High Prevalence of Hypovitaminosis D in Morocco: Relationship to Lifestyle, Physical Performance, Bone Markers, and Bone Mineral Density

Fadoua Allali, MD,* Sihame El Aichaoui, MD,[†] Hamza Khazani, MD,[†] Boubker Benyahia, MD,[†] Bouchra Saoud, MD,[†] Saâd El Kabbaj, MD,[‡] Rachid Bahiri, MD,[§] Redouane Abouqal, MD,[¶] and Najia Hajjaj-Hassouni, MD[∥]

Objectives: We undertook this study to determine the prevalence of hypovitaminosis D, its determinants, and its relationships to physical performance, serum parathyroid hormone (PTH) concentration, bone mineral density, and biochemical markers of bone turnover in healthy, ambulatory, pre- and postmenopausal women.

Methods: The group studied included 415 women aged 24 to 77 years. Between July and September, we assessed calcium intake and measured serum calcium, phosphorus, albumin, alkaline phosphate, 25-hydroxyvitamin D (25(OH)D), PTH, osteocalcin, and C-terminal cross-linking telopeptide of Type I collagen. We also measured bone mineral density (BMD) by dual-energy radiograph absorptiometry in the spine and total femur. Three tests were used to assess physical performance: timed get-up-and-go test, 5-times-sit-to-stand test, and 2.4 m speed walk.

Results: The prevalence of vitamin D insufficiency (<30 ng/mL) was 91%. In multiple logistic regression, the main determinants of hypovitaminosis D were age >55 years (OR 2.14 [95% IC, 1.1-4.1; P = 0.026)], wearing a veil [OR 2 (95% IC, 1.1-4; P = 0.04)], time spent outdoors less than 30 min/d [OR 2.8 (95% IC: 1.4-5.7; P = 0.003)], and daily calcium intake less than 700 mg [OR 2.39 (95% IC, 1.2-4.7; P < 0.01)]. A significant inverse correlation between 25 OH and osteocalcin (r = -0.18, P < 0001), 25 OH, and Type I collagen (r = -0.15, P = 0.003) were observed. By Locally Weighted Regression and Scatterplot Smoothing technique, there was an increase in PTH level when S-25(OH)D was below 30 ng/mL. After adjustment for age, both spine BMD and total femoral BMD failed to show any significant correlation with serum 25(OH)D and PTH. There was no correlation between any physical performance tests and 25(OH)D levels.

Conclusions: Our study showed that during the summer season, vitamin D insufficiency is very common in healthy adult Moroccan women. Lack of sun exposure and veiled clothing style were the most important factors that influenced hypovitaminosis D. Patients with hypovitaminosis D had a high bone turnover, whereas there was no effect on BMD and physical performance. Further research is needed to evaluate the clinical impact of the above findings.

© 2008 Elsevier Inc. All rights reserved. Semin Arthritis Rheum xx:xxx

Keywords: hypovitaminosis D, veil wearing, physical performance, bone markers, bone mineral density

†Residents of Rheumatology, University Hospital, Rabat-Salé, Morocco.

‡Head of Laboratory of Royal Military Force (Gendarmerie), Rabat, Morocco.

^{*}Professor of Rheumatology, Department of Rheumatology, University Hospital, Rabat-Salé, Morocco. Member of the Laboratory of Biostatistical, Clinical and Epidemiological Research. Faculty of Medicine and Pharmacy, Rabat, Morocco.

[§]Professor of Rheumatology, Department of Rheumatology, University Hospital, Rabat-Salé, Morocco.

Professor of medical care. Head of the Laboratory of Biostatistical, Clinical and Epidemiological Research. Faculty of Medicine and Pharmacy, Rabat, Morocco.

^{||}Professor of Rheumatology, head of the Department of Rheumatology, University Hospital, Rabat-Salé, Morocco. Dean of the faculty of Medicine and Pharmacy, Rabat, Morocco.

Address reprint requests to: Fadoua Allali, Department of Rheumatology (Pr N.Hajjaj-Hassouni), El Ayachi University Hospital, Salé, Morocco. E-mail: fadouaallali@yahoo.fr

ARTICLE IN PRESS

Vitamin D deficiency causes rickets in children and myopathy, osteopenia, secondary hyperparathyroidism, and osteomalacia in adults. Vitamin D insufficiency, which corresponds to a smaller decrease of 25hydroxyvitamin D (25(OH)D) than vitamin D deficiency, may lead to secondary hyperparathyroidism. Moreover, vitamin D insufficiency may increase postural instability through increased body sway and muscle weakness and reduced ability to counteract falls, further increasing the risk of fracture (1-3). The repletion of vitamin D stores by dietary supplementation has been shown to decrease levels of parathyroid hormone (PTH) and increase bone mineral density (BMD) of the lumbar spine and hip (4,5).

