ELSEVIER

Bone 39 (2006) 268–275

BONE

www.elsevier.com/locate/bone

Hypovitaminosis D osteopathy: Is it mediated through PTH, lean mass, or is it a direct effect?

Asma Arabi [a], Rafic Baddoura [b], Hassane Awada [b], Mariana Salamoun [a], Ghazi Ayoub [b], Ghada El-Hajj Fuleihan [a,*]

[a] *Calcium Metabolism and Osteoporosis Program, American University of Beirut-Medical Center, Bliss street, Beirut 113-6044, Lebanon*
[b] *Division of Rheumatology, Saint Joseph University, Beirut, Lebanon*

Received 21 October 2005; revised 16 December 2005; accepted 3 January 2006
Available online 21 Ferbruary 2006

Abstract

Hypovitaminosis D is increasing worldwide and is associated with low bone mass. The effects of hypovitaminosis D on bone might be direct or mediated through decreased muscle mass and function and/or secondary hyperparathyroidism. This study systematically investigated the relative contribution of lean mass, PTH, and the direct effect of vitamin D as predictors of vitamin D mediated osteopathy in elderly individuals.

460 ambulatory subjects aged 65–85 years had their bone mass and lean body mass measured by a dual-energy X-ray absorptiometry. Serum calcium, phosphorus and alkaline phosphatase, intact parathyroid hormone (PTH) and 25-hydroxyvitamin D (25 OHD) were also measured.

Serum 25 OHD correlated with lean body mass in men, $r = 0.24$, $P = 0.002$, but not in women; and with bone mass at all skeletal sites in men, $r = 0.20–0.30$, $P < 0.02$. Correlations were also noted at all skeletal sites in women except for the spine, $r = 0.13–0.18$, $P < 0.04$. In both genders, BMD at sites enriched in cortical bone was 0.4–0.7 SD lower in the group with the lowest vitamin D tertile than that in the group in the highest tertile. After controlling for PTH, the magnitude of the correlations between BMD and 25 OHD remained significant in both genders. After controlling for lean body mass, the magnitude of these correlations did not change in women and decreased but remained significant in men. After adjustment for age and height, both lean body mass and PTH had significant independent contributions to BMD variance at all skeletal sites. After adjustment for age, height, lean mass, and PTH, 25 OHD did not have any significant residual contribution to BMD variance except at the trochanter in men.

This study demonstrates that vitamin D osteopathy in the elderly is in large part mediated through lean mass in men and through PTH levels in both genders, with a greater contribution of PTH in women than in men. There was little demonstrable independent relation between serum 25 OHD and bone mass.

Keywords: Elderly; Lean mass; BMD; Vitamin D osteopathy; PTH

Introduction

Osteoporosis is the commonest metabolic bone disorder in the elderly. Osteoporosis has been proposed as a latent manifestation of hypovitaminosis D [1].

Despite the availability of preventive and therapeutic strategies, the prevalence of vitamin D deficiency is increasing worldwide and notoriously in community dwelling elderly [2–5]. This is particularly important in view of the effect of vitamin D on muscle, bone, and ultimately fracture risk.

Studies have shown positive association between 25-hydroxyvitamin D (25 OHD) levels and bone mineral density (BMD) [6–8]. Insufficient levels of vitamin D have been associated with decreased muscle strength and increased fall risk, both increasing the risk of osteoporotic fractures [9–12]. Vitamin D supplementation has been shown to increase bone mineral density, reduce falls, and decrease the incidence of hip fracture [13–18]. Lean mass is an independent predictor of BMD [19,20]. Low vitamin D levels are associated with secondary hyperparathyroidism and bone loss. It has been

* Corresponding author. Fax: +961 1 744464.
E-mail address: gf01@aub.edu.lb (G. El-Hajj Fuleihan).

8756-3282/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2006.01.140

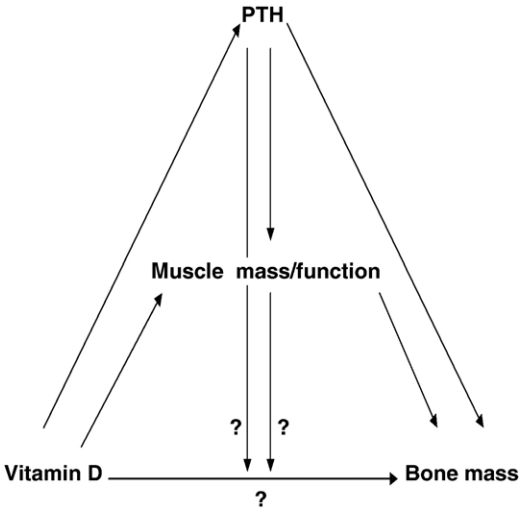

Fig. 1. Suggested physiological pathways for the relationship between vitamin D and bone mass as tested in the study.

suggested that secondary hyperparathyroidism mediates muscle weakness and the high risk of falling associated with hypovitaminosis D [21]. Therefore, the detrimental effect of low vitamin D on bone may possibly be direct or more likely mediated through changes in lean mass and muscle function and/or through secondary hyperparathyroidism and its effect on both muscle and bone (Fig. 1). However, studies evaluating causal pathways for the effect of vitamin D on bone are scarce, have for the most part only evaluated PTH, and have been conducted mostly in women [22,23].

This study investigated the relationship between vitamin D and bone mass and aimed at dissecting the relative contribution of known mediators lean mass and PTH for the deleterious effect of hypovitaminosis D on musculoskeletal health, in elderly community dwelling men and women. Elderly individuals with a spectrum of sub-optimal 25 OHD levels were studied, a group where the relationship between vitamin D and bone mass is the steepest, and the risk of osteoporotic fractures the highest [6].

