

Relationship between Vitamin D, Parathyroid Hormone, and Bone Health

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Context: There is a controversy regarding the definition of vitamin D insufficiency as it relates to bone health.

Objective: The objective of the study was to examine the evidence for a threshold value of serum 25-hydroxyvitamin D (25OHD) that defines vitamin D insufficiency as it relates to bone health.

Design and Participants: This was a cross-sectional analysis of baseline data in 488 elderly Caucasian women, mean age 71 yr, combined with a literature review of 70 studies on the relationship of serum PTH to serum 25OHD.

Setting: The study was conducted in independent-living women in the midwest United States.

Main Outcome Measure: The relationship between serum 25OHD, serum PTH, and serum osteocalcin and 24-h urine N-telopeptides was evaluated.

Results: Serum PTH was inversely correlated with serum 25OHD ($r = -0.256$, $P < 0.0005$), but no threshold as defined by suppression of serum PTH was found within the serum 25OHD range 6–60 ng/ml (15–150 nmol/liter). However, in contrast, there was a threshold for bone markers, serum osteocalcin and urine N-telopeptides, that increased only below a serum 25OHD of approximately 18 ng/ml (45 nmol/liter). Calcium absorption was not correlated with serum PTH and serum 25OHD, and no threshold was found. A literature review of 70 studies generally showed a threshold for serum PTH with increasing serum 25OHD, but there was no consistency in the threshold level of serum 25OHD that varied from 10 to 50 ng/ml (25–125 nmol/liter).

Conclusions: Vitamin D insufficiency should be defined as serum 25OHD less than 20 ng/ml (50 nmol/liter) as it relates to bone. (*J Clin Endocrinol Metab* 96: 0000–0000, 2011)

Serum 25-hydroxyvitamin D (25OHD) is considered to be the best indicator of overall vitamin D status of an individual. Severe vitamin D deficiency [serum 25OHD < 10 ng/ml (25 nmol/liter)] is associated with malabsorption of calcium, secondary hyperparathyroidism, leading to increased bone resorption, accelerated cortical bone loss, and increased fractures. Very low levels of serum 25OHD can also cause osteomalacia.

The definition of vitamin D insufficiency is less clear-cut. The World Health Organization originally defined it

as a serum 25OHD less than 20 ng/ml (50 nmol/liter) (1). There have been many publications describing the relationship of serum PTH to serum 25OHD, and some but not all papers defined a level of serum 25OHD at which serum PTH levels decreased and reached a plateau. This threshold has been used to define vitamin D insufficiency. Recent influential reviews stated that a serum 25OHD level of 30 ng/ml (75 nmol/liter) should be used as the defining level for vitamin D insufficiency because in the studies analyzed in those reviews, serum PTH showed a

plateau at serum 25OHD of approximately 30 ng/ml (75 nmol/liter) (2, 3). Clinical chemistry laboratories now define vitamin D insufficiency as a serum 25OHD level less than 30 ng/ml (75 nmol/liter) and normal values as greater than 30 ng/ml (75 nmol/liter) on their report forms and have adopted this view unquestioningly. This has led to an epidemic of vitamin D insufficiency, and it has been suggested that 1 billion people worldwide have vitamin D deficiency or insufficiency (3).

A critical question, however, is what levels of serum PTH are harmful to bone because it was assumed that elevated serum PTH was related to bone loss (3). Independent of vitamin D metabolism, there is an age-related decrease in calcium absorption that probably contributes to secondary hyperparathyroidism (4).

We examined the relationship between serum PTH and serum 25OHD in our own data for evidence of a threshold or plateau and also examined the relationship between bone markers and serum 25OHD. A literature review of studies relating serum PTH and serum 25OHD was performed to determine whether serum PTH showed a plateau or was maximally suppressed in relation to serum 25OHD and whether we could find supporting evidence for a threshold value of serum 25OHD of 30 ng/ml (75 nmol/liter) (3).

Materials and Methods

Literature review

We did an extensive search on MEDLINE using terms vitamin D (title/abstract) or 25-hydroxyvitamin D (title/abstract) or cholecalciferol (title/abstract) and parathyroid hormone (title/abstract) or PTH (title/abstract). This search retrieved 4092 studies that were conducted from January 1988 to June 2010. We then screened abstracts that assessed the relationship between serum 25OHD and serum PTH and stated a level of serum 25OHD at which serum PTH plateaus and/or is maximally suppressed either by statistical methods or by interpretation from graphical depictions. There were a total of 70 studies that showed a relationship between serum 25OHD and PTH (5–57, 59–66, 82, 83). Seven of these studies were counted twice: one study mentioned two thresholds based on different statistical models (6), three studies found different thresholds based on participants' age (17, 62, 82), one found different thresholds based on race (23), and two studies found different thresholds based on gender (28, 48).

Study subjects

Information on our study subjects was derived from the baseline data of a 3-yr intervention trial [Sites Testing Osteoporosis Prevention or Intervention (STOP IT)] in elderly women, described previously in detail (58). They were 488 healthy independent women living at home. Excluded were women who had any disease or took medication that affected calcium, vitamin D metabolism, and bone.

