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Evidence of an Age-Related Decrease in Intestinal Responsiveness to Vitamin D: Relationship between Serum 1,25-Dihydroxyvitamin D_3 and Intestinal Vitamin D Receptor Concentrations in Normal Women*

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ABSTRACT

Although aged rats reportedly have reduced intestinal vitamin D receptor (VDR) concentrations, it is unclear whether an analogous age-related defect occurs in man. Thus, we assessed the interrelationship among serum 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], calcium absorption and intestinal VDR in 44 healthy, ambulatory women, ages 20–87 yr. Fractional calcium absorption was measured after oral administration of ^{45}Ca (20 mg $CaCl_2$ as carrier); serum 1,25-$(OH)_2D_3$, by the calf thymus binding assay; and serum intact PTH, by a two-site immunochemiluminometric assay. Vitamin D receptor concentration was measured, by a new immunoradiometric assay, in biopsy specimens taken from the second part of the duodenum during gastroduodenoscopy in 35 of the women. Despite an age-related increase in serum PTH ($r = 0.48$; $P < 0.001$) and in serum 1,25-$(OH)_2D_3$ concentration ($r = 0.32$; $P < 0.05$), intestinal VDR concentration decreased with age ($r = -0.38$; $P = 0.03$) and fractional calcium absorption did not change with age. Although a contribution of decreased 25-hydroxyvitamin D 1α-hydroxylase activity to the blunting of the increase in serum 1,25-$(OH)_2D_3$ concentration late in life is not excluded, the data are far more consistent with impaired intestinal responsiveness to 1,25-$(OH)_2D_3$ action. This defect could lead to compensatory increases in PTH secretion and 1,25-$(OH)_2D_3$ production which maintain calcium absorption and serum ionic calcium, but at the expense of increased bone loss. (*J Clin Endocrinol Metab* **75:** 176–182, 1992)

IMPAIRED calcium absorption is the likely cause of the secondary hyperparathyroidism present in elderly women (1). This, in turn, may contribute to age-related bone loss. As assessed by absorption of radiocalcium from a mixture with a fixed amount of calcium carrier (2–6), intestinal calcium absorption decreases with age. The main hormonal regulator of calcium absorption is 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$]. Active calcium transport is vitamin D-dependent, whereas passive calcium transport is vitamin D-independent (7).

In a previous study (8), our group demonstrated that serum 1,25-$(OH)_2D_3$ concentration increased with age in women between ages 30 and 80, but true calcium absorption did not change. This suggested intestinal resistance to 1,25-$(OH)_2D_3$ action with aging. Intestinal vitamin D receptor (VDR) concentration has been reported to decrease in aging rats (9), and we hypothesize that an analogous defect might be present in aging women. To test this hypothesis, we measured intestinal concentrations of VDR in a group of healthy women ranging from young adulthood to old age using a newly developed, sensitive immunoradiometric assay and compared these data with variables relating to bone and calcium metabolism.

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Subjects and Methods

Experimental subjects

The experimental subjects were paid volunteers who responded to posted notices at the Mayo Clinic or who had previously taken part in ongoing epidemiological studies. Of the 44 healthy, ambulatory women (ages 20–87 yr; mean ± SD, 52 ± 19 yr) studied, 20 were premenopausal and 24 were postmenopausal (19 ± 9 yr after menopause; range, 4–44 yr). None had any detectable disease or was taking any drugs known to affect calcium metabolism. As assessed by dual-energy x-ray absorptiometry of the lumbar spine (10), all had values within the age-adjusted 95% confidence intervals for normal women. None of the postmenopausal women had vertebral fractures on thoracic and lumbar radiographs. The studies were approved by the Mayo Institutional Review Board, and signed informed consent was obtained from each of the women.

Study protocol

The study diet was matched to each subject's habitual intake of calcium and phosphorus as assessed by a 7-day diet record and an interview by a dietician. The distribution of calcium among the meals was also matched to that in the habitual diet, and each subject ate these meals in the Clinical Research Center for 4 days prior to commencement of the study, to ensure a constant calcium intake before the study. Blood was withdrawn through an indwelling catheter at 0700 h; the serum was separated and stored at −70 C until analysis. A 24-h urine collection was started at 0700 h.

