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The relationship of 1,25-dihydroxyvitamin D and radial bone mass

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Summary

We assessed the association between radial bone mass and vitamin D considering age, estrogen sufficiency and thiazide use in 373 women, aged 20–80 years in a geographically defined area. We measured cortical bone mass of the radius, using photon absorptiometry, and serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, using preliminary chromatography and protein-binding assay.

We found that 1,25-dihydroxyvitamin D levels were higher in premenopausal women and postmenopausal women using estrogen replacement as compared to postmenopausal women ($P < 0.02$). Users of thiazide-based antihypertensive medications had significantly lower 1,25-dihydroxyvitamin D than their age-matched peers ($P < 0.02$). Dietary calcium intake was negatively associated with 1,25-dihydroxyvitamin D levels. Estimates of vitamin D intake from diet or sunlight were not associated with 1,25-dihydroxyvitamin D leveis; nor were levels of 1,25-dihydroxyvitamin D related to 25-hydroxyvitamin D levels.

1,25-Dihydroxyvitamin D was negatively and significantly associated with radial bone mass, contributing approximately 6% of the explained variability of bone mass measurements. Together age, body size, thiazide use and 1,25-dihydroxyvitamin D accounted for about 47% of the explained variability in radial bone mass measurement. 1,25-Dihydroxyvitamin D was not associated with bone mass in women currently using a thiazide medication.

Key words: Bone mass; 1,25-Dihydroxyvitamin D; Vitamin D; Thiazide antihypertensive; Dietary calcium

Introduction

It has been suggested that an age-related decline of quantity or efficiency of 1,25-dihydroxyvitamin D is of sufficient magnitude to contribute to negative calcium balance and potentially promote osteoporosis [1–3]. Renal function declines with age

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[4–5] and may be causally related to the observed impairment of intestinal calcium absorption with age [6–8]. One mechanism for diminished calcium absorption might be impaired 1-α hydroxylation of 25-hydroxyvitamin D, in the kidney, to its hormonally-active form, substantially impeding the promotion of a calcium-binding protein. Other mechanisms might include alteration in secondary factors related to the 1,25-dihydroxyvitamin D response to serum calcium [9].

This paper characterizes the factors associated with different levels of 1,25-dihydroxyvitamin D including measures of menopause and estrogen sufficiency, age, and use of thiazide medication in a large population of women who reside in a geographically-defined area. The levels of 1,25-dihydroxyvitamin D are related to radial bone mass, considering important factors previously observed to influence bone mass in this population [10,11].

Materials and Methods

Women from a single rural community who were aged 20–80 years were invited to participate in a study of bone mass. Women who were institutionalized in nursing homes or diagnosed with cancer, kidney disease, liver disease or other debilitating conditions which resulted in immobilization or loss of capacity to climb three stairs were excluded from the study recruitment (n = 108). To minimize effects of in- or

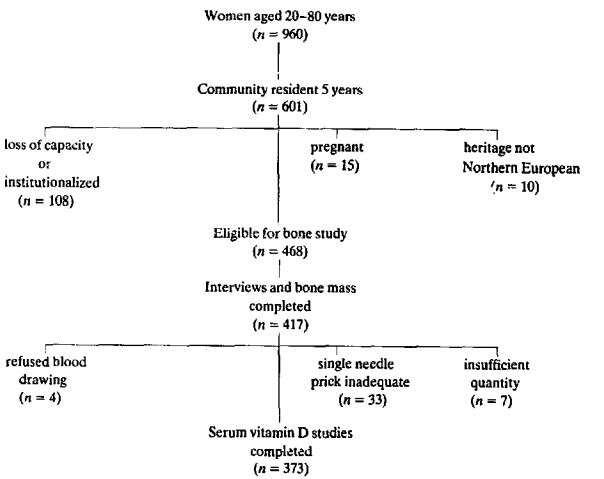

Fig. 1. Characterization of the population from which the study sample was secured.

out-migration, participants eligible for recruitment had to have resided in the community at least 5 years. Pregnant women ($n = 15$) were excluded from study participation, as were women who reported their heritage as being other than Northern European ($n = 10$). Four hundred sixty-eight women ($n = 468$) met these eligibility criteria and, of these, 89% ($n = 417$) participated in the study, as shown in Fig. 1. All measurements were collected during the two month period from June 1 to August 1.

