Age-Related Changes in the 25-Hydroxyvitamin D *Versus* Parathyroid Hormone Relationship Suggest a Different Reason Why Older Adults Require More Vitamin D

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Vitamin D requirements are thought to vary with age, but there is little comparative evidence for this. One goal in establishing a vitamin D requirement is to avoid secondary hyperparathyroidism. We studied 1741 euthyroid, thyroid clinic outpatients without evidence of calcium abnormalities, ranging in age from 19 to 97 yr, whose serum and urine had been analyzed for calcium, vitamin D, and parathyroid status. We found no effect of age on the 25-hydroxyvitamin D [25(OH)D] concentration associated with specific vitamin D intakes, and there was no relationship between 25(OH)D and 1,25-hydroxyvitamin D [1,25(OH)$_2$D]. In every age group, serum 1,25(OH)$_2$D declined with increasing creatinine ($P < 0.001$). What changed with age included creatinine, which correlated with 25(OH)D ($r = 0.146$, $P < 0.001$) only in the youngest age group (19–50 yr) but not in the older age groups ($P > 0.1$). Creatinine did not correlate with PTH in the youngest age group, but the relationship became significant as age increased (*e.g.* for the elderly, $r = 0.365$, $P < 0.001$). Linear regression of log PTH *vs.* log 25(OH)D agreed with the natural shape of the relationship observed with scatterplot smoothing, and this showed no plateau in PTH as 25(OH)D increased. We compared PTH concentrations among age groups, based on 20 nmol/liter increments in 25(OH)D. Mean PTH in adults older than 70 yr was consistently higher than in adults younger than 50 yr ($P < 0.05$ by ANOVA and Dunnett's t test). PTH levels of the elderly who had 25(OH)D concentrations greater than 100 nmol/liter matched PTH of younger adults having 25(OH)D concentrations near 70 nmol/liter. This study shows that all age groups exhibit a high prevalence of 25(OH)D insufficiency and secondary hyperparathyroidism. Older adults are just as efficient in maintaining 25(OH)D, but they need more vitamin D to produce the higher 25(OH)D concentrations required to overcome the hyperparathyroidism associated with their diminishing renal function. (*J Clin Endocrinol Metab* 88: 185–191, 2003)

DIETARY INTAKE RECOMMENDATIONS for several nutrients, including vitamin D, are now referred to as adequate intake (AI), a term that replaces the recommended dietary allowance when data are deemed inadequate (1). The Food and Nutrition Board, National Academy of Sciences, tripled its AI vitamin D intake recommendation, for adults over 70 yr to 15 μg (600 IU/d), it doubled the previous AI for those between ages 50 and 70 yr to 10 μg (400 IU/d). Because 25-hydroxyvitamin D [25(OH)D[is the objective measure of vitamin D nutritional status (1) and nutrient recommendations assume no other input of nutrient (2), current recommendations imply that young adults need less vitamin D than older adults to maintain the same circulating 25(OH)D concentration. Although several studies show that older adults benefit from higher vitamin D intakes (3–5), we are not aware of direct comparisons showing that younger adults need any less vitamin D than the elderly.

Many reports show an inverse correlation between PTH and 25(OH)D for the elderly (6–10), and although the same phenomenon is true for young adults (11) and adolescents (12), there have been no systematic comparisons of this relationship across age groups.

Several reports have assumed that the decay function for PTH *vs.* 25(OH)D must reach a plateau and concluded this plateau occurs when 25(OH)D concentration is at least 72 nmol/liter (4, 6, 7, 9, 10). We are not aware that the assumption of a plateau relationship is consistently valid or similar for all age groups.

To address these issues, we analyzed laboratory data from a cross-section of thyroid clinic outpatients who had been evaluated for vitamin D and parathyroid status as part of their routine follow-up visits. The aims were to determine whether the relationships among 25(OH)D concentration, vitamin D intake, and PTH concentration differ among adult age groups. We will show that for similar 25(OH)D concentrations, PTH concentrations are consistently higher in the elderly than in younger adults. Our data also support the need for higher intake of vitamin D in older adults, not because they are less efficient at maintaining 25(OH)D concentrations, but because they need higher 25(OH)D concentrations to prevent secondary hyperparathyroidism.

