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Prevalence of Vitamin D Inadequacy among Postmenopausal North American Women Receiving Osteoporosis Therapy

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Purpose: To evaluate serum 25-hydroxyvitamin D [25(OH)D] concentrations and factors related to vitamin D inadequacy in postmenopausal North American women receiving therapy to treat or prevent osteoporosis.

Methods: Serum 25(OH)D and PTH were obtained in 1536 community-dwelling women between November 2003 and March 2004. Multivariate logistic regression was used to assess risk factors for suboptimal (<30 ng/ml) 25(OH)D.

Results: Ninety-two percent of study subjects were Caucasian, with a mean age of 71 yr. Thirty-five percent resided at or above latitude 42° north, and 24% resided less than 35° north. Mean (SD) serum 25(OH)D was 30.4 (13.2) ng/ml: serum 25(OH)D was less than 20 ng/ml in 18%; less than 25 ng/ml in 36%; and less than 30 ng/ml in 52%. Prevalence of suboptimal 25(OH)D was significantly higher in subjects who took less than 400 *vs.* 400 IU/d or more vitamin D. There was a significant negative correlation between serum PTH concentrations and 25(OH)D. Risk factors related to vitamin D inadequacy included age, race, body mass index, medications known to affect vitamin D metabolism, vitamin D supplementation, exercise, education, and physician counseling regarding vitamin D.

Conclusions: More than half of North American women receiving therapy to treat or prevent osteoporosis have vitamin D inadequacy, underscoring the need for improved physician and public education regarding optimization of vitamin D status in this population. **(*J Clin Endocrinol Metab* 90: 3215–3224, 2005)**

VITAMIN D IS ESSENTIAL for maintaining calcium homeostasis and optimizing bone health. 1,25-Dihydroxyvitamin D, the active vitamin D metabolite, binds to a specific nuclear hormone receptor thereby increasing intestinal calcium absorption and regulating bone turnover (1). Low concentrations of vitamin D lead to alterations in calcium and phosphorus homeostasis, secondary hyperparathyroidism, bone loss, osteoporosis, and an increase in fracture risk (2–4). More severe degrees of vitamin D deficiency lead to impairment of bone mineralization and osteomalacia (5). The standard method of assessing vitamin D status is by measuring serum concentration of the major circulating metabolite of vitamin D, 25-hydroxyvitamin D [25(OH)D] (6).

Adequate vitamin D and calcium intake is considered an essential component of osteoporosis management (http://consensus.nih.gov/cons/111/111_statement.htm; http://www.nof.org//physguide/risk_assessment.htm; http://www.osteofound.org/osteoporosis/index.html). Currently, in the United States, the recommended daily vitamin D intake is 400 IU (10 μg) for individuals aged 51–70 yr and 600 IU (15 μg) for those aged 70 yr and older (7). In Europe, 400 IU is recommended for people aged 65 yr or older. Although vitamin D is produced in the skin with exposure to sunlight, there is an age-related decline in cutaneous synthesis making older individuals more dependent on dietary intake (8, 9).

Numerous epidemiological studies have assessed the prevalence of low serum 25(OH)D concentrations (10–12) and have indicated that vitamin D inadequacy is a common problem worldwide (13). Differences in the prevalence of vitamin D inadequacy have been related to a variety of factors, including physiological changes with age, race, body mass index (BMI), sun exposure, latitude, and dietary vitamin D intake. Opinions regarding the optimal concentration of serum 25(OH)D vary widely. Several studies have shown that serum concentrations of at least 20–30 ng/ml are necessary to maximize intestinal calcium absorption and minimize perturbations in PTH, calcium, and phosphorus homeostasis (5, 12, 14, 15). The majority of epidemiological studies that have assessed vitamin D status of postmenopausal women have been performed outside the United States. In addition, although past studies have included both osteoporotic and nonosteoporotic women, none have specifically evaluated those receiving osteoporosis therapies. Therefore, we conducted

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Abbreviations: BMI, Body mass index; CI, confidence interval; LC-MS/MS, liquid chromatography-mass spectrometry; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; RR, relative risk.

an epidemiological study in North America to determine the vitamin D status of postmenopausal women currently receiving antiresorptive or anabolic therapies to treat or prevent osteoporosis. Furthermore, we sought to identify key risk factors associated with suboptimal 25(OH)D concentrations within this population.

Subjects and Methods

Study subjects

From November 2003 to March 2004, a cohort of women over the age of 55 yr and postmenopausal for a minimum of 2 yr who were receiving osteoporosis medications was recruited from 61 study sites that were evenly distributed across geographic regions by latitude (42° north or more, Boston, MA; between 35 and 42° north, and less than 35° north, Memphis, TN). Subjects could be taking vitamin D supplementation and were required to be taking therapy to treat or prevent osteoporosis, including: alendronate, calcitonin, etidronate, raloxifene, risedronate, or teriparatide for a minimum of 3 months to qualify. Subjects were excluded if they had participated in a clinical trial for osteoporosis within the 3 yr before study start or if they had increased and/or decreased use of over-the-counter supplements or prescription forms of vitamin D within 3 months. The study sites received institutional review board approval and signed informed consent from each subject before enrollment.

Study conduct

Subjects attended a single study visit during which past medical history, height, and weight were recorded. The subject's most recent dual x-ray absorptiometry, if available, was provided. Concurrent medication use and use of any bone-related therapies (including hormone therapies ever taken) were collected. A specific request was made to record medications that could potentially affect vitamin D metabolism, *e.g.* glucocorticoids, rifampin, and anticonvulsants (*i.e.* phenytoin, phenobarbital, carbamazepine). All calcium, vitamin D, and multivitamin supplements taken within 3 months before the study visit were recorded.

A single blood sample was collected and sent to a central laboratory (Quest Laboratories, Van Nuys, CA) to assess 25(OH)D, calcium, intact PTH, phosphorus, albumin, creatinine, magnesium, alkaline phosphatase, and total bilirubin concentrations; fasting was not required.

A self-administered questionnaire was completed by all study subjects and reviewed by study coordinators for completeness and legibility. If changes or additions were required, these modifications were made at the time of the visit by the subject only.

Laboratory studies

Calcium, phosphorus, albumin, creatinine, magnesium, alkaline phosphatase, and total bilirubin were measured by automated standard laboratory methods. Serum 25(OH)D samples collected at the study sites were measured using the Nichols Advantage system (Nichols Institute Diagnostics, San Clemente, CA). The Nichols Advantage system is a commercially available chemiluminescent assay, in which 25(OH)D is released from vitamin D-binding proteins by a releasing agent and the competitive assay is completed automatically by the Nichols Advantage system. This assay has a normal range in adults of 10–68 ng/ml, with an intraassay coefficient of variation of 3.0–4.5% and an interassay coefficient of variation of 7.1–10.0%.

