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Serum 25 HydroxyVitamin D Concentrations and the Risk of Hip Fractures: The Women's Health Initiative

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Abstract

Background—The association between and vitamin D levels and fractures is uncertain.

Objective—To test the hypothesis that serum 25-hydroxyVitamin D (25(OH) vitamin D) levels are associated with the risk of hip fracture in community dwelling women.

Design—Nested case-control study.

Setting—40 US clinical centers.

Participants—We studied 400 cases of incident hip fractures and 400 controls matched on age, race/ethnicity and date of blood draw (average follow-up time, 7.1 years). Subjects were selected from 39,795 postmenopausal women without previous hip fractures, not using estrogens or other bone-active therapies.

Measurements—Serum 25(OH) vitamin D was measured on baseline serum using radioimmunoassay with DiaSorin reagents and divided into quartiles. Conditional logistic regression was used to estimate the odds ratio with 95% confidence intervals (CI). Multivariable models included age, body mass index, parental and personal history of fractures, smoking, alcohol and calcium intake, geographic location and corticosteroid use.

Results—The mean **(**standard deviation, SD) 25(OH) vitamin D (nM) was lower in cases, 56.2 (20.3) compared to controls, 59.7(18), p=0.007. A 25 nM (10ng/ml) decrease in 25(OH) vitamin D was associated with a 33% increased risk of hip fracture (odds ratio=1.33; 95%CI,1.06, 1.68) in multivariable models. Compared to women with 25(OH) vitamin D ≥70.7 nM (Quartile 4), the odds ratio of hip fracture was 1.71 (1.05, 2.79), 1.09 (0.70, 1.71) and 0.82 (0.51, 1.31) in women with 25 (OH) vitamin D <47.5 nM, 47.5 to 60 nM, 60 to <70 nM, respectively, p trend =0.015. This association was in part mediated by a marker of bone resorption but remained statistically significant. Adjustment for falls, physical function, frailty, renal function, or sex steroid hormones had no effect on this association.

Limitations—No measure of bone density.

Conclusion—Low serum 25(OH) vitamin D concentrations are associated with a higher risk of hip fracture. Measurement of 25(OH) vitamin D may be useful in identifying women at high risk of hip fracture.

Introduction

Vitamin D deficiency is common in older adults, especially in home bound populations(1), during the winter(2), in general medical inpatients(3) and in community dwelling women admitted to the hospital with acute hip fracture(4) . An evidence based report on vitamin D and bone health has recently been published(5). The level of evidence for an association between levels of serum 25(OH) vitamin D and fracture was considered inconsistent(5). Since publication of this review, one prospective study reported no relationship between 25(OH) vitamin D and fractures (6), while a second reported a significantly lower risk of hip fracture with 25(OH) vitamin D levels >60 nM(7).

Vitamin D could contribute to fractures by influencing muscle strength and balance, both of which contribute to falls and disability(8-10). The association between 25(OH) vitamin D and

fracture could also be influenced by renal function, since renal insufficiency has been linked to fracture(11) and to vitamin D deficiency(12). Several interactions between vitamin D and estrogen receptors have been described(13), and hormone therapy has been shown to reverse abnormalities in vitamin D metabolism(14). Low vitamin D has also been linked to higher bone turnover(15,16). Thus, sex steroid hormone and bone turnover levels could contribute to the association between 25(OH) vitamin D and fractures.

We conducted a nested case control study within the Women's Health Initiative Observational Study (WHI-OS) among 400 cases of incident adjudicated hip fracture and 400 controls. We tested the following hypotheses: 1) low serum 25(OH) vitamin D concentrations are associated with a higher risk of hip fractures in community dwelling women; 2) this relationship maybe mediated by poor physical functioning, frailty, falls, sex steroid hormones, renal function and bone turnover.

Methods

Study Population

The study population comes from the WHI-OS, a prospective cohort study that enrolled 93,676 women ages 50−79 years from 1994−1998 at 40 US clinical centers. Study methods have been described in detail elsewhere(17). Briefly, women were eligible if they were postmenopausal, unlikely to move or die within three years, not enrolled in the WHI Clinical Trials and not currently participating in any other clinical trial. The study was approved by Human Subjects Review Committees at each participating institution.