Materials and methods

Subjects

460 home-dwelling ambulatory subjects, aged 65–85 years (73 ± 5.2), were randomly recruited between November 2002 and March 2003 from the Greater Beirut area, based on geographical maps for a study aiming at characterizing bone mass in a population-based sample of elderly Lebanese subjects. All participants were resident in the greater Beirut area which is located at 33.5° North, with both parents of Middle Eastern origin.

Exclusion criteria included the following: any medical condition likely to affect bone metabolism such as the history of major chronic disease, the intake of medications that affect bone metabolism, history of steroid intake for more than 6 months, treatment with bisphosphonates, SERM, calcitonin or hormone replacement therapy for more than 1 year during the last 5 years. Also excluded were subjects with history of bed rest for more than 1 month within 6 months prior to the study, subjects with previous surgery on the spine, both forearms or both hips, and those with history of radiotherapy or chemotherapy.

11 subjects (2 men and 9 women) were excluded from the analyses because of suspicion of primary hyperparathyroidism, based on a serum calcium ≥10.5 mg/dl and PTH levels above the upper limit of normal (76 pg/ml). Serum 25 OHD levels were missing in 6 subjects; thus, data from 443 subjects (286 women and 157 men) were included in the analyses.

The study was approved by the Institutional Review Board of the American University of Beirut, and written informed consent was obtained from all study participants.

Assessments

Clinical and biochemical data collection

At baseline, physical examination included height and weight assessment. The subject's standing height, using a wall stadiometer, was recorded in centimeters to the nearest 0.1 cm. Weight was recorded in kilograms, to the nearest 0.5 kg, with the participants wearing light clothes without shoes and using a standard clinical balance. The body mass index (BMI kg/m^2) was calculated. The estimated time that the study subjects spent outdoors was assessed by asking them about the time spent outdoors during the week preceding the screening visit. Supplementation with calcium and/or vitamin D was assessed. Dairy calcium intake was assessed using a food frequency questionnaire that evaluated the consumption of calcium enriched foods, mostly from dairy products.

Serum calcium, phosphorus, and alkaline phosphatase levels were measured by standard colorimetric methods, using the Hitachi 912 analyzer (Hitachi, Mannheim, Germany). Serum 25 OHD level was measured by RIA using the IDS (Immunodiagnostic System Limited, UK). The IDS RIA kit picks up 100% of the 25-hydroxyvitamin D3 and 75% of the 25-hydroxyvitamin D2 ([25] (OH) Vitamin D, IDS kit package). The normal range as reported in the kit was 10–60 ng/ml; the intra-assay and inter-assay variability are below 10%. Serum PTH was measured by ELISA-PTH immunoradiometric assay (CisBio International, Gif-Sur-Yvette, Cedex, France). The normal range of the kit is 8–76 pg/ml. The detection limit of the assay is 0.7 pg/ml, and the intra-assay and inter-assay variability are below 10%. Serum for 25 OHD and PTH measurements were frozen at −70 F and assayed within 6 months after recruitment of the first patient. Our center participates in the international quality assurance program for the vitamin D assays: Vitamin D External Quality Assessment Scheme (DEQAS), London, UK.

Bone mineral density and body composition measurements:

Areal bone mineral content (BMC, grams) of the total body, bone mineral density BMD (g/cm^2) of the antero-posterior lumbar spine (L1–L4), the left femur (total hip, femoral neck and trochanter), the left 1/3 radius as well as lean mass (grams) were measured by dual-energy X-ray absorptiometry (DXA), using a Hologic 4500 A device [n = 219] and a Hologic 4500 W [n = 230] (Hologic, Bedford, MA, USA) in two centers.

Cross-calibration of densitometers

30 subjects had BMD at all skeletal sites simultaneously measured in both centers. Linear regression analyses were performed to allow conversion from one device to another using cross-calibration formulas that were consistent with those reported in the literature [25] and were as follows:

Spine: BMD QDR 4500 A = 0.004 + 1.02*BMDQDR 4500 W (R^2 = 0.95)
Total hip: BMD QDR 4500 A = 0.05 + 0.98*BMD QDR 4500 W. (R^2 = 0.97)
Femoral neck: BMD QDR 4500 A = 0.21 + 0.67*BMDQDR 4500 W. (R^2 = 0.94)
Trochanter: BMD QDR 4500 A = 0.03 + 0.96*BMDQDR 4500 W. (R^2 = 0.94)
Forearm: BMD QDR 4500 A = 0.01 + 0.98*BMDQDR 4500 W. (R^2 = 0.89)
Total body: BMC QDR 4500 A = 9.97 + 1*BMCQDR 4500 W. (R^2 = 0.99)
Lean mass: lean QDR 4500 A = −405 + 1.03*lean QDR 4500 W. (R^2 = 0.99).

The mean ± SD BMD, BMC data presented in this paper were those as if all subjects were measured on the Hologic 4500 A densitometer, using the above cross-calibration formulas.

The mean ± SD for precision, expressed as the coefficient of variation (CV %) for 83 serial duplicate scans performed in vivo at the time of the study, were

as follows: Lumber spine = 0.90 ± 0.79%, Total hip = 0.84 ± 0.70%, Femoral neck = 1.35 ± 1.14%, Trochanter = 1.08 ± 0.84%, Forearm = 1.02 ± 0.72%.

Statistical analyses

Outcome measures

The main objective of the study was to test whether PTH and/or lean mass mediate the effect of vitamin D on BMD. We conducted several sets of analyses, in order to check if these two variables satisfy all the conditions that should be met by a mediator [24]. First, we checked whether the determinant 25 OHD was associated with the potential mediators, i.e., PTH and/or lean mass. We then checked the relationship between the determinant 25 OHD and the outcome, i.e., BMD or BMC. In the third step, we checked the relationship between the outcomes BMD or BMC and the mediators. In the fourth step, we assessed the correlations between the outcomes BMD or BMC and the determinant 25 OHD after adjustment for each of the mediators. Finally, we implemented multivariate analyses to assess the strength of the associations between 25 OHD and BMD or BMC after adjustment for both mediators and other determinants of BMD such as age and height.