Subjects and Methods

Subjects

Subjects were euthyroid outpatients who were being treated for various thyroid conditions, as presented in Table 1. The analyses were done because their endocrinologist (P.G.W.) regarded them as relevant to his routine surveillance of his patients' endocrine and nutritional health; furthermore, our laboratory is a regional referral laboratory for the vitamin D analyses, and the incremental cost was marginal. The hos-

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Abbreviations: 1,25(OH)$_2$D, 1,25-Hydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; AI, adequate intake; LOWESS, locally weighted regression and scatterplot smoothing technique.

TABLE 1. Details of study subjects, from 558 reviewed charts

Female	497
Male	61
Age (yr + SD)	56.9 ± 14.8
Diagnosis (no. of patients)	
Hyperthyroid	118
Hypothyroid	358
Goiter	200
Thyroid cancer	90
Thyroidits	153
Osteoporosis	87

The patients are treated, euthyroid outpatients of the endocrine clinic of co-author P.G.W. More than one diagnosis may apply per subject. The data set excludes patients with parathyroid disease. This is representative of the full 1741 subjects for whom biochemical data are presented.

pital's ethics review committee approved this descriptive study. Data for patients assessed for serum PTH, 25(OH)D, 1,25-hydroxyvitamin D [$1,25(OH)_2D$], and biochemical parameters related to calcium metabolism were captured, using a structured query, from the hospital's computer database system for laboratory data and patient demographics (SoftLab, Boca Raton, FL). We limited the data assembled for the present report to a time window during which our laboratory made no modifications to the pertinent methods. In total, the study included biochemical data from 1741 adult endocrine outpatients who were not suffering from parathyroid or calcium-related disease based on biochemical measures and clinical assessment (12 patients had been removed from the data set because of serum calcium >2.7 mmol/liter). Data were collected from patient visits between May 1997 and the end of December 1998; of these, 1049 (60%) of 1741 samples were obtained during the summer half of the year, May through October inclusive, when sunshine at our latitude (42 degrees north) increases 25(OH)D concentrations (11). Of the 568 consecutive patient charts reviewed, we had recorded vitamin D

FIG. 1. Serum 25(OH)D concentrations of thyroid clinic outpatients grouped according to age and further divided according to information available about their vitamin D intake. These results are presented as box plots, in which the *box* indicates quartile values, the *whiskers* indicate the upper and lower values not classified as statistical outliers, and *circles* denote 25(OH)D concentrations that were statistically outliers. The groups labeled here as Missing show 25(OH)D concentrations of patients in whom the vitamin D intake was not retrieved from the patients' files.
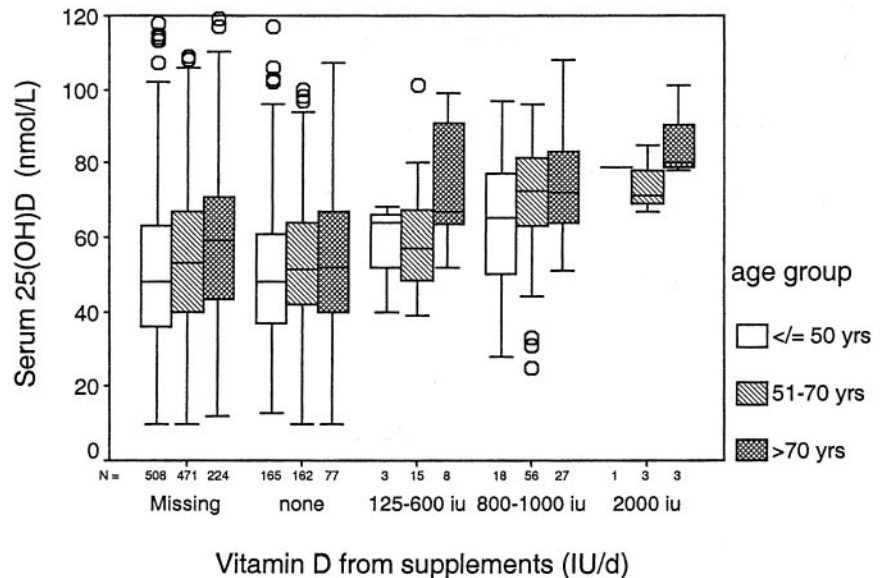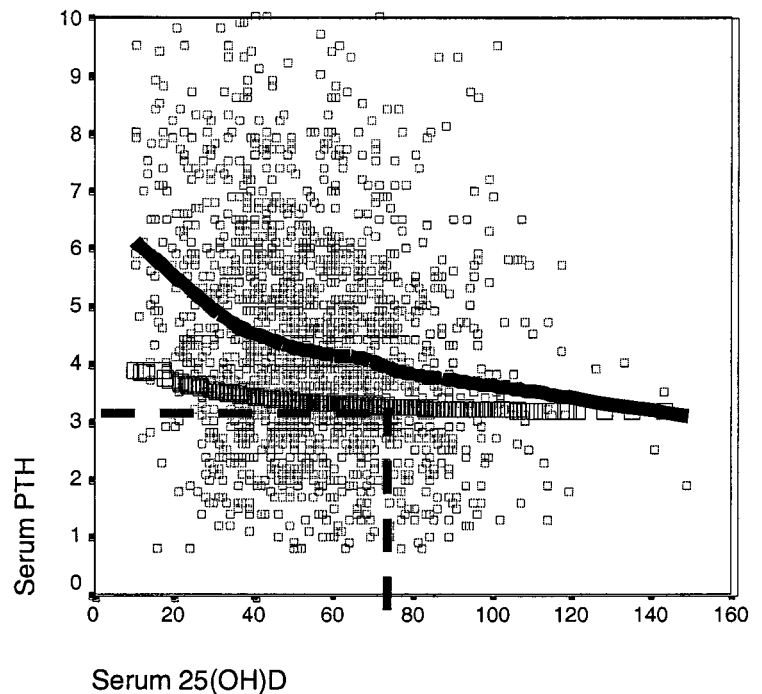

