantly associated with higher serum osteocalcin (+0.6 μg/l for a doubling of SHBG, $p = 0.004$) and urinary Hp/Cr ratios (+15.7% for a doubling of SHBG, $p = 0.002$), but not to the levels of serum PTH(1–84), alkaline phosphatase, and the fasting urinary Ca/Cr ratio (all $p > 0.56$).

TABLE 2. MEAN (SD), MEDIAN, 5TH, AND 95TH PERCENTILES FOR BIOCHEMICAL PARAMETERS IN BLOOD AND 2 H FASTING URINE

Parameter	*Mean (SD)*	*Median*	*5th and 95th percentile*	*Reference values**
25(OH)D (nmol/l)	28.1 (13.0)	25.0	13.0–50.0	30–100
$1,25(OH)_2D$ (pmol/l)	114 (34)	111	63–177	60–160
SHBG† (nmol/l)	61.8 (29.9)	56.0	23.0–120.0	20–140
PTH(1–84) (pmol/l)	3.9 (2.4)	3.4	1.7–8.7	<0.5–4.0
Ca/Cr_{urine}‡ (mmol/mmol)	0.33 (0.22)	0.28	0.07–0.82	<0.45
Hp/Cr_{urine}§ (μmol/mmol)	22 (9)	21	12–38	<25
Osteocalcin (μg/l)	3.9 (1.8)	3.5	1.7–7.0	1.8–6.6
Alkaline phosphatase (U/l)	68 (20)	65	42–107	<90
Calcium (corrected) (mmol/l)	2.38 (0.10)	2.37	2.21–2.56	2.20–2.60
Phosphate (mmol/l)	1.05 (0.13)	1.05	0.84–1.26	0.70–1.40
Creatinine (μmol/l)	86.9 (19.0)	83.0	65–120	60–110
Albumin (g/l)	36.5 (2.6)	36.0	32–41	35–50

* Local, in healthy adults.
† Sex hormone binding globulin.
‡ Calcium creatinine ratio.
§ Hydroxyproline creatinine ratio.

TABLE 3. MEDIAN SERUM 25(OH)D IN nmol/l* AND NUMBER OF PARTICIPANTS† (n), BY SUNSHINE PREFERENCE SCORE AND OUTDOOR FREQUENCY SCORE IN THE SUMMER AND THE DIFFERENCES FOR THE OTHER SEASONS

	Outdoor frequency				
Estimated for summer sunshine preference	*<1 time/ week*	*1–2 times/ week*	*≥3 times/ week*	*Difference in*	
Avoids sunshine	19.7 (n = 13)	22.5 (n = 7)	25.3 (n = 58)	winter	−26.0%
Prefers some sunshine	22.1 (n = 14)	25.4 (n = 14)	28.5 (n = 97)	spring	−12.2%
Prefers sunshine	26.3 (n = 2)	30.1 (n = 8)	33.9 (n = 117)	autumn	−5.5%

* Estimated by multiple regression analysis corrected for age: values for the other seasons can be obtained by subtracting the appropriate percentage from median 25(OH)D levels.
† n indicates the total number of participants in a particular category.

Relation of serum PTH, 25(OH)D, and SHBG with BMD

Serum PTH was negatively related to BMD at all measurement sites (all $p < 0.001$). The correlation coefficient ranged from −0.19 for the distal radius to −0.27 for the left femoral neck. Figure 2 shows the relationship of 25(OH)D with BMD at the left femoral neck, adjusted in an MLR model for mean age, age at menopause, body weight, and SHBG. For the BMD of the hip, the best fit was obtained with a threshold at 30, similar to the relation of serum 25(OH)D to serum PTH(1–84) and osteocalcin. In Table 4, the differences in BMD, for every 10 nmol/l of higher 25(OH)D up to the threshold, are shown. There was a significant relation of 25(OH)D with BMD at the left and right femoral neck and the right trochanter, a borderline significant relation at the left trochanter, and no significant relation at the distal radius. As shown in Table 4, a higher SHBG level was significantly associated with lower BMD at all measurement sites but the left femoral neck. The strongest relation of SHBG with BMD, however, was found for the distal radius (Fig. 3).

Interaction of serum 25(OH)D and SHBG

Interaction was tested by entering the product-term 25(OH)D * SHBG in the regression models. Low serum 25(OH)D in combination with high serum SHBG was associated with higher levels of PTH(1–84) than was accounted for by its independent relation with 25(OH)D and SHBG, as shown in Fig. 4 ($p = 0.04$). No significant interaction of serum 25(OH)D and SHBG was observed for $1,25(OH)_2D$, parameters of bone turnover, nor BMD at all measurement sites (data not shown).