Follow-up and Outcome Ascertainment

Women were sent questionnaires annually to report any hospitalization and other outcomes including fractures. Follow-up time ranged from 0.7 − 9.3 years as of August, 2004 with a median duration of 7.1 years. At that time, 3.7% of participants had withdrawn or were lost to follow-up and 5.3% had died. Hip fractures were verified by review of medical records and confirmed by blinded central adjudicators(18). Pathological hip fractures were excluded.

Nested Case-Control Study Design

The present study is a case-control study nested within the prospective design of the WHI-OS. Participants were excluded if they had a prior history of hip fracture, were taking hormones up to one year prior to enrollment, or were currently taking androgens, selective estrogen receptor modulators, antiestrogens, or other osteoporosis treatments (bisphosphonates, calcitonin, parathyroid hormone). Women without sufficient serum stored or with unknown ethnicity were also excluded leaving a final study group of 39,793 eligible participants. From among 38,793 eligible women, a total of 404 women suffered a hip fracture. We randomly selected 400 of these women to comprise the incident hip fracture case group. One control per case was selected with individual matching by age at screening (+/− one year), race/ethnicity, and date of blood draw (+/− 120 days).

Baseline Clinical Variables

Clinical centers were divided into three geographical regions based on latitude: Northern, >40 degrees N; Middle, 35−40 degrees N; and Southern; <35 degrees N. All covariates were ascertained at baseline. Current use of prescription medications was recorded by clinic interviewers by direct inspection of medicine containers. Prescription names were entered into the WHI database and assigned drug codes using Medispan software. Average amounts of elemental calcium and vitamin D preparations were entered directly from supplement containers. Dietary intakes of calcium and vitamin D were assessed using a semi-quantitative

food frequency questionnaire(19). Total calcium and vitamin D intake was defined as the sum of diet and supplements.

Questionnaires ascertained information on date of birth, race/ethnicity, age at menopause, history of any fracture after age 55, smoking, parental history of hip fracture, self-rated health status and alcohol consumption. Physical activity was classified on the basis of frequency and duration of walking and mild, moderate and strenuous activities in the prior week. Kilocalories of energy expended in a week was calculated (metabolic equilivant (MET), score=kcal hours/week/kg)(20). Physical function was measured using the 10-item Rand-36 physical function scale(21). The physical function scale includes 10 items measuring whether health now limits physical function in moderate/vigorous activity (2 items); strength to lift, carry, stoop, bend, stair climb (4 items); ability to walk various distances without difficulty (3 items); and self-care (1 item). The scale is scored from 0 to 100, with higher scores indicating better physical function. We compared women with a score >90 versus ≤ 90; this cutoff corresponded to the median score. A frailty score was computed and included self-reported muscle weakness and impaired walking speed (RAND- 36 Physical Function Scale <75), exhaustion (RAND- 36 Vitality Scale <55), low physical activity (lowest quartile of physical activity) and unintended weight loss between baseline and three years of follow-up(22). A woman was considered "frail" if she reported 3 or more of these indicators. Weight was measured on a balance beam scale with the participant dressed in indoor clothing without shoes. Height was measured using a wall-mounted stadiometer. Body mass index was calculated as weight (kg)/height (m^2).

Laboratory Procedures

A 12 hour fasting sample was obtained at the baseline visit, processed and stored at − 80° C according to strict quality control procedures(23). Laboratory personnel were blinded to case-control status. Serum 25(OH) vitamin D and sex steroid hormone levels were measured at the Reproductive Endocrine Research Laboratory at the University of Southern California. For 25 (OH) vitamin D, a radioimmunoassay was used with DiaSorin reagents (Stillwater, MN). The sensitivity of the assay was 3.75 nM. The inter-assay coefficient of variation were 11.7%, 10.5%, 8.6% and 12.5% at 14.0, 56.8, 82.5 and 122.5 nM, respectively.

Estradiol and testosterone concentrations were quantified using sensitive and specific radioimmunoassays following organic solvent extraction and celite column partition chromatography(24-27). For estradiol, the intra-assay and inter-assay coefficients of variation were 7.9% and 8−12%, respectively and for testosterone, 6% and 10−12%, respectively. Bioavailable hormone concentrations were calculated using mass action equations(28-30). Sex hormone binding globulin (SHBG) was measured using a solid phase two site chemiluminescent immunoassay. The intra-assay and inter-assay coefficients of variation were 4.1−7.7% and 5.8−13%, respectively. Serum levels of cystatin-C levels, a marker of renal function that is independent of age and weight, were measured with the Dade Behring BN-II nephelometer and Dade Behring reagents(Ramsey, MN) using a particle-enhanced immunonepholometric assay at Medical Research Laboratories International in Highland Heights, Kentucky. Serum C-terminal telopeptide of Type 1 collagen and aminoterminal procollagen extensions propeptide were measured by immunoassay (Synarc Inc., Lyon, France).