FIG. 2. Serum PTH concentrations *vs.* 25(OH)D concentrations for all 1741 patients, overlaid with the LOWESS plot (*dash-dot line*) and exponential decay function fitted to the data (*dashed line*). The *arrow* indicates the point at which PTH concentrations theoretically attain the plateau value, based on the exponential function.
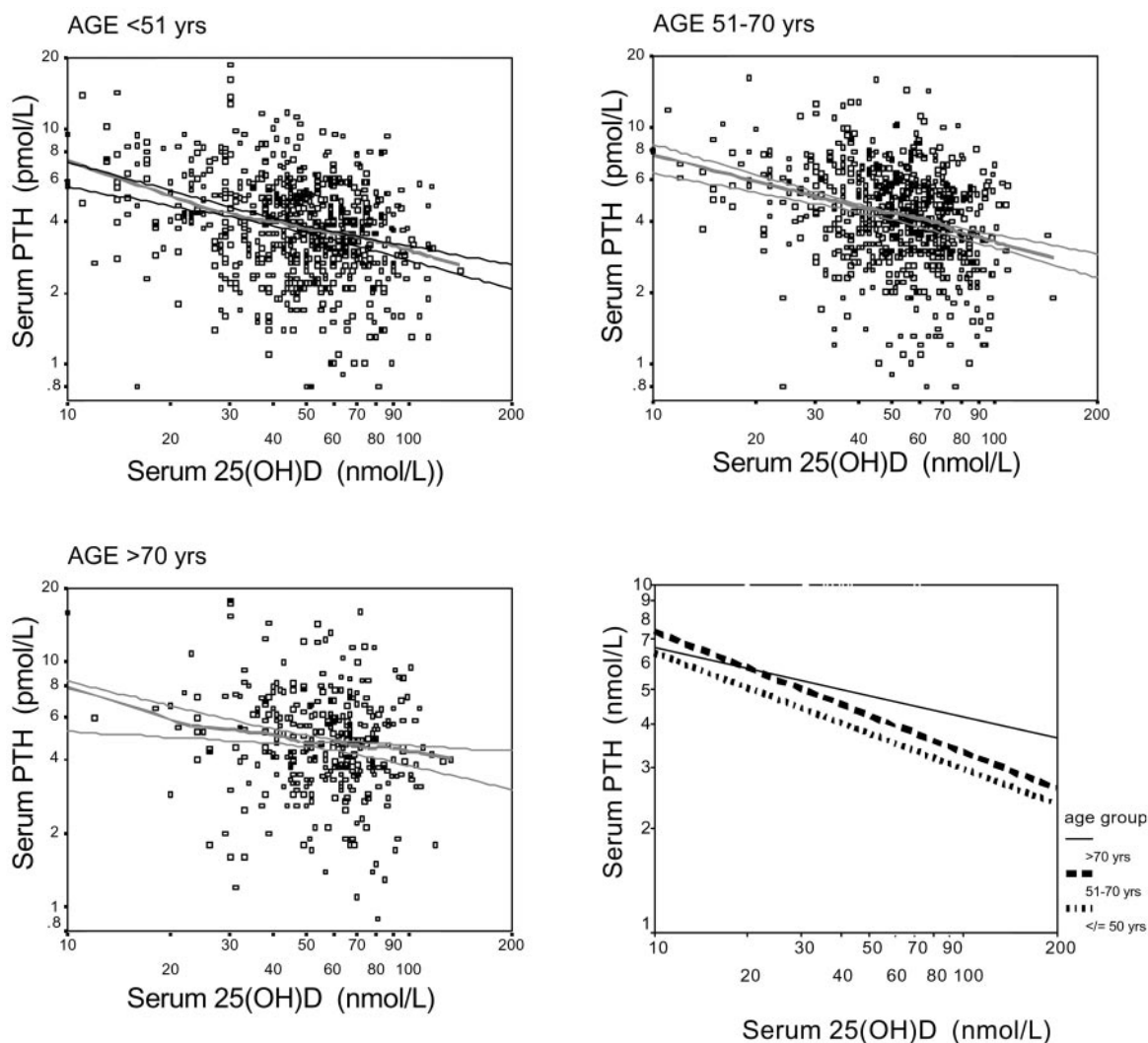
FIG. 3. Effect of age on the relationship between log 25(OH)D *vs.* log of PTH concentrations. The *light curves* show the 95 percent confidence limits for the linear regression line for each age group. The *heavy line* between these curves is the LOWESS plot, inserted instead of the linear regression line, to show that linear regression on log-log axes agrees with the natural pattern of the underlying data. The lower right panel shows the linear regression lines by themselves, for the three age groups.