Serum biointact PTH concentrations were measured using the Nichols Advantage chemiluminescence intact immunoassay; the normal range in adults is 6–40 pg/ml, with an interassay coefficient of variation of 7.0–9.2%.

Liquid chromatography-mass spectrometry (LC-MS/MS) was performed on a random subset of 296 subject samples to determine the contribution of $25(OH)D_2$ and $25(OH)D_3$ to serum 25(OH)D measured in the study population. $25(OH)D_2$ and $25(OH)D_3$ calibration control solutions (eight per set, spanning 1–200 ng/ml) were generated from standards provided by Calbiochem (San Diego, CA). 25(OH)D samples from study subjects were prepared and analyzed through a turbulent flow LC system (Cohesive Technologies, Franklin, MA) followed by traditional laminar flow chromatography. The study samples were then analyzed relative to the control solutions for detection and quantification of the $25(OH)D_2$ and $25(OH)D_3$ component of each sample. The analysis was performed using a TSQ Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, CA). The intraassay coefficient of variation was 6.0%.

Outcome measures

Vitamin D inadequacy and secondary hyperparathyroidism. Various cut points of serum 25(OH)D were used to define vitamin D inadequacy (<9 ng/ml, <15 ng/ml, <20 ng/ml, <25 ng/ml, or <30 ng/ml). Serum 25(OH)D concentrations less than 9 ng/ml have previously been used to define overt vitamin D deficiency (1). A range of higher values was chosen to define vitamin D insufficiency in the absence of a consensus value (5, 6). The percent of subjects with biochemical evidence of secondary hyperparathyroidism (as defined by normal serum calcium with a PTH value above the upper limit of the manufacturer's normal reference range) was determined.

Predictor variables. Risk factors evaluated for association with 25(OH)D included demographic and baseline variables collected from patient history and physical examination and health-related variables obtained from the subject questionnaire.

The demographic and baseline variables were age, race, BMI (kilograms per square meter), latitude of primary residence, use of daily vitamin D supplementation, history of a medical condition within the past 5 yr known to affect vitamin D metabolism (gastric surgery, chronic liver disease, chronic renal disease, intestinal malabsorption, morbid obesity, hyperparathyroidism, chronic granulomatous disease, malnutrition), and recent use (within the past 4 wk) of concomitant medications known to affect vitamin D metabolism.

Subject questionnaire. The 28-item questionnaire evaluated factors potentially influencing serum 25(OH)D concentrations including general health, limitation of daily activities, amount of exercise per week, discussions with physician of the importance of vitamin D to bone health, highest level of education obtained, annual household income, fall or fracture history, weekly consumption of vitamin D-rich foods, and weekly sun exposure.

Subjects were asked to provide the number of servings on a daily, weekly, or monthly basis for vitamin D containing foods, such as milk, fortified orange juice and cereals, fish, eggs, cod liver oil, and nutritional supplements.

Subjects were asked to record the number of hours per week spent outside without sun protection and the number of body parts exposed. A sun exposure index was calculated using the number of hours outside without sun protection multiplied by the percentage of the body exposed to sunlight (9% for the face, 1% for each hand, 9% for each arm, and 18% for each leg). Exposure of the chest, back, and abdomen were not included.

Statistical analysis

A sample size of 1500 subjects was estimated based on the assumption that the prevalence of low serum 25(OH)D (<15 ng/ml) was 12% and the half-width of the 95% confidence interval (CI) for the true prevalence was 1.6% (16).

The analysis population included all subjects with valid results of serum 25(OH)D. The distribution (mean, SD, median, range, *etc.*) of serum 25(OH)D and the percent of patients less than 9, 15, 20, 25, and 30 ng/ml and associated 95% CIs were calculated.

Univariate logistic regression models were used to assess the relative risk for vitamin D inadequacy (<30 ng/ml) for each potential risk factor (such as age, race, BMI). $P \leq 0.05$ was considered statistically significant. The unadjusted odds ratio (OR), 95% CI, and P value were estimated for each risk factor. Only those with $P \leq 0.10$ were included in a multivariate stepwise logistic regression analysis. The final logistic regression model included only risk factors with $P \leq 0.05$. Because a 25(OH)D concentration less than 30 ng/ml was not a rare event, the OR directly estimated from the logistic regression would overestimate the relative risk (RR) if OR was more than 1 or underestimate the RR if OR was less than 1 (17).

Therefore, the RR for the risk factors was calculated based on the OR and predicted prevalence of low vitamin D in the reference groups. A risk cohort was a subgroup of patients who had the same number of significant risk factors identified from the multivariate logistic regression analysis. The prevalence of vitamin D inadequacy [25(OH)D < 30 ng/ml] was evaluated within each risk cohort.

Pearson's correlation coefficient was calculated to assess the relationship between serum 25(OH)D and serum PTH. The means ± SE of PTH was plotted by 10 serum 25(OH)D subgroups (0–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40–49, 50–59, or ≥60 ng/ml). An exponential model [$PTH = \beta_0 + \alpha * \exp^{\beta * 25(OH)D}$] was previously used to describe the relationship between serum 25(OH)D and serum PTH by Chapuy *et al.* (15). We used a second, similar model (the quadratic fit with plateau model) to describe the relationship between serum 25(OH)D and serum PTH (18). That is, for 25(OH)D less than the certain concentration (X_0), the equation relating 25(OH)D and PTH was quadratic, and for 25(OH)D greater than X_0, the equation was constant. SAS PROC NLIN (18) can fit such a model when X_0 is unknown.

All analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC).

Results

Subject sample

One thousand five hundred fifty-four subjects met inclusion criteria. A total of 1536 (98.8%) subjects provided a blood sample for determination of serum 25(OH)D concentration and completed the questionnaire.

Subject characteristics

Subject characteristics are summarized in Table 1. All subjects were female, 91.5% were Caucasian, and 57% were 70 yr of age, or older. The majority (76%) lived at or above 35° latitude, and 35% lived at or above 42° north (Fig. 1). Fifty-nine percent of subjects reported use of at least 400 IU of vitamin D supplementation daily; 4.2% of subjects had a medical condition associated with vitamin D inadequacy; and 13.4% reported prior and/or concomitant medication use known to affect vitamin D metabolism.