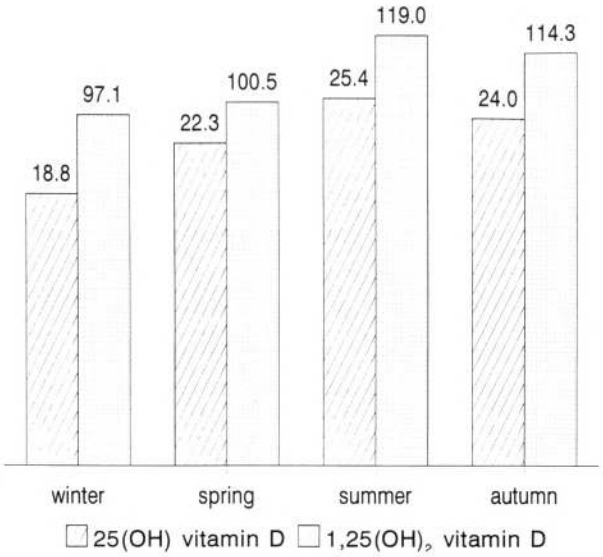

FIG. 1. Seasonal variation of serum 25(OH)D, nmol/l ($p < 0.0001$), adjusted for age, sunshine preference score and outdoor frequency score, and serum $1,25(OH)_2D$, pmol/l ($p < 0.0001$) adjusted for age and serum creatinine.

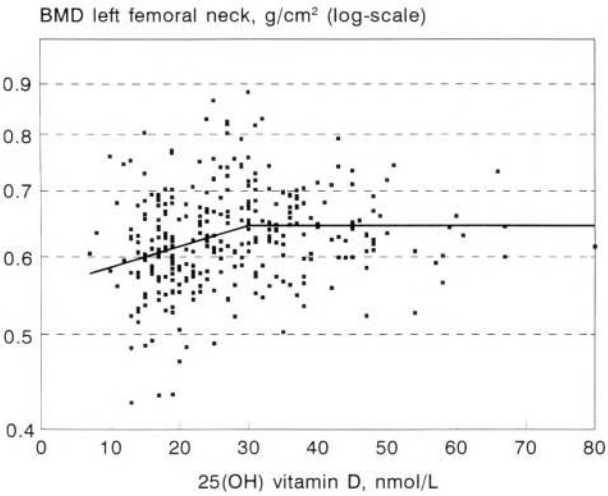

FIG. 2. Relation of BMD of the left femoral neck with serum 25(OH)D ($p = 0.001$), adjusted for serum SHBG, age, age at menopause, and body weight.

DISCUSSION

We studied the influence of the vitamin D status and the remaining estrogen activity on bone metabolism and BMD in a group of elderly women. Although the results are based on cross-sectional data, the validity of the results was enhanced by correction for important potential confounders with multiple regression. The average vitamin D status of the subjects was poor, similar to what has been found in other studies in the elderly,[1,7,8] which is caused by the decreasing capacity of the skin for vitamin D synthesis with aging,[7] as well as lesser exposure to sunshine. In our study, this is illustrated by the separate relations of the serum 25(OH)D concentrations with age, outdoor frequency, and sunshine preference scores. It has been suggested that vitamin D deficiency may contribute to hip fracture risk in the elderly by decreasing calcium absorption, with subsequent mineralization defects and secondary hyperparathyroidism, leading to increased bone turnover and cortical bone loss.[5,26] Our study supports this hypothesis. The parallel variation over the seasons and the positive relation of $1,25(OH)_2D$ with 25(OH)D indicate that the production of $1,25(OH)_2D$ is substrate-dependent in the case of vitamin D deficiency. This has been observed in other studies as well.[10,12] Low serum 25(OH)D concentrations were associated with higher PTH and osteocalcin concentrations and lower BMD of the hip. Higher serum PTH levels were associated with lower BMD at all measurement sites, which suggests that the lower BMD in the case of vitamin D deficiency may be due to secondary hyperparathyroidism. Similar relations have been found in other groups of elderly people with comparable 25(OH)D concentrations.[11,12,27,28] In a recent cross-sectional study in the U.K., a positive relation between serum 25(OH)D concentrations and BMD of the lumbar spine, the femoral neck, and trochanter was observed in a group of middle-aged women, but these women had low 25(OH)D concentrations as well.[29] Because these relations are improbable in a vitamin D–replete status, and inspection of the data suggested a clearly nonlinear relation, we examined the possibility of a threshold phenomenon. It appeared that the vitamin D status was negatively related to PTH and osteocalcin levels and positively to the BMD of the hip, but only when serum 25(OH)D was lower than approximately 30 nmol/l. Above this threshold no relationship was found. This suggests that the boundary between a deficient and a replete vitamin D status in Dutch elderly women is at 30 nmol/l. Dawson–Hughes et al.[30] observed in a group of North American postmenopausal women that a vitamin D plus calcium supplement (377 mg/day) decreased wintertime bone loss in the spine and the whole body when compared with the calcium supplement alone. The mean 25(OH)D concentrations in the placebo group in that study ranged, depending on the period, from 61–81 nmol/l (95% CI 56–66 and 77–86 nmol/l, respectively). This is well above the threshold observed in our study and seems to preclude vitamin D deficiency. This may be explained by differences in the 25(OH)D assays.[31] Another explanation might be that in that study the mean calcium intake from supplements and food was approximately 700–800 mg/day. The dietary calcium intake of the Dutch elderly women in our study was much higher: 921 mg/day from dairy products alone, implicating that the total mean calcium intake will be well above 1100 mg/day.[21] In populations with a lower calcium intake, the calcium absorption from the gut will depend more on vitamin D–mediated active absorption than on passive diffusion.[32] Serum $1,25(OH)_2D$ is inversely related to calcium intake[33] and, therefore, the threshold may be higher when the calcium intake is low.