Bivariate analyses

All variables except 25 OHD and PTH were normally distributed. Therefore, Spearman correlation coefficients were used to determine the strength of relationships between 25 OHD, PTH, and other variables such as BMD, BMC, and lean mass. Partial correlation coefficients were computed to assess the relationship between 25 OHD and BMD, controlling for either PTH or lean mass.

The subjects were subdivided into three groups according to tertiles of vitamin D levels, and the difference in BMD values between the three groups were assessed using one-way analysis of variance ANOVA. When significance was observed, least significant difference post hoc *t* tests were performed to identify the groups that contributed to the overall difference.

Regression

Because of the impact of vitamin D on lean mass, PTH and BMD, and the effect of PTH on both BMD and lean mass, and because age, height, PTH levels, and lean mass are all known correlates of areal BMD, we assessed the independent effect of vitamin D on BMD after adjustment for these correlates using multiple regression models (Fig. 1). This allowed the dissection of the physiological pathways mediating the impact of vitamin D on BMD or BMC. The outcome was BMD or BMC, and the independent predictors were age, height, lean mass, PTH, and 25 OHD levels. The models were constructed based on all enter selection procedure. The independent variables were entered by sequential blocks, and the R^2 due to the additional entry of each block was calculated, thus allowing the determination of the amount of variance in the outcome measure (BMD or BMC) explained by each independent block. In the first model, these blocks were entered as follows: Anthropometric variables (age, height) as a first block, lean mass as a second block, and PTH as a third block. PTH was entered after lean mass because the impact of PTH on bone may be direct but could also be indirect through effects on lean mass and muscle function [21]. In the second models, PTH was entered before lean mass to explore the independent effect of lean mass on bone mass. Serum 25 OHD levels were entered last in the model in order to investigate the strength of its association with bone mass after correcting for all other predictors that are on the causal pathway between vitamin D and BMD, BMC (Fig. 1).

Values were expressed as mean ± SD. Because 25 OHD and PTH were not normally distributed, median [min–max] was reported. P value < 0.05 was considered as statistically significant. No adjustment was made for multiple testing.

Results

Clinical characteristics

The clinical characteristics of the study population are shown in Table 1. The mean age of the study group was 73.6 ± 5.2. Women had higher BMI and lower areal BMD at all skeletal sites than men ($P < 0.001$).

Daily calcium intake was low in both genders, lower in women as compared to men ($P = 0.006$). Only 18 subjects (12 women and 6 men) were receiving vitamin D supplementation. The estimated time spent outdoors by the study subjects was (mean ± SD): 3.2 ± 2.8 h per week in women and 6.0 ± 2.6 h per week in men.

The mean calcium, phosphorus, and alkaline phosphatase were normal in both genders. The median serum 25 OHD was low in both genders. 158 women (55%) and 58 men (37%) had 25 OHD <10 ng/ml. Only 5 subjects had 25 OHD level ≥30 ng/ml. Three subjects (two women and one man) had

Table 1
Clinical characteristics of the study population

Variable	All ($N = 443$)	Women ($N = 286$)	Men ($N = 157$)	P value[a]
Age (years)	73.6 ± 5.2	73.4 ± 5.2	74.1 ± 5.08	NS
Weight (kg)	70 ± 14	69 ± 16	73 ± 12	0.01
Height (cm)	155 ± 8.8	150 ± 6	163 ± 6	<0.001
BMI (kg/m^2)	29.4 ± 5.9	30.5 ± 6.5	27.2 ± 3.9	<0.001
Dietary calcium intake (mg/days)	310 ± 231	286 ± 242	355 ± 262	0.006
Estimated time spent outdoors (h/week)	4.2 ± 3.0	3.2 ± 2.8	6.0 ± 2.6	<0.001
Calcium (mg/dl)	9.3 ± 0.5	9.4 ± 0.6	9.3 ± 0.4	0.001
Phosphorus (mg/dl)	3.4 ± 0.5	3.6 ± 0.5	3.1 ± 0.5	<0.001
Alkaline phosphatase (IU/l)	88.4 ± 32.0	91.5 ± 33.3	82.5 ± 29.2	0.004
25 (OH) Vitamin D (ng/ml)	10.3 [4.0–38.7]	9.6 [4.7–38.7]	11.3 [4.0–36.2]	0.008
PTH (pg/ml)	36.2 [1.9–647]	37.4 [1.9–647]	35.3 [6.8–385.0]	NS
L1–L4 BMD (g/cm^2)	0.832 ± 0.16	0.790 ± 0.15	0.910 ± 0.16	<0.001
Total hip BMD (g/cm^2)	0.772 ± 0.14	0.730 ± 0.12	0.850 ± 0.13	<0.001
Femoral neck BMD (g/cm^2)	0.633 ± 0.09	0.613 ± 0.09	0.669 ± 0.09	<0.001
Trochanter BMD (g/cm^2)	0.550 ± 0.11	0.520 ± 0.10	0.611 ± 0.11	<0.001
1/3 Radius BMD (g/cm^2)	0.566 ± 0.11	0.512 ± 0.08	0.663 ± 0.08	<0.001
Total body BMC (g)	1311 ± 372	1150 ± 266	1612 ± 354	<0.001
Total body lean mass (g)	41,368 ± 19,925	36,969 ± 5948	47,119 ± 6300	<0.001

Values are mean ± SD except for 25 (OH) vitamin D and PTH: median [min–max].
[a] P value for difference between male and female by independent *t* test.

serum 25 OHD levels below 5 ng/ml. Serum calcium, phosphorus, and alkaline phosphatase were normal in all the three, and PTH level was at the upper limit of normal in only one of them.