Dietary intake of calcium and vitamin D was collected from 7-d food diaries.

Biochemical measurements

Serum 25OHD was measured by a competitive binding assay after chromatographic separation and purification on Sep-Pak cartridges (Waters Associates, Milford, MA). The limit of detection was 5 ng/ml, and the interassay variation was 5%. Serum PTH was measured as the intact molecule 1–84 by immunometric assay (Nichols Institute, San Juan Capistrano, CA). The limit of detection was 1 pg/ml, and the interassay coefficient of variation was 3.5%. Serum osteocalcin was measured with RIA (INCSTAR Corp., Stillwater, MN); the limit for the assay was 0.78 ng/ml, and the interassay coefficient of variation was 4.5%. Twenty-four hour urine N-telopeptides (NTx/Cr) were measured by an ELISA (INCSTAR). Calcium absorption was measured fasting; 100 mg elemental calcium was mixed with 5 μ Ci Ca^{45} in 200 ml distilled water. Blood samples were collected at 1, 2, and 3 h for estimation of Ca^{45} , and calcium absorption was expressed as the percentage absorbed per liter after 2 h.

Statistical methods

All baseline demographic and clinical variables were continuous and presented as mean and SD, and all bivariate associations were evaluated by Pearson's correlation (r). Four multivariate linear regression analyses were conducted to assess the association between serum 25OHD and serum PTH, serum osteocalcin, NTx/Cr, and calcium absorption after adjusting for age, body mass index (BMI), or weight, calcium intake, and serum creatinine or glomerular filtration rate (GFR). An additional covariate 1,25-dihydroxyvitamin D [1,25(OH)₂D] was added to the calcium absorption analysis. The decision to use BMI *vs.* body weight and serum creatinine *vs.* GFR was determined by the largest bivariate association with the outcome variable of interest. Calcium absorption was corrected for body size using a 15% of total body weight (67), BMI was used for serum osteocalcin and urine NTx/Cr and total body weight used for serum PTH. Serum creatinine was used for calcium absorption, serum PTH, and urine NTx/Cr, whereas GFR was used for serum osteocalcin. The unique effect of serum 25OHD after adjustment for covariates is presented by the semipartial correlation (sr) and the squared sr (sr^2). The sr^2 is interpreted similarly to model R^2 except that instead of overall variance explained, sr^2 indicates the amount of variance in the outcome that is explained uniquely by serum 25OHD.

For each outcome variable, subjects with missing data and those with outlying (univariate or multivariate) data were identified and removed before analysis. Because the sample size was large, only clearly disconnected data points with z greater than 3.29 were considered univariate outliers. Data were considered multivariate outliers with Mahalanobis distance at $P < 0.001$. Furthermore, square root transformations were performed on serum PTH, and natural log transformations were performed on vitamin D intake and urine NTx/Cr (uNTx/Cr) to normalize the distribution and reduce the influence of outliers.

To assess whether a threshold within serum 25OHD exists, nonlinear and piecewise linear regression were used. These analyses were conducted only for outcomes significantly related to serum 25OHD, as indicated by multivariate linear regression analyses described above. For all piecewise analysis, a single threshold was hypothesized. The search for the threshold began using the constrained nonlinear regression procedure with a serum 25OHD threshold of 20 ng/ml and starting values (*i.e.* the intercept and slopes) based on the results of the bivariate linear regression. Because the convergence of nonlinear regression is