TABLE 1. Demographics and baseline factors for vitamin D inadequacy

Characteristic	Total (n = 1536)
Age (yr) [mean (SD)]	71.1 (9.0)
Race [n (%)]	
White	1406 (91.5)
Black	41 (2.7)
Hispanic	39 (2.5)
Asian	32 (2.1)
Other	18 (1.2)
BMI (kg/m^2) [mean (SD)]	26.4 (5.5)
Geographic region by latitude [n (%)]	
≥42°	532 (34.6)
35–41°	642 (41.8)
<35°	362 (23.6)
Vitamin D supplementation [n (%)]	
<400 IU daily	622 (40.5)
≥400 IU daily	914 (59.5)
T-score [mean (SD)]	
Spine (n = 1119)	−1.8 (1.5)
Total hip (n = 671)	−1.7 (1.0)
Significant prior medical condition [n (%)]	
Gastric surgery	8 (0.5)
Chronic liver disease	3 (0.2)
Chronic renal diseases	9 (0.6)
Intestinal malabsorption	24 (1.6)
Morbid obesity	2 (0.1)
Hyperparathyroidism	13 (0.8)
Chronic granulomatous diseases	2 (0.1)
Malnutrition	5 (0.3)
Any of the above	64 (4.2)
Significant prior/concomitant medication [n (%)]	
Glucocorticoids	135 (8.8)
Anticonvulsants	88 (5.7)
Antimycobacterials (rifampin)	1 (0.07)
Any of the above	206 (13.4)

Subject questionnaire

A total of 84.4% of subjects reported being in good to excellent health (Table 2), 18.7% reported a fracture related to a fall within the past 5 yr, 22% reported a fracture of the hip, spine, or wrist since the age of 45 yr, and the majority (66%) reported that they had previously discussed the importance of vitamin D to bone health with their physician. Subjects had a broad range of educational and financial backgrounds; 51% reported at least some college education. Dietary consumption of vitamin D-rich foods was low (including intake of fatty fish; salmon, mackerel, herring, and sardines); the mean and median milk consumption was one 8-oz glass per day.

Distribution of serum 25(OH)D

The mean (SD) serum 25(OH)D was 30.4 ng/ml (13.2) and the distribution was nearly symmetric. The median value was 29.0 ng/ml. The 25th percentile value was 22 ng/ml and the 75th percentile value was 37 ng/ml. The discrepancy between the mean and median was due in large part to a single subject with a 25(OH)D level of 212.0 ng/ml who was consuming approximately 18,000 IU of supplemental vitamin D daily and had a high serum calcium of 10.4 mg/dl and a low-normal biointact PTH of 14 pg/ml. The next highest serum 25(OH)D concentration was 100 ng/ml in a subject with a normal serum calcium and PTH (9.5 mg/dl and 28 pg/ml, respectively).

Prevalence of low serum 25(OH)D concentration

Several cut points were used to describe the prevalence of vitamin D inadequacy in the study population (Fig. 2). Fifty-two percent (95% CI 50, 55%) of subjects had a serum 25(OH)D less than 30 ng/ml, and 18% (95% CI 16, 20%) had values less than 20 ng/ml. Regardless of the level used to define inadequacy, the prevalence was significantly greater in the group that reported daily vitamin D supplementation of less than 400 IU, compared with the group that used 400 IU or more (Fig. 3). For example, at a cut point of 30 ng/ml, the prevalence of vitamin D inadequacy in those using less than 400 IU vitamin D supplementation daily was 63%, compared with 45% among those using at least 400 IU/d.

Based on the results of LC-MS/MS, the prevalence of vitamin D inadequacy, as defined by various 25(OH)D cut points, in a random subset of 296 subject samples was nearly identical with the prevalence in the overall population, as determined by the Nichols Advantage assay (<30 ng/ml: 52.7 *vs.* 52.0%, and <20 ng/ml: 18.9 *vs.* 18.2%, respectively). The correlation coefficient (r) was 0.698. The

FIG. 1. Distribution of study sites by latitude. The map presents the geographical location of the study sites within the United States relative to latitudes of 42° north and 35° north. The number and percentage of subjects residing within each region classified by latitude is provided.

median 25(OH)D_2 concentration in the subgroup of 296 subjects was 0.8 ng/ml. The median ratio of D_2/total 25(OH)D was 0.03, indicating that 50% of subjects had less than 3% of total 25(OH)D contributed as D_2. Very few subjects (n = 12) reported use of large doses of vitamin D_2 (≥50,000 IU weekly).

TABLE 2. Summary of key results of the subject questionnaire

Question	n (%)
General health (n = 1530)	
Excellent	183 (12.0)
Very good	526 (34.4)
Good	582 (38.0)
Fair	208 (13.6)
Poor	31 (2.0)
Fracture of the hip, spine, or wrist since the age of 45[a]	
Hip	75 (5.2)
Spine	138 (9.5)
Wrist	183 (12.6)
Any of the above	333 (21.8)
Fall during past 5 yr resulting in a broken bone of any type (n = 1488)	
Yes	278 (18.7)
No	1210 (81.3)
Frequency of exercise/week (n = 1528)	
Never	319 (20.9)
1–2 times	378 (24.7)
3–5 times	579 (37.9)
6 times or more	252 (16.5)
Physician discussion regarding the importance of vitamin D to bone health (n = 1244)	
Yes	821 (66.0)
No	423 (34.0)
Highest level of education completed (n = 1532)	
Less than grade 12	197 (12.9)
High school graduate or GED	561 (36.6)
Some college	413 (27.0)
College graduate	205 (13.4)
Postgraduate degree	156 (10.2)
Annual household income (n = 1173)	
Under $15,000	215 (18.3)
$15,000–$24,999	257 (21.9)
$25,000–$34,999	200 (17.1)
$35,000–$49,999	194 (16.5)
$50,000–$74,999	172 (14.7)
$75,000 or more	135 (11.5)

[a] n values are hip (n = 1448), spine (n = 1452), wrist (n = 1454), any of above (n = 1529).