Sunlight exposure is by far the most important source of vitamin D (6,7). Hence, limited skin exposure to sunlight is presumed to be the cause of vitamin D deficiency (8-10). The reports of a greater prevalence of the disease in the northern regions of China where the intensity of summer ultraviolet (UV) light is less than in the south further suggest that vitamin D deficiency is simply the consequence of inadequate exposure of the skin to the sun. Regarding this significant role of sunlight in vitamin D synthesis, it is quite logical to suggest low prevalence of vitamin D deficiency in tropical countries. However, studies performed in the preceding 2 decades have shown a high prevalence of vitamin D deficiency in tropical countries such as Turkey, India, Iran, and Saudi Arabia (9,11-13). UV-B radiation depends on many factors, for instance latitude, season and time of day sunlight exposure (8-16).

Sunlight is abundant in Morocco throughout the year. However the extent of solar-UV exposure is determined primarily by lifestyle rather than outdoor UV irradiance. We have already shown that in Moroccan postmenopausal women, wearing an Islamic veil or traditional clothing style covering arms, legs, and head, was an independent factor for increasing osteoporosis risk (17). The influence of clothing style on vitamin D status has been the subject of a few studies (18-21). In a study on multinational subjects in the United Arab Emirates (22), mean serum 25(OH)D was 21.5 nmol/l in United Arab Emirates, 31.5 nmol/l in non-gulf Arabs, and 160.5 nmol/l in Europeans. In this study, wearing a veil was an independent predictor of hypovitaminosis D.

Because of the absence of any data on the vitamin D status in the general adult Moroccan population, we undertook this study to determine the prevalence of hypovitaminosis D, its determinants, and its relationships to physical performance. PTH concentration, BMD, and biochemical markers of bone turnover in healthy, ambulatory pre- and postmenopausal women were measured.

METHODS

Subjects

Between June and August, 415 healthy Moroccan volunteer women were recruited from the city of Rabat through advertisements in local hospitals. Informed consent was obtained from all subjects and the study was approved by the Ethics Committee of our university hospital. We excluded from the study all patients with a history of the following: (1) taking drugs known to influence bone metabolism in the past 2 years, such as vitamin D, calcium, corticosteroids, bisphosphonates, and hormone replacement therapy; (2) musculoskeletal, thyroid, parathyroid, adrenal, hepatic, or renal disease; (3) malignancy; and (4) hysterectomy.

Data Collection and Measurements

Each patient completed a questionnaire on sociodemographic parameters and osteoporosis risk factors. Information regarding veil use was recorded. The definition of veil was that of a concealing clothing, ie, a clothing style not only limited to the Islamic dressing habits but also including the Moroccan traditional clothing, which covers most of the body including the arms, legs, and head. We also collected data related to age at menopause, the time since menopause, the number of pregnancies, and the personal history of peripheral osteoporosis fractures (including proximal femoral fractures).

Anthropometric Data

Weight and height were measured without clothes or shoes at the time of bone densitometry measurements. The body mass index (BMI) was calculated as body weight (kg)/height (m²).

Physical Performance Measures

Three measures to assess physical performance were used: timed get-up-and-go test (TGUG), 5-times-sit-to-stand test (TSTS), and 2.4 m speed walk. Time was measured in seconds with a stopwatch and rounded to the nearest hundredth of a second.

TGUG—Time Taken to Complete the Test "Get-Up-and-Go"

The subject rises from a chair, walks 3 m, turns around, returns to the chair, and sits down. The subject was instructed to "Sit with your back against the chair and your arms on the arm rests. On the word 'go', stand upright, then walk at your normal pace to the line on the floor, turn around, return to the chair, and sit down." The stopwatch was started on the word "go" and stopped when the subject returned to the starting position.

5 TSTS

Participants were asked to stand up and sit down 5 times as quickly as possible without the use of hands and were timed from their initial sitting position to the final standing position at the end of the fifth stand.

8-feet (2.4 m) Speed Walk (8 FSW)

The women were instructed to walk as fast as they could in their ordinary shoes for 2.4 m. Participants used the footwear they normally used. The distance was marked on the floor with red tape and the participant stood just behind the starting line before the test. A digital stopwatch was started when the participant started to walk and stopped when the first foot crossed the finishing line.

Dietary Calcium Questionnaire

The self-questionnaire of Fardellone and coworkers (23) has been modified, simplified, and adjusted to the Moroccan food habits. After translation and back translation, it was administered to 62 volunteers women, aged between 30 and 60 years. To test its validity, the questionnaire was compared with the weekly docket system, chosen as a reference method. To test its reproducibility, the questionnaire was administered again after a 1-week interval. The coefficient of correlation was 0.91. The questionnaire correctly classified women with daily calcium intake less than 800 mg with 77% specificity, while its sensitivity was 87%.