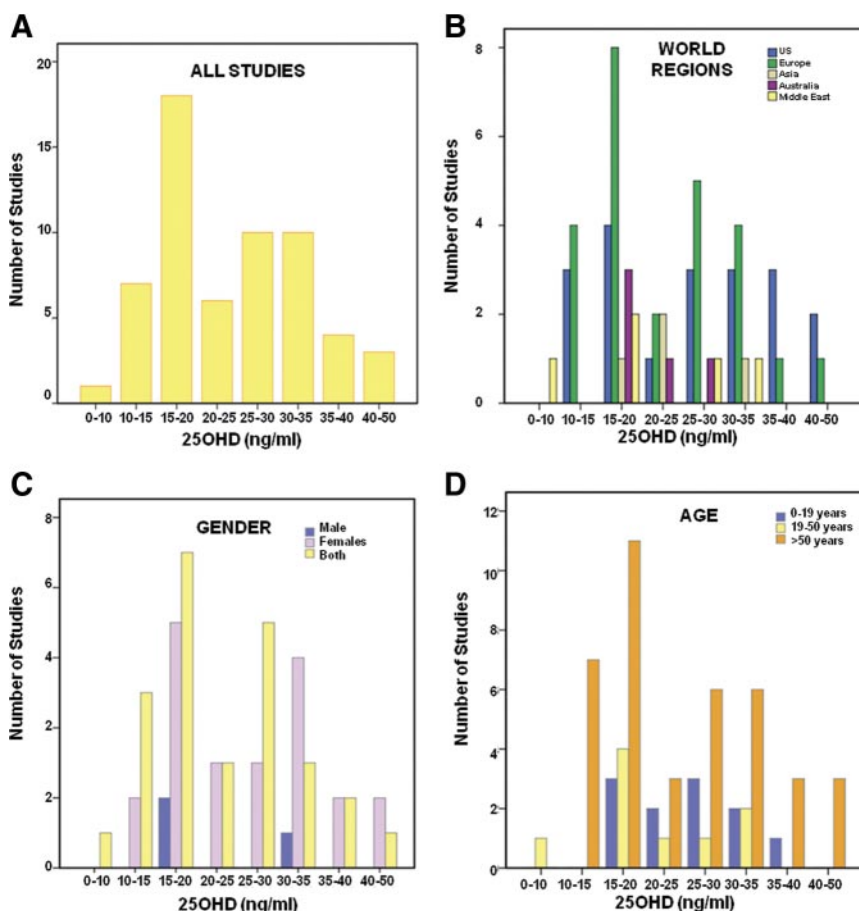


FIG. 1. Various levels of serum 25OHD (nanograms per milliliter) at which serum PTH (picograms per milliliter) plateaus and/or is maximally suppressed. A, Data here are presented from 59 studies (not shown, eight studies in which serum PTH continually decreased with increasing serum 25OHD and three studies that found no relationship between serum 25OHD and serum PTH). B–D, Location, age, and gender of study populations included in these studies.

based heavily on the particular starting values, a series of piecewise linear regression analyses followed using threshold values 10 ng/ml above and below (at 0.5 increments) the converged serum 25OHD threshold value. The optimal threshold value was chosen based on adjusted R^2 , the F statistic, model SE, and the t value and associated P value for the threshold variable (68). Overall model fit for all regression analyses (both linear and piecewise linear) was assessed by the distribution of residuals and added-variable plots.

Finally, the interaction effect between calcium intake and 25OHD on serum PTH was assessed using a linear regression analysis. Unless indicated otherwise, two-tailed $P < 0.05$ was considered statistically significant. All analyses were performed using IBM SPSS Statistics (version 18.0.2; SPSS Inc., Chicago, IL).

Results

Literature review

There was a large variability in the reported level of serum 25OHD at which serum PTH reached a plateau or was maximally suppressed (Fig. 1A): 10 ng/ml or less in one study (5), 10–15 ng/ml in seven studies (6–11, 62), 15–20 ng/ml in 18 studies (12–27, 48, 82), 20–25 ng/ml in

six studies (28–33), 25–30 ng/ml in 10 studies (6, 34–39, 41–43), 30–35 ng/ml in 10 studies (17, 23, 44–50, 83), 35–40 ng/ml in four studies (51–54), 40–50 ng/ml in three studies (55–57), no plateau in eight studies, (28, 40, 59–64), and no relationship at all between serum 25OHD and serum PTH in three studies (65, 66, 82).

Subgroup analysis

Subgroup analysis based on geographical location revealed that most of the studies were conducted in the United States and Europe (Fig. 1B) with some from the Middle East, Australia, and Asia. Subgroup analysis based on age and gender of study population is shown in Fig. 1, C and D, respectively. A majority of studies included both men and women or women alone, and three studies included men alone. Most studies (65%) had data on older populations (>50 yr age).

Study subjects

The baseline characteristics of our own study subjects are shown in Table 1. The mean age of subjects was 71.4 yr (range 65–78 yr).

Correlations between serum PTH, serum osteocalcin, 24 h urine NTx/Cr, and serum 25OHD

The results of the multivariate linear regression analysis are presented in Table 2. After adjusting for baseline demo-

TABLE 1. Baseline characteristics of study population

Variables	Mean	SD
n = 488		
Age (yr)	71.4	3.6
BMI	26.8	4.7
Blood urea nitrogen (mg/dl)	15.3	4.3
Serum creatinine (mg/dl)	0.87	0.16
Serum calcium (mg/dl)	9.3	0.3
Serum PTH (pg/ml)	37.2	14.8
Serum 25OHD (ng/ml)	31.3	10.5
Serum 1,25(OH) ₂ D (pg/ml)	34.4	8.0
Serum osteocalcin (ng/ml)	3.8	1.3
24-h urine NTX (nmol BCE/mmol Cr)	50.8	26.7
Calcium absorption (% actual dose/liter)	2.6	0.7
GFR (mL/min per 1.73 m ²)	71.8	19.3
Vitamin D intake (μg/d)	3.5	2.0
Calcium intake (mg/d)	739	310

NTX, 24-h urine N-telopeptide/creatinine; BCE, bone collagen equivalent.