Serum vitamin D and bone measurements

Serum vitamin D analysis was completed for 373 women which represents 80% of the eligible 468 women. Of the 417 study participants, four refused the blood drawing, seven had samples of insufficient quantity for analysis of all products specified in the protocol, and blood from 33 women could not be drawn using a single venipuncture probe specified in the protocol. Serum was drawn off from the centrifuged blood, aliquoted and stored in vials frozen at –20 °C for a time period of 1–63 days and then stored at –70 °C until analysis. The serum was protected from light oxidation and had a single thaw at time of analysis. Levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were quantified following extraction and preliminary chromatography by competitive protein binding assay using labelled calf thymus or radio-receptor assay as previously described [12].

Radial bone mass was measured using a Norland 278 Photon Absorptiometer (Norland Corporation; Fort Atkinson, WI) whose system is based on the method of Cameron [13,14]. Bone mass was measured at a site one-third the distance between the styloid process and the olecranon and expressed as the amount of bone mineral, in grams, relative to bone width, reported in centimeters. Our precision, expressed as the coefficient of variation from two measurements of 30 persons and relocation of the measurement site, was 2.7%.

Other variables

The interviewer observed labels of currently used nutrition supplement preparations for nutrient quantity; the participant was asked to recall the number, frequency and duration of use. A 15-item food frequency instrument was limited to selected foods high in calcium and vitamin D. Its time frame was the previous 2 years. Participants were asked to recall the consumption frequency of selected foods and shown color photographs to help quantify serving sizes as large, medium or small. Calcium values were assigned to foods using USDA Handbook #8. Vitamin D values were assigned to food based upon the McCance and Widdowson Food Composition Tables [15] or nutrient composition information to reflect food fortification. All nutrient values were transformed to common logarithms to normalize their distributions.

An interviewer-administered questionnaire was used to gather information about factors which might affect bone mineralization. That questionnaire was the information source for variables about menopause and sunlight exposure. Current medications were observed by the interviewers for drug name, dose and prescribed frequency of use, with actual use based on participant's self-report. For purposes of analysis, women were defined as postmenopausal if they reported no longer having menses because of surgery or natural history. If menopause was uncertain, a wo-

man was classified as postmenopausal if she did not experience menses in the 3 months prior to bone mass measurement and was not pregnant or lactating. Information was also reported about current use of estrogen replacement. Women were classified as users of thiazides if their current medications included any thiazide derivative.

Participants were measured for height and weight. Midarm circumference and triceps skinfolds were also measured and muscle area was calculated from these values [16]. Informed consent was obtained in accord with the ethical standards of the Committee on Human Experimentation of the University of Iowa.

Statistical analyses

Normality of variable distributions was evaluated from univariate analysis, and those variables whose distribution was highly skewed were either categorized or transformed. Regression analysis with adjustment for multiple comparisons was used to assess relationships between variables. Models which describe the relationship of serum vitamin D to bone mass were developed by assessing the bivariate relationships and then adding variables in a stepwise manner using multiple variable regression analyses to observe the nature and strength of that relationship [17].

Results

Correlates with 1,25-dihydroxyvitamin D

The mean values (± SD) for 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in women aged 20–80 years are shown in Table 1, according to 5-year intervals. There was no significant correlation ($r = 0.06$) between values for 25-hydroxyvitamin D

Table 1

Mean (± SD) values in 5-year age intervals of radial bone mass (g/cm^2), serum 25-hydroxyvitamin D (ng/ml) and 1,25-hydroxyvitamin D (pg/ml) of 373 women