Bone Mineral Density Measurements

Lumbar spine, trochanter, femoral neck, and total hip BMD were measured by dual-energy radiograph absorptiometry with a Lunar prodigy densitometer. Daily quality control was performed by measurement of a Lunar phantom. At the time of the study, phantom measurements showed stable results. The phantom precision expressed as the coefficient of variation (%) was 0.08. Both *T*- and *Z*-scores were obtained. In the *T*-score calculations, the manufacturer's ranges for European reference population were used because of the absence of a Moroccan database.

Biochemical Measurements

Biological samples were collected between June and August. Morning fasting blood and random urine samples were collected from every subject for the measurement of the following parameters: serum calcium, phosphorus, albumin, creatinine, 25(OH)D, PTH, osteocalcin, and Cterminal cross-linking telopeptide of type I collagen (CTX). Serum calcium, phosphorus, creatinine, and albumin were measured by automated standard laboratory methods. Serum 25(OH)D was measured by chemiluminescence (Liaison, Diasorin). The intra- and interassay coefficient of variation were 5 and 11%, respectively, and the normal range was 20 to 60 ng/mL. Intact PTH was measured by immunochemoluminometric assay (Elecsys; Roche Diagnostics, Mannheim, Germany). Intra- and interassay variances were 5 and 7% and the normal range was 15 to 65 pg/mL. Osteocalcin and CTX were both measured by immunochemoluminometric assay (Elecsys; Roche Diagnostics). Intra- and interassay variances were 5

and 7% and the normal ranges were 15 to 46 ng/mL for osteocalcin, and 0.3 to 0.6 ng/mL for CTX.

Statistical Analysis

Hypovitaminosis D or vitamin D insufficiency was defined as a circulating level of 25(OH)D below 30 ng/mL, based on published data showing that serum immunoreactive parathyroid hormone (iPTH) is increased in patients who have 25(OH)D concentrations equal to or below 30 ng/mL (23-25). Results for continuous variables are expressed as mean \pm SD; comparison was made by the *t*-test. Categorical variables were compared by using the χ^2 .

Logistic regression was used to estimate crude and adjusted odds ratio (OR) for the dichotomous outcomes of hypovitaminosis D. Analyses were performed with adjustment for age, BMI, parity, the wearing of a veil, sun exposure less than 30 min/d, and dietary calcium intake. The age was entered in the model in the following categories: <40 years, between 40 and 50 years, between 50 and 55 years, and >55 years. The BMI variable was entered in the model in 2 categories, with (BMI > 30) or without (BMI \leq 30) obesity, with the BMI \leq 30 group as the reference category. The dietary calcium intake variable was entered in the model in 4 categories: below 500 mg/d, between 500 and 700 mg/d, between 700 and 800 mg/d, and over 800 mg/d, with the higher category as the reference. Wearing the veil and sun exposure less than 30 minutes were entered in the model in dichotomous variable (yes/no). The number of parity in was measured in 4 categories: nuliparous, 1 to 2, 3 to 5, and 6 and more children, the nuliparous group being the reference group. Model calibration was assessed by using the Hosmer and coworkers goodness-of-fit test (26).

Correlations between variables were estimated by using Pearson's coefficient of correlation. The Locally Weighted regression and Scatterplot Smoothing (LOWESS regression plot) was conducted to assess the association between 25(OH)D and PTH. The LOWESS method is a technique for determining the shape of the function that best summarizes the scatter plot between 2 continuous variables. The method does require the input of a "smoothing parameter," which is the fraction of the data that is used around each point.

Statistical significance was defined as a value of P < 0.05. All analyses were performed in SPSS, version 13 (SPSS Inc., Chicago, IL).

RESULTS

Clinical Characteristics

The characteristics of the patients are shown in Table 1. The mean age of the patients in the entire population was 50 ± 9.3 years. All patients were residing in urban areas. Seventy-four percent were menopausal with a mean age of 56 ± 9.6 years. Fifty-six percent of patients had a paid