TABLE 2. Multivariate linear regression results

	Slope	SE	P	95% CI for slope	
				Lower	Upper
Square root-transformed serum PTH (n = 478) ^a					
Serum 25OHD (ng/ml)	-0.0311	0.0050	<0.0005	-0.0410	-0.0212
Age (yr)	0.0009	0.0128	0.9420	-0.0242	0.0261
Weight (kg)	0.0168	0.0038	<0.0005	0.0094	0.0242
Calcium intake (mg/d)	0.0003	0.0002	0.0820	-0.0006	0.0000
Serum creatinine (mg/dl)	1.0733	0.2922	0.0003	0.4992	1.6475
Constant	4.9945	0.9964			
Serum osteocalcin (n = 475) ^b					
Serum 25OHD (ng/ml)	-0.0146	0.0060	0.0153	-0.0263	-0.0028
Age (yr)	-0.0026	0.0154	0.8867	-0.0328	0.0276
BMI (kg/m ²)	-0.0691	0.0125	<0.00005	-0.0938	-0.0445
Calcium intake (mg/d)	0.00002	0.0002	0.9263	-0.0004	0.0004
GFR (ml/min per 1.73 m ²)	-0.0053	0.0029	0.0715	-0.0110	0.0005
Constant	6.6426	1.2092			
Natural log-transformed uNTx/Cr (n = 475) ^c					
Serum 25OHD (ng/ml)	-0.0062	0.0023	0.0065	-0.0106	-0.0017
Age (yr)	0.0002	0.0058	0.9697	-0.0111	0.0115
BMI (kg/m ²)	-0.0220	0.0047	<0.0005	-0.0312	-0.0127
Calcium intake (mg/d)	-0.0001	0.0001	0.3139	-0.0002	0.0001
Serum creatinine (mg/dl)	-0.4620	0.1309	0.0005	-0.7193	-0.2048
Constant	5.0372	0.4439			
Calcium absorption (n = 470) ^d					
Serum 25OHD (ng/ml)	0.0036	0.0031	0.2472	-0.0025	0.0096
Age (yr)	-0.0214	0.0078	0.0066	-0.0367	-0.0060
15% body weight (kg)	-0.0667	0.0154	<0.0005	-0.0970	-0.0364
Calcium intake (mg/d)	-0.0002	0.0001	0.0227	-0.0004	0.00003
Serum creatinine (mg/dl)	-0.1276	0.1811	0.4814	-0.4835	0.2283
Serum 125OH2D (pg/ml)	0.0194	0.0038	<0.0005	0.0120	0.0268
Constant	4.2719	0.6368			

^a Overall model ($F_{5,472} = 21.820$; $P < 0.0005$; adjusted $R^2 = 0.179$).

^b Overall model ($F_{5,469} = 8.034$; $P < 0.0005$; adjusted $R^2 = 0.069$).

^c Overall model ($F_{5,469} = 9.258$; $P < 0.0005$; adjusted $R^2 = 0.080$).

^d Overall model ($F_{5,467} = 8.327$; $P < 0.0005$; adjusted $R^2 = 0.072$).

graphic and clinical variables, serum 25OHD was inversely associated with serum PTH ($sr = -0.256$, $P = 0.0066$; $sr^2 = 0.066$), serum osteocalcin ($sr = -0.108$, $P = 0.015$; $sr^2 = 0.012$), and urine NTx/Cr ($r = -0.120$, $P = 0.007$; $sr^2 = 0.014$).

In addition, increasing body weight or BMI was associated with an increase in serum PTH and a decrease in urine NTx/Cr and serum osteocalcin and calcium absorption (all $P < 0.0005$). Serum creatinine correlated with increased serum PTH and decreased urine NTx/Cr (both $P < 0.0005$).

Identification of serum 25OHD threshold for serum PTH, bone markers, and role of calcium intake

The derivations of regression equations are provided below.

Piecewise linear regression equations

Generic form is: $\hat{Y} = \beta_0 + \beta_1(25OHD) + \beta_2(25OHD - \text{threshold})\text{dummy}$ for threshold.

Serum osteocalcin (Fig. 2C)

Predicted serum osteocalcin = $6.163 - 0.141(25OHD) + 0.141(25OHD - 17)$ (dummy), where the dummy is 1 if 25OHD is 17 or greater and the dummy is 0 if 25OHD is less than 17.

However, as you can see in Fig. 2C, the intercept is dependent on whether the individual has serum 25OHD before or after the threshold (*i.e.* intercept before is 6.163 and intercept after is 3.765). Thus, to provide the reader with this type of information, the exact same equation as above is written as: predicted serum osteocalcin = $[6.163 - (0.141)(17)$ (dummy)] $- 0.141(25OHD) + (0.141)(17)$ (dummy), where the dummy is 1 if 25OHD is 17 or greater and the dummy is 0 if 25OHD is less than 17.

Natural log uNTx/Cr (Fig. 2D)

Predicted LN uNTx/Cr = $4.989 - 0.068(25OHD) + 0.068(25OHD - 17.5)$ (dummy), where the dummy is 1 if 25OHD is 17.5 or greater and the dummy is 0 if 25OHD is less than 17.5.