Our data and the fact that vitamin D supplementation has been shown to increase serum $1,25(OH)_2D$ and suppress PTH secretion in vitamin D–deficient elderly,[12,32]

TABLE 4. DIFFERENCES (%) FOR BMD FOR EACH 10 nmol/l OF HIGHER SERUM 25(OH)D UP TO THE THRESHOLD, AND FOR A DOUBLING OF SERUM SEX HORMONE BINDING GLOBULIN (SHBG), CORRECTED IN REGRESSION MODELS FOR AGE, AGE AT MENOPAUSE, AND BODY WEIGHT

	25(OH)D			*SHBG*	
BMD	*Threshold*	*% difference/ 10 nmol/l*	p*	*% difference/ doubling*	p*
Left femoral neck	30	5.0	0.001	−1.4	0.43
Right femoral neck	30	3.8	0.004	−3.4	0.05
Left femoral trochanter	30	2.5	0.13	−4.8	0.02
Right femoral trochanter	30	3.2	0.05	−4.5	0.03
Dominant distal radius	—	−1.2	0.32	−10.5	0.001

* Two-sided p values of 25(OH)D and SHBG in the multiple regression model.

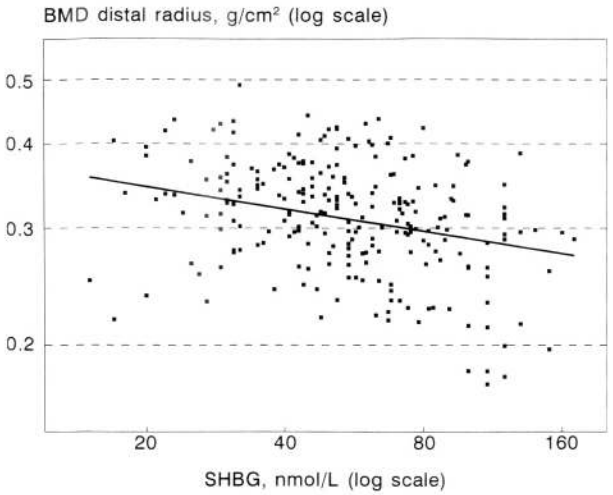

FIG. 3. Relation of BMD of dominant distal radius with serum sex hormone binding globulin (SHBG, $p = 0.001$), adjusted for serum 25(OH)D, age, age at menopause, and body weight.