Table 1 Clinical, Laboratory, and Osteodensitometric Characteristics for all the Sample, Pre- and Postmenopausal Women						
	Whole Population $(n - 415)$	Premenopausal Women $(n - 108)$	Post menopausal Wom $(n - 307)$	en P		
	(11 = 413)	(n = 108)	(n - 307)	F		
Age (y)	50 ± 9	42.8 ± 6.2	55.9 ± 6.8	< 0.001		
BMI (kg/m²)	$\textbf{27.8} \pm \textbf{4.3}$	27.2 ± 4.4	$\textbf{28.4} \pm \textbf{4.2}$	0.23		
Number of children	3.3 ± 2.4	2.4 ± 1.8	4.1 ± 2.6	< 0.001		
Calcium intake (mg/d)	719 ± 221	737 ± 232	704 ± 211	0.06		
Veil wearing (%)	56	54	55	0.64		
Veil wearing duration (y)	10 ± 11	7.7 ± 7.1	13.4 ± 13	< 0.001		
Sunlight exposure less than 30 min/d (%)	53	40	60	0.53		
Spine BMD (g/cm ²)	1.02 ± 0.17	1.1 ± 0.13	0.97 ± 0.14	< 0.001		
Femoral neck BMD (g/cm ²)	0.89 ± 0.14	0.97 ± 0.6	0.85 ± 0.12	< 0.001		
Trochanter BMD (g/cm ²)	0.73 ± 0.12	0.79 ± 0.11	0.70 ± 0.11	< 0.001		
Femoral total BMD (g/cm ²)	0.94 ± 0.14	1.02 ± 0.16	0.90 ± 0.12	< 0.001		
Total calcium (mg/l)	96.6 ± 4.8	95.5 ± 4.8	97.6 ± 4.6	0.006		
Phosphorus (mg/l)	35.4 ± 5.3	34.2 ± 4.6	36.4 ± 5.7	< 0.001		
25 OH vitamin D (ng/mL)	18.1 ± 7.9	18.6 ± 7.7	17.7 ± 8.1	0.3		
PTH (pg/mL)	60.5 ± 4.1	56.2 ± 19.9	63.9 ± 26.6	0.002		
Osteocalcin (ng/mL)	21.7 ± 10.8	18.2 ± 8.8	24.8 ± 11.4	< 0.001		
CTX (ng/mL)	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	<0.001		
<i>P</i> < 0.05; significant difference between pre- and postmenopausal women.						

job. Eighty-five percent had never practiced any sporting activity, but 90% walked over 30 minutes at least once a week.

The mean \pm SD serum 25(OH)D concentration for all 415 patients was 18.4 \pm 7.9 ng/mL. The prevalence of vitamin D insufficiency [circulating 25(OH)D equal or less than 30 ng/mL] was 91%. Values of 25(OH)D lower than 15 ng/mL were found in 43% of the women and levels lower than 5 ng/mL were found in 4%. Hypovitaminosis D was observed in 80% of premenopausal women versus 92% in menopausal women. The mean daily dietary calcium intake was 719 \pm 221 (range, 190 to 1800) mg. Sixteen percent of the subjects had a daily intake of calcium less than 500 mg.

Among menopausal women, 31% of patients were osteoporotic and 12% had peripheral fractures, all in the vitamin D insufficiency group. The mean \pm SD ageadjusted 25 OH values were lower in women who sustained a peripheral fracture (16.6 \pm 5.8 ng/mL) than others (18.2 \pm 8.2 ng/mL), but this difference was not statistically significant. However, patients with prevalent peripheral fractures had a significantly higher level of PTH than those without fractures (73.6 \pm 22 pg/mL versus 60.5 \pm 24 pg/mL, P = 0.04).

Risk Factors of Hypovitaminosis D

Risk factors of hypovitaminosis D are shown in Table 2. In univariate analysis, hypovitaminosis D was highly prevalent in women >55 years (P = 0.025) and in veiled women (P = 0.004). Hypovitaminosis D was also associated with the time spent outdoors less than 30 min/d (P =0.007). There was no association between hypovitaminosis D and dietary calcium intake nor with parity level and BMI. In multiple logistic regression, the main determinants of hypovitaminosis D were age >55 years [OR 2.14 (95% IC, 1.1-4.1; P = 0.026)], wearing a veil [OR 2 (95% IC, 1.1-4; P = 0.04)], time spent outdoors less than 30 min/d [OR 2.8 (95% IC: 1.4-5.7; P = 0.003)], and daily calcium intake less than 700 mg [OR 2.39 (95% IC. 1.2-4.7; P < 0.01)].

25(OH)D and Physical Performances

There was no correlation between any physical performance tests and 25(OH)D levels: serum 25(OH)D versus 8 FSW (r = -0.06, P = 0.8), serum 25(OH)D versus 5 TSTS (r = -0.08, P = 0.1), and serum 25(OH)D versus TGUG (r = -0.03, P = 0.6) (P, age-adjusted).

25(OH)D, iPTH, and BMD

A moderate significantly inverse correlation between PTH and 25(OH)D was observed (r = -0.13; P = 0.03). The LOWESS regression showed a "breaking point" around 30 ng/mL (Fig. 1).

