Plasma 25-Hydroxyvitamin D Levels and Fracture Risk in a Community-Based Cohort of Elderly Men in Sweden

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Context: Blood levels of 25-hydroxyvitamin D [25(OH)D] is the generally accepted indicator of vitamin D status, but no universal reference level has been reached.

Objective: The objective of the study was to determine the threshold at which low plasma 25(OH)D levels are associated with fractures in elderly men and clarify the importance of low levels on total fracture burden.

Design and Participants: In the Uppsala Longitudinal Study of Adult Men, a population-based cohort (mean age, 71 yr, n = 1194), we examined the relationship between 25(OH)D and risk for fracture. Plasma 25(OH)D levels were measured with high-pressure liquid chromatography-mass spectrometry.

Setting: The study was conducted in the municipality of Uppsala in Sweden, a country with a high fracture incidence.

Main Outcome Measure: Time to fracture was measured.

Results: During follow-up (median 11 yr), 309 of the participants (26%) sustained a fracture. 25(OH)D levels below 40 nmol/liter, which corresponded to the fifth percentile of 25(OH)D, were associated with a modestly increased risk for fracture, multivariable-adjusted hazard ratio 1.65 (95% confidence interval 1.09–2.49). No risk difference was detected above this level. Approximately 3% of the fractures were attributable to low 25(OH)D levels in this population.

Conclusions: Vitamin D insufficiency is not a major cause of fractures in community-dwelling elderly men in Sweden. Despite the fact that cutaneous synthesis of previtamin D during the winter season is undetectable at this northern latitude of 60°, only one in 20 had 25(OH)D levels below 40 nmol/liter, the threshold at which the risk for fracture started to increase. Genetic adaptations to limited UV light may be an explanation for our findings. (*J Clin Endocrinol Metab* 95: 2637–2645, 2010)

The importance of adequate vitamin D status in maintaining bone health has been recognized for a long time, but its role in reducing fracture risk is still unclear. 25-hydroxyvitamin D [25(OH)D] is the major circulating metabolite of vitamin D, and plasma levels of this metabolite have been the generally accepted indicator of vitamin D status. A plasma 25(OH)D less than 25 nmol/liter has been regarded an index of true vitamin D deficiency (1),

Abbreviations: BMD, Bone mineral density; CI, confidence interval; HR, hazard ratio; MS, mass spectrometry; 25(OH)D, 25-hydroxyvitamin D.

which is associated with rickets in children and osteomalacia in adults. Both these diseases are relatively uncommon. Moderate vitamin D deficiency is more frequent and might cause secondary hyperparathyroidism that increases the susceptibility to fractures in the elderly (2). Attempts to define such vitamin D insufficiency using plasma 25(OH)D have most often been based on the identification of the point at which plasma PTH starts to rise. These threshold levels have varied greatly, from 20 to 110 nmol/liter (3). This wide range may in part be due to different settings, study designs, or analytical methods used to measure 25(OH)D.

Additionally, an insufficiency threshold level should preferably be based on a clinical end point. The results from previous large prospective cohort studies that have tried to define the inflection point using plasma 25(OH)D and the more relevant outcome fracture instead of PTH levels are also conflicting. Except for a recent nested case-control study in men (4), none of the previous studies used the gold standard HPLC or mass spectrometry (MS) to measure 25(OH)D or presented data for older men (5–11). We therefore used data from a population-based cohort of elderly men to determine at what levels plasma 25(OH)D, measured with HPLC-MS, are associated with future elevated fracture risk. Additionally, we aimed to ascertain the relative importance of low vitamin D levels on total fracture burden in a setting with a high incidence of osteoporotic fractures and no detectable cutaneous synthesis of previtamin D during the winter season (12).

Subjects and Methods

Study population

The Uppsala Longitudinal Study of Adult Men has been described previously (13–15). Briefly, in 1970 all 50-yr-old men (born 1920–1924) living in Uppsala, Sweden, were invited to participate in a health survey. The baseline for the present study is the third examination (1991–1995), when the mean age of the participants was 71 yr. Of the 1221 participants, 1194 (98%) had valid measurements of plasma 25(OH)D and 965 (79%) of plasma PTH. We also analyzed data from the fifth survey (2003–2005, n = 530) at the mean age 82 yr. Fasting blood samples were collected at each investigation in addition to a questionnaire survey regarding medical history, lifestyle habits, and regular medication. All participants gave written consent and the study was approved by the Ethics Committee of Uppsala University.