Thirty-two subjects (23 women and 9 men) had high PTH levels, all of whom except for two had normal serum calcium level.

Serum calcium was low (<8.5 mg/dl) in 10 subjects (6 women and 4 men), PTH and alkaline phosphatase were high in 2 of them.

Correlations between vitamin D and the potential mediators PTH, and lean mass

As expected, 25 OHD levels correlated negatively with PTH ($r = -0.38$ in men and $r = -0.45$ in women, $P < 0.001$). In addition, in both genders, subjects who were in the lowest tertile of vitamin D had higher PTH levels compared to those in the highest tertile ($P < 0.05$) (Table 3).

25 OHD correlated with lean mass in men ($r = 0.23$, $P = 0.002$). In addition, those who were in the lowest tertile of vitamin D had lower lean mass compared to those in the highest tertile ($P < 0.05$) (Table 3). There was no correlation between lean mass and 25 OHD in women.

Correlations between vitamin D and the outcomes BMD or BMC

In both genders, serum 25 OHD showed significant correlations with BMD at all skeletal sites, except at the spine in women. The correlation coefficients tended to be higher in men as compared to those in women ranging from 0.12 to 0.17 in women versus 0.20 to 0.30 in men (Table 2).

In both genders, there was a consistent increase in BMC, BMD at almost all skeletal sites going from the lowest to the highest vitamin D tertile (Table 3). This was significant at the three hip sites and total body BMC in both genders and in addition at the 1/3 radius in women (Table 3).

Correlations between BMC or BMD and the potential mediators PTH and lean mass

PTH showed significant negative correlations with BMD at cortical sites, i.e., the total hip, the femoral neck ($r = -0.19$ to -0.21, $P < 0.05$), and at the trochanter in both genders, $r = -0.19$ to -0.23, $P < 0.05$, and with 1/3 radius BMD in women, $r = -0.16$, $P = 0.006$ (Table 2).

Lean mass correlated with total body BMC and with BMD at all skeletal sites in both genders ($r = 0.38$–0.52; $P < 0.001$). There were no correlations between PTH and lean mass in either gender.

Adjusted correlations

In women, the magnitude of the correlations between 25 OHD and BMD decreased after controlling for PTH and decreased slightly or remained unchanged after controlling for lean mass. In men, the magnitude of these correlations decreased after controlling for lean mass and to a lesser degree after controlling for PTH (Table 2).

Table 2
Coefficients of simple and adjusted correlations (*P* values) between BMD/BMC, 25 (OH) vitamin D, PTH, and lean mass in women and men

	Women			Men		
	25 OHD	PTH	Lean	25 OHD	PTH	Lean
BMD/BMC						
BMD L1–L4	0.03 (0.5)	−0.03 (0.5)	0.41 (<0.001)	0.20 (0.02)	−0.2 (0.7)	0.38 (<0.001)
	0.03 (0.5) [a]	–	–	0.20 (0.01) [a]	–	–
	0.04 (0.4) [b]	–	–	0.14 (0.07) [b]	–	–
BMD total hip	0.17 (0.004)	−0.21 (<0.001)	0.51 (<0.001)	0.25 (0.001)	−0.21 (0.007)	0.50 (0.04)
	0.11 (0.06) [a]	–	–	0.22 (0.005) [a]	–	–
	0.17 (0.005) [b]	–		0.20 (0.01) [b]	–	–
BMD femoral neck	0.18 (0.002)	−0.18 (0.002)	0.44 (<0.001)	0.21 (0.006)	−0.19 (0.017)	0.47 (0.05)
	0.13 (0.02) [a]	–	–	0.18 (0.02) [a]	–	–
	0.18 (0.004) [b]	–	–	0.16 (0.05) [b]	–	–
BMD trochanter	0.17 (0.003)	−0.19 (0.001)	0.49 (<0.001)	0.30 (<0.01)	−0.23 (0.003)	0.48 (0.01)
	0.12 (0.04) [a]	–	–	0.26 (0.001) [a]	–	–
	0.18 (0.004) [b]	–	–	0.27 (0.001) [b]	–	–
BMD 1/3 radius	0.12 (0.04)	−0.16 (0.006)	0.46 (<0.001)	0.21 (0.007)	−0.08 (0.2)	0.56 (0.006)
	0.09 (0.1) [a]	–	–	0.20 (0.01) [a]	–	–
	0.10 (0.1) [b]	–	–	0.14 (0.08) [b]	–	–
BMC total body	0.13 (0.03)	−0.10 (0.09)	0.52 (<0.001)	0.25 (0.002)	−0.06 (0.4)	0.47 (0.007)
	0.13 (0.1) [a]	–	–	0.20 (0.1) [a]	–	–
	0.13 (0.03) [b]	–	–	0.13 (0.1) [b]	–	–
Lean mass	0.012 (0.7)	−0.03 (0.5)	–	0.24 (0.002)	−0.05 (0.4)	–
	0.007 (0.8) [a]	–	–	0.23 (0.004) [a]	–	–
PTH	−0.45 (<0.001)	–	−0.03 (0.5)	−0.38 (<0.001)	–	−0.05 (0.4)

[a] Partial coefficients of correlations (*P* value), after controlling for PTH.
[b] Partial coefficients of correlations (*P* value), after controlling for lean mass.