Statistical Methods

Baseline characteristics were compared between hip fracture cases and matched controls, using chi-square tests and t-tests. 25(OH) vitamin D levels were divided into quartile categories defined on the basis of the distribution in the control subjects. To further assess confounding, baseline characteristics were compared across quartiles of 25(OH) vitamin D levels in cases and controls combined. The p-values for trend were calculated using logistic regression by

coding the variable of interest as a continuous variable. Associations between serum 25(OH) vitamin D levels and incident hip fracture were assessed in conditional logistic regression models retaining the matched case-control design. Associations were first examined unadjusted and then adjusted for age, body mass index, parental history of hip fracture, previous fractures, smoking, alcohol use and total calcium intake, oral corticosteroid use and geographic location. We had information on 25(OH) vitamin D levels in 799 individuals.

Odds ratios and 95% confidence intervals were calculated from the conditional logistic regression models per 2.5 nM: (1ng/ml) and per 25 nM (10 ng/ml) decrease in 25(OH) vitamin D and across quartiles. The highest quartile of 25(OH) vitamin D formed the referent group. We separately examined the association in women < age 70 and age ≥70 and the interaction of age × 25(OH) vitamin D. Nonparametric smoothing techniques were used to test whether the relationship was linear or a threshold one, at a pre-specified threshold of 50 nM (20ng/mL).

To investigate mechanisms by which 25(OH) vitamin D might be associated with hip fracture, we constructed a base multivariate model and then added the following variables one at a time to determine their impact on the associations between 25(OH) vitamin D and hip fracture: 1) markers for deteriorating health status (poor physical function, frailty score(22); 2) number of falls; 3) sex steroid hormones; 4) renal function (Cystatin-C); and 5) bone turnover (Serum C-terminal telopeptide of Type 1 collagen and aminoterminal procollagen extensions propeptide). We hypothesized that the association between 25(OH) vitamin D and hip fracture would be reduced after adjusting for these factors, if they are in the causal pathway. A summary multivariate model included physical function score, falls, sex steroid hormones, renal function and bone turnover markers. The Hosmer-Lemeshow Goodness-of-Fit Test was used to evaluate the models(31). All models indicated excellent fit for the data.

The study had a power of 90% (with a two sided alpha of 0.05) to detect a 0.14 standard deviation difference in 25(OH) vitamin D standard deviation difference in 25(OH) vitamin D between cases and controls.

Results

Comparison of Cases and Controls

The average age of the cases was 71± 6.15 years; one third were older than 70 and 95% were white, Table 1. Cases also had a lower body mass index and physical activity and were more likely to report oral corticosteroid use, fair/poor health status, poor physical function and smoking and were more likely to be considered frail. Serum 25(OH) vitamin D levels were 6% lower in cases than controls (p=0.007). Calcium intake and sex steroid hormones were lower and Cystatin C and bone resorption marker, higher in cases than controls. There was no difference in hormone therapy use, alcohol intake, use of vitamin D supplements or dietary vitamin D, personal or family history of fracture, geographic location or bone formation between cases and controls.

Comparisons Across Quartiles of 25(OH) Vitamin D

The percent of non white, obese, frail and subjects with fair/poor health status decreased with increasing 25(OH) vitamin D, Table 1. Physical function and physical activity increased with increasing concentration. Use of vitamin D supplements, vitamin D intake and calcium intake also increased across quartiles of 25(OH) vitamin D. A lower percentage of subjects from the Northern region were in the highest vitamin D quartile. Sex steroid, Cystatin C and bone resorption markers decreased with increasing vitamin D.