25(OH)D was correlated with lumbosacral BMD (r = 0.13 P = 0.03) and trochanter (r = 0.14, P < 0.03) but not with femoral neck or total femoral BMD. After adjustment for age, serum 25(OH)D levels were not significantly associated with BMD in any measured site. As shown in Table 3, there was a significant negative linear correlation between iPTH and femoral total BMD but not with other sites.

Bone Turnover Markers

The mean values of biochemical markers of bone turnover were higher in the vitamin D insufficiency group (osteocalcin: 23.9 ± 12.3 ng/mL versus 20.2 ± 9.5 ng/mL; P < 0.001, and CTX: 0.46 ± 0.25 ng/ml

Variables	Hypovitaminosis D Prevalence (%)	Univariate			Multivariate		
		O.R	CI 95%	P value	OR	CI 95%	P value
Age (y)							
<40	30	1.00			1.00		
40 to 50	41	1.31	0.79 to 2.16	0.29	1.28	0.35 to 4.52	0.74
50 to 55	40	1.36	0.82 to 2.26	0.22	1.11	0.28 to 4.54	0.83
>55	52	2.36	1.35 to 4.12	0.002	2.14	1.15 to 4.15	0.026
BMI (kg/m ²)							
<30	45	1.00			1.00		
>30	37	0.72	0.46 to 1.12	0.14	1.05	0.49 to 2.23	0.91
Parity							
0	43	1.00			1.00		
1 to 2	30	0.56	0.29 to 1.05	0.07	1.1	0.26 to 4.54	0.81
3 to 5	43	1.03	0.55 to 1.8	0.99	1.34	0.36 to 4.92	0.63
>6	58	1.70	0.73 to 4.52	0.26	1.34	0.26 to 6.18	0.74
Calcium intake (mg/j)							
>800	33	1.00			1.00		
700 to 800	42	1.32	0.71 to 2.34	0.2	1.6	0.9 to 3.1	0.93
500 to 700	44	1.52	0.89 to 2.41	0.1	1.8	1.06 to 3.2	0.03
<500	46	1.61	0.81 to 3.34	0.1	2.39	1.25 to 4.61	0.01
Veil							
No	33	1.00			1.00		
Yes	47	1.81	1.24 to 2.71	0.004	2.01	1.15 to 4.02	0.04
Sunlight exposure							
<30 mn							
No	34	1.00			1.00		
Yes	47	1.74	1.15 to 2.61	< 0.001	2.8	1.43 to 5.71	0.003

versus 0.40 \pm 0.22 ng/ml; P < 0.01) and correlated with 25(OH)D (r = -0.18; P < 0.05 and r = -0.15; P < 0.05, respectively). However this correlation, even though significant, was moderate. Also, there were significant correlations between PTH and osteocalcin and between PTH and CTX (r = 0.19, P < 0.05 and r =



Figure 1 LOWESS plot of parathyroid hormone as a function of 25-hydroxyvitamin D with a break point between 25 and 30 ng/mL. (Color version of figure is available online.)

0.16, P < 0.04), respectively. Those correlations persisted after adjustment for age.

DISCUSSION

This study suggests that during the summer season, vitamin D insufficiency is very common in adult healthy Moroccan women. Age, the lack of sun exposure, veiled

Table 3 Linear Correlation Between PTH, 25 (OH) D, Bone Mineral Density, and Bone Turnover Markers						
	25 OH VIT D3	PTH	Osteocalcin	СТХ		
250H VIT D3		-0.13*				
PTH	-0.13*	—				
Osteocalcin	-0.18*	0.19†				
СТХ	-0.15*	0.16†	0.72†			
BMD spine	0.13*	-0.1	-0.24†	-0.26*		
BMD trochanter	0.14*	-0.08	-0.15^{\dagger}	-0.11		
BMD						
Femoral neck	0.1	-0.05	-0.15†	-0.07		
BMD femoral total	0.08	-0.17*	-0.16^{\dagger}	-0.08		
OR, odds ratio; CI 95%, 95% confidence interval. For multivariate analysis, Hosmer–Lemeshow's Goodness of Fit Tests: $P = 0.65$. * $P < 0.05$. † $P < 0.001$.						

clothing style, and a daily calcium intake less than 700 mg are the most important factors that influence hypovitaminosis D. We defined hypovitaminosis D as a circulating level of 25(OH)D below 30 ng/mL. The choice of 30 ng/mL as a cutoff value agrees with previous studies that demonstrated secondary hyperparathyroidism, increased bone turnover, and decreased bone density at the hip at 25(OH)D serum concentrations below this level (24-26).