Table 3
PTH, lean mass, bone mineral density (BMD), and bone mineral content (BMC) by 25 (OH) vitamin D tertiles in women and men

	Women (n = 286)			Men (n = 157)		
	1st tertile (n = 95)	2nd tertile (n = 95)	3rd tertile (n = 96)	1st tertile (n = 53)	2nd tertile (n = 52)	3rd tertile (n = 52)
25 (OH) vitamin D (ng/ml)	7.0 (4.7–8.2)	9.6 (8.3–11.3)	14.6 (11.3–38.7)	8.4 (4.0–9.6)	11.3 (9.7–12.9)	16.0 (13.0–36.2)
PTH (pg/ml)	48.2 [a, b] (11.2–244.7)	37.9 (4.4–647.5)	28.3 [c] (1.9–83.8)	42.6 [a, b] (13.2–364.0)	35.4 (12.7–385.0)	29.1 (6.8–56.5) [c]
Lean mass (g)	36,987 ± 6062	36,884 ± 5639	37,017 ± 6308	45,640 ± 6407 [a, b]	46,864 ± 6016	48,722 ± 6265 [c]
Bone mass						
Lumbar spine BMD (g/cm^2)	0.772 ± 0.13	0.803 ± 0.17	0.791 ± 0.14	0.899 ± 0.13	0.882 ± 0.16	0.950 ± 0.17
Total hip BMD (g/cm^2)	0.687 ± 0.12 [a, b]	0.743 ± 0.12 [c]	0.760 ± 0.12 [c]	0.814 ± 0.13 [a, b]	0.848 ± 0.13 [b]	0.890 ± 0.11 [c]
Femoral neck BMD (g/cm^2)	0.587 ± 0.09 [a, b]	0.619 ± 0.08 [c]	0.633 ± 0.08 [c]	0.643 ± 0.08 [a, b]	0.678 ± 0.10 [b]	0.690 ± 0.08 [c]
Trochanter BMD (g/cm^2)	0.484 ± 0.10 [a, b]	0.527 ± 0.10 [c]	0.540 ± 0.10 [c]	0.574 ± 0.11 [a, b]	0.609 ± 0.11 [b]	0.653 ± 0.11 [c]
1/3 Radius BMD (g/cm^2)	0.494 ± 0.08 [a, b]	0.519 ± 0.09	0.523 ± 0.07 [c]	0.637 ± 0.08	0.659 ± 0.09	0.684 ± 0.08
Total body BMC (g)	1098 ± 255 [a, b]	1174 ± 273 [c]	1205 ± 272 [c]	1511 ± 289 [a, b]	1541 ± 383 [b]	1718 ± 383 [c]

Values are mean ± SD, median [min–max].
[a] $P < 0.05$ by ANOVA between the three tertile groups by gender.
[b] PTH values were log transformed for ANOVA.
[c] Variables in the same row with different superscript are significantly different from each others.

Regression analyses

In women, lean mass accounted for 8.4–18.8% of BMD or BMC variance after adjustment for age and height, whereas PTH accounted for only 1.7–5% of such variance, after adjustment for age, height, and lean mass (Table 4). In men, lean mass accounted for 6.2–19.6% of BMD or BMC variance after adjustment for age and height, whereas PTH independently accounted for up to 3.5% of such variance (Table 4). The magnitudes of the R2 for lean mass and for PTH were essentially unchanged when PTH was entered before lean mass in the model (model 2, Table 5).

After adjustment for age, height, lean mass, and PTH levels, 25 OHD level did not have residual significant contribution to BMD at any skeletal site except the trochanter in men, $R^2 = 3\%$ (Table 4).

Because 32 subjects had high PTH levels and 10 had low serum calcium, we repeated the analyses after excluding these subjects. The overall results obtained did not differ in terms of R^2 and significance of the results obtained as outlined above (data not shown).

Discussion

In this group of community dwelling elderly individuals with hypovitaminosis D, the effect of vitamin D seemed to be in large part mediated though lean mass and PTH levels in men and through PTH in women, with a greater contribution of PTH in women than in men. In the adjusted analyses, we could detect very little independent relationship, if any, between serum 25 OHD and bone mass in either gender.

Most studies except for one [26] revealed a possible association between vitamin D and BMD at several skeletal sites [2,6–8]. The magnitude of correlations between vitamin D and BMD in the current study was comparable to those previously reported [2,6–8]. Associations seemed to be stronger at cortical than trabecular sites [2,7,8], similarly to what we observed. Interestingly, whereas the correlation coefficient was lower for the spine compared to other skeletal sites in men, no correlation was observed in women at this site.

Lean mass correlates with bone density [19,20], and vitamin D correlates with muscle strength and muscle function [27]. Vitamin D supplementation improves muscle function and reduces the risk of falling among elderly [18]. In this study, there was a correlation between lean mass and serum 25 OHD in men only. This is in agreement with a previous study where muscle strength correlated with 25 OHD levels in men only, and with 1,25 dihydroxyvitamin D ([1,25] $(OH)_2D$) in both genders [28]. Men had higher levels of serum 25 OHD than women, and it is possible that there may be a threshold level for vitamin D above which the relationship with lean mass becomes clinically significant. Alternatively, it may be that local vitamin D rather than circulating levels of 25 OHD or 1,25 $(OH)_2D$ levels are keys for detecting a relationship between vitamin D and muscle. Finally, lean mass is only one parameter reflecting muscle strength, and the absence of a detectable correlation between lean mass and serum 25 OHD in women does not preclude the presence of a relationship between 25 OHD and muscle strength, in both genders.