25(OH) Vitamin D and Hip Fracture

The unadjusted odds ratio for incident hip fracture per 25 nM (10ng/ml) decrease in serum 25 (OH) vitamin D was 1.30 (95% CI, 1.07. 1.58), Table 2. Further multivariate adjustment had little effect. The increased risk of hip fracture was primarily confined to women with the lowest 25(OH) vitamin D (Quartile 1). Compared to women in Quartile 4, the multivariate adjusted odds ratio of hip fracture for women in Quartile 1 was 1.71 (1.05, 2.79). We tested a threshold model at 50.0 nM, but the threshold model was not a significant improvement over the linear model (p=0.78). There was no difference in the relationship of 25(OH) vitamin D and hip fracture by age, p interaction= 0.62.

Potential Mediators

The average number of falls over the follow-up did not differ in cases (mean=2.36 ± 2.75) or controls (mean=2.82 ± 3.5), p=0.09 and did not differ over quartiles of 25(OH) vitamin D. We tested several factors that could contribute to the association between 25(OH) vitamin D and subsequent hip fractures, Table 3. Adjustment for frailty, physical functioning and falls resulted in similar attenuations in the association between 25(OH) vitamin D and hip fractures. Addition of the sex steroid hormones to the models tended to increase the odds ratio slightly. Inclusion of serum C-terminal telopeptide of Type 1 collagen in the model resulted in the greatest attenuation in the odds ratio for hip fracture in women with the lowest 25(OH) vitamin D. Nevertheless, the overall trend between 25(OH) vitamin D and hip fracture remained statistically significant. In our summary multivariable model, women with the lowest 25(O(H) vitamin D (Quartile 1) had a 72% increased odds of experiencing a hip fracture in comparison to women with the highest 25(OH) vitamin D, p trend=0.029. The area under the curve for our multivariate model was 0.69.

Discussion

In our prospective nested case-control study, we found that women with low vitamin D levels, 25(OH) vitamin D<47.6 nM (<19ng/ml), at entry to the study had a significantly greater increased risk of subsequent hip fracture over the next seven years compared to women with levels >70.7 nM (≥23ng/ml) . Testing specific thresholds of < 50 nM (20ng/ml) did not differ significantly from the linear model suggesting a continuous linear relationship between serum 25(OH) vitamin D and hip fracture, at least within the ranges of 25(OH) vitamin D found in this study. The association between 25(OH) vitamin D and hip fracture was observed in both younger (<70) and older (age≥70) women. This observation is clinically important because hip fractures are the most serious consequence of osteoporosis, resulting in substantial morbidity, loss of independence, institutionalism and mortality(32). Our results suggest that measurement of 25(OH) vitamin D may be useful for identifying women at high risk of hip fracture.

Using an English language Medline search until January 2008, we identified five prospective studies of vitamin D and fracture. Our results are consistent with a recent report from the Third National Health and Nutrition Examination Survey (NHANES III), where the relative risk of hip fracture was 0.64 (0.46−0.89) among subjects with 25(OH) vitamin D levels >60 nM (24 ng/ml) compared to those with lower levels. Similarly, Swedish women with 25(OH) vitamin D <52.5 nM had a two-fold increased risk of fracture(33). Previous cohort studies which failed to find a significant association between 25(OH) vitamin D and fracture were limited by small sample size and high lost to follow rate(34) or use of an older assay(35). A nested case-control study of 730 incident fracture cases and 1,445 controls found no evidence of an association between 25(OH) vitamin D and fracture(6). However, they studied a heterogeneous group of fractures, including only 22 hip fractures and studied a younger population (mean age ≈ 50). Vitamin D may be more strongly linked to frailty related fractures like hip fractures which tend to occur in much older women.

Vitamin D deficiency can cause secondary hyperparathyroidism, high bone turnover, low bone mineral density and mineralization defects(1), all of which could contribute to an increased fracture risk. In our study, C-terminal telopeptide of Type I, a marker of bone resorption, tended to be higher among hip fracture cases with the lowest 25(OH) vitamin D , an association that may be driven by higher parathyroid hormone levels in this group(1). Adjustment for bone resorption resulted in a 18% relative reduction in the odds ratio, suggesting that high bone resorption may in part mediate this association.

The increased fracture risk could also be related to impaired muscle strength and balance, poor physical function, all of which could lead to an increased risk of falls(8-10). Hip fracture cases had lower physical function scores and were more likely to be frail. Higher 25(OH) vitamin D was association with a lower likelihood of being frail and higher physical function, but the association between vitamin D and hip fracture was independent of these factors.