Associations with serum 25(OH)D concentration

Factors associated with the prevalence of serum 25(OH)D concentrations less than 30 ng/ml were evaluated using univariate logistic regression analyses (Table 3). Factors found to have a significant relationship ($P < 0.05$) to vitamin D inadequacy were older age (>80 yr), higher BMI (>25 kg/m^2), use of concomitant medications known to affect vitamin D metabolism, vitamin D supplementation (<400 IU daily), poor general health, limitation in daily activities, exercise level (less than six times per week), absence of previous discussion with a physician regarding the importance of vitamin D to bone health, lower education level and annual income, and lower number of weekly servings of vitamin D-containing foods. All of these factors remained significantly associated with vitamin D inadequacy when D inadequacy was defined as a 25(OH)D less than 20 ng/ml (data not shown). When age and BMI were analyzed as categorical variables (with more than two categories), the tests for trend were significant, $P < 0.05$ (data not shown). With advancing age, there was an increased risk of vitamin D inadequacy; however, even among the youngest age groups (51–60 yr), the prevalence of vitamin D inadequacy was relatively high (51%).

In a multivariate analysis, eight variables were significantly associated with vitamin D inadequacy. They included age (>80 yr), race (nonwhite), BMI (>30 kg/m^2), use of concomitant medicines known to affect vitamin D metabolism, use of vitamin D supplementation (<400 IU daily), lack of exercise, absence of a previous discussion with a physician regarding the importance of vitamin D to bone health, and education level (less than grade 12). The estimates of the relative risks for these eight variables in the multivariate analysis were similar to those seen in the univariate analysis. The number of risk factors present in a single patient was positively correlated with the risk of vitamin D inadequacy (r = 0.893, $P < 0.001$). The prevalence of vitamin D inadequacy ranged from 43% for patients with one risk factor (n = 366) to 90% for patients with five or more risk factors (n = 31). Even among subjects with no identifiable risk factors (n = 203), there remained a 32% prevalence of vitamin D inadequacy.

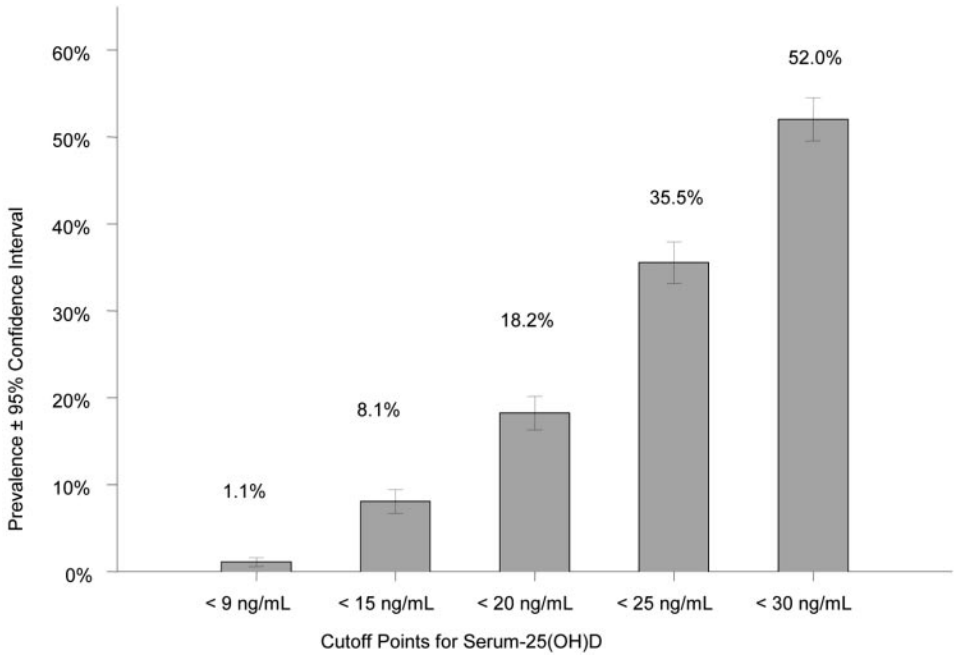

FIG. 2. Prevalence of vitamin D inadequacy in all subjects. The percentage of subjects with serum 25(OH)D concentrations below predefined cutoffs of less than 9, less than 15, less than 20, less than 25, and less than 30 ng/ml.

Prevalence of secondary hyperparathyroidism

Among the overall study population, 1246 subjects had been on chronic (≥1 yr) therapy for osteoporosis. Of these, 206 (16.5%) had biochemical evidence of secondary hyperparathyroidism. There was a significant inverse correlation between serum PTH and 25(OH)D; $r = -0.283$, $P < 0.001$. This correlation was also performed for the overall study population and identified a significant and consistent correlation between serum PTH and 25(OH)D ($r = -0.290$, $P < 0.001$). The strength of the relation between PTH and 25(OH)D may be underestimated by this r value because of their nonlinear association (Fig. 4A). When the relationship between serum PTH and 25(OH)D was analyzed using a quadratic model, it was found that PTH values began to increase with 25(OH)D concentrations less than 29.8 ng/ml. At 25(OH)D concentrations above 29.8 ng/ml, PTH values appeared to reach a plateau level of 27.2 pg/ml (data not shown). Consequently, the prevalence of secondary hyperparathyroidism was significantly higher among subjects with lower serum 25(OH)D concentrations ($P < 0.001$ for trend test) (Fig. 4B).

Discussion

In this study of 1536 postmenopausal women living in North America and receiving osteoporosis therapy, we found a high prevalence of vitamin D inadequacy. Although