The effect of vitamin D on lean mass and ultimately bone mass may be direct or mediated by secondary hyperparathyroidism [21]. Hyperparathyroidism is associated with increased bone resorption and low bone mass at cortical sites [29]. In view of the positive effect of muscle on bone, and the negative effect of PTH on lean mass and on bone metabolism, the detrimental effect of 25 OHD on bone may be mediated by the direct effect of PTH on the skeleton and/or its effect on lean mass (Fig. 1). In men, the magnitude of the correlations between vitamin D and BMD decreased after adjustment for lean mass and also but to a lesser extent after adjustment for PTH. In women, the magnitude of these correlations decreased after adjustment for PTH, but not for lean mass. This suggests an important effect of lean mass as a mediator of vitamin D effect on bone in men and is consistent with the weaker correlations noted between lean mass and BMD in women. In the multivariate analyses, both lean mass and PTH accounted for a significant contribution to the variance of bone mass in both genders, and the relative contribution of PTH was higher in women than men at multiple skeletal sites

Table 4
Linear regression models with bone mineral density (BMD) or bone mineral content (BMC) as outcome, age and height, lean mass, PTH, and 25 OHD as predictors

Site	Predictors	Women		Men	
		R^2 (%)	P value	R^2 (%)	P value
L1–L4 BMD	Age (years)	–	0.5	–	0.4
	Height (cm)	4.9	0.6	8.2	0.6
	Lean mass (g)	10.8	<0.001	7.8	0.005
	PTH (pg/ml)	5.0	<0.001	0.1	0.7
	25 OHD (ng/ml)	0.1	0.5	1.7	0.08
Total hip BMD	Age (years)	–	0.001	–	0.04
	Height (cm)	20.0	0.1	8.1	0.4
	Lean mass (g)	12.6	<0.001	19.6	<0.001
	PTH (pg/ml)	3.7	0.001	3.5	0.02
	25 OHD (ng/ml)	0.4	0.1	1.7	0.06
Femoral neck BMD	Age (years)	–	<0.001	–	0.04
	Height (cm)	20.0	0.3	8.3	0.5
	Lean mass (g)	8.4	<0.001	6.2	0.001
	PTH (pg/ml)	2.1	0.03	2.9	0.04
	25 OHD (ng/ml)	0.8	0.07	1.0	0.1
Trochanter BMD	Age (years)	–	0.005	–	0.03
	Height (cm)	18.7	0.04	10.6	0.9
	Lean mass (g)	12.9	0.001	15.6	<0.001
	PTH (pg/ml)	3.0	0.005	4.5	0.01
	25 OHD (ng/ml)	0.6	0.1	3.1	0.01
1/3 Radius BMD	Age (years)	–	0.002	–	0.03
	Height (cm)	17.0	0.2	6.0	0.4
	Lean mass (g)	10.1	<0.001	9.8	<0.001
	PTH (pg/ml)	1.7	0.07	0.3	0.6
	25 OHD (ng/ml)	0.3	0.3	1.5	0.1
Total body BMC	Age (years)	–	0.2	–	0.3
	Height (cm)	9.1	0.8	4.9	0.3
	Lean mass (g)	18.8	<0.001	18.3	<0.001
	PTH (pg/ml)	1.7	0.01	0.5	0.3
	25 OHD (ng/ml)	0.1	0.9	0.1	0.8

R^2 (%) = the proportion of variance in BMD or BMC explained by each variable.

(Table 4). The similarity of the R^2 for PTH and lean mass regardless of which variable was entered first in the model suggests an independent effect of both predictors on bone mass. There was no clinically significant residual independent effect of vitamin D on BMD or BMC in either gender. In view of these observations, the effect of 25 OHD on BMD may be in large part mediated through lean mass and PTH in men and through PTH in women, with higher contribution of PTH in women compared to men. Although regression analyses showed that lean mass is an independent determinant of BMD in both genders, we cannot conclude from the current study that lean mass mediates the effect of vitamin D on bone in women because of the lack of relationship between lean mass and 25 OHD in this gender. The above noted gender differences reflect the stronger effect of lean mass on BMD at weight bearing sites in men as compared to women, as previously reported [30] and similar to what our group has reported in adolescents [31]. It also reflects the higher proportion of subjects with hypovitaminosis D and secondary hyperparathyroidism in women compared to men.

A previous study showed that postmenopausal women who had hypovitaminosis D and hyperparathyroidism had lower BMD as compared to those who had hypovitaminosis D but without hyperparathyroidism [22]. Another study showed that after adjustment for BMI, years since menopause and PTH, 25 OHD accounted for only 2.5% of BMD variance at the femoral neck [23]. The above suggests that the negative effect of low vitamin D on bone density in women may be at least in part, if not in its totality, mediated through PTH. However, an independent effect of lean mass was not explored in either study. Pluijm et al. showed that in women, neither 25 OHD nor PTH levels had significant effect on hip BMD after adjustment for other determinants, such as age, weight changes, walking activities, and sex-hormone binding globulin. Less attention has been given to the mediators of vitamin D effect on bone in men. In the current study, both lean mass and PTH seemed to mediate the effect of vitamin D in men. Pluijm et al. also showed that in men, serum 25 OHD did not have any significant effect on hip BMD, after adjustment for PTH, participation in sportive activities, and smoking [30]. They also showed that lean mass was an independent determinant of hip BMD but was not a mediator for the effect of any other determinant of bone density, in either gender [30].