In vitro and in vivo studies have also shown that vitamin D may modulate the activity of estrogen compounds in bone cells(36). Thus, we hypothesized that sex steroid hormones could influence the relationship between 25(OH) vitamin D and hip fracture. Bioavailable estradiol decreased across quartiles of 25(OH) vitamin D in the controls, but this was largely explained by obesity. There was no relationship with testosterone and 25(OH) vitamin D. However, addition of either sex hormone to our models had no effect on our results.

Renal function could affect bone metabolism directly through its effects on vitamin D and parathyroid hormone metabolism(12). Cystatin C levels were significantly higher in hip fracture cases compared to controls and decreased with increasing 25(OH) vitamin D. Nevertheless, Inclusion of cystatin C in our models did not attenuate the association between 25(OH) vitamin D and hip fracture.

We matched cases and controls on blood draw data, +/−120 days and thus, seasonal variability in 25(OH) vitamin D could have confounded our results. However, the mean difference in blood draw date between cases and controls was 0.27 ± 6.12 days; 99% of cases and controls were matched within one month. Geographic variation in 25(OH) vitamin D has been reported with greater vitamin D deficiency observed in northern latitudes(37). Women from the northern region (>40 degrees N), had lower 25(OH) vitamin D levels but the relationship between 25 (OH) vitamin D and hip fracture was independent of geographic location.

The prevalence of obesity was significantly lower in subjects with the highest 25(OH) vitamin D, consistent with the lower bioavailability of vitamin D reported in obese subjects(38) and with reports of an inverse association between 25(OH)D and adiposity (39,40). Lower 25(OH) vitamin D in obese subjects may reflect lower physical activity (less sunlight exposure) and deposition in body fat compartments(38). Nevertheless, the association between 25(OH) vitamin D and hip fracture was independent of obesity.

The optimal serum 25(OH) vitamin D needed to maintain bone health is not established. Optimal 25(OH) vitamin D levels have been defined as the level in which serum parathyroid hormone levels plateau in the normal range but this approach has led to a wide range of optimal thresholds (20−115 nM) (41). More recently, the optimal threshold of 25(OH) vitamin D based on bone mineral density levels was found to be at least 78 nM with a target of 92−105 nM (42). Randomized trials of vitamin D supplementation (with or without calcium) that brought mean serum 25(OH) vitamin D levels up to 75 nM −102.5 nM found significantly lower fracture rates(43). Trials in which the mean serum 25(OH) vitamin D did not reach this threshold showed no overall effect on fractures (44,45). However, in the largest study to date, a 29% reduction in hip fractures was observed among adherent women, despite not achieving this 25 (OH) vitamin D threshold(45).

In the WHI Calcium-Vitamin D Trial, no relationship was found between 25(OH) vitamin D and fracture. The characteristics of women in the Calcium-Vitamin D Trial differed from the women in our study. Of importance, over 50% of the women in the Calcium-Vitamin D Trial were on hormone therapy while we excluded women on any bone active agents from this analysis. In addition, different cut points were used and their results were unadjusted.

Strengths of our study include the control for numerous confounders, elimination of hormone use as a confounder, and the ability to explore several mechanisms underlying this association. We used the currently recommended 25(OH) vitamin D assay in a research endocrine laboratory. There are however, several limitations. Minority women are more likely to be vitamin D deficient(46), but only 20 hip fractures occurred in minority women. Bone mineral density was measured in only three WHI clinics and thus we were unable to test whether the association between low 25(OH) vitamin D and hip fracture is mediated by bone mineral density. In the NHANES III study, the association between 25(OH) vitamin D and hip fracture was independent of bone density(7). We measured total 25(OH) vitamin D and were unable to distinguish 25(OH) vitamin D_2 from 25(OH) vitamin D_3. However, reporting D_2 and D_3 separately has been shown to cause some clinical confusion(47). We did not measure parathyroid hormone, which could contribute to the relationship between 25(OH) vitamin D and hip fracture. We have few women with 25(OH) vitamin D levels >75 nM so we could not test whether even higher levels offer greater protection against hip fracture risk. We estimated dietary intake of vitamin D using a food frequency questionnaire but few foods contain or are fortified with vitamin D. Circulating 25(OH) vitamin D is standardly used to determine an individual's vitamin D nutritional stores(46). Finally, we used an observational study design and adjusted for many factors that could confound the association. However, there may be residual confounding by unmeasured factors.