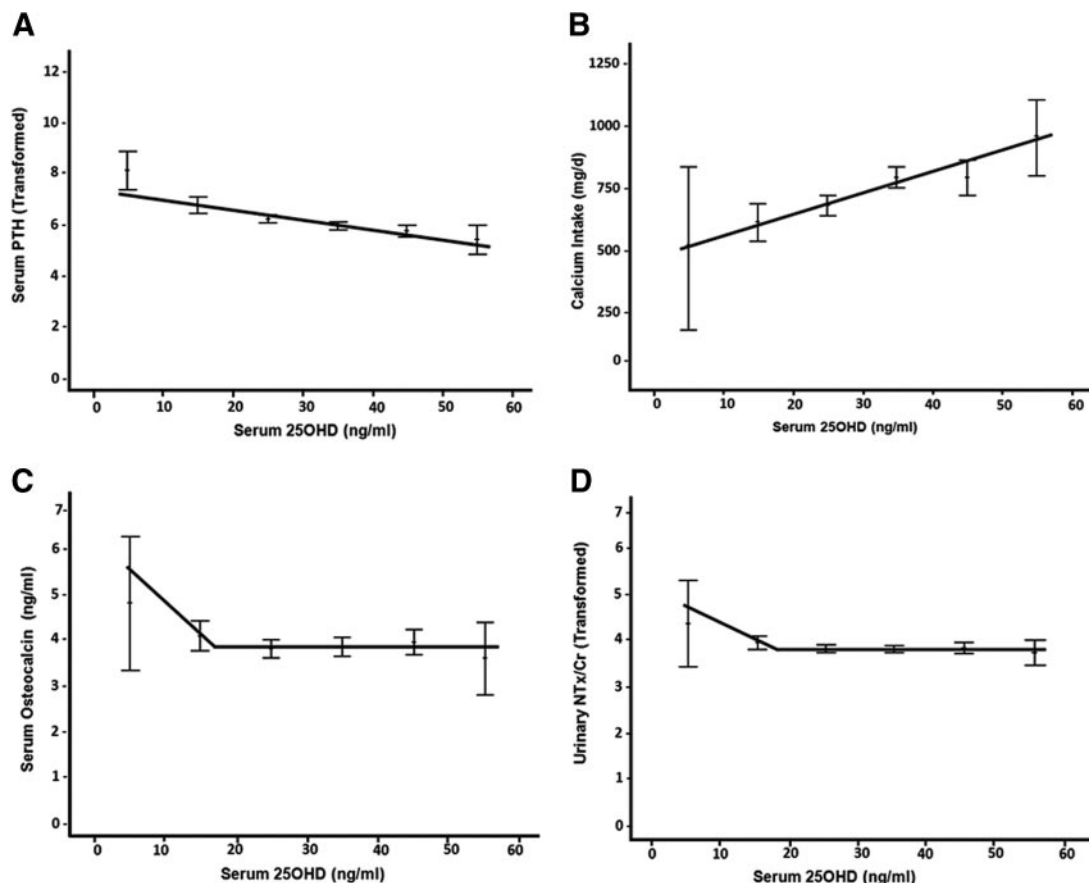


FIG. 2. Relationship between serum 25OHD, serum PTH, bone markers, and calcium intake. A, Serum PTH was inversely correlated with serum 25OHD ($r = -0.338$, $P < 0.0005$) and continually decreasing with increasing serum 25OHD. Nonlinear regression failed to identify a threshold for serum PTH. Calcium intake was significantly positively correlated with serum 25OHD ($r = 0.295$, $P < 0.0005$; B). For serum osteocalcin, nonlinear regression converged on a threshold of 17.034 ng/ml (95% CI 12.61–21.46). Piecewise linear regression indicated an optimal threshold of 17 ng/ml (42.4 nmol/liter; $P = 0.007$; adjusted $R^2 = 0.016$). Before this threshold value, statistically significant decreases in serum osteocalcin were indicated with increases in serum 25OHD; however, this effect plateaued after threshold (C). For uNTx/Cr, nonlinear regression converged on a threshold of 18.0 ng/ml (95% CI 14.347–21.653), with an optimal threshold of 17.5 ng/ml (43.7 nmol/liter; $P = 0.0002$; adjusted $R^2 = 0.036$). Similarly, a plateau effect was evidenced after the threshold (D).

Similar to above, the intercept is dependent on whether the individual has serum 25OHD before or after the threshold; thus, the predicted LN uNTx/Cr = $[4.989 - (.068)(17.5) (\text{dummy})] - 0.068 (25\text{OHD}) + (.068)(17.5) (\text{dummy})$, where the dummy is 1 if 25OHD is 17.5 or greater and the dummy is 0 if 25OHD is less than 17.5.

Statistically significant thresholds (*i.e.* change in slope before and after threshold) were indicated for bone markers at serum 25OHD levels less than 18 ng/ml (45 nmol/liter). For serum osteocalcin, nonlinear regression converged on a threshold of 17.034 ng/ml [95% confidence interval (CI) 12.61–21.46]. Piecewise linear regression indicated an optimal threshold of 17 ng/ml (42.4 nmol/liter; $P = 0.007$; adjusted $R^2 = 0.016$). Before this threshold value, statistically significant decreases in serum osteocalcin were indicated with increases in serum 25OHD; however, this effect plateaued after threshold (Fig. 2C). For natural log-trans-

formed uNTx/Cr, nonlinear regression converged on a threshold of 18 ng/ml (95% CI 14.347–21.653), with an optimal threshold of 17.5 ng/ml (43.7 nmol/liter; $P = 0.0002$; adjusted $R^2 = 0.036$). Similarly, a plateau effect was evidenced after the threshold (Fig. 2D). Finally, nonlinear regression failed to identify a threshold for square root-transformed serum PTH (Fig. 2A). Slopes, SES, and 95% CIs of the piecewise regression analyses are provided in Table 3.