Assessment of fractional calcium absorption

Intestinal calcium absorption was assessed by the method of Bullamore *et al.* (4). Radiocalcium (^{45}Ca) was purchased from the Atomic Energy Commission (Oak Ridge, TN) and was administered orally with 20 mg calcium carrier (as $CaCl_2$) in 200 mL deionized water. Heparinized blood samples were obtained at baseline and 15, 30, 45, 60, 90, and 120 min after the dose and fractional calcium absorption (FCA) was calculated.

Procurement of duodenal mucosal biopsies

Multiple mucosal biopsy specimens were obtained from the second part of the duodenum in 35 of the women by gastroduodenoscopy at least 30 min after completion of the calcium absorption study. Each biopsy specimen was immediately frozen by immersion in methanol and dry ice, stored at −70 C, and sent on dry ice to the University of Wisconsin for VDR assays.

Assay of vitamin D receptors

The concentration of VDR was measured by an immunoradiometric assay using purified porcine intestinal receptor as a standard and two high-affinity monoclonal antibodies directed against two different epitopes on the receptor (11). The extracts of human intestinal mucosa were prepared as described (12). Based on experiments with nuclear extracts of porcine intestinal mucosa, coefficients of variation were 8.6% intraassay and 18.2% interassay. Sensitivity (the lowest amount of receptor detectable with 95% confidence, Student's *t* test) was 3 fmol VDR per crude biopsy extract.

We also measured $1,25(OH)_2[26,27\text{-}^3H]D_3$-binding activity in human intestinal mucosal extracts by a modified (13) hydroxyapatite binding assay (14, 15). Pellets were assayed for radioactivity in BioSafe II counting solution (RPI, Mount Prospect, IL) in a Prias 400 CL/D liquid scintillation counter (Packard Instruments, Downers Grove, IL).

Biochemical methods

The serum $1,25(OH)_2D_3$ concentration was measured in two to four replicate microassays by the method of Reinhardt *et al.* (16) and the mean of these results was used. This method uses rapid extraction and preliminary purification on a silica Sep-Pak column with hexane/isopropanol followed by nonequilibrium competitive protein binding assay with $1,25(OH)_2D_3$ receptor from calf thymus and $1,25(OH)_2[26,27\text{-}^3H]D_3$ as tracer (Incstar Corporation, Stillwater, MN). Serum 25-hydroxyvitamin D [25(OH)D] was measured by high-pressure liquid chromatography (17). Serum intact PTH was measured by duplicate immunochemiluminometric assays (18); intraassay variation was 9%, and interassay variation was 14%.

We measured serum osteocalcin (also called bone Gla-protein) by RIA (19) with antiserum raised in rabbits to bovine osteocalcin; homogeneous bovine osteocalcin was used for tracer and standard. Antibody-bound and free ^{125}I-labeled osteocalcin were separated by the double-antibody method. Intraassay variation was less than 7% and interassay variation was less than 10%. All measurements were made in duplicate.

We measured serum bone alkaline phosphatase activity by a solid-phase immunoassay method using two monoclonal antibodies and serum isoenzyme standards (20). The intraassay variation was 3.3% and interassay variation was 13%.

We measured serum and urinary calcium and dietary calcium content by atomic absorption spectroscopy (model 2380, Perkin-Elmer Instruments, Norwalk, CT). Serum and urinary creatinine (21) and serum and urinary phosphorus (22) were measured with a centrifugal analyzer (Multistat Plus System, Instrumentation Laboratory, Inc., Spokane, WA). We measured urinary hydroxyproline by high-pressure liquid chromatography (23), and the results were normalized to 100 mL glomerular filtration based on creatinine clearance.

Statistical methods

Correlations among age, intestinal VDR, and variables relating to bone and calcium metabolism were assessed through the calculation of either Pearson's Product Moment correlation coefficient or Spearman's Rank correlation coefficient as appropriate. The independent effects of age and variables related to bone and calcium metabolism on VDR and FCA were assessed using multiple regression models. While developing the models, the use of higher order terms and interactions among the independent variables was investigated as was the use of transformations to normalize residuals, stabilize variances, and reduce the influence of outliers. In no instance did the existence and nature of the relationships depend on the transformation, although the natural logarithm transformation was sometimes used because it successfully stabilized the variance. All analyses were performed by using the Statistical Analysis System software program (24).