Baseline examinations

At baseline, venous blood samples were drawn after an overnight fast and stored at −70 C for 12.6 ± 1.1 yr until analysis (16). Plasma 25(OH)D was determined with HPLC-atmospheric pressure chemical ionization-MS at Vitas, Oslo, Norway (www.vitas.no). Vitas has a Vitamin D External Quality Assessment Scheme certificate. One hundred fifty microliters of human plasma were diluted with 450 μL 2-propanol containing butylated hydroxytoluene as an antioxidant. After thorough mixing (15 min) and centrifugation (10 min, 4000 $\times$ g at 10 C), an aliquot of 35 μL was injected from the supernatant into the HPLC system. HPLC was performed with a HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) interfaced by atmospheric pressure chemical ionization to an HP mass spectrometric detector operated in single ion monitoring mode. Vitamin D analogs were separated on a 4.6- $\times$ 50-mm reversed-phase column with 1.8 μM particles. The column temperature was 80 C. A two-point calibration curve was made from an analysis of albumin solution enriched with known vitamin D concentration. Recovery was 95%. The method was linear from 5 to 400 nmol/liter and the limit of detection was 1–4 nmol/liter. The interassay coefficient of variation was 7.6% at 47.8 nmol/liter and 6.9% at 83.0 nmol/liter. 25(OH)D is stable in stored plasma (17).

Intact plasma PTH was measured with solid-phase two-site chemiluminescent immunoassay using an Immulite 2500 (Diagnostics Product Corp., Los Angeles, CA). Serum cystatin C was measured by latex enhanced reagent (N Latex Cystatin C; Dade Behring, Deerfield, IL) using a Behring BN ProSpec analyzer (Dade Behring) (18). Glomerular filtration rate can be calculated from serum cystatin C results in milligrams per liter by the formula, $y = 77.24 \times {}^{-1.2623}$, which has been shown to be closely correlated with iohexol clearance (19). Height and weight were measured under standardized conditions. Participants reported their leisure-time physical activity (categorized as low, medium, and high) and number of falls the previous year (0, 1–2, 3 or more) on a standardized questionnaire (15). Coding of smoking was based on interview and questionnaire data (14), and smoking status was categorized as current, former, or never smoker. A dietitian instructed participants to record their dietary intake in a 7-d precoded food diary. Daily intakes of vitamin D, calcium, and other nutrients were calculated using a computer program and the Swedish National Food Administration database (SLV Database, 1990) (13, 20). Information on perceived health (classified as good or not good) and alcohol use at ages 60 and 77 yr [classified according to the Michigan alcoholism screening test as abstainer, normal, suspected alcohol dependence (21)] was retrieved from the questionnaires. By linkage to the National Patient Registry, we collected information on any musculoskeletal, endocrine, hematological, infectious, psychiatric, neurological, respiratory kidney, urinary, or gastrointestinal disorder and any dermatoses. The census from 1970 provided information on educational level. Socioeconomic group at age 50 yr was interview based and categorized as low, medium, and high.

Follow-up investigation

At the fifth investigation at age 82 yr, *i.e.* on average 11 yr after the analysis of 25(OH)D at baseline, 507 of the 530 men agreed to undergo measurements of bone mineral density (BMD; grams per square centimeter) of the total body, femoral neck region of the hip, total proximal femur, and the lumbar spine (vertebrae L2-L4) by dual-energy x-ray absorptiometry (DPX Prodigy; Lunar Corp., Madison, WI). When applicable, both extremities were used in the calculation. By triple measurements in 15 subjects, the precision error of the dual-energy x-ray absorptiometry measurements in our laboratory has been calculated to be between 0.8 and 1.5% for BMD, depending on site. Daily scans of a lumbar spine phantom were performed. The

long-term precision error coefficient of variation percentage was less than 1% during the study period.

Analyses of stored plasma from the fifth investigation were performed in 2007 for 25(OH)D and in 2009 for PTH and cystatin C as described above.

Identification of fractures

We sought to identify all fractures that occurred in study participants after enrollment. Using the Swedish personal identification number of every participant, we linked the study cohort to the National Patient Registry to identify all cases of fractures admitted to hospital. Fractures were also confirmed by linkage, with use of the personal identification number, to radiographic records and county outpatient registries. All orthopedic records at the local hospitals in areas in which the participants in the initial investigation resided were reviewed to identify fractures according to the type and circumstances of the injury as previously described (13, 15).

Statistical analysis

All statistical calculations were performed using SAS (SAS 9.1; SAS Institute, Cary, NC). Fracture risk associated with plasma 25(OH)D was analyzed using Cox proportional hazards models to estimate hazard ratio with 95% confidence intervals (CIs) as a measure of the association. For each man, the number of years of follow-up was calculated from baseline until the date of a first fracture, the date of death, or the end of the follow-up period (December 31, 2007). Dates of deaths were based on data from the continuously updated Swedish National Population Register. We first assessed the linear association between 25(OH)D and rate of fracture. To gain additional insights into potential nonlinearity, we modeled the nonlinear trend in the risk of fracture by a restricted cubic-spline Cox regression analysis with five knots [25(OH)D percentiles 5, 27.5, 50, 72.5, and 95] (22) and 80 nmol/liter as reference, a normal level suggested by expert opinion (23). The results of this analysis are presented as a smoothed plot with 95% CI for the overall risk of fracture.