In previous studies of elderly housebound people, medical inpatients, and nursing home residents, one-quarter to one-half had low vitamin D levels (27-30). However, in our study, with ambulatory subjects without secondary causes or medications that might affect bone density, the prevalence of vitamin D insufficiency was 91%. This prevalence might be higher if 25(OH)D assessment was made in the winter season. The high prevalence of severe hypovitaminosis D we observed in our young healthy population with a high potential for sun exposure is a matter of concern. It is related to cultural requirements that limit sun exposure in many women as well as to low vitamin D intake. In our country, food is not supplemented with vitamin D. Hence, cutaneous synthesis would be the major source of the vitamin in ambulatory women of this age group.

It has been estimated that a 10-minute exposure of head and arms (unprotected) 3 times per week would be sufficient to prevent vitamin D insufficiency. Many factors influence the intensity and duration of exposure to UV light, including geographic location, season, atmospheric conditions, and the daily length of time spent outdoors (31-35). To detect the influence of clothing style on 25(OH)D level, we performed this study in the summer season. After a multilinear regression analysis, wearing a veil was found to be an independent predictor of hypovitaminosis D.

The influence of clothing on vitamin D status has been the subject of a few studies. Three reports from Saudi Arabia (13,35) and Kuwait (18) revealed the presence of vitamin D insufficiency among people from the Arabian Gulf. This insufficiency was more common in veiled Kuwaiti women compared with nonveiled women. Severe hypovitaminosis D (25(OH)D \leq 5 ng/mL) affected 42% of Lebanon women particularly those who wore veils (62%) (7). In this study, a higher degree of parity was found to affect negatively vitamin D status. This was observed mainly in the veiled women, therefore, contributing to their vitamin D insufficiency. In our study, the parity was not associated to the risk of hypovitaminosis D.

More surprisingly, while 4% of our subjects had vitamin D deficiency (<5 ng/mL), none of them had symptoms suggesting hypovitaminosis D. Abnormal biochemical parameters such as hypocalcemia and hypophosphatemia also were rarely observed. It would be interesting to perform bone biopsies in subjects with very low 25(OH)D levels looking for histological osteomalacia.

Secondary hyperparathyroidism is a well-known consequence of vitamin D insufficiency (36-40). Our data show a significant, although moderate, negative age independent correlation between iPTH and 25(OH)D. This association was well described by the exponential curve with an inflection when serum 25(OH)D levels fell below 30 ng/mL. It should be mentioned that there is disagreement in the value of the inflection point between studies that reported a plateau effect, the level required for optimal PTH suppression varying from 30 to 99 nmol/l (37,40).

As reported in several previous studies (7,8,41), we found a significant, age-independent correlation between 25 OH and bone turnover markers and a significant correlation among iPTH and bone turnover markers. The biochemical picture suggests that increased concentrations of PTH might play an important role in the development of increased bone remodeling and bone loss in women with vitamin D insufficiency. In our study, correlation between osteocalcin and 25(OH)D was PTH independent, suggesting that hypovitaminosis D may contribute directly to increase bone formation. In untreated postmenopausal women, Lukert and coworkers (42) found that decreased serum 25(OH)D levels were associated with faster bone loss at the distal radius in small populations of healthy early or late postmenopausal women. However, other studies indicated that vitamin D is unlikely to play a major role in regulating systemic levels of bone turnover markers (5,43).

No significant age-independent association was detected between 25(OH)D levels and BMD at any measured site. This is in agreement with some, but not all, studies (12,39,44). In the Os des Femmes de Lyon (OFELY) study, Garnero and coworkers (43) showed that, after adjustment for age, there was no significant difference in incidence of fracture, BMD, radius BMD loss, or bone turnover markers, among women with 25(OH)D levels below or above 75, 50, or 30 nmol/l. Investigators in the Multiple Outcomes of Raloxifene Evaluation (MORE) study (39) showed that a 25(OH)D level in the lowest tertile (less than 25 nmol/l) was associated with a 4% lower trochanteric BMD, although there was no difference in lumbar spine or femoral neck BMD. Villareal and coworkers have reported low serum 25(OH)D levels in women referred for osteoporosis screening (44). These women had no symptoms of vitamin D deficiency except low BMD. In our opinion, these discordances in skeletal site BMD correlation with 25(OH)D are likely to be linked to differences in the sample size, sample selection criteria, country of origin, and perhaps dietary calcium and vitamin D intake among the different studied populations. Nevertheless, as reported by Bischoff-Ferrari and coworkers (25), altogether these data indicate that a relationship exists between these 2 parameters, thus suggesting an influence of 25(OH)D on BMD. The relatively low degree of this correlation and its variability among different skeletal sites found by various authors also support the concept that the action of vitamin D on BMD is a part of the combined action of multiple factors.