intake in a subset of 531 consecutive based on intake of mineral and vitamin supplements reported by the patients. Focus on patients seen in spring and summer was used to increase the proportion of elderly in this subset. Patients who previously had low 25(OH)D (<50 nmol/liter) had been advised to take vitamin D_3, as 25-μg (1000 IU) pills available over the counter.

Biochemical methods

We measured the intact PTH molecule, as implemented on the DPC Immulite 2000 (DPC, Los Angeles, CA). Serum 25(OH)D was measured with a RIA (DiaSorin, Inc., Stillwater, MN) with which our laboratory consistently reported close to the mean of the DEQAS international proficiency survey for this analyte (13). Serum 1,25(OH)$_2$D was measured with the classic, calf-thymus receptor assay, involving purification of analyte on Bond Elut C18OH cartridges (Varian, Harbor City, CA) and an internal standard to correct for losses during purification (14).

Statistical analysis and graphical presentation were carried out using SPSS version 10 (SPSS, Inc., Chicago, IL). Analysis was based on data available through clinical practice, and because of this, there are variations in sample size, as we note throughout this report. The locally weighted regression and scatterplot smoothing (LOWESS) technique was applied to allow the graphed data to reveal their own patterns (15);
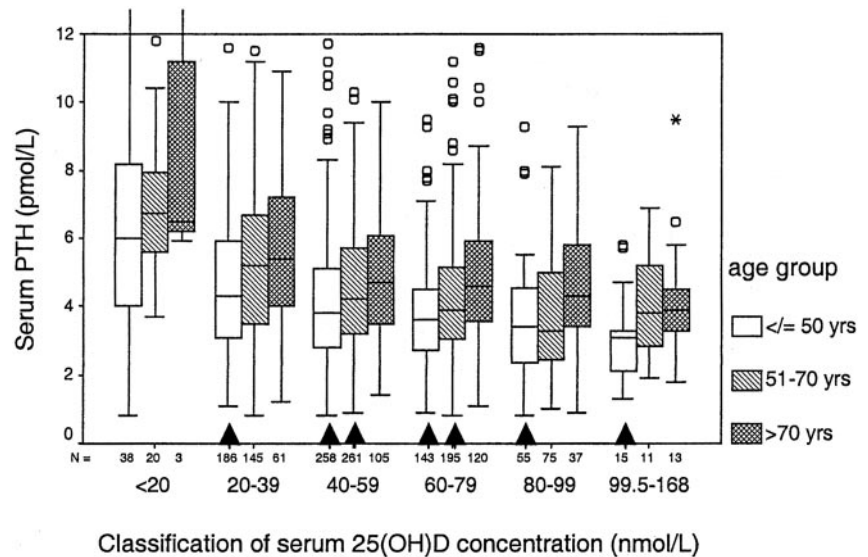
LOWESS is available as part of the SPSS software. The exponential decay function was determined by Dr. K. Norwich (Department of Biomedical Engineering, University of Toronto), fitting of the data to three parameters of an exponential decay equation, using iterative regression analysis to optimize the fit.