Categorization of a continuous variable is often based on quantiles, *e.g.* quartiles. This approach is, however, not suitable if the level of risk varies substantially within a quantile and therefore ordinary percentile categories can do poorly in this regard (24, 25). Plasma 25(OH)D was therefore divided into biologically more meaningful categories using the method of Contal and O'Quigley (25, 26), which is based on a log-rank test statistics. With this new but previously used (27) method, we found the optimal category boundary of plasma 25(OH)D to be 40 nmol/liter. Accordingly, based on this value, two categories of plasma 25(OH)D were constructed with the high-level category used as reference in the Cox proportional hazards models. The proportional hazards assumptions for the Cox models were confirmed formally by Shoenfeld's tests and graphically by comparing Nelson-Aalen plots. Kaplan-Meier curves with log log-rank tests for fracture probability were constructed by the categories of plasma 25(OH)D.

We considered three separate models: a crude- and two multivariable models. The first multivariable model included age, weight, height, cystatin C, calcium intake (all continuous), physical activity (low, medium, high), smoking (never, former, current), and blood draw season (winter, summer). The winter season was defined as November 1 to April 30 (28). We extended this model to evaluate the influence of comorbid conditions for the association between vitamin D status and fracture risk. Based on data from the National Patient Registry, we included the following variables diagnosed after the age of 50 yr and before the baseline examination: dermatoses, diabetes mellitus, other endocrine disease, hematologic, infectious, musculoskeletal, psychiatric, neurological, respiratory kidney, urinary, or gastrointestinal disease (yes/no for each of all these). Diabetes mellitus was defined as fasting plasma glucose of 7.0 mmol/liter or greater, 2-h postload glucose levels of 11.1 mmol/liter or greater, or the use of oral hypoglycemic agents or insulin. Further adjustment was made for energy intake, alcohol intake, number of falls the previous year, self-perceived health, and socioeconomic class. All these additional adjustments influenced our fracture risk only marginally, and they were thus not retained in the final full multivariable model.

The population-attributable risk of fracture among those with a low plasma vitamin D (<40 nmol/liter) was calculated as

$$\frac{p(HR-1)}{[p(HR-1) + 1]}$$

where p is the prevalence of low plasma vitamin D in the cohort at baseline (29).

To further investigate the mechanisms behind the association between low vitamin D levels and fracture risk, we analyzed the associations between vitamin D and BMD, PTH, and risk of falls. The association between plasma 25(OH)D at age 71 yr (in two categories, <40 and >40 nmol/liter) and BMD at age 82 yr was examined, also taking into account plasma 25(OH)D at the time of the BMD examination, seasons of blood draw, and the covariates included in the first, less comprehensive, multivariable Cox model. We further considered the influence on BMD by change in plasma 25(OH)D between ages 71 and 82 yr.

The correlation between vitamin D and PTH was examined with the variables in continuous form, but the average PTH values with 95% CI by the categories of vitamin D (<40, >40 nmol/liter) was also determined.

Differences in the number of falls (0, 1, 2, 3 or more) during the previous year among men with 25(OH)D levels below or above 40 nmol/liter were compared with the χ^2 test.

Results

Characteristics of the participants are displayed in Table 1. Only 40 men (3%) reported use of supplemental vitamin D. Approximately half of the men had blood samples taken during the winter season (November-April). At baseline, mean 25(OH)D was 68.7 nmol/liter. Only 10 individuals (0.8%) had levels less than 25 nmol/liter at baseline and four (0.8%) at 82 yr (Fig. 1). Because all fortified foods and dietary supplements in Sweden currently contain vitamin D_3, almost all circulating 25(OH)D was derived from vitamin D_3.

During the follow-up period (median 11 yr), 309 of the 1194 participants had a fracture. Mean 25(OH)D levels did not differ between men with and without fracture, 68.7 nmol/liter (95% CI 66.5–70.9) *vs.* 68.7 nmol/liter (95% CI 67.5–70.0). We did not observe any statistically

TABLE 1. Baseline characteristics of the total Uppsala Longitudinal Study of Adult Men cohort and by fracture status during follow-up