There was a significant correlation between serum PTH and calcium intake ($r = -0.181$, $P < 0.0005$). Serum 25OHD was positively correlated with total calcium intake ($r = 0.303$, $P < 0.0005$; Fig. 2B) and vitamin D intake ($r = 0.289$, $P < 0.0005$). Thus, the subjects with a higher total calcium intake had higher serum 25OHD levels. However, the results of a linear regression analysis indicated that after controlling for main effects, the interaction between the calcium intake and serum 25OHD had no significant ($P = 0.116$) effect on serum PTH.

TABLE 3. Piecewise linear regression results^a

	Slope	SE	P	95% CI for slope	
				Lower	Upper
Serum osteocalcin (n = 475)					
Slope change at threshold	0.141	0.052	0.0070	0.039	0.242
Before threshold (serum 25OHD <17)	-0.141	0.049	0.0040	-0.237	-0.045
After threshold (serum 25OHD ≥17)	0.000	0.007	0.9290	-0.013	0.120
Intercept before threshold	6.163	0.792			
Natural log uNTx/Cr (n = 475)					
Slope change at threshold	0.068	0.018	0.0002	0.033	0.103
Before threshold (serum 25OHD <17.5)	-0.068	0.017	0.00006	-0.101	-0.035
After threshold (serum 25OHD ≥17.5)	0.000	0.003	0.8980	0.005	-0.104
Intercept before threshold	4.989	0.277			

^a The effects in piecewise linear regression are additive. Thus, whereas the slopes before threshold were -0.141 and -0.068, for serum osteocalcin and uNTx/Cr, respectively, the slopes after the threshold are 0 for both. To calculate the slope after the threshold, the slope change at the threshold must be considered. That is, the calculation of slope after the threshold for serum osteocalcin is the sum of the slope before the threshold and the slope change at the threshold (i.e. -0.141 + 0.141 = 0). This zero slope, or plateau, after threshold for serum osteocalcin is evidenced clearly when evaluating Fig. 2C. For urine NTx/Cr, the calculation for slope after threshold is identical (-0.068 + 0.068 = 0; Fig. 2D).

Serum 25OHD, 1,25(OH)₂D, and calcium absorption

The results of the multivariate linear regression analyses are presented in Table 2. There was no significant association between serum 25OHD and calcium absorption for both unadjusted and adjusted models ($r = 0.068$, $P = 0.071$; $sr = 0.050$, $P = 0.247$, respectively; Fig. 3A shows adjusted model) and thus no threshold. There was a positive correlation between calcium absorption and serum 1,25(OH)₂D for both unadjusted and adjusted models ($r = 0.266$; $P < 0.0005$ and $sr = 0.224$; $P < 0.0005$; $sr^2 = 0.050$, respectively; adjusted model Fig. 3B). An increase in age and body weight was associated with decreased calcium absorption ($P = 0.0066$ and $P < 0.0005$, respectively).

The correlation between calcium absorption and serum PTH was not statistically significant ($r = 0.046$, $P = 0.324$).

Discussion

In our own study of 488 postmenopausal Caucasian women, we found that serum PTH decreased continuously as serum 25OHD increased from 6 to 60 ng/ml (15–150 nmol/liter) without evidence of a plateau, whereas the bone markers serum osteocalcin and uNTx/Cr initially decreased between a serum 25OHD of 6–18 ng/ml (15–45 nmol/liter) but then plateaued between serum 25OHD of 18 to 60 ng/ml (45–150 nmol/liter).

We did not find any significant effect of the interaction of calcium intake and serum 25OHD on serum PTH. This might be explained by the relatively few number of subjects ($n = 69$) with serum 25OHD levels less than 20 ng/ml. However, the few studies that looked at the interaction between calcium intake and serum 25OHD in terms of serum PTH suppression found that at low levels of serum