Results

The characteristics of the study population are presented in Table 1, which shows that aside from the expected, chance occurrence of osteoporosis, the group did not exhibit other abnormalities of calcium homeostasis.

Comparisons of 25(OH)D concentration, vitamin D intake, and age group showed distinct increases in serum 25(OH)D with higher vitamin D intake. However, there was none of the expected evidence that the young exhibit higher 25(OH)D concentrations than older adults when consuming similar amounts of vitamin D (Fig. 1). Among the 404 patients who were not taking vitamin supplements based on what they reported to us, there was no relationship between age group

FIG. 4. Effect of age on serum PTH concentrations associated with ranges of 25(OH)D concentrations. These are the data from Fig. 3 presented in 20 nmol/liter increments of 25(OH)D, with data clustered according to age group. The *box* indicates quartile values, the *whiskers* indicate the upper and lower values not classified as statistical outliers, and *circles* denote PTH concentrations that were statistically outliers. The *black triangles* point up to groups whose mean PTH concentration was statistically different from that of patients who were older than 70 yr and in the same category of 25(OH)D concentration. These differences are based on a significant ANOVA test, followed two-tail Dunnett *t* test ($P < 0.05$).

and serum 25(OH)D concentration, based on one-way ANOVA ($P > 0.4$). The median 25(OH)D concentration associated with each level of vitamin D intake was actually higher for the oldest age group than the younger groups, but in each case this was not statistically significant. Because the bottom of each box in the figure indicates the 25th percentile, the figure shows that for adults who do not take vitamin D supplements, the prevalence of vitamin D insufficiency is about 25%, regardless of age. Figure 1 also shows that for patients reporting vitamin D intake of at least 800-1000 IU/d, there is reasonable assurance (based on the bottom whisker of the box plots) that 25(OH)D concentration exceed 40 nmol/liter.

The 25(OH)D and PTH concentrations for all 1741 adults were fit to a three-parameter exponential decay function using the approach used by Chapuy *et al.* (9). The resulting exponential decay formula was as follows:

$$PTH = 3.54 \; (-0.027 \times 25(OH)D) + 3.20$$

where PTH and 25(OH)D represent the molar, SI-unit concentrations of these analytes.

With this approach, the PTH concentration approached a theoretical low plateau at 25(OH)D concentrations more than 73 nmol/liter (Fig. 2). However, application of the LOWESS plot to the same data revealed a substantial divergence of the data (based on natural shape) from the exponential plot of the above equation (based on investigator expectation of a plateau).

When we applied the LOWESS approach to graphs of log PTH concentration *vs.* log 25(OH)D concentration, the technique produced lines that fell entirely within the 95% confidence limits of the lines obtained by linear regression (Fig. 3). In other words, the LOWESS regression plot that involved no assumptions about the eventual shape of the relationship produced lines not statistically different from those determined by linear regression on log-*vs.*-log axes. Slopes and intercepts of these regression lines were individually not quite significantly different among age groups ($P < 0.10$, two-tail); part of the regression line for the elderly lay outside

the 95% confidence limits of for each of the younger age groups, indicating a combined slope and intercept effect because of age.

To compare in a different way, the effects of 25(OH)D concentration on PTH, patients were grouped according to their age category and then further grouped, according to their 25(OH)D concentration, in 20 nmol/liter increments. This approach produced the box plots of serum PTH concentrations shown in Fig. 4. For all age categories, there was a substantial and progressive decrease in each quartile value of PTH concentration as 25(OH)D increased. The PTH concentrations of the oldest age group were consistently higher than those of younger adults in the same category of 25(OH)D concentration, *i.e.* PTH differed (Dunnett *t* test, $P < 0.05$) among ages, for each category of 25(OH)D beyond 19 nmol/liter. For elderly subjects with 25(OH)D greater than 99 nmol/liter, the median 25(OH)D was comparable to the PTH concentration of younger adults whose 25(OH)D ranged between 40 and 79 nmol/liter.