	Total cohort (n = 1194) Mean (SD)	Men without fracture during follow-up (n = 885) Mean (SD)	Men with fracture during follow-up (n = 309) Mean (SD)
Age (yr)	71.0 (0.6)	71.0 (0.6)	71.0 (0.6)
Weight (kg)	80.4 (11.5)	80.9 (11.6)	78.9 (11.2)
Height (cm)	174.8 (6.0)	174.8 (6.1)	174.6 (5.7)
Body mass index (kg/m^2)	26.3 (3.4)	26.4 (3.5)	25.9 (3.2)
P 25-hydroxyvitamin D			
Total (nmol/liter)	68.7 (19.1)	68.7 (18.9)	68.7 (19.7)
D$_3$ (nmol/liter)	67.9 (19.1)	67.9 (18.9)	67.6 (19.5)
D$_2$ (nmol/liter)	0.8 (3.8)	0.8 (3.5)	1.1 (4.2)
Dietary vitamin D intake (μg/d)	5.7 (2.2)	5.8 (2.2)	5.7 (2.2)
Total vitamin D intake (μg/d)	5.9 (2.4)	5.9 (2.3)	5.9 (2.4)
Dietary calcium intake (mg/d)	965 (343)	962 (333)	976 (371)
Energy intake (kcal/d)	1741 (459)	1748 (460)	1723 (457)
Alcohol intake (g/d)	6.4 (7.4)	6.4 (7.4)	6.5 (7.1)
P cystatin C (mg/liter) (reference < 1.55 mg/liter)	1.25 (0.27)	1.24 (0.24)	1.27 (0.34)
P PTH (pmol/liter) (reference 1.1–6.9 pmol/liter)	4.3 (2.1)	4.3 (2.0)	4.5 (2.4)
Season			
Winter (November–April), n (%)	645 (54)	477 (54)	174 (56)
Summer (May–October), n (%)	549 (46)	408 (46)	135 (44)
Vitamin D supplement users, n (%)	40 (3)	28 (3)	12 (4)
Smoking status			
Never, n (%)	458 (39)	353 (40)	105 (34)
Former, n (%)	563 (47)	405 (46)	158 (51)
Current, n (%)	173 (14)	127 (14)	46 (15)
Leisure physical activity			
Low, n (%)	65 (5)	40 (5)	21 (7)
Medium, n (%)	432 (36)	331 (37)	101 (33)
High, n (%)	699 (59)	514 (58)	187 (61)
Number of falls during the previous year			
1–2, n (%)	80 (7)	58 (7)	22 (8)
3 or more, n (%)	16 (1)	13 (2)	3 (1)
Self-perceived health			
Good, n (%)	947 (79)	721 (81)	226 (73)
Diabetes mellitus, n (%)	199 (17)	147 (17)	52 (17)
Cardiovascular disease, n (%)	357 (30)	257 (29)	100 (32)
Cancer, n (%)	81 (7)	60 (7)	21 (7)
Gastrointestinal disease, n (%)	368 (31)	257 (29)	111 (36)
Kidney-urinary disease, n (%)	238 (20)	169 (19)	69 (22)
Respiratory disease, n (%)	70 (6)	54 (6)	16 (5)
Psychiatric disorder, n (%)	41 (3)	23 (3)	18 (6)
Muscular-skeletal disorder, n (%)	163 (14)	118 (13)	45 (15)
Infection, n (%)	188 (16)	131 (15)	57 (18)
Skin disease, n (%)	37 (3)	21 (2)	16 (5)
Blood disease, n (%)	18 (2)	11 (1)	7 (2)
Neurological disease, n (%)	43 (4)	28 (3)	15 (5)
Socioeconomic class			
Low, n (%)	693 (58)	510 (58)	183 (59)
Medium, n (%)	233 (20)	173 (20)	60 (19)
High, n (%)	268 (22)	202 (23)	66 (21)

significant association between 25(OH)D in continuous form and fracture risk, hazard ratio (HR) 0.98 (95% CI 0.87–1.09) per SD increase in plasma 25(OH)D. As seen in Fig. 2, in a multivariable Cox proportional hazard model, the adjusted HR for fracture was higher than the reference for 25(OH)D values only below approximately 40 nmol/liter, which corresponded to the fifth percentile of 25(OH)D.

When we compared the fracture probability for individuals with 25(OH)D less than 40 nmol/liter with those

having levels greater than 40 nmol/liter, there appeared to be a divergence of the Kaplan Meier curves throughout the follow-up period (Fig. 3). For individuals with 25(OH)D levels less than 40 nmol/liter compared with those with greater than 40 nmol/liter (Table 2), the crude HR for fracture was 1.71 (95% CI 1.13–2.57). The HR decreased slightly after adjustment for age, weight, height, cystatin C, calcium intake, physical activity, smoking, and blood draw season to 1.65 (95% CI 1.09–2.49) and after addi-
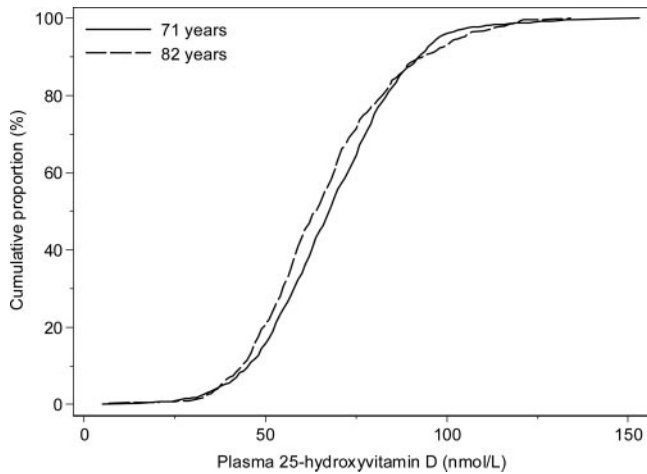
FIG. 1. Cumulative proportion of plasma 25(OH)D in participants at 71 (*solid line*) and 82 (*hatched line*) yr of age.