We conclude that low serum 25(OH) vitamin D concentrations are associated with an increased risk of hip fracture in community dwelling women. Measurement of 25(OH) vitamin D may be useful in identifying women at high risk of hip fracture.

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Table 1

Baseline characteristics by case-control status and across quartiles of 25(OH) vitamin D (nM).

	Control	Case		1st Quartile Median (range) 37.1 (9.23 − <47.6)	2nd Quartile Median (range) 54.0 (47.6 − < 60.2)	3rd Quartile Median (range) 65.1 (60.2 − < 70.7)	4th Quartile Median (range) 80.4 (70.7 − 121.5)	
	N (%) Mean (Std)	N (%) Mean (Std)	P-value	N (%) Mean (Std)	N (%) Mean (Std)	N (%) Mean (Std)	N (%) Mean (Std)	P-value
Age (>70)								
Yes	132 (33.0)	132 (33.0)	1.00	84 (34.43)	57 (29.23)	54 (32.34)	68 (35.23)	0.58
Ethnicity (white)								
Yes	380 (95.0)	380 (95.0)	1.00	224 (91.80)	184 (94.36)	163 (97.60)	188 (97.41)	0.02
BMI (kg/m^2)								
<25	144 (36.09	193 (48.61)	0.001	91 (37.45)	70 (36.27)	77 (46.11)	98 (51.04)	0.002
25−<30	150 (37.59)	127 (31.99)		80 (32.92)	72 (37.31)	63 (37.72)	62 (32.29)	
≥30	105 (26.32)	77 (19.40)		72 (29.63)	51 (26.42)	27 (16.17)	32 (16.67)	
BMI (kg/m^2)	27.41 (5.13)	26.04 (5.15)	<0.001	27.70 (5.72)	27.04 (4.76)	26.14 (4.87)	25.75 (25.75)	<0.001
HT usage status								
Past user	98 (24.50)	95 (23.75)	0.80	57 (23.36)	44 (22.56)	48 (28.74)	43 (22.28)	0.45
Oral corticosteroid use								
Yes	3 (0.75)	14 (3.50)	0.007	7 (2.87)	3 (1.54)	1 (0.60)	6 (3.11)	0.29
RAND 36 Physical Function (>90)								
Yes	117 (29.77)	86 (21.66)	0.009	47 (19.50)	56 (29.02)	38 (23.17)	61 (31.94)	0.02
Total physical activity (MET-hrs/wk)	13.86 (15.32)	10.71 (12.67)	0.002	10.31 (15.09)	10.69 (10.37)	12.88 (12.08)	15.92 (17.0)	<0.001
Frailty[†]								
Yes	66 (16.50)	89 (22.25)	0.04	65 (26.64)	29 (14.87)	31 (18.56)	30 (15.54)	0.005
Self-reported health status								
Fair/Poor	42 (10.69)	61 (15.37)	0.05	44 (18.26)	24 (12.44)	17 (10.30)	18 (9.47)	0.03
Smoking								
Past smoker	171 (43.18)	144 (36.55)	<0.001	92 (37.86)	70 (36.65)	72 (43.90)	81 (42.41)	0.12
Current smoker	10 (2.53)	36 (9.14)		22 (9.05)	10 (5.24)	5 (3.05)	8 (4.19)	
Alcohol intake (drinks/wk)	3.40 (1.67)	3.37 (1.60)	0.84	3.29 (1.62)	3.39 (1.67)	3.35 (1.63)	3.51 (1.60)	0.58
25 VITD (nM)	59.60 (18.05)	55.95 (20.28)	0.007	35.83 (8.33)	53.95 (3.73)	65.13 (3.05)	83.00 (10.80)	<0.001