For every age group, there was no evidence of a relationship between serum concentrations of 25(OH)D and $1,25(OH)_2D$ (Fig. 5). Serum 25(OH)D correlated positively with plasma creatinine for all 1741 patients (r value, 0.117; $P < 0.001$). For the 533 subjects for whom vitamin D intake had been recorded from patient charts, partial correlation was done, controlling for both age and vitamin D intake. This resulted in a significant correlation coefficient between 25(OH)D and creatinine (partial r = 0.139; $P = 0.001$). There were age-related differences in the relationships between plasma creatinine *vs.* PTH, $1,25(OH)_2D$, and 25(OH)D; the correlations for these are summarized in Table 2. In particular, increasing creatinine (diminishing kidney function) correlated with a higher 25(OH)D only in the youngest age group ($P < 0.001$), but for the older age groups creatinine showed no relationship with 25(OH)D ($P > 0.1$). In contrast, increasing creatinine did not correlate with PTH in the youngest age group ($P = 0.738$), but in the oldest age group creatinine showed a strong
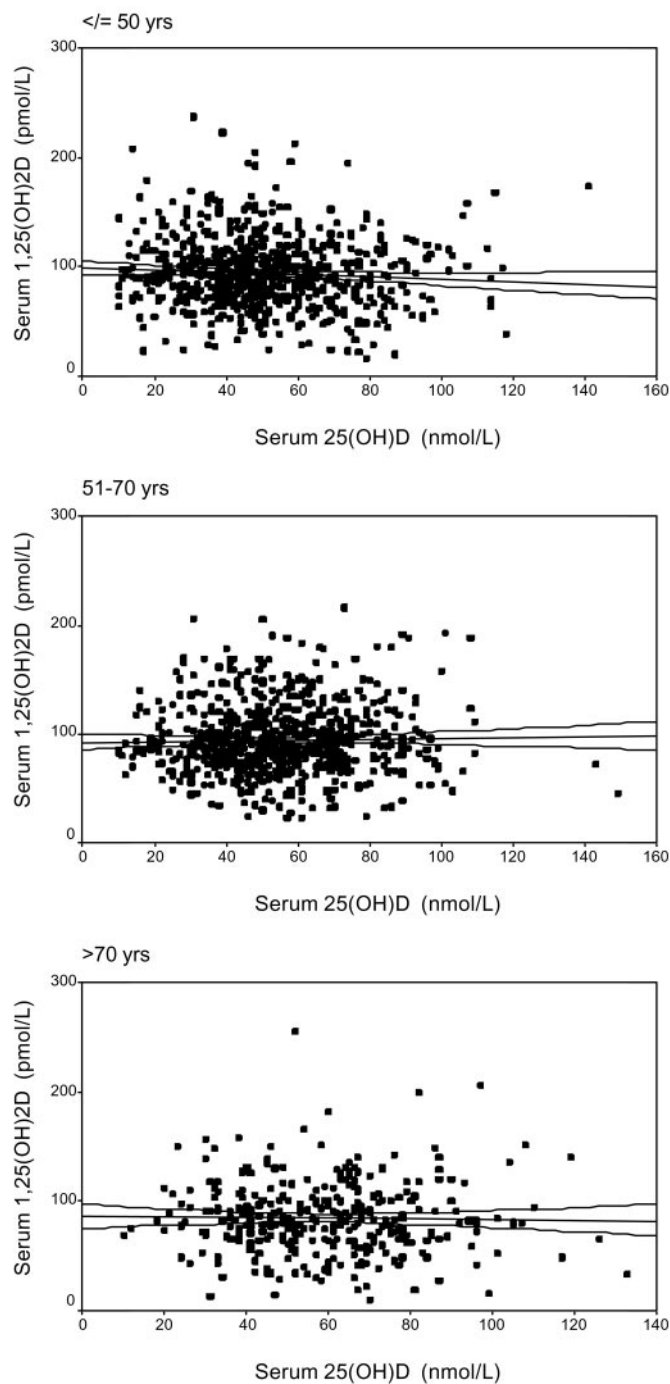
FIG. 5. Serum 1,25(OH)2D *vs.* serum 25(OH)D concentrations for each age group. The *lines* show the linear regression line and its 95 percent confidence limits, indicating that across the range of 25(OH)D concentrations relevant to each age group, there is no relationship between circulating concentrations of these metabolites.

relationship with PTH (r = 0.365; $P < 0.001$). In all age groups, creatinine consistently correlated negatively with 1,25(OH)$_2$D levels ($P < 0.001$). The relationship between age and creatinine was not significant for the group younger than 50 yr but became progressively stronger and significant for the older age groups (Table 2).