F. Allali et al.

Muscle weakness and increased risk of falling are associated with vitamin D deficiency mainly in elderly frail subjects (1), and vitamin D supplementation reduces the risk of falling in ambulatory older women over the age of 65 years (45). In our study, after adjusting for age, preand postmenopausal women with vitamin D insufficiency, physical performance was similar to women with normal vitamin D levels. This finding is in agreement with the OFELY study, which did not find a significant association between serum 25(OH)D levels and grip strength.

Limitations of our study include those associated with cross-sectional studies. All the subjects were volunteers; so our sample is not representative of the general population.

In conclusion, we found that, despite abundant sunshine, because of poor sunlight exposure and excessive outdoor clothing due to sociocultural and religious reasons, the prevalence of vitamin D insufficiency is very high in Morocco. Hypovitaminosis D was associated with an increase in PTH levels and in bone turnover markers. Although women with vitamin D insufficiency had a lower spine and trochanter BMD than the other individuals, this difference disappeared after adjustment for age. Further studies are needed to confirm our results. Encouraging a more active outdoor lifestyle within the framework of our religious and cultural commitments and vitamin D fortification of foods might be the optimal approach to manage this disorder especially in women whose clothing covers their body.

ACKNOWLEDGMENTS

This work was supported by grants from the University Mohammed V, Souissi, Rabat-Morocco. The Laboratory of Royal Gendarmerie supported the Biochemical measures. We wish to express special thanks to its director, Dr. Saâd El Kabbaj.

REFERENCES

- 1. Prabhala A, Garg R, Dandona P. Severe myopathy associated with vitamin D deficiency in western New York. Arch Intern Med 2000;160:1199-203.
- 2. Sakuma M, Endo N, Oinuma T, Hayami T, Endo E, Yazawa T, et al. Vitamin D and intact PTH status in patients with hip fracture. Osteoporos Int 2006;17:1608-14.
- Leboff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with hip fracture. JAMA 1999;281:1505-11.
- Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, et al. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 2006;354:669-83.
- Hunter D, Major P, Arden N, Swaminathan R, Andrew T, MacGregor AJ, et al. A randomized controlled trial of vitamin D supplementation on preventing postmenopausal bone loss and modifying bone metabolism using identical twin pairs. J Bone Miner Res 2000;15:2276-83.
- Brustad M, Alsaker E, Engelsen O, Aksnes L, Lund E. Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. Public Health Nutr. 2004;7:327-35.

- Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res 2000;15:1856-62.
- Diamond TH, Levy S, Smith A, Day P. High bone turnover in Muslim women with vitamin D deficiency. Med J Aust 2002;177: 139-41.
- 9. Alagol F, Shihadeh Y, Boztepe H. Sunlight exposure and vitamin D in Turkish women. J Endocrinol Invest 2000;23:173-7.
- Falch JA, Steihaug S. Vitamin D deficiency in Pakistani premenopausal women living in Norway is not associated with evidence of reduced skeletal strength. Scand J Clin Lab Invest 2000;60:103-9.
- Harinarayan CV. Prevalence of vitamin D insufficiency in postmenopausal south Indian women. Osteoporos Int 2005;16:397-402.
- Rassouli A, Milanian I, Moslemi-Zadeh M. Determination of serum 25-hydroxyvitamin D3 levels in early postmenopausal Iranian women: relationship with bone mineral density. Bone 2001; 29:428-30.
- Ghannam NN, Hammami MM, Bakheet SM, Khan BA. Bone mineral density of the spine and femur in healthy Saudi females: relation to vitamin D status, pregnancy, and lactation. Calcif Tissue Int 1999;65:23-8.
- McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. Am J Med 1992;93:69-77.
- 15. Azizi F, Rais-Zadeh F, Mir Said Ghazi A. Vitamin D deficiency in a group of Tehran Population. Res Med 2000;4:291-303.
- Grover SR, Morley R. Vitamin D deficiency in veiled or darkskinned pregnant women. Med J 2001;175:251-2.
- Allali F, El Aichaoui S, Saoud B, Maaroufi H, Abouqal R, Hajjaj-Hassouni N. The impact of clothing style on bone mineral density among post menopausal women in Morocco: a case-control study. BMC Public Health 2006;19;6:135.
- Guzel R, Kozanoglu E, Guler-Uysal F, Soyupak S, Sarpel T. Vitamin D status and bone mineral density of veiled and unveiled Turkish women. J Womens Health Gend Based Med 2001;10: 765-70.
- Hashemipour S, Larijani B, Adibi H, Sedaghat M, Pajouhi M, Bastan-Hagh MH, et al. Vitamin D deficiency and causative factors in the population of Tehran. BMC Public Health 2004;4:38.
- Hatun S, Islam O, Cizmecioglu F, Kara B, Babaoglu K, Berk F, et al. Subclinical vitamin D deficiency is increased in adolescent girls who wear concealing clothing. J Nutr 2005;135:218-22.
- El Sonbaty R, Abdul-Ghaffar NU. Vitamin D deficiency in veiled Kuwaiti women. Eur J Clin Nutr 1996;50:315-8.
- Dawodu A, Absood G, Patel M, Agarwal M, Ezimokhai M, Abdulrazzaq Y, et al. Biosocial factors affecting vitamin D status of women of childbearing age in the United Arab Emirates. J Biosoc Sci 1998;30:431-7.
- Fardellone P, Sebert JL, Bouraya M, Bonidan O, Leclercq G, Doutrellot C, et al. Evaluation of the calcium content of diet by frequential self-questionnaire. Rev Rhum Mal Osteoartic 1991; 58:99-103.
- Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713-6.
- Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.
- Hosmer DW, Lemeshow S, Klar J. Goodness of fit testing for the logistic regression model when the estimated probabilities are small. Biomed J 1988;30:911-24.
- Aloia JF, Talwar SA, Pollack S, Feuerman M, Yeh JK. Optimal vitamin D status and serum parathyroid hormone concentrations in African American women Am J Clin Nutr 2006;84:602-9.
- Gloth FM, Gundberg CM, Hollis BW, Haddad JG, Tobin JD. Vitamin D deficiency in homebound elderly persons. JAMA 1995;274:1683-6.