tional adjustment for comorbid conditions to 1.58 (95% CI 1.04–2.41). The adjusted population-attributable risk of fracture was 0.03 (95% CI 0.01–0.08). If we analyzed only first fractures (n = 207), crude HR was 1.84 (95% CI 1.12–3.02) and adjusted HR 1.69 (95% CI 1.03–2.80). Seventy-three men sustained a hip fracture during follow-up, but only five of these had 25(OH)D levels less than 40 nmol/liter. There appeared to be an association also between 25(OH)D and hip fracture rate, but the precision in this estimate was low (crude HR 1.45; 95% CI 0.59–3.60).

To investigate possible mechanisms behind the association between plasma 25(OH)D and fracture risk, we examined the influence of baseline 25(OH)D levels on BMD in 507 of the men at 82 yr. Compared with individuals with 25(OH)D levels greater than 40 nmol/liter, those with levels less than 40 nmol/liter had a lower adjusted BMD at the lumbar spine (adjusted BMD 1.20 (95% CI 1.09–1.32) *vs.* 1.32 (95% CI 1.30–1.35) g/cm^2, P = 0.04) but not at the proximal femur (adjusted BMD 0.96 (95% CI 0.89–1.04) *vs.* 0.99 (95% CI 0.98–1.01) g/cm^2, P = 0.48) or total body (adjusted BMD 1.15 (95% CI 1.10–1.20) *vs.* 1.19 (95% CI 1.18–1.19) g/cm^2, P = 0.15).
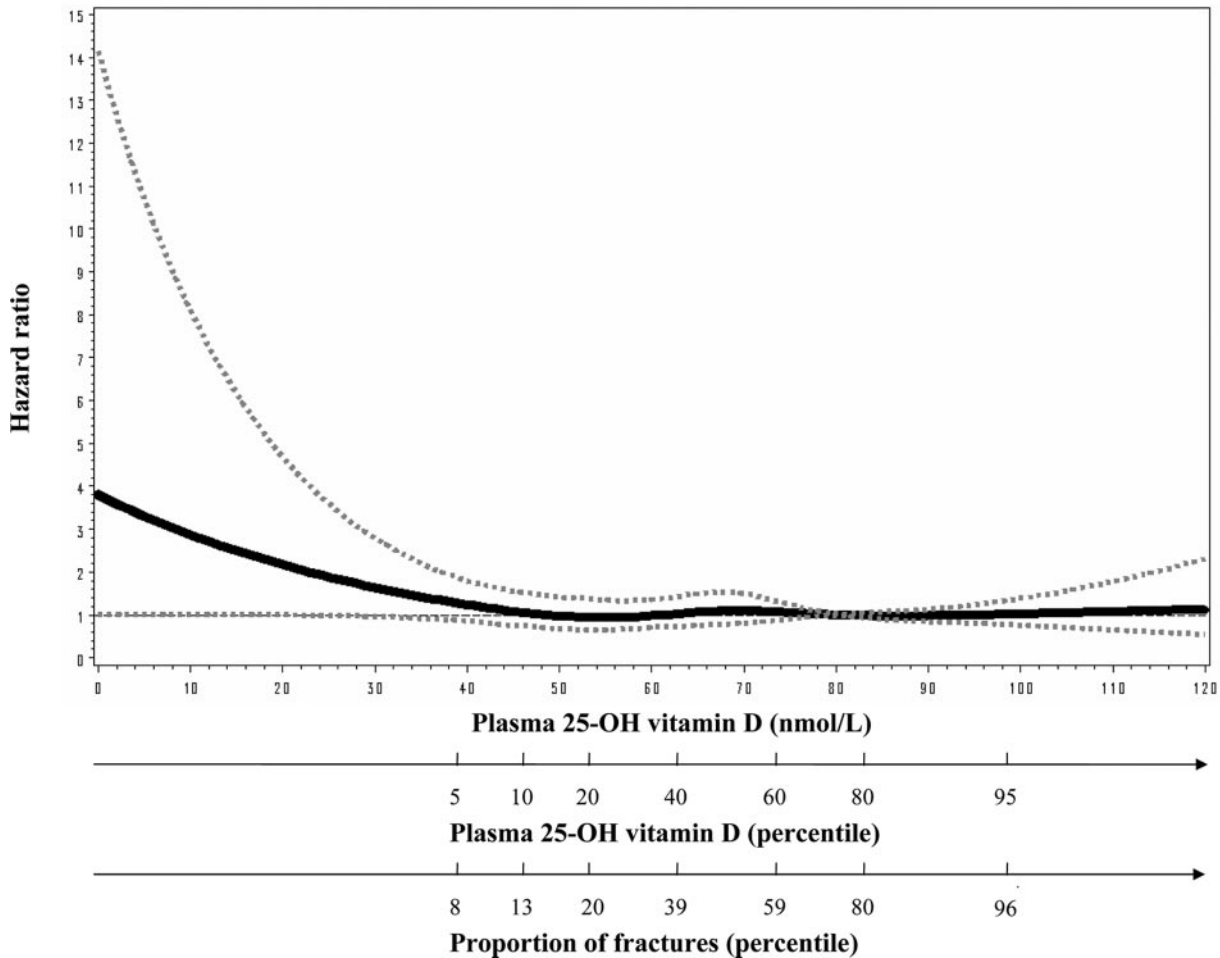
FIG. 2. Smoothed plot of HRs for fracture according to the plasma 25(OH)D level. The HRs (*solid line*) and 95% CIs (*dotted lines*) were estimated by restricted cubic-spline Cox regression analysis, with the plasma 25(OH)D percentile 80 as the reference value, adjusted by weight, height, age, cystatin C, calcium intake (all continuous), season (winter, summer), physical activity (low, medium, high), and smoking (never, former, current).
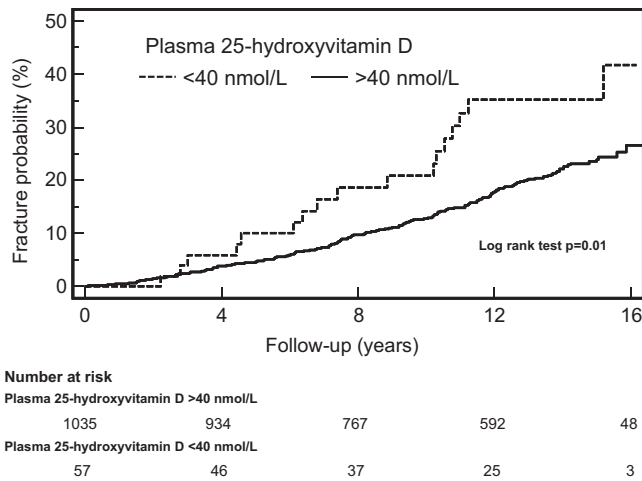
FIG. 3. Cumulative fracture probability for men with plasma 25(OH)D levels less than 40 nmol/liter (*dashed line*) *vs.* greater than 40 nmol/liter (*solid line*) throughout a 17-yr observation by a Kaplan Meier analysis.