Discussion

Most authors attempting to establish requirements for vitamin D have focused on the relationship between PTH and 25(OH)D concentrations and have assumed that PTH reaches a plateau as 25(OH)D rises. We asked whether the relationship between PTH and 25(OH)D might be different among the age groups used for nutritional recommendations in adults. This forced us to reevaluate the nature of the PTH *vs.* 25(OH)D relationship. Although the use of an exponential curve optimized to all our data produced essentially the same outcome reported by Chapuy *et al.* (9), the curve we obtained did not agree with the pattern generated with a nonparametric graphical approach to regression.

Cleveland (15) developed the locally weighted scatterplot smoother line (LOWESS) to offer investigators a way to determine what he regarded as the true shape of scatterplot relationships without an investigator-driven bias. We found that regression lines obtained using log PTH *vs.* log 25(OH)D concentrations were a close match to LOWESS plots of the data, indicating that for our subjects, there was no evidence of a plateau relationship.

There are several possible explanations for why we saw a different quality of 25(OH)D *vs.* PTH relationship than what others have reported. Differences in the biochemical test methods used, the sample population tested, and investigator expectations could all play a role. The lack of a 25(OH)D-related plateau in PTH concentrations was consistently seen in all three age groups, based on both the regression (Fig. 3) and progressive declines in mean PTH concentrations whenever 25(OH)D was higher (Fig. 4). We think that the lack of a PTH plateau would also apply to patients without thyroid disease because extremely high 25(OH)D concentrations are known to cause severe suppression of PTH in normal subjects (16), and high vitamin D doses can be used to treat hypoparathyroidism without a requirement for PTH therapy. Although it cannot be excluded that the present results are unique to this patient cohort, all of these patients were euthyroid when these biochemical tests were ordered and very probably not different from what would have been observed if we had access to similar data for healthy volunteers. Use of volunteer subjects would also have been a selective sampling, and such an approach would have yielded a smaller proportion of elderly subjects.

If there is no plateau in the relationship between PTH *vs.* 25(OH)D, then the choice of an optimal or desirable 25(OH)D concentration, based only on PTH, becomes arbitrary. If lower PTH is a goal, then a higher 25(OH)D concentration will always lower PTH further.

PTH concentrations were consistently highest in the oldest adults, for whom it declined more gradually than in the young, as 25(OH)D increased. This is reminiscent of the relative resistance to 1,25(OH)$_2$D previously reported for the elderly (17).

It is often assumed that there is a positive relationship between the circulating concentrations of the theoretically inactive compound, 25(OH)D, and 1,25(OH)2D. This is as if to say consumption of vitamin D is equivalent to providing a higher circulating 1,25(OH)$_2$D concentration. Need *et al.* (18) emphasized that a 25(OH)D concentration of 40 nmol/

TABLE 2. Correlations for creatinine in relation to 25(OH)D, 1,25(OH)2D, PTH, and age analyzed for our patients grouped according to the age classifications used for dietary guidelines

Age group (mean creatinine ± SD) [n]	Pearson correlation			
	Serum 25(OH)D	Serum 1,25(OH)2D	Serum PTH	Age
≤50 yr (68 ± 11 μmol/liter) [682–695]	0.146[a]	−0.203[a]	0.013	0.017
51–70 yr (70 ± 12 μmol/liter) [699–707]	0.048	−0.172[a]	0.124[a]	0.077[b]
>70 yr (81 ± 20 μmol/liter) [329–339]	0.046	−0.203[a]	0.365[a]	0.161[a]

Results presented are Pearson correlation coefficients, r-values, for bivariate analyses done for the indicated age groups, between serum creatinine and the variables shown at the top of each column. Sample sizes are indicated by n. The mean ± SD creatinine concentration for each age group is shown in *parentheses*.
[a] $P < 0.001$.
[b] $P < 0.05$.