	n	25-hydroxyvitamin D (mean ± SD)	1,25-dihydroxyvitamin D (mean ± SD)	Bone mass (mean ± SD)
20–24	6	29.8 ± 6.5	19.7 ± 5.0	0.74 ± 0.06
25–29	31	25.4 ± 7.9	25.1 ± 7.8	0.73 ± 0.05
30–35	30	23.6 ± 8.3	25.6 ± 9.4	0.76 ± 0.05
36–39	21	23.6 ± 7.3	26.5 ± 7.1	0.73 ± 0.05
40–44	28	23.7 ± 5.8	23.1 ± 8.3	0.76 ± 0.05
45–49	33	22.0 ± 6.8	24.0 ± 8.1	0.74 ± 0.06
50–54	42	28.0 ± 10.3	24.5 ± 10.2	0.72 ± 0.07
55–59	28	22.3 ± 6.9	23.6 ± 9.1	0.65 ± 0.10
60–64	34	24.3 ± 7.2	24.4 ± 7.2	0.64 ± 0.09
65–69	41	24.6 ± 8.4	23.4 ± 7.5	0.60 ± 0.11
70–74	45	22.1 ± 7.4	22.8 ± 5.8	0.55 ± 0.09
75–80	34	24.1 ± 8.8	20.9 ± 6.1	0.57 ± 0.10

and 1,25-dihydroxyvitamin D whether data were or were not stratified by age or menopause status.

The level of serum 1,25-dihydroxyvitamin D was influenced by menopausal status, including estrogen replacement, as well as age, of the woman. As shown in Table 2, premenopausal women or postmenopausal women taking estrogen replacement had a significantly greater mean 1,25-dihydroxyvitamin D level than estrogen deficient postmenopausal women. To confirm this association, the data were limited to values of 1,25-dihydroxyvitamin D observed in women aged 30–55 years. The mean (± SEM) 1,25-dihydroxyvitamin D levels were similar in premenopausal women (25.5 pg/ml ± 0.8, $n = 112$) and postmenopausal women taking estrogen (26.3 pg/ml ± 1.1, $n = 12$), values which were significantly different ($P < 0.05$) than those postmenopausal women, aged 30–55 years, who were not taking estrogen replacement (22.9 pg/ml ± 1.2, $n = 55$).

There was a limited association with 1,25-dihydroxyvitamin D and age among postmenopausal women. There was a weak trend for postmenopausal women, aged 70–80 years, to have lower mean 1,25-dihydroxyvitamin D level than those postmenopausal women less than 70 years who were not taking an estrogen supplement ($P < 0.11$).

Postmenopausal women currently using thiazide antihypertensive medications ($n = 53$) had significantly lower 1,25-dihydroxyvitamin D values than postmenopausal non-users of thiazides (21.4 ± 1.1 pg/ml vs. 24.2 ± 0.4 pg/ml, $P < 0.02$), before and after age and adjustment.

Estimates of dietary calcium intake were associated with 1,25-dihydroxyvitamin D. Calcium intake, as supplement alone ($r = 0.15$), as diet alone ($r = 0.12$), and the combination of diet and supplement ($r = 0.20$), were significantly correlated with 1,25-dihydroxyvitamin D levels. The correlations, though not high, were consistent in direction, suggesting that as calcium intake levels rose, levels of 1,25-dihydroxyvitamin D fell. Estimates of precursor forms of vitamin D available from food, vitamin D supplement use, the combination of diet and supplement, or degree of sunlight exposure were not related to the level of 1,25-dihydroxyvitamin D.

Table 2

The comparison of 1,25-dihydroxyvitamin D (pg/ml) levels in premenopausal women vs. postmenopausal women who are described according to current estrogen replacement status

Menopausal status	Number	Mean ± SEM	Menopausal status	Number	Mean ± SEM	*P*-value
Premenopausal	154	25.05 ± 0.69	Postmenopausal			
			overall	263	23.1 ± 0.50	0.02
			replacement[a]	20	24.6 ± 1.80	NS
			w/o replacement[b]	243	22.9 ± 0.52	0.01

[a] With current estrogen replacement

[b] Without estrogen replacement.

Bone mass and 1,25-dihydroxyvitamin D levels

Because we observed differences in 1,25-dihydroxyvitamin D levels based on menopausal status, the relationships between 1,25-dihydroxyvitamin D and bone mass are reported according to the menopausal status of the women. Furthermore, we evaluated the relationship singly and after considering the influence of other factors we had previously observed to be associated with bone mass measurement as presented in Table 3 [10,11].

In bivariate analyses, the levels of 1,25-dihydroxyvitamin D were significantly and negatively associated with bone mass in premenopausal women ($r = -0.23$, $P = 0.0054$) and postmenopausal women ($r = -0.20$, $P = 0.0048$).