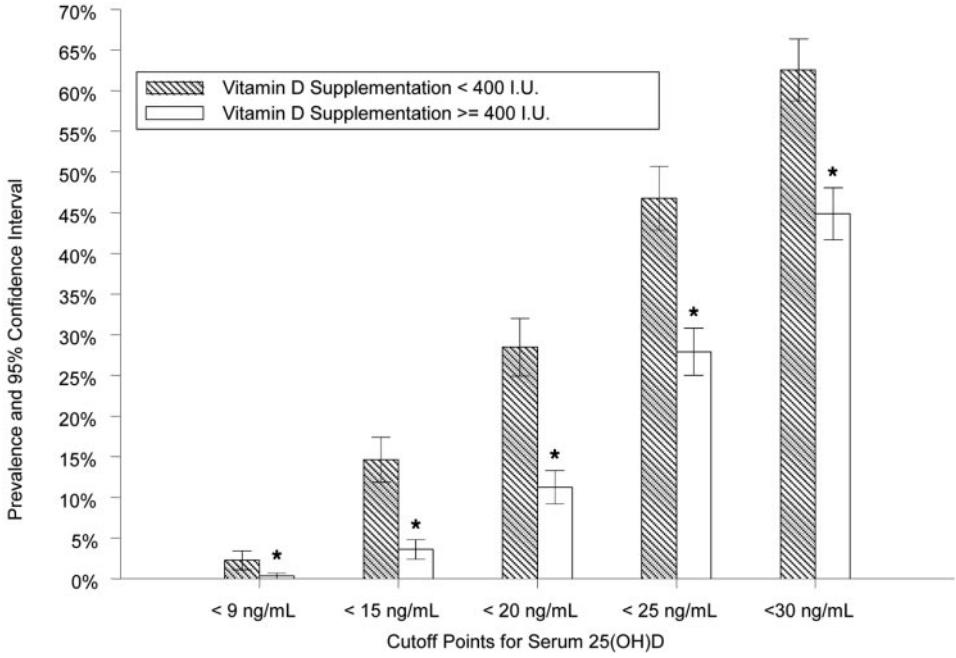

FIG. 3. Subgroup analysis of vitamin D inadequacy relative to daily vitamin D supplementation less than 400 IU or 400 IU or more. The percentage of subjects with serum 25(OH)D concentrations below predefined cutoffs of less than 9, less than 15, less than 20, less than 25, and less than 30 ng/ml relative to their daily vitamin D supplementation of either less than 400 IU or 400 IU or more. A *P* value is provided for the difference between subjects with less than 400 IU or 400 IU or more daily vitamin D supplementation at each cutoff. *, $P < 0.001$ for comparison of patients with vitamin D supplementation <400 IU *vs.* ≥400 IU.

TABLE 3. Prevalence and unadjusted RR for vitamin D inadequacy less than 30 ng/ml in the univariate analysis

Risk factor	n	Prevalence (%)	RR (95% CI)	*P* value
Age (yr)				
≤80	1276	50.7	Ref	
>80	260	58.5	1.13 (1.02, 1.22)	0.023
Race				
Nonwhite	130	60.6	1.17 (0.99, 1.33)	0.058
White	1406	51.3	Ref	
BMI (kg/m^2)				
<25	646	44.1	Ref	
25–30	459	50.1	1.12 (1.00, 1.24)	0.049
>30	317	69.4	1.25 (1.20, 1.29)	<0.001
Latitude (°N)				
≥42	532	48.7	0.89 (0.77, 1.01)	0.078
35–42	642	53.3	0.97 (0.85, 1.09)	0.664
<35	362	54.7	Ref	
Significant medical history[a]				
No	1472	51.9	Ref	
Yes	64	54.7	1.05 (0.82, 1.26)	0.664
Significant concomitant medication[b]				
No	1329	50.5	Ref	
Yes	207	61.8	1.16 (1.06, 1.26)	0.003
Vitamin D supplements (IU daily)				
<400	622	62.5	1.39 (1.28, 1.50)	<0.001
≥400	914	44.9	Ref	
General health				
Excellent/very good	709	44.3	Ref	
Good	582	56.7	1.21 (1.12, 1.29)	<0.001
Fair/poor	239	63.6	1.25 (1.16, 1.32)	<0.001
Activity limitation				
Limited a lot	294	60.9	1.22 (1.09, 1.34)	0.001
Limited a little/not at all	1236	50.0	Ref	
Climbing stairs				
Limited a lot	345	58.3	1.26 (1.12, 1.40)	<0.001
Limited a little	504	56.0	1.17 (1.07, 1.26)	0.001
Not limited at all	679	46.1	Ref	
Exercise/week				
Never	319	64.6	1.56 (1.37, 1.74)	<0.001
1–5 times	957	50.8	1.19 (1.05, 1.31)	0.007
6 times or more	252	41.3	Ref	
Discussed vitamin D with doctor				
No	423	58.6	1.24 (1.12, 1.36)	<0.001
Yes	821	47.3	Ref	
Osteoporosis diagnosed by doctor				
No	257	52.9	1.03 (0.90, 1.16)	0.617
Yes	1211	51.2	Ref	
Education				
Less than 12 grades	197	61.9	1.38 (1.19, 1.56)	<0.001
No college degree	974	52.5	1.14 (1.03, 1.25)	0.014
College degree or above	361	44.9	Ref	
Annual income				
Up to $24,999	472	58.7	1.65 (1.37, 1.91)	<0.001
$25,000–$74,999	566	51.8	1.31 (1.13, 1.46)	0.001
$75,000 or more	135	35.6	Ref	
Fracture since age 45				
No	875	51.5	Ref	
Yes	654	52.9	1.03 (0.93, 1.12)	0.598
Fracture of hip, spine, wrist since age 45				
No	1196	51.3	Ref	
Yes	333	55.3	1.07 (0.96, 1.18)	0.196
Nontraumatic fracture since age 45				
No	1246	52.6	Ref	
Yes	283	50.2	0.95 (0.83, 1.08)	0.467
Fall in past 5 yr				
No	641	52.4	Ref	
Yes	860	51.6	0.98 (0.89, 1.08)	0.762
Fall causing injury				
No	1014	50.6	Ref	
Yes	480	55.0	1.08 (0.98, 1.17)	0.111
Fall causing broken bone				
No	1210	51.0	Ref	
Yes	278	56.1	1.09 (0.98, 1.20)	0.124

TABLE 3. *Continued*

Risk factor	n	Prevalence (%)	RR (95% CI)	*P* value
Servings of vitamin D containing food/week				
≤14	701	54.9	1.11 (1.01, 1.21)	0.037
>14	835	49.6	Ref	
Sun exposure (h/wk)				
0	430	54.0	1.10 (0.97, 1.24)	0.144
>0 to 0.8	439	50.8	1.04 (0.91, 1.17)	0.582
>0.8	415	48.9	Ref	
Skin tone				
Light	707	52.5	Ref	
Medium	792	50.9	0.97 (0.87, 1.07)	0.538
Dark	35	65.7	1.17 (0.94, 1.33)	0.130
Month when blood sample drawn				
Nov/Dec	205	48.8	Ref	
Jan	616	50.0	1.02 (0.87, 1.18)	0.762
Feb/March	715	54.7	1.11 (0.97, 1.24)	0.136

Ref, Serves as reference value.
[a] Significant preexisting medical history in the past 5 yr with the potential to alter vitamin D absorption or metabolism.
[b] Significant concomitant medications in the past 4 wk with the potential to alter vitamin D absorption or metabolism.

similar findings have been reported in previous studies, our study was the first to evaluate a population that consisted solely of women currently on pharmacological therapy to treat or prevent osteoporosis. In addition, the majority of women in the study were healthy, ambulatory, community-dwelling, Caucasian women who were well educated. Despite these apparent advantages, more than half of these women (52%) had suboptimal (<30 ng/ml) 25(OH)D concentrations, and the prevalence of vitamin D inadequacy was even higher in those who reported supplemental vitamin D use of less than 400 IU/d (63%). A high prevalence of vitamin D inadequacy was seen across all age groups and latitudes studied.