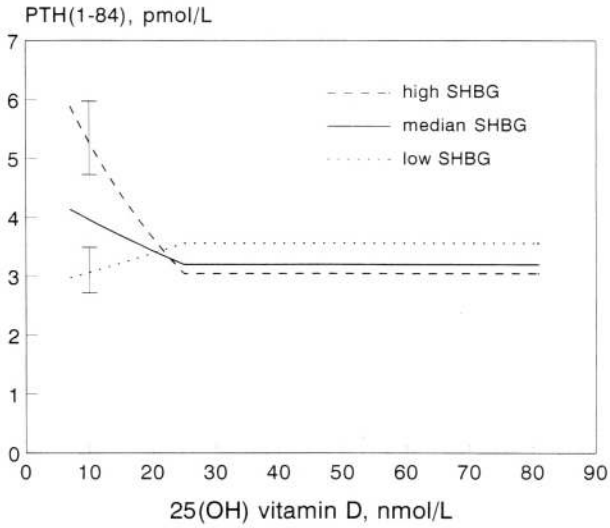

FIG. 4. Relation of serum PTH(1–84) with serum 25(OH)D, for a low, median, and high serum sex hormone binding globulin (SHBG; 23, 56, and 120 nmol/l, respectively), adjusted for age, age at menopause, and serum creatinine. The error bars indicate the standard error for the effect of SHBG on the slope of the regression lines ($p = 0.04$).

suggest that vitamin D supplementation may prevent bone loss from the hip and hip fractures in the elderly. At a serum 25(OH)D level of 10 nmol/l the BMD of the left femoral neck was 9.3% lower, which is 0.6 SD below the average BMD for an adequate vitamin D status, i.e. above 30 nmol/l. According to the recent data of Cummings et al.,[34] this would result in a relative risk of hip fracture of 1.8.

SHBG has been found to be a predictor of BMD and bone loss, superior to endogenous estrogen levels in younger groups of women.[17,19] SHBG is the principal determinant of the level of free (active) estrogen and testosterone. SHBG was considerably higher in older women and lower in heavier women. The control of SHBG concentrations is very complex but probably involves estrogens (+), testosterone (−), insulin levels (−), and the nutritional status, especially lipids (−). With aging the androgen levels decrease, which might account for the increase in the SHBG levels. Obesity is often associated with higher lipid and insulin levels, which may be an explanation for the lower SHBG levels in the heavier subjects.[16,35] In this study in elderly women, a higher SHBG level was associated with higher bone turnover and lower BMD at the femoral trochanter and the distal radius. The fact that age and body weight are related to BMD as well as SHBG may account for this.[21] However, even after adjusting for age and body weight, the relation of SHBG with BMD remained. It has been proposed that the loss of calcium from the skeleton due to oestrogen deficiency will decrease PTH secretion.[36] This might counteract the PTH elevating effect of the decreased calcium absorption caused by vitamin D deficiency. In our study, however, higher SHBG levels were associated with higher PTH levels in vitamin D–deficient elderly women only, which is in contradiction with the former. There are some data, however, that indicate that estrogen

deficiency, i.e. high SHBG, may decrease calcium absorption directly[37] or cause resistance of the gut to $1,25(OH)_2D$.[38] In vitamin D–deficient elderly, compensation for decreased calcium absorption by increasing $1,25(OH)_2D$ levels may be impaired due to substrate deficiency, resulting in a further increase of PTH levels.

Although the relation of SHBG with BMD may be explained by its effect on bone loss during previous years, the higher osteocalcin and Hp/Cr ratio in participants with high SHBG reflect a currently increased bone turnover. Although we did not measure free estrogen and androgen levels, this suggests that even in the very old estrogen activity plays a role in bone metabolism and osteoporosis. While a stronger relationship of 25(OH)D levels with BMD was found at the femoral neck, SHBG was related more strongly to the BMD of the femoral trochanter and the distal radius. The femoral neck predominantly consists of cortical bone,[39] which is more susceptible to bone loss due to secondary hyperparathyroidism.[5,28] The femoral trochanter and the distal radius have a higher content of trabecular bone, which is more sensitive to estrogen deficiency.[4,39,40]

We conclude that 25(OH)D levels below 30 nmol/l are associated with secondary hyperparathyroidism, increased bone turnover, and decreased BMD at the hip. SHBG is positively related to bone turnover and negatively to trabecular bone mass, which may indicate that estrogen activity influences bone turnover and trabecular bone loss even in the very old. When vitamin D deficiency is combined with a high SHBG the secondary hyperparathyroidism is more severe, while it is almost lacking when SHBG is low. This suggests that low oestrogen activity causes decreased sensitivity of the gut to $1,25(OH)_2D$, leading to higher serum PTH levels. This increases the impact of vitamin D deficiency. Since vitamin D deficiency according to the 30 nmol/l limit is common, it may be an important risk factor for hip fractures in the elderly in The Netherlands.

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