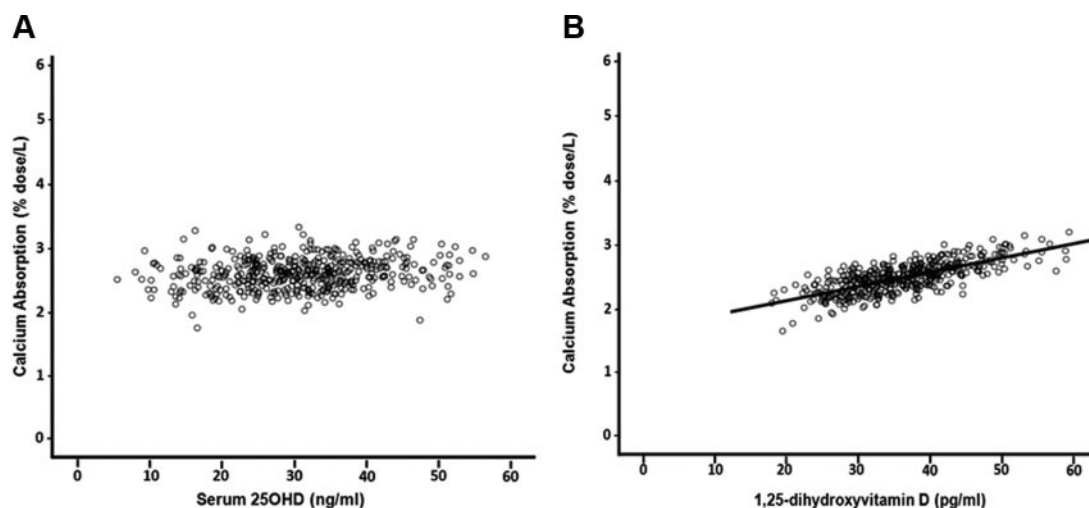


FIG. 3. Relationship between calcium absorption and vitamin D metabolites. A, Calcium absorption was not associated with serum 25OHD after adjustment for age, body weight, calcium intake, serum 1,25(OH)₂D, and serum creatinine ($r = 0.05$, $P = 0.247$). B, Calcium absorption was significantly correlated positively with serum 1,25(OH)₂D after adjustment for age, body weight, calcium intake, serum 25OHD, and serum creatinine ($r = 0.224$, $P < 0.0005$).

25OHD (<20 ng/ml), calcium intake was associated with serum PTH (13, 69–71). In a study from Iceland, subjects with low serum 25OHD less than 10 ng/ml (25 nmol/liter) had significantly higher serum PTH associated with calcium intake less than 800 mg/d but above a serum 25OHD of 18 ng/ml (45 nmol/liter), calcium intake had no effect on serum PTH (13). In a study of elderly subjects (85 yr), vitamin D 400 IU/d increased serum 25OHD from 19 to 25 ng/ml (47.4–62.4 nmol/liter) but failed to produce a significant change in serum PTH and serum osteocalcin; however, the mean calcium intake was low (450 mg/d) (69). In a study from Australia of postmenopausal women, 1000 IU/d of vitamin D3 and 1000 mg/d calcium significantly increased serum 25OHD from 18.5 to 23 ng/ml (46.2–57.4 nmol/liter) and significantly suppressed serum PTH and serum C-terminal telopeptides (70). Neither calcium nor vitamin D alone suppressed serum PTH, although calcium alone but not vitamin D alone significantly suppressed serum C-terminal telopeptides.

In a placebo-controlled study of healthy subjects, aged 55 yr, with baseline serum 25OHD level of 26.8 ng/ml (67 nmol/liter), calcium 1200 mg/d alone significantly reduced the bone turnover markers serum C-terminal telopeptides of type 1 collagen and serum amino-terminal propeptide of type I procollagen compared with placebo (71), whereas vitamin D alone (4000 IU/d) had no significant effect on markers; there was no significant effect of calcium or vitamin D on serum PTH. In a study of healthy females aged 47 yr from England and a mean calcium intake of 570 mg/d, vitamin D3 800 IU/d increased serum 25OHD from 29 to 39 ng/ml (72.4–97.3 nmol/liter) with no change in serum PTH and bone markers, but serum PTH decreased significantly only in the lowest quartile of serum 25OHD (<24 ng/ml (60 nmol/liter; n = 18) (36).

In a 4-yr randomized, placebo-controlled trial of elderly women aged 75 yr and baseline serum 25OHD of 23.6 ng/ml (59 nmol/liter), treatment with 750 mg/d of calcium or 15 μ g of 25OH vitamin D3 significantly reduced serum PTH. Calcium intervention was more effective than 25-hydroxyvitamin D3 with the effect of latter seen only at lower calcium intake (<716 mg/d) but not at higher calcium intake (>716 mg/d) (72). Also, bone markers serum osteocalcin and uNTx/Cr did not decrease in any groups; on the contrary, bone markers increased in the vitamin D and placebo groups and stayed the same in the calcium group.

Many other studies in which serum PTH declined significantly after vitamin D and calcium intervention started with low baseline serum 25OHD levels less than 20 ng/ml (50 nmol/liter) (73–75).

Calcium absorption was not associated with serum PTH or serum 25OHD but was associated significantly

with serum 1,25(OH)₂D. Another recent study showed no correlation between calcium absorption and serum 25OHD levels [mean serum 25OHD 21 ng/ml (52.4 nmol/liter)] (76). In contrast, Heaney (77) suggested that calcium absorption increased with increasing serum 25OHD and did not reach a plateau until 32 ng/ml (80 nmol/liter); these results were derived from a heterogeneous group of five studies, three of which did not directly measure calcium absorption. One study showed that calcium absorption was positively correlated with serum 25OHD only when serum 25OHD level was less than 4 ng/ml (10 nmol/liter), and absorption did not increase with higher serum 25OHD (78). So in studies in which serum 25OHD was greater than 5 ng/ml (12.5 nmol/liter), a threshold effect might have been missed.