Values of 1,25-dihydroxyvitamin D were negatively and significantly associated with radial bone mass measurement among postmenopausal women after considering previously reported significant associations [11]. A multiple variable model, including age, body size (humeral muscle area), thiazide use and serum 1,25-dihydroxyvitamin D was tested. Levels of 1,25-dihydroxyvitamin D were negatively and significantly associated with bone mass, after considering the contribution of the other variables in the model. The complete model explained approximately 46–47% of the variability associated with bone mass measurements. After considering the influence of the other factors, 1,25-dihydroxyvitamin D accounted for approximately 5% of the explained variability (see Table 3).

Table 3

Multivariate models which characterize the association between radial bone mass and 1,25-dihydroxyvitamin D in three groups of women restricted by menopausal status, estrogen replacement and thiazide use

	beta coefficients ± SEM	*P*-value
A. *Postmenopausal women without estrogen replacement (n = 223)*		
Intercept	1.0590 ± 0.0487	0.0001
Age (years)	−0.0071 ± 0.0005	0.0001
Muscle area (cm^2)	0.0012 ± 0.0003	0.0002
Thiazide (yes, no)	−0.0190 ± 0.0452	0.6800
1,25-Dihydroxyvitamin D (pg/ml)	−0.0036 ± 0.0009	0.0001
Interaction of thiazide and $1,25(OH)_2D$	0.0034 ± 0.0020	0.1000
B. *Postmenopausal women without estrogen replacement or current thiazide use (n = 171)*		
Intercept	1.0650 ± 0.0561	0.0001
Age (years)	−0.0067 ± 0.0007	0.0001
Muscle area (cm^2)	0.0013 ± 0.0004	0.0015
1,25-Dihydroxyvitamin D (pg/ml)	−0.0036 ± 0.0009	0.0001
C. *Postmenopausal women without estrogen replacement and who currently use thiazide (n = 52)*		
Intercept	0.0105 ± 0.0928	0.0001
Age (years)	−0.0061 ± 0.0012	0.0001
Muscle area (cm^2)	0.0011 ± 0.0005	0.0404
1,25-Dihydroxyvitamin D (pg/ml)	−0.0012 ± 0.0016	0.4800

The full model included an important interaction term between thiazide use and 1,25-dihydroxyvitamin D, indicating that the magnitude of the association between bone mass and 1,25-dihydroxyvitamin D differed according to thiazide status (see Table 3, part A). To more fully explore the relationship, bone mass and 1,25-dihydroxyvitamin D levels were evaluated in estrogen-deficient postmenopausal women according to current thiazide use. Among women not using thiazide, serum 1,25-dihydroxyvitamin D was associated negatively with bone mass (see Table 3, parts B and C) after considering other explanatory factors. However, among the 52 thiazide users, the 1,25-dihydroxyvitamin D measurement was not significantly related to bone mass after considering other explanatory factors.

Among premenopausal women, the influence of 1,25-dihydroxyvitamin D accounted for 5% of the explained variability in a model that had included the influence of alcohol consumption, age of first pregnancy and calcium intake. This model explained approximately 19% of the variability of radial bone mass measurements observed in premenopausal women, and was developed in previous work of associating life style factors with maximal bone mass [10].

Discussion

Because decrease in bone mass with age is an aggregation of several processes [18], studies of 1,25-dihydroxyvitamin D are important for understanding those factors which may influence calcium absorption and calcium metabolism with aging. We have described some relationships between 1,25-dihydroxyvitamin D, bone mass, estrogen status, age, and thiazide use, cross-sectionally, in a large population of non-institutionalized women from a geographically defined area across a substantial age range.

Several hypotheses have been advanced to describe the contribution of 1,25-dihydroxyvitamin D to bone loss and osteoporosis. At menopause, bone tissue may become more sensitive to mobilization by parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [19]. This could result in suppression of PTH and 1,25-dihydroxyvitamin D production, decrease in calcium absorption and increased calcium turnover from bone. Another hypothesis suggests that declining renal function due to aging may impede appropiate 1-α-hydroxylation, including parathyroid phosphate response [1,20].