Among the potential risk factors identified, only high BMI, lack of exercise, absence of a previous discussion with a physician regarding the importance of vitamin D to bone health, vitamin D supplementation less than 400 IU daily, and education level below grade 12 were consistently found to be associated with vitamin D inadequacy (when defined as either <30 or <20 ng/ml) in both the univariate and multivariate analyses. The association of vitamin D inadequacy with obesity is well documented (19). Although obesity and lack of exercise are linked to additional health risks, modifications to diet and activity level are difficult to achieve (20, 21). However, two measures that could be readily implemented in clinical practice to reduce the prevalence of vitamin D inadequacy are physician-initiated discussions regarding the importance of vitamin D to bone health and use of vitamin D supplementation. This study suggests that the prevalence of vitamin D inadequacy could be reduced if subjects took at least 400 IU of vitamin D supplementation per day. This is not an excessive recommendation as the results of this study suggest that some women need more vitamin D to obtain optimal serum concentrations. Moreover, the current recommended daily allowance of 400 IU/d is based on the dose of vitamin D necessary to prevent osteomalacia.

Previous epidemiological studies have shown a wide variation in the prevalence of low 25(OH)D concentrations, but comparisons between studies are difficult because of differences in 25(OH)D cutoffs and 25(OH)D assays used. Thomas *et al.* (22) reported that 57% of hospitalized patients on a general medical service had 25(OH)D less than 15 ng/ml. Even higher rates of vitamin D deficiency have been reported for institutionalized and inactive elderly individuals (23, 24). Low 25(OH)D concentrations are not invariably associated with the sick or elderly because suboptimal 25(OH)D concentrations (≤20 ng/ml) have been reported in healthy adolescents (25). The prevalence in our study was consistent with the results of a previously reported multinational osteoporosis study (13). Indeed, vitamin D inadequacy is being recognized as an increasingly significant public health problem.

The optimal serum concentration of 25(OH)D remains the subject of much debate. Heaney *et al.* (14) concluded that serum 25(OH)D concentrations of 32–36 ng/ml (80 to 90 nmol/liter) may be required to attain maximum intestinal calcium absorption, whereas Malabanan *et al.* (5) concluded that a serum 25(OH)D concentration of 20 ng/ml (50 nmol/liter) was the minimum required to optimize serum PTH levels and prevent secondary hyperparathyroidism. We included a cut point of 30 ng/ml based on the findings of Chapuy *et al.* (15), who showed that mean serum PTH concentrations began to rise when serum 25(OH)D concentrations fell below 31 ng/ml. Our findings are consistent with Chapuy *et al.* demonstrating an increase in serum PTH at 25(OH)D concentrations less than 29.8 ng/ml. Recognition of functional hypoparathyroidism in up to one third of patients with hypovitaminosis D suggests that biochemical evidence of secondary hyperparathyroidism may not be the most sensitive indicator of vitamin D inadequacy in an individual patient (26). A recent cross-sectional study of 4100 ambulatory elderly (>60 yr) suggested that serum 25(OH)D concentrations greater than 40 ng/ml were the minimum needed for optimal musculoskeletal function of the lower extremities (27). Given the above, it is clear that the lower limit of normal for current laboratory 25(OH)D ranges is set too low, yet the optimum serum concentration of 25(OH)D remains uncertain.

Vitamin D deficiency has long been recognized to lead to rickets in children and osteomalacia in adults. There is growing evidence that less severe degrees of vitamin D insuffi-