In vitro 1,25 $(OH)_2$D induces osteoclastogenesis but may also play an anti-apoptotic effect on osteoblasts after activation of the Fas-ligand pathway [32,33]. In this clinical study, we could demonstrate very little direct effect serum 25 OHD on bone, after adjusting for known determinants of BMD that is PTH and lean

Table 5
Linear regression models with bone mineral density (BMD) or bone mineral content (BMC) as outcome, age and height, PTH, lean mass, and 25 OHD as predictors

Site	Predictors	Women		Men	
		R^2 (%)	P value	R^2 (%)	P value
L1–L4 BMD	Age (years)	–	0.5	–	0.4
	Height (cm)	4.9	0.6	8.2	0.6
	PTH (pg/ml)	3.8	0.7	0.0	0.8
	Lean mass (g)	10.9	<0.001	7.8	0.001
	25 OHD (ng/ml)	0.1	0.5	1.7	0.08
Total hip BMD	Age (years)	–	0.001	–	0.04
	Height (cm)	20.0	0.1	8.1	0.4
	PTH (pg/ml)	2.5	0.004	4.2	0.009
	Lean mass (g)	13.8	<0.001	18.8	<0.001
	25 OHD (ng/ml)	0.4	0.1	1.7	0.06
Femoral neck BMD	Age (years)	–	<0.001	–	0.04
	Height (cm)	20.0	0.3	8.3	0.5
	PTH (pg/ml)	1.4	0.03	3.4	0.01
	Lean mass (g)	9.4	<0.001	15.7	<0.001
	25 OHD (ng/ml)	0.8	0.07	1.0	0.1
Trochanter BMD	Age (years)	–	0.005	–	0.03
	Height (cm)	18.7	0.04	10.6	0.9
	PTH (pg/ml)	2.0	0.01	3.4	0.01
	Lean mass (g)	13.9	<0.001	15.7	<0.001
	25 OHD (ng/ml)	0.6	0.1	3.1	0.01
1/3 Radius BMD	Age (years)	–	0.002	–	0.03
	Height (cm)	17.0	0.2	6.0	0.4
	PTH (pg/ml)	0.8	0.1	0.5	0.3
	Lean mass (g)	10.4	<0.001	9.6	<0.001
	25 OHD (ng/ml)	0.3	0.3	1.5	0.1
Total body BMC	Age (years)	–	0.2	–	0.3
	Height (cm)	9.1	0.8	4.9	0.3
	PTH (pg/ml)	1.3	0.05	0.9	0.2
	Lean mass (g)	19.2	<0.001	18	<0.001
	25 OHD (ng/ml)	0.1	0.9	0.1	0.8

R^2 (%) = the proportion of variance in BMD or BMC explained by each variable.

mass. The relationship between vitamin D and bone may be mediated by 25 OHD or $1,25(OH)_2D$. The latter could be produced locally, thus rendering it impossible to dissect the above possibilities and exclude a direct effect of vitamin D on BMD, especially at low circulating levels of 25 OHD [34].

It may be argued that the vitamin D levels in the study group are quite low, thus limiting the clinical implications of the study findings to other populations. However, the vitamin D values obtained in this age group span over a clinically relevant range of low vitamin D levels that are actually prevalent in elderly subjects worldwide [2–5,35,36]. Indeed, not only do they range over similarly observed values in other community dwelling elderly individuals but, as importantly, comprise the range over which the relationship between vitamin D and BMD seems to be the steepest as demonstrated in the NHANES study [6].

Although histomorphometric examination is needed to rule out possible osteomalacia in some subjects, it is unlikely to have been prevalent in the study population. Indeed, only 3 subjects had 25 OHD levels below 5 ng/ml, and 10 subjects had low serum calcium levels. Concomitant low serum phosphorus levels and high alkaline phosphatase and PTH levels, the biochemical hallmark of osteomalacia, were present only in 2 out of these 10 subjects only. Furthermore, the results of our analyses were unchanged when these subjects were excluded. We believe that the clinical and laboratory profile of study subjects is consistent with the first degree and possibly the second degree of "hypovitaminosis D osteopathy" described by Heaney et al. [1]. The principal clinical manifestations of these entities are indeed osteoporosis and not osteomalacia [1].

This study has some limitations. The study population may not representative of the whole population since Greater Beirut represents only one-third of the total Lebanese population at large. 25 OHD levels were below 10 ng/ml. Nevertheless, a much small proportion of subjects had elevated PTH levels. 25 OHD was measured by IDS, and it has been shown that IDS has a diminished capacity to detect 25-hydroxyvitamin D_2 compared to Diasorin RIA [37]. It is therefore possible that 25 OHD was underestimated. However, we do not think that this would not affect the multivariate analyses and the major conclusion of the study. Renal function which might influence PTH, BMD, and possibly serum 25 OHD was not assessed. However, all participants were healthy elderly with no history of kidney disease. Finally, it is a descriptive cross-sectional study, and whereas linear regression models can be used to explore causal pathway mechanisms for relationships between predictors and outcomes, they certainly do not prove a cause–effect relationship.

In conclusion, this study suggests a causal pathway for the deleterious impact of vitamin D on bone health in the elderly. This effect seems to be in large part mediated by PTH and lean mass in men and through PTH levels in women, with higher contribution of PTH in women compared to men. The implementation for Vitamin D supplementation would be anticipated to have a salutary effect on all mediators of the deleterious effect of hypovitaminosis D on bone and ultimately a multifaceted beneficial impact on osteoporotic fractures.

Acknowledgments

The authors thank the study subjects for making the study possible. The authors also thank Mrs. S. Mroueh for her technical assistance in the acquisition and analyses of the bone mineral density scans and Mrs. C. Hajj-Chahine for running the PTH and vitamin D assays. The study was supported by a grant from the World Health Organization–Eastern Mediterranean Region Organization (WHO-EMRO) and from Aventis pharma Lebanon and by institutional funds from the American University of Beirut and Saint Joseph University.