Our data suggests that decline in the measurable quantity of 1,25-dihydroxyvitamin D may be, in part, associated with estrogen status, with lower 1,25-dihydroxyvitamin D levels more likely to be associated with estrogen deficiency. Increased levels of 1,25-dihydroxyvitamin D_3 have been reported in studies of estradiol administration of postmenopausal women [16,21] and in premenopausal women contrasted to postmenopausal women [22]. Additionally, a finding of lower 1,25-dihydroxyvitamin D levels in menopause without estrogen replacement is not inconsistent with the animal literature which suggests that estrogen deficiency may decrease 1-α-hydroxylase activity directly [23,24] or indirectly via the parathyroid gland [25]. In contrast, a prospective study of 1,25-dihydroxyvitamin D and vitamin

D binding protein showed no association with the hormone and carrier with postmenopausal bone loss; however, the small sample size would have limited the investigators to a significant observation only when change in 1,25-dihydroxyvitamin D was greater than 25% [26]. Though the basis for an estrogen effect remains unclear, the apparent consistency of this observation in animal studies, clinical populations and a geographically defined general population suggests that association is not artifactual.

There are inconsistencies among studies describing levels of circulating 1,25-dihydroxyvitamin D in older persons, an issue of importance since decline in calcium absorption with aging may contribute to bone loss with age. Some studies indicate reduction in mean 1,25-dihydroxyvitamin D levels [1–3], other reports suggest minimal age-related reduction [21,27–30]. Some of the inconsistencies in findings may be attributable to differences in criteria for study selection, age of the study subjects, numbers of patients evaluated, and assay variability. Initial examination of our data suggested that 1,25-dihydroxyvitamin D levels were lower in older members of this female population. However, closer examination of the findings suggested menopausal change was a potent explanatory factor of 1,25-dihydroxyvitamin D levels. Thus, when postmenopausal women are compared with premenopausal women, there was an observed effect on 1,25-dihydroxyvitamin D levels. However, when one examined the levels among postmenopausal women without estrogen replacement, age-associated decline is less prominent. We, like Tsai et al. [2], observed a modest decline in 1,25-dihydroxyvitamin D levels among postmenopausal women (without current estrogen replacement) which is more pronounced after approximately age 70. This is consistent with the reports of decreases in calcium absorption with aging, especially after age 70 [1,6,7].

We demonstrated a negative and significant association between bone mass and 1,25-dihydroxyvitamin D values in a geographically defined population. Even following adjustment for age, hormonal vitamin D levels rose, cross-sectionally, as bone mass values declined. Greater 1,25-dihydroxyvitamin D levels associated with a lower bone mass is not indicative of a primary reduction in 25-hydroxyvitamin D–1-α-hydroxylase. This is consistent with the findings of Riggs et al. [9] and Sorensen et al. [3], whose experimental administration of parathyroid hormone to osteoporotic and age-comparable normal postmenopausal women did not support the hypothesis that patients with osteoporosis have a primary reduction in 25-hydroxyvitamin D–1-α-hydroxylase reserve capacity. Prince et al. [25] observed similar renal reserve synthetic capacity in comparing postmenopausal women to premenopausal women. However, the negative relationship with bone does suggest that either calcium absorption response in the gut is not efficient, that calcium excretion is excessive, that a new PTH-vitamin D set point has been generated which is associated with estrogen deficiency [31], or that there is potent stimulation of bone resorption independent of calcium absorption.

Though bone mass decline was associated with a rise in 1,25-dihydroxyvitamin D levels, this finding is not observed among postmenopausal women using thiazide anti-hypertensive medication. In that group, there is no relationship between bone mass and 1,25-dihydroxyvitamin D values. Furthermore, these thiazide-using post-

menopausal women, as a group, had lower 1,25-dihydroxyvitamin D values. Previous investigators have reported that thiazide use increases renal conservation of calcium and use of thiazide medication has been associated with greater bone mass in both women [11] and men [32].

We cannot address the issue whether the cause of bone loss and fracture in osteoporosis is associated with decreased bone formation or increased bone resorption. Our study is cross-sectional in nature and measures radial bone mass. In spite of these limitations, evidence from this examination of 1,25-dihydroxyvitamin D levels and bone mass is consistent with increased bone resorption rather than decreased osteoblast formation in bone loss of aging, particularly in women more likely to be estrogen deficient. The evidence includes the observation that greater vitamin D levels were associated with lower bone mass except in women using a calcium-conserving medication, thiazide. Furthermore, calcium intake was also negatively associated with 1,25-dihydroxyvitamin D levels.

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