It has been suggested that because serum PTH is still elevated at a serum 25OHD of 30 ng/ml (75 nmol/liter), it is an indicator of secondary hyperparathyroidism and because PTH resorbs bone, it must be an adverse finding (2, 3), but many of the studies did not measure bone resorption, bone loss, or fractures. In our study increased bone resorption was associated only with serum 25OHD less than 18 ng/ml (45 nmol/liter). There are other bone studies that confirm this threshold. A nested case-control study from the Women's Health Initiative found that the risk of hip fractures was significantly greater in women with mean serum 25OHD 19 ng/ml or less (47.5 nmol/liter) compared with the group with mean serum 25OHD 28.3 ng/ml or greater (70.7 nmol/liter) [odds ratio 1.71, 95% confidence limits (CL) 1.05–2.79] (79). In another case-cohort study, 436 men with incident nonspine fractures *vs.* 1608 controls, the group in the lowest quartile of serum 25OHD less than 20 ng/ml (50 nmol/liter) had more hip fractures compared with men in the top quartile of serum 25OHD (\geq 28 ng/ml) (70 nmol/liter) [hazard ratio (HR) 2.36, 95% CL 1.08–5.15] (80). In the National Health and Nutrition Examination Survey III study consisting of 1917 white men and women 65 yr of age or older, the risk of hip fracture was significantly lower in the group with serum 25OHD 25 ng/ml or greater (62.5 nmol/liter) than those with serum 25OHD less than 25 ng/ml (62.5 nmol/liter) [relative risk 0.64, 95% CL 0.46–0.89] (81). In another recent study of fractures in 1194 men with a median follow-up of 11 yr, it was found that serum 25OHD levels of less than 16 ng/ml (40 nmol/liter) were associated with an increased risk of fracture (HR 1.65, 95% CL 1.09–2.49) (82). In another Swedish study involving 986 ambulatory women, aged 75 yr, the HR for sustaining a fracture was 2.04 (95% CL 1.04–4.04) in the group with serum 25OHD less than 20 ng/ml (50 nmol/liter) (84).

In a longitudinal study of bone density from the Osteoporotic Fractures in Men study, the rate of bone loss in the hip was higher in the group with serum 25OHD levels less than 20 ng/ml (50 nmol/liter) compared with greater than 20 ng/ml (50 nmol/liter) (85). A bone biopsy study in Germany performed in 675 autopsies of men and women showed that about 96.5% of osteomalacia cases occurred at a serum 25OHD level of less than 20 ng/ml (50 nmol/liter), and almost 99% occurred at serum 25OHD levels of less than 25 ng/ml (62.4 nmol/liter) (86).

The accumulation of these findings support the concept that a serum 25OHD greater than 20 ng/ml (50 nmol/liter) is adequate for bone health, and there are no bone data supporting the need for a higher serum 25OHD of 30 ng/ml (75 nmol/liter).

It is not clear what is the clinical significance of higher serum PTH at serum 25OHD levels greater than 20 ng/ml (50 nmol/liter) when bone markers are not increased. Age-related decreases in calcium absorption and renal function and low calcium intake are some of the explanations. Several other factors affect serum PTH levels including race (23), gender (48), weight (20), serum leptin (45), and serum SHBG (8). These factors may play a role in the range of serum 25OHD levels at which serum PTH is maximally suppressed.

We saw a continuous decline in serum PTH over the normal range of serum 25OHD up to 60 ng/ml (150 nmol/liter), and these are levels that none would suggest represents vitamin D insufficiency. Possibly the decreasing serum PTH is a sign of the pharmacological effect of higher serum 25OHD or 1,25(OH)₂D acting on the vitamin D response element in the PTH gene.

With regard to the literature review, there are some limitations in defining a threshold serum 25OHD from various studies. There are different methods for measurement of serum 25OHD and serum PTH among the 70 papers. The study populations are variable in terms of age, race, location, diet, gender, and culture, and these factors may influence the relationship between serum 25OHD and serum PTH. The role of calcium intake might be important in suppressing serum PTH, particularly at low serum 25OHD levels, as discussed above. Most of the studies did not analyze the effect of calcium intake while assessing the relationship between serum PTH and serum 25OHD. Twenty-four studies showed a threshold level for serum PTH at a serum 25OHD between 10 and 20 ng/ml (25–50 nmol/liter), and six studies showed a threshold between 20 and 25 ng/ml (50–62.4 nmol/liter). Thirty studies showed a threshold level of serum 25OHD of less than 25 ng/ml (62.4 nmol/liter) in terms of serum PTH suppression. Several studies including our own failed to find any threshold level of serum 25OHD at which serum PTH plateaus. What is very clear is that of the 70 studies

we reviewed, a serum 25OHD threshold varying between 10 and 50 ng/ml (25–150 nmol/liter) was found.

In summary, the practice of defining vitamin D insufficiency as a serum 25OHD less than 30 ng/ml (75 nmol/liter) (3) based on serum PTH suppression is not supported by the literature review. Taking into account our own results on bone markers, the five large hip fracture studies and the bone loss data from Osteoporotic Fractures in Men, the totality of the data show that vitamin D insufficiency as it relates to bone occurs at a serum 25OHD less than 20 ng/ml (50 nmol/liter). Whether this threshold is higher for diseases other than bone remains to be established by clinical trials.

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