	Control	Case		1st Quartile Median (range) 37.1 (9.23 − <47.6)	2nd Quartile Median (range) 54.0 (47.6 − < 60.2)	3rd Quartile Median (range) 65.1 (60.2 − < 70.7)	4th Quartile Median (range) 80.4 (70.7 − 121.5)	
	N (%) Mean (Std)	N (%) Mean (Std)	P-value	N (%) Mean (Std)	N (%) Mean (Std)	N (%) Mean (Std)	N (%) Mean (Std)	P-value
Vitamin D supplement ever								
Yes	189 (47.25)	195 (48.75)	0.67	63 (25.82)	96 (49.23)	102 (61.08)	123 (63.73)	<0.001
Total vitamin D intake (IU/d)	373 (275)	383 (396)	0.71	262 (332)	361 (263)	439 (403)	480 (307)	<0.001
Total calcium intake (mg/d)	1167.01 (683.85)	1072.47 (694.23)	0.05	906.38 (567.86)	1087.03 (702.50)	1215.80 (731.58)	1341.99 (703.77)	<0.001
History of fracture on/after age 55								
Yes	82 (20.50)	96 (24.0)	0.23	49 (20.08)	35 (17.95)	39 (23.35)	55 (28.50)	0.07
Parents broke hip								
Yes	64 (16.0)	80 (20.0)	0.14	48 (19.67)	30 (15.38)	28 (16.77)	37 (19.17	0.63
Geographic region								
Southern < 35 degrees M	108 (27.0)	106 (26.50)	0.25	56 (22.95)	48 (24.62)	48 (28.74)	62 (32.12)	0.01
Middle 35−40 degrees N	95 (23.75)	115 (28.75)		59 (24.18)	64 (32.82)	32 (19.16)	54 (27.98)	
Northern > 40 degrees N	197 (49.25)	179 (44.75)		129 (52.87)	83 (42.56)	87 (52.10)	77 (39.90)	
Bioavailable estradiol (pg/ml)	7.54 (4.54)	6.57 (4.34)	0.002	7.79 (4.82)	7.01 (4.68)	6.50 (4.0)	6.64 (4.05)	0.01
Bioavailable testosterone (pg/ml)	12.59 (6.98)	10.90 (6.26)	<0.001	12.43 (7.21)	11.27 (5.68)	11.43 (6.50)	11.66 (7.04)	0.26
Cystatin-C (mg/L)	1.06 (0.24)	1.10 (0.30)	0.02	1.12 (0.37)	1.06 (0.21)	1.07 (0.23)	1.04 (0.20)	0.02
PINP (ng/ml)	49.64 (23.71)	51.00 (23.03)	0.42	49.04 (21.44)	51.65 (21.41)	51.68 (51.68)	49.34 (27.89)	0.53
CTx (ng/ml)	0.41 (0.19)	0.45 (0.21)	0.02	0.45 (0.22)	0.44 (0.19)	0.43 (0.21)	0.39 (0.16)	0.02

* Abbreviations: SD, standard deviation; HT, hormone therapy; MET, metabolic equilivant; PINP, amino proterminal procollagen extension propeptide; CTx, serum c terminal telopeptide of type I collagen

† Frailty was defined a 3 or more of the following criteria: muscle weakness, slow walking speed, exhaustion, low physical activity and unintentional weight loss (25).

Table 2

Odds ratio for the risk of hip fracture

Vitamin D[*]	**Unadjusted**	**MV-adj**[†]
Number of missing pairs (total =400)	18[**]	y18[**]
Per 2.5 nM (1 ng/ml) decrease[‡]	1.03 (1.01, 1.05)	1.03 (1.01, 1.05)
Per 25 nM (10 ng/ml) decrease	1.30 (1.07, 1.58)	1.33 (1.06, 1.68)
p for linear trend	p = 0.009	p = 0.015
Quartiles (according to control group)		
1st Quartile (<47.6 nM)	1.73 (1.13, 2.66)	1.71 (1.05, 2.79)
2nd Quartile (47.6− <60.2 nM)	1.08 (0.72, 1.63)	1.09 (0.70, 1.71)
3rd Quartile (60.2−<70.7 nM)	0.78 (0.50, 1.20)	0.82 (0.51, 1.31)
4th Quartile (70.7+ nM) (ref)	1 (ref)	1 (ref)

[*] Hip fractures case and controls selection matched on age, ethnicity and blood draw date.

[**] Because of deletion of cases and controls due to missing values, 18 case-control pairs were missing from our multivariable models. We also excluded these pairs from our unadjusted models to ensure the same analytic sample.

[†] Multivariate adjustment includes age, body mass index, parental history of hip fracture, history of fracture, smoking, alcohol use, and total calcium intake, oral corticosteroid use and geographic region.

[‡] To convert values for 25-hydroxyvitamin D to ng/ml divide by 2.5.