ARTICLE IN PRESS

- Goldray D, Mizrahi-Sasson E, Merdler C, Edelstein Singer M, Algoetti A, Eisenberg Z, et al. Vitamin D deficiency in elderly patients in a general hospital. J Am Geriatr Soc 1989;37:589-92.
- Thomas MK, Lloyd Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. N Engl J Med 1998;338:777-783.
- 31. Meyer HE, Falch JA, Sogaard AJ, Haug E. Vitamin D deficiency and secondary hyperparathyroidism and the association with bone mineral density in persons with Pakistani and Norwegian background living in Oslo. Norway, The Oslo Health Study. Bone 2004;35:412-7.
- 32. Vecino-Vecino C, Gratton M, Kremer R, Rodriguez-Manas L, Duque G. Seasonal variance in serum levels of vitamin D determines a compensatory response by parathyroid hormone: study in an ambulatory elderly population in Quebec. Gerontology 2006; 52:33-9.
- Bettica P, Bevilacqua M, Vago T, Norbiato G. High prevalence of hypovitaminosis D among free-living postmenopausal women referred to an osteoporosis outpatient clinic in northern Italy for initial screening. Osteoporos Int 1999;9:226-9.
- 34. Bhattoa HP, Bettembuk P, Ganacharya S, Balogh A. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women. Osteoporos Int 2004;15:447-51.
- Sedrani SH, Elidrissy AW, Arabi KM. Sunlight and vitamin D status in normal Saudi subjects. Am J Clin Nutr 1983;38:129-32.
- Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ. Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. J Clin Endocrinol Metab 1996;81: 1129-33.
- Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001;22:477-501.

- Serhan E, Holland MR. Relationship of hypovitaminosis D and secondary hyperparathyroidism with bone mineral density among UK resident Indo-Asians. Ann Rheum Dis 2002;61: 456-8.
- 39. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. 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 2001;86:1212-21.
- 40. Need AG, o'Loughlin PD, Morris HA, Horowitz M, Nordin BEC. The effects of age and other variables on serum parathyroid hormone in postmenopausal women attending an osteoporosis center. J Clin Endocrinol Metab 2004;89:1646-9.
- 41. Scharla SH, Scheidt-Nave C, Leidig G, Woitge H, Wüster C, Seibel MJ, et al. Lower serum 25OHD is associated with increased bone resorption markers and lower bone density at the proximal femur in normal females: a population-based study. Exp Clin Endocrinol Diabetes 1996;104:289-92.
- Lukert B, Higgins J, Stoskopf M. Menopausal bone loss is partially regulated by dietary intake of vitamin D. Calcif Tissue Int 1992;51:173-9.
- 43. Garnero P, Munoz F, Sornay-Rendu E, Delmas PD. Associations of vitamin D status with bone mineral density, bone turnover, bone loss and fracture risk in healthy postmenopausal women. The OFELY study. Published online November 15, 2006, doi: 10.1016/j.bone.2006.09.026.
- 44. Villareal DT, Civitelli R, Chines A, Avioli LV. Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. J Clin Endocrinol Metab 1991;72:628-34.
- Bischoff-Ferrari HA, Orav EJ, Dawson-Hughes B. Effects of cholecalciferol on falling in ambulatory older men and women: a 3 year randomised controlled trial. Arch Intern Med 2006;166: 424-30.