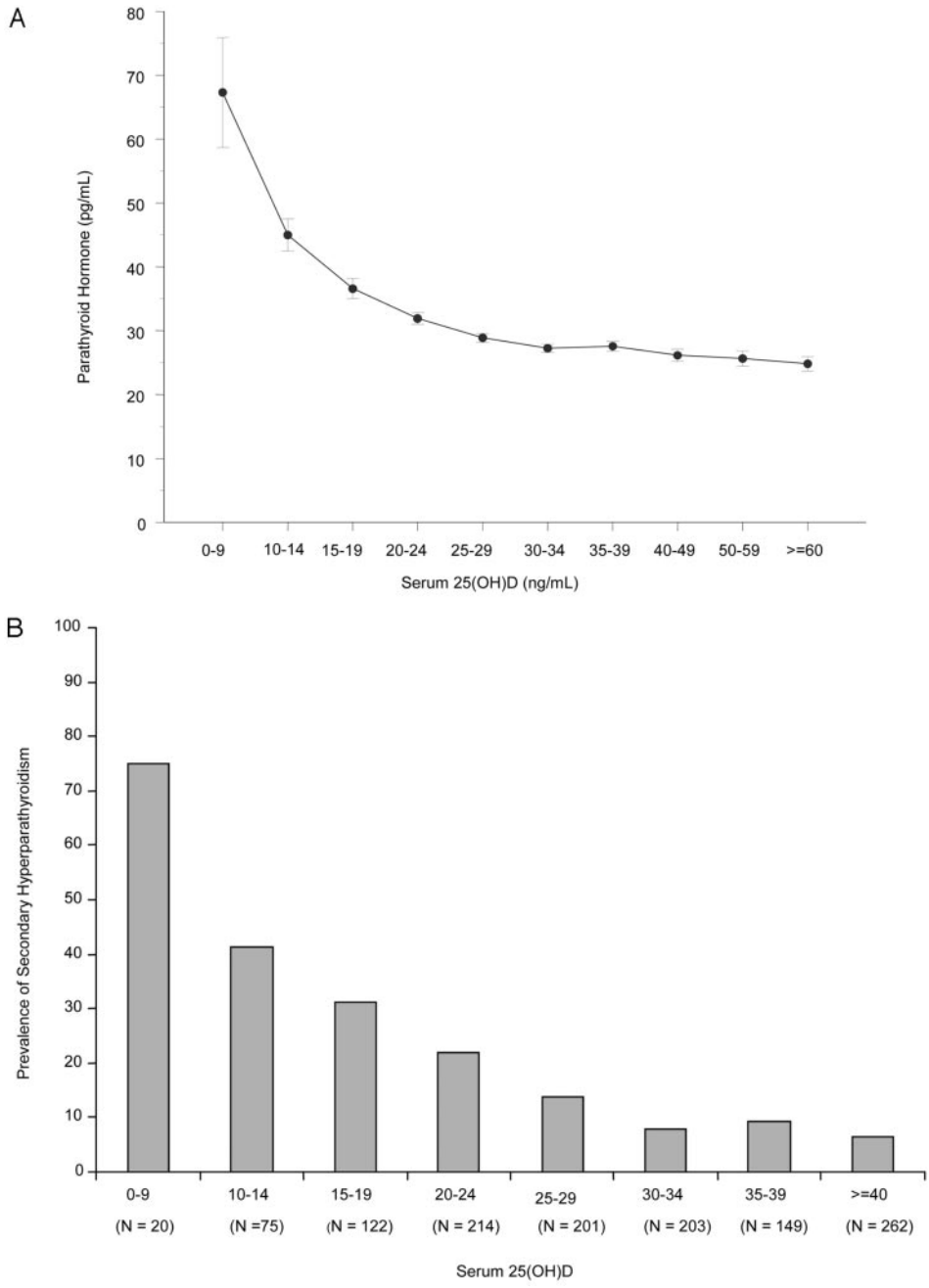

FIG. 4. A, Mean (±SE) serum PTH (picograms per milliliter) by serum 25(OH)D subgroups. Subject PTH concentrations (picograms per milliliter) relative to serum 25(OH)D concentrations sorted by subgroups delineated by predefined cutoffs for analyses of 25(OH)D inadequacy. Serum PTH values began to increase with 25(OH)D concentrations less than 29.8 ng/ml. B, Percent of subjects with secondary hyperparathyroidism by 25(OH)D level. The percent of subjects with secondary hyperparathyroidism (PTH > 40 pg/ml) sorted by subgroups with serum 25(OH)D concentrations delineated by predefined cutoffs for analyses of 25(OH)D inadequacy.

ciency also lead to skeletal disease. There is a positive association between serum 25(OH)D concentrations and total hip bone mineral density (28), and lower 25(OH)D concentrations have been reported in hip fracture patients, compared with controls (29). Moreover, a placebo-controlled study by Chapuy *et al.* (30, 31) demonstrated a significant reduction in hip and nonvertebral fractures in elderly women living in retirement homes after daily supplementation with vitamin D_3 and elemental calcium. A reduction in nonvertebral fracture incidence was also observed in a study of elderly community-dwelling men and women by Dawson-Hughes *et al.* (32) with daily use of vitamin D_3 and calcium. Intermittent administration of vitamin D has been shown to be as effective as daily use, in that 100,000 IU of oral vitamin D_3 given every 4 months for 5 yr in an elderly British population significantly reduced the incidence of osteoporotic fractures (33).

Vitamin D also plays an important role in the maintenance of proximal muscle strength, and reduction of falls is a potential benefit of vitamin D supplementation (34–37). A 3-month clinical trial of 122 patients in long-term care facilities demonstrated that daily vitamin D_3 and calcium provided a 49% reduction in fall rates, compared with calcium alone (38). A recently published metaanalysis demonstrated that vitamin D supplementation reduced the likelihood of falling by more than 20%, compared with calcium or placebo (39). Thus, the above-noted reduction in fracture risk with vitamin D supplementation may reflect skeletal and extraskeletal effects.

Serum 25(OH)D assays are widely available from commercial laboratories throughout the world. The wide interlaboratory variation as well as the even greater intermethod variation in serum 25(OH)D measurement has been the subject of several reports (40, 41). It has recently been reported that most of the commercial assays tested substantially overestimated serum 25(OH)D when compared with a gold standard HPLC method (42, 43). As such, the assay used in the present study (the Nichols Advantage system) may be reporting a higher 25(OH)D concentration than is actually present. Thus, the prevalence of vitamin D inadequacy might be even higher than reported here. This assay was chosen because of its proven precision, ease of use, and widespread availability. Conversely, this specific assay has been reported to underestimate circulating $25(OH)D_2$ (43, 44). However, because milk is routinely supplemented with vitamin D_3, and less than half of the available multivitamin preparations contain vitamin D_2, it is unlikely that assay-dependent underestimation of vitamin D_2 contributed in a major way to the results of this study. In addition, in this study few subjects reported taking large doses of vitamin D_2. Furthermore, LC-MS/MS on a subset of subjects confirmed that very few had $25(OH)D_2$ as the major component of their serum 25(OH)D concentrations. LC-MS/MS is currently among the most accurate measures of the separate contributions of both $25(OH)D_2$ and $25(OH)D_3$ to total 25(OH)D concentrations. Results using LC-MS/MS and the Nichols Advantage assay were almost identical for the prevalence of vitamin D inadequacy in this subgroup of patients.

The major strength of our study is that it provides estimates of the prevalence of vitamin D inadequacy among women receiving therapy to treat or prevent osteoporosis. These women were expected to be more aware of the importance of vitamin D to bone health than the general population. There are several limitations of our study. First, our study was performed during the winter months only, when serum 25(OH)D concentrations are typically near the lowest levels of the year. Very little vitamin D is made in the skin after November at latitudes above 35° north (45). For this reason, we may not have seen a significant variation across latitudes, although an inverse trend was observed as reported by Lips *et al.* (13). Even with adequate sun exposure during the summer and fall, subjects are at risk of low serum 25(OH)D concentrations because of the relatively short half-life of 25(OH)D (~2 wk). Second, our study population was confined to North American women only. The great majority of subjects in our study were Caucasian; thus, we could not fully assess the prevalence of vitamin D inadequacy in non-Caucasians, although, as reported by others, non-Caucasians are likely to have an even higher prevalence of vitamin D inadequacy (46, 47).

Conclusions

There is an unacceptably high level of vitamin D inadequacy among postmenopausal North American women receiving therapy to treat or prevent osteoporosis. Even in the absence of traditional risk factors for vitamin D deficiency, vitamin D inadequacy was highly prevalent. In our study, supplemental use of vitamin D less than 400 IU/d and lack of physician counseling regarding the importance of vitamin D were two risk factors independently and significantly associated with vitamin D inadequacy that could easily be addressed through physician and patient education. These results underscore the need for better education of the public and physicians regarding the optimization of vitamin D status in the care of postmenopausal women with osteoporosis.