Table 3

Adjusted odds ratios for hip fractures according to vitamin D quartiles

	1st (9.23 − <47.6 nM) (N = 244)	2nd (47.6 − <60.2 nM) (N = 195)	3rd (60.2 − < 40.7 nM) (N =167)	4th (70.7 − 121.5 nM) (N =193)	P for linear trend
Base Analysis†					
Odds Ratio & 95% CI	1.71 (1.05 − 2.79)	1.09 (0.70 − 1.71)	0.82 (0.51 − 1.31)	1.00 (ref)	0.016
P	0.032	0.760	0.453		
Base Analysis† + Adjusted for Frailty †† Score					
Odds Ratio & 95% CI	1.65 (1.01 − 2.69)	1.08 (0.68 − 1.70)	0.81 (0.50 − 1.30)	1.00 (ref)	0.022
P	0.047	0.744	0.375		
Base Analysis† + Adjusted for RAND 36 Physical Functioning >90					
Odds Ratio & 95% CI	1.68 (1.02 − 2.76)	1.15 (0.73 − 1.83)	0.81 (0.50− 1.31)	1.00 (ref)	0.016
P	0.041	0.550	0.392		
Base Analysis† + Adjusted for Total Number of Falls during follow-up					
Odds Ratio & 95% CI	1.66 (1.00 − 2.77)	1.14 (0.70 − 1.85)	0.86 (0.52 − 1.42)	1.00 (ref)	0.018
P	0.039	0.594	0.551		
Base Analysis† + Adjusted for Bioavailable Estradiol					
Odds Ratio & 95% CI	1.75 (1.06 − 2.87)	1.01 (0.64 − 1.59)	0.75 (0.46 − 1.23)	1.00 (ref)	0.022
P	0.028	0.976	0.253		
Base Analysis† + Adjusted for Bioavailable Testosterone					
Odds Ratio & 95% CI	1.72 (1.05 − 2.83)	1.05 (0.67 − 1.66)	0.82 (0.50 − 1.34)	1.00 (ref)	0.021
P	0.031	0.830	0.422		
Base Analysis† + Adjusted for cystatin-C					
Odds Ratio & 95% CI	1.68 (1.02 − 2.76)	1.18 (0.70 − 1.76)	0.81 (0.50 − 1.31)	1.00 (ref)	0.019
P	0.042	0.658	0.389		
Base Analysis† + Adjusted for PINP* Odds Ratio & 95% CI	1.69 (1.04 − 2.77)	1.05 (0.67 − 1.67)	0.84 (0.52 − 1.35	1.00 (ref)	0.022
P	0.036	0.822	0.472		
Base Analysis† + Adjusted for CTx* Odds Ratio & 95% CI	1.58 (0.97 − 2.60)	1.03 (0.66 − 1.64)	0.83 (0.51 − 1.34)	1.00 (ref)	0.040
P	0.069	0.890	0.441		

	1st (9.23 − <47.6 nM) (N = 244)	2nd (47.6 − <60.2 nM) (N = 195)	3rd (60.2 − < 40.7 nM) (N =167)	4th (70.7 − 121.5 nM) (N =193)	P for linear trend
Summary MV model** Odds Ratio & 95% CI	1.72 (0.98 − 3.02)	1.21 (0.71 − 2.05)	0.84 (0.48 − 1.46)	1.00 (ref)	0.029
P	0.060	0.481	0.533		

*Abbreviations: PINP, amino proterminal procollagen extension propeptide; CTx, serum c terminal telopeptide of type I collagen.

†Matched on age, ethnicity, blood draw date, controlled for age, BMI, parental history of hip fracture, history of fracture, smoking, alcohol use, and total calcium intake (from diet, supplement and medications, per 100 mg increase), oral corticosteroid use, geographic region.

**Matched on age, ethnicity, blood draw date, controlled for age, BMI, parental history of hip fracture, history of fracture, smoking, alcohol use, and total calcium intake (from diet, supplement and medications, per 100 mg increase), oral corticosteroid use, geographic region, frailty, total number of falls, bioavailable estradiol, bioavailable testosterone, cystatin-C, PINP, and CTx.

††Frailty was defined a 3 or more of the following criteria: muscle weakness, slow walking speed, exhaustion, low physical activity and unintentional weight loss (23).