Because the 25(OH)D levels may change between 70 and 82 yr, we also analyzed the effect of such changes on BMD in a multivariable model. To further take into account season of blood take, we also analyzed individuals with blood sampling ±15 calendar days between the two different occasions (n = 67), but changes in plasma 25(OH)D still had no significant association with BMD at any site (data not shown).

Valid PTH measurements were available in a subgroup of 965 men, and there were 246 fractures among these. As expected, PTH levels were higher [5.6 pmol/liter (95% CI 5.5–6.1)] in individuals with 25(OH)D less than 40 nmol/liter than in those with 25(OH)D greater than 40 nmol/liter [4.2 pmol/liter (95% CI 4.0–4.5), $P < 0.001$ for difference]. However, there was no association between PTH and fracture risk. The 85 men with PTH values above the reference interval (1.1–6.9 pmol/liter) did not have a higher risk of fracture than men with less than 6.9 pmol/liter, adjusted HR 1.18 (95% CI 0.78–1.80). The risk of fracture in the PTH subgroup, adjusted HR 1.85 (95% CI 1.19–2.89), was similar to that found among all 1194 men [adjusted HR 1.65 (95% CI 1.09–2.49)], and HR was essentially unchanged after adjustment for plasma PTH levels 1.78 (95% CI 1.14–2.79).

We also investigated which 25(OH)D level corresponded to the PTH inflection point. As seen in Fig. 4, the correlation between 25(OH)D and PTH was weak (at age 71 yr, $R^2 = 0.02$ determined by a 3-degree polynomial to account for a nonlinear association, $P < 0.001$ and at age 82 yr, $R^2 = 0.002$, $P = 0.32$). There was no clear inflection point, especially not at 82 yr, but at 71 yr, it appeared to occur somewhere less than 25(OH)D concentrations of 50 nmol/liter.