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References

1. **Holick MF** 1999 Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. Primer on the metabolic bone diseases and disorders of mineral metabolism. 5th ed. Philadelphia: Lippincott; 92–98
2. **Kinyamu HK, Gallagher JC, Balhorn KE, Petranick KM, Rafferty KA** 1997 Serum vitamin D metabolites and calcium absorption in normal young and elderly free-living women and in women living in nursing homes. Am J Clin Nutr 65:790–797
3. **Aaron JE, Gallagher JC, Anderson J, Stasiak L, Longton EB, Nordin BE, Nicholson M** 1974 Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. Lancet 1:229–233
4. **Holick MF** 2004 Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 79:362–371
5. **Malabanan A, Veronikis IE, Holick MF** 1998 Redefining vitamin D insufficiency. Lancet 351:805–806
6. **Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF** 1997 Calcium absorptive effects of vitamin D and its major metabolites. J Clin Endocrinol Metab 82:4111–4116
7. **Holick MF** 1998 Vitamin D requirements for humans of all ages: new increased requirements for women and men 50 years and older. Osteoporos Int 8:S24–S29
8. **MacLaughlin J, Holick MF** 1985 Aging decreases the capacity of human skin to produce vitamin D_3. J Clin Invest 76:1536–1538
9. **Holick MF, Matsuoka LY, Wortsman J** 1989 Age, vitamin D, and solar ultraviolet. Lancet 2:1104–1105
10. **Bettica P, Bevilacqua M, Vago T, Norbiato G** 1999 High prevalence of hypovitaminosis D among free-living postmenopausal women referred to an osteoporosis outpatient clinic in northern Italy for initial screening. Osteoporos Int 9:226–229
11. **Aguado P, del Campo MT, Garces MV, Gonzalez-Casaus ML, Bernad M, Gijon-Banos J, Martin Mola E, Torrijos A, Martinez ME** 2000 Low vitamin D levels in outpatient postmenopausal women from a rheumatology clinic in Madrid, Spain: their relationship with bone mineral density. Osteoporos Int 11:739–744
12. **Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ** 1996 Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. J Clin Endocrinol Metab 81:1129–1133
13. **Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, Nickelsen T** 2001 A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab 86:1212–1221
14. **Heaney RP, Dowell MS, Hale CA, Bendich A** 2003 Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 22:142–146
15. **Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, Meunier PJ** 1997 Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 7:439–443
16. **Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH, Rush D** 1997 Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr 66:929–936
17. **Zhang J, Yu KF** 1998 What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 280:1690–1691

18. **SAS Institute** 1994 The NLIN procedure: SAS/STAT user's guide. Vol 2, version 6, ed 4:1135–1193
19. **Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF** 2000 Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 72:690–693
20. **Berke EM, Morden NE** 2000 Medical management of obesity. Am Fam Physician 62:419–426
21. **Council on Scientific Affairs** 1988 Treatment of obesity in adults. JAMA 260:2547–2551
22. **Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS** 1998 Hypovitaminosis D in medical inpatients. N Engl J Med 338:777–783
23. **Bischoff H, Stahelin HB, Vogt P, Friderich P, Vonthein R, Tyndall A, Theiler R** 1999 Immobility as a major cause of bone remodeling in residents of a long-stay geriatric ward. Calcif Tissue Int 64:485–489
24. **Theiler R, Stahelin HB, Kranzlin M, Tyndall A, Bischoff HA** 1999 High bone turnover in the elderly. Arch Phys Med Rehabil 80:485–489
25. **Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ** 2004 Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 158:531–537
26. **Sahota O, Mundey MK, San P, Godber IM, Lawson N, Hosking DJ** 2004 The relationship between vitamin D and parathyroid hormone: calcium homeostasis, bone turnover, and bone mineral density in postmenopausal women with established osteoporosis. Bone 35:312–319
27. **Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, Dawson-Hughes B** 2004 Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged ≥60 y. Am J Clin Nutr 80:752–758
28. **Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B** 2004 Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116:634–639
29. **Weatherall M** 2000 A meta-analysis of 25 hydroxyvitamin D in older people with fracture of the proximal femur. N Z Med J 113:137–140
30. **Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ** 1992 Vitamin D_3 and calcium to prevent hip fractures in the elderly women. N Engl J Med 327:1637–1642
31. **Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, Garnero P, Meunier PJ** 2002 Combined calcium and vitamin D_3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. Osteoporos Int 13: 257–264
32. **Dawson-Hughes B, Harris SS, Krall EA, Dallal GE** 1997 Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 337:670–676
33. **Trivedi DP, Doll R, Khaw KT** 2003 Effect of four monthly oral vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 326:469–474
34. **Janssen HC, Samson MM, Verhaar HJ** 2002 Vitamin D deficiency, muscle function, and falls in elderly people. Am J Clin Nutr 75:611–615
35. **Pfeifer M, Begerow B, Minne HW, Schlotthauer T, Pospeschill M, Scholz M, Lazarescu AD, Pollahne W** 2001 Vitamin D status, trunk muscle strength, body sway, falls, and fractures among 237 postmenopausal women with osteoporosis. Exp Clin Endocrinol Diabetes 109:87–92
36. **Sato Y, Inose M, Higuchi I, Higuchi F, Kondo I** 2002 Changes in the supporting muscles of the fractured hip in elderly women. Bone 30:325–330
37. **Dhesi JK, Bearne LM, Moniz C, Hurley MV, Jackson SH, Swift CG, Allain TJ** 2002 Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status. J Bone Miner Res 17:891–897
38. **Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA, Conzelmann M** 2003 Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. J Bone Miner Res 18:343–351
39. **Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB** 2004 Effect of vitamin D on falls: a meta-analysis. JAMA 291:1999–2006
40. **Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF** 1999 An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporos Int 9:394–397
41. **Hollis BW** 2000 Comparison of commercially available ^{125}I-based RIA methods for the determination of circulating 25-hydroxyvitamin D. Clin Chem 46:1657–1661
42. **Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, DeLuca HF, Drezner MK** 2004 Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. J Clin Endocrinol Metab 89:3152–3157
43. **Gemar DM, Binkley N, Krueger D, Engelke J, Drezner MK** 2004 Clinical assessment of vitamin D status: an imposing dilemma. J Bone Miner Res 19(Suppl 1):S343
44. **Holick MF, Chen TC, Jamieson D, Lu Z, Mathieu J** 2004 Evaluation of precision and accuracy of Nichols Advantage 25-hydroxyvitamin D assay for 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3: comparison to four other assay methods including liquid chromatography-mass spectrometry. J Bone Miner Res 19(Suppl 1):S343
45. **Webb AR, Kline L, Holick MF** 1988 Influence of season and latitude on the cutaneous synthesis of vitamin D_3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D_3 synthesis in human skin. J Clin Endocrinol Metab 67:373–378
46. **Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Doughertly C, Gunter EW, Bowman BA** 2002 Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 76:187–192
47. **Plotnikoff GA, Quigley JM** 2003 Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clin Proc 78:1463–1470

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