References

[1] Heaney RP. Long-latency deficiency disease: insights from calcium and vitaminD. Am J Clin Nutr 2003;78:912–9.
[2] 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.
[3] 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.
[4] Isaia G, Giorgino R, Rini GB, Bevilacqua M, Maugeri D, Adami S. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. Osteoporos Int 2003;14:577–82.
[5] Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439–43.
[6] Bischoff-Ferrari HA, Dietrich T, Orav J, Dawson-Hughes B. Positive association between 25-hydroxyvitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 2004;116:634–9.
[7] Collins D, Jasani C, Fogelman I, Swaminathan R. Vitamin D and bone mineral density. Osteoporos Int 1998;8:110–4.
[8] Fradinger E, Zanchetta J. Vitamin D and bone mineral density in ambulatory women living in Buenos Aires, Argentina. Osteoporos Int 2001;12:24–7.
[9] Parfitt AM, Gallagher JC, Heaney RP, Johnston CC, Neer R, Whedon GD. Vitamin D and bone health in the elderly. Am J Clin Nutr 1982;36: 1014–31.
[10] Boonen S, Vanderschueren D, Cheng XG, Verbeke G, Dequeker J, Geusens P, et al. Age-related (type II) femoral neck osteoporosis in men: biochemical evidence for both hypovitaminosis D- and androgen deficiency-induced bone resorption. J Bone Miner Res 1997;12:2119–26.
[11] LeBoff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. JAMA 1999;281:1505–11.
[12] Flicker L, Mead K, MacInnis RJ, Nowson C, Scherer S, Stein MS, et al. Serum vitamin D and falls in older women in residential care in Australia. J Am Geriatr Soc 2003;51:1533–8.
[13] Dukas L, Bischoff HA, Lindpaintner LS, Schacht E, Birkner-Binder D, Damm TN, et al. Alfacalcidol reduces the number of fallers in a community-dwelling elderly population with a minimum calcium intake of more than 500 mg daily. J Am Geriatr Soc 2004;52:230–6.
[14] Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, et al. Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. Osteoporos Int 2002;13: 257–64.
[15] Ooms ME, Roos JC, Bezemer PD, van der Vijgh WJ, Bouter LM, Lips P. Prevention of bone loss by vitamin D supplementation in elderly women: a randomized double-blind trial. J Clin Endocrinol Metab 1995;80:1052–8.
[16] Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and

vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337:670–6.
[17] Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. J Bone Miner Res 2003;18:343–51.
[18] Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effect of Vitamin D on falls: a meta-analysis. JAMA 2004;291:1999–2006.
[19] Chen Z, Lohman TG, Stini WA, Ritenbaugh C, Aickin M. Fat or lean tissue mass: which one is the major determinant of bone mineral mass in healthy postmenopausal women? J Bone Miner Res 1997;12:144–51.
[20] Khosla S, Atkinson EJ, Riggs BL, Melton III LJ. Relationship between body composition and bone mass in women. J Bone Miner Res 1996;11:857–63.
[21] Visser M, Deeg DJ, Lips P. Longitudinal Aging Study Amsterdam low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): The Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab 2003;88:5766–72.
[22] Sahota O, Mundey MK, San P, Godber IM, Lawson N, Hosking DJ. The relationship between vitamin D and parathyroid hormone: calcium homeostasis, bone turnover, and bone mineral density in postmenopausal.
[23] Mezquita-Raya P, Munoz-Torres M, Luna JD, Luna V, Lopez-Rodriguez F, Torres-Vela E, et al. Relation between vitamin D insufficiency, bone density, and bone metabolism in healthy postmenopausal women. J Bone Miner Res 2001;16:1408–15.
[24] Baron RM, Kenny DA. The moderator variable distinction in social psychological research: conceptual strategic and statistical considerations. J Pers Soc Psychol 1986;51:1173–82.
[25] Genant HK, Grampp S, Gluer CC, Faulkner KG, Jergas M, Engelke K, et al. Universal standardization for dual energy X-ray absorptiometry: patient and phantom cross-calibration result. J Bone Miner Res 1994;10:1004–14.
[26] Sigurdsson G, Franzson L, Steingrimsdottir L, Sigvaldason H. The association between parathyroid hormone, vitamin D and bone mineral density in 70-year-old Icelandic women. Osteoporos Int 2000;11:1031–5.
[27] Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. Osteoporos Int 2002;13:187–94.
[28] Bischoff H, Stahelin H, Ursheler N, Ehrsam R, Vonthein R, Perring-Chiello P, et al. Muscle strength in the elderly: Its relation to vitamin D metabolites. Arch Phys Med Rehabil 1999;80:54–8.
[29] 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.
[30] Pluijm SMF, Visser M, Smit JH, Popp-Snijders C, Roos JC, Lips P. determinants of bone mineral density in older men and women: body composition as mediator. J Bone Miner Res 2001;16:2142–51.
[31] Arabi A, Tamim H, Nabulsi M, Maalouf J, Khalifé H, Choucair M, et al. Sex differences in the effect of body-composition variables on bone mass in healthy children and adolescents. Am J Clin Nutr 2004;80:1428–35.
[32] Duque G, Abdaimi K, Henderson J, Lomri A, Kremer R. vitamin D inhibits Fas ligand-induced apoptosis in human osteoblasts by regulating components of both the mitochondrial and Fas-related pathways. Bone 2004;35:57–64.
[33] O'Brien C, Gubrij I, Lin SC, Saylors R, Manolagas S. STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-kB ligand and stimulation of osteoclastogenesis by gp 130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. J Biol Chem 1999;274:19301–8.
[34] Fleet JC. Genomic and proteomic approaches for probing the role of vitamin D on health. Am J Clin Nutr 2004;80(Suppl 6):S1730–4.
[35] 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;338:777–83.
[36] Gloth III FM, Gundberg CM, Hollis BW, Haddad Jr JG, Tobin JD. Vitamin D deficiency in homebound elderly persons. JAMA 1995;274:1683–6.
[37] Hollis B. Comparison of commercially available I-based RIA methods for the determination of circulating 25-D. Hydroxyvitamin. Clin Chem 2000;46:1657–61.