We finally analyzed the association between 25(OH)D and number of falls. Men with 25(OH)D levels less than

TABLE 2. HR and population attributable risk (PAR) with 95% CIs of first fracture by plasma 25(OH)D level

Plasma 25(OH)D	Fractures (n)	Person-years of observation	Rate of fracture (n/person-years)	HR (95% CI)	Adjusted HR (95% CI)[a]	Adjusted HR (95% CI)[b]	PAR (95% CI)	Adjusted PAR (95% CI)[a]	Adjusted PAR (95% CI)[b]
Greater than 40 nmol/liter	284	12,598	22/1,000	1.0	1.0	1.0	NA	NA	NA
Less than 40 nmol/liter	25	653	38/1,000	1.71 (1.13–2.57)	1.65 (1.09–2.49)	1.58 (1.04–2.41)	0.04 (0.01–0.08)	0.03 (0.01–0.08)	0.03 (0.01–0.07)

Other endocrine disease, hematological diseases, dermatoses, infectious disease, musculoskeletal disease, psychiatric disease, respiratory disease, and kidney or urinary disease were identified by matching to the National Patient Registry by *International Classification of Diseases*, seventh through 10th revision codes from 1970. NA, Not available.

[a] Adjusted by weight, height, age, cystatin C, calcium intake (all continuous), season (winter, summer), physical activity (low, medium, high), and smoking (never, former, current).

[b] Adjusted by weight, height, age, cystatin C, calcium intake (all continuous), season (winter, summer), physical activity (low, medium, high), smoking (never, former, current), diabetes mellitus (yes, no), other endocrine disease (yes/no), hematological diseases (yes/no), dermatoses (yes/no), infectious disease (yes/no), musculoskeletal disease (yes/no), psychiatric disease (yes/no), neurological disease (yes/no), respiratory disease (yes/no), kidney or urinary disease (yes/no), gastrointestinal disease (yes/no).
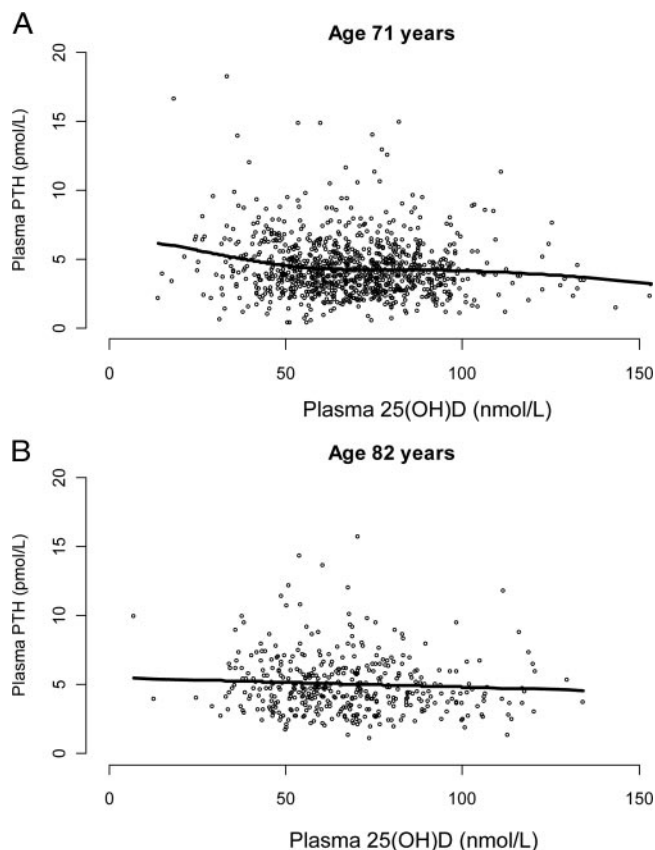
FIG. 4. Plasma PTH levels according to plasma 25(OH)D levels at 71 (A) and 82 yr (B).

40 nmol/liter did not have more falls during the previous year than men with higher levels ($P = 0.86$). The mean 25(OH)D level for men with and without falls was the same [68.8 (95% CI 65.2–72.5) vs. 68.8 (95% CI 67.6–70.0) nmol/liter].

Discussion

In this population-based cohort study in men, we used plasma 25(OH)D, determined with the gold standard HPLC-MS, as a continuous variable and fracture risk as the clinical end point to try to define the lowest required vitamin D level needed to avoid an increased fracture risk. Using a restricted cubic spline analysis together with a subsequent categorization of 25(OH)D by a novel approach (25), we found that 25(OH)D levels less than 40 nmol/liter were associated with a higher risk for fracture, whereas no risk difference was detected above this level. We also wanted to clarify the relative importance of these low vitamin D levels on total fracture burden. The population-attributable risk of fracture was as low as 3%.

In a previous Swedish cohort study of 75-yr-old women (6), a doubled risk for fracture was seen at 25(OH)D levels less than 50 nmol/liter, and 4.4% of the women had these

low levels. The observed association was, however, not adjusted for any confounding variables. Comparison of absolute 25(OH)D levels is difficult because a different method was used, but the proportion of subjects below the identified threshold levels is strikingly similar.