liter was important because above this cut-point the concentration of $1,25(OH)_2D$ increased along with rising 25(OH)D concentrations. Likewise, Devine *et al.* (19) reported that in elderly women, there is a strong positive correlation between 25(OH)D and $1,25(OH)_2D$. Our results did not confirm this aspect of either of those reports. No matter how we analyzed our data, they exhibited no association or cut-point between 25(OH)D and concentrations of $1,25(OH)_2D$ (Fig. 5). In the rat, there is a strong negative relationship between 25(OH)D and $1,25(OH)_2D$ concentrations (20), so it makes little sense to us why the opposite should occur in man. We suggest that reports showing a positive correlation between these metabolites in man reflect either a selective focus on a subset of results to confirm an expectation (18) or interference in the $1,25(OH)_2D$ assay by another metabolite (19). Hollis (21), originator of the $1,25(OH)_2D$ method used by Devine *et al.* (19), showed that the method was interfered with by $24,25(OH)_2D$ (a metabolite whose levels parallel those of 25(OH)D). Therefore, he adopted the modifications included in the $1,25(OH)_2D$ method we used for the present work (14). Our findings confirm those of Barger-Lux *et al.* (22) showing that 25(OH)D concentrations in healthy adults do not affect circulating $1,25(OH)_2D$.

If it is not through circulating $1,25(OH)_2D$, then by what mechanism does 25(OH)D suppress PTH? The parathyroid gland does possess vitamin D receptor (23, 24), and recently Segersten *et al.* (25) demonstrated expression of 25(OH)D-1-α-hydroxylase in human parathyroid tissue by RT-PCR and immunohistochemical analysis. Therefore, circulating 25(OH)D can affect PTH secretion and parathyroid growth by $1,25 (OH)_2D$ generated within parathyroid tissue. 25(OH)D is not a hormone, but it serves as the substrate required for normal, paracrine control of parathyroid tissue.

The authors of the Food and Nutrition Board guidelines for calcium and related nutrients suggested that because cutaneous vitamin D production in the elderly is diminished (26), the elderly require more vitamin D intake to compensate for that (1). Therefore, one should expect that for adults who do not take supplementary vitamin D, the elderly group should have lower 25(OH)D concentrations than younger adults. However, in agreement with other published reports that have compared 25(OH)D levels among age groups (27–29), the 25(OH)D concentrations in the elderly patients were certainly not lower than those in the younger ones. The paradox for the elderly is that although cutaneous vitamin D production is diminished (26), 25(OH)D concentrations are not affected. This can be explained by the age-related decline in kidney function. As kidney function declines in rats, so does $1,25(OH)_2D$ production (30) and metabolic clearance of 25(OH)D. Likewise, the most consistent relationship that we saw across adult age groups was that $1,25(OH)_2D$ correlated inversely with creatinine (Table 2). Thus, although vitamin D production in the skin decreases with age, so does the utilization of 25(OH)D in the kidney; these effects cancel each other out so that aging *per se* has no effect on 25(OH)D levels. This interpretation is suggested by our results, but because we are not aware of any studies reporting on the effect of age on biological turnover of 25(OH)D, more conclusive studies are needed. The effect of age was complex, and the quality of several relationships changed with the age group being analyzed. In particular, the relationship between diminishing kidney function and increasing PTH became stronger with each older age group, as did the relationship between creatinine and age itself. These observations show that the age-related differences among these relationships cannot be explained on the basis of vitamin D nutrition alone. It appears that the elderly become somewhat resistant to the effects of 25(OH)D in a manner reminiscent of the relative resistance to $1,25(OH)_2D$ previously reported in elderly adults (17).

One issue that remains is whether individuals of all ages require similar 25(OH)D concentrations and vitamin D intakes. We conclude that if the aim of vitamin D supplementation in adults is to suppress PTH, then the elderly need higher intakes because they are resistant to effects of the nutrient. However, if the aim of vitamin D supplementation is to ensure a minimum target level of 25(OH)D, then the daily consumption of vitamin D should be the same for all adults, regardless of age. The present cross-sectional study confirms a previous intervention study (31), showing that if one wants to ensure that all adults have 25(OH)D of at least 40 nmol/liter, they must consume at least 20–25 μg (800–1000 IU) vitamin D_3 daily.

Acknowledgments

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