Few previous studies included men and the results are conflicting (8, 9, 11, 30). Except for a recent nested case-control study (4), 25(OH)D has not been determined with HPLC or MS. In that study, no association was found between 25(OH)D and nonspine fractures, but compared with the top quartile (>70 nmol/liter), the HR of hip fracture was 2.36 (95% CI 1.08–5.15) for men in the lowest quartile (<50 nmol/liter). How the risk changed within the lowest quartile of 25(OH)D was not determined. Compared with our study, these men had lower 25(OH)D levels; about one fourth had values less than 50 nmol/liter despite a higher intake of vitamin D supplements. PTH was not measured.

Results regarding vitamin D and fracture risk from studies in women are also contradictory. In contrast to the Swedish study (6), no association with fracture risk was found in the Os des Femmes de Lyon cohort (7) or the Study of Osteoporotic Fractures (5), but in a more recent study within the Women's Health Initiative Observational Study, 25(OH)D levels less than 47 nmol/liter were associated with a higher risk of hip fracture (10).

The mechanism for the association between low 25(OH)D and increased fracture risk that we observed is unclear. Comorbidity, including renal dysfunction, is unlikely to be a major mechanism because adjustment for comorbidity and cystatin C had only minor effects on the risk estimates. The fact that we found reduced lumbar spine BMD and higher PTH levels but not increased number of falls in individuals with 25(OH)D less than 40 nmol/liter suggested that vitamin D insufficiency increased the risk of fracture due to minor disturbances in the calcium homeostasis leading to increased bone turnover rather than via muscle weakness and increased susceptibility to falls. However, PTH was not associated with fracture risk and the increased fracture risk at 25(OH)D levels less than 40 nmol/liter was essentially unaltered after adjustment for PTH.

Because we do not find support for major mediating effects of comorbidity, secondary hyperparathyroidism, or increased risk of falls, the association may be a consequence of direct effects of vitamin D on bone. 25(OH)D is converted to the active form $1,25(OH)_2D_3$, which has direct effects on osteoblasts and is involved in osteoblast differentiation, control of osteoblast activity, bone formation, and bone resorption (31).

Although Sweden is a country with limited sunlight during the winter and an incidence of osteoporotic frac-

tures that is one of the world's highest (32), both Gerdhem *et al.* (6) and we find that vitamin D insufficiency is rare in community-dwelling elderly. These results are consistent with previous comparisons of serum vitamin D concentrations among populations in Europe, which have shown that the highest levels are found in the Nordic countries (12, 33).

The lack of UV light in these countries has through evolution led to two important genetic adaptations to vitamin D deficiency: a much lighter skin color and lactose tolerance. The milk consumption is higher in Nordic countries (34) due to lactose tolerance (35), and low-fat milk products were fortified with vitamin D in Sweden during the study period. Nevertheless, we have previously shown that the dietary intake of vitamin D is a poor predictor of 25(OH)D (12), and this is also true in this study ($R^2 < 0.01$ independent of season).) By studying twins, we have recently shown that genetic factors are an important determinant of vitamin D status, and the key genetic effect appears to be on the cutaneous synthesis of vitamin D (36).

A main strength of our study is the method used for 25(OH)D analysis (37). Except for the recent MrOS study (4), there are no longitudinal studies that have used this gold standard method of measuring vitamin D. Additional strengths are the setting in a single geographic area, the prospective population-based design, the relatively large number of fractures, and the detailed characterization of study participants regarding risk factors for fractures. We studied a homogenous population in which all individuals had the same age. This should be an important strength because 25(OH)D levels change through life due to endogenous or exogenous factors and usually decrease with age (38). Few participants used vitamin D supplements. Our analytical approach is a further advantage of our study. An ordinary epidemiological approach using, for example, quartiles, would not have revealed the association patterns that we can demonstrate using the restricted cubic spline analysis with a subsequent categorization of vitamin D (26).

There are also limitations of this study. The BMD measurements at 82 yr of age were performed a decade after the 25(OH)D levels were analyzed, but we included the 25(OH)D levels at 82 yr as a covariate. The optimal time point for evaluating low 25(OH)D levels as a risk factor for reduced BMD is not known but may be considerably shorter because the adult human skeleton is estimated to be completely regenerated every 10 yr. Plasma PTH was available for only four fifths of the cohort, but the proportion of men with fractures was the same as in the entire cohort (25%). A single baseline measurement may not adequately reflect long-term vitamin D status. Nevertheless, a strong correlation (r = 0.7) between 25(OH)D val-

ues measured 3 yr apart has been reported (39), and we also find a correlation (r = 0.5) for the measurements 11 yr apart. Finally, we cannot completely exclude the possibility that the excess of fractures observed in the lowest category may be a reflection of poor health not captured by our adjustments.

Despite these limitations, we conclude that vitamin D insufficiency is not a major cause of fractures in community-dwelling elderly men in Sweden.

Acknowledgments

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