Results

The variables measuring bone and calcium metabolism as well as those under investigation as potentially related to such measures are summarized in Table 1. Their individual association with age as assessed by Spearman's Rank correlation coefficient are also summarized in Table 1, whereas their individual regression equations on age are summarized in Table 2. Only three of the variables—serum PTH, creatinine clearance, and lumbar spine bone mineral density (BMD)—were highly associated with age ($P < 0.001$) in both analyses, while a few other variables show a significant ($P < 0.05$) relationship with age.

As seen in Table 1, FCA ranged from 0.35%/h to 1.05%/h with a mean of 0.62 ± 0.28%/h (mean ± SD). A 95% confidence interval estimate of the true mean value of FCA would be the interval from 0.54%/h to 0.70%/h. Although FCA was not directly related to age, it was positively correlated with serum $1,25\text{-}(OH)_2D_3$ (Fig. 1) which is related to age. FCA was also positively correlated with urinary calcium ($r = 0.34$, $P < 0.05$) and negatively with dietary calcium intake ($r = -0.33$, $P = 0.03$) and not significantly with either intestinal VDR concentration or intestinal $1,25\text{-}(OH)_2D_3$ binding.

Multiple regression analyses to determine which variables maintained an independent association worked best when the natural logarithm of FCA was used. The best fitting regression equation (Table 3) involves three variables, *viz*, the reciprocal of serum $1,25\text{-}(OH)_2D_3$, dietary calcium and age ($R^2 = 29\%$, $P = 0.003$). Thus, age which was not significantly related to FCA has a significant negative association with FCA (coefficient negative, $P = 0.031$) after adjusting for serum $1,25\text{-}(OH)_2D_3$ and diet calcium. In the stepwise process of building this model, it was observed that the previous positive correlation between FCA and urinary calcium disappeared after adjusting for serum $1,25\text{-}(OH)_2D_3$.

The intestinal VDR concentration decreased with age (Fig. 2, *upper*). In one woman the VDR concentration was undetectable, presumably because of receptor degradation during transport, and her value was excluded from statistical analyses. The intestinal VDR concentration was not related to serum $1,25\text{-}(OH)_2D_3$ concentration but correlated positively with creatinine clearance corrected for surface area ($r = 0.39$, $P = 0.03$) and with VDR binding activity ($r = 0.35$; $P < 0.05$). Although there was no overall correlation between intestinal VDR and serum $1,25\text{-}(OH)_2D_3$, there was a trend for a positive correlation between these two variables in women older than 60 yr (Fig. 3, *upper*). Using intestinal VDR as the

TABLE 1. Variables related to bone and calcium metabolism and their association with age in 44 women aged 20–87 yr

	Mean ± SD	Median (range[a])	Spearman correlation with age	
			r	*P*
Age	52 ± 19	55 (29,73)		
FCA, %/h	0.62 ± 0.28	0.56 (0.35,1.05)	−0.14	≥0.20
Intestinal VDR[b], fmol/mg protein	323 ± 66	336 (234,390)	−0.35	0.04
Intestinal 1,25-$(OH)_2D_3$ binding,[b] fmol/mg protein	89 ± 53	72 (39,159)	0.01	≥0.20
Serum 1,25-$(OH)_2D_3$, pmol/L	64 ± 16	63 (45,87)	0.28	0.06
Serum 25-(OH)D, pmol/L	72 ± 22	76 (46,99)	−0.28	0.06
Serum PTH, pmol/L	2.8 ± 0.9	2.6 (1.9,4.2)	0.38	0.01
Serum calcium, mmol/L	2.32 ± 0.06	2.32 (2.25,2.40)	0.23	0.12
Osteocalcin, ng/mL	8.1 ± 2.3	7.5 (5.5,11.5)	0.29	0.05
Serum bone alkaline phosphatase, μkat/L	0.31 ± 0.09	0.31 (0.20,0.43)	0.34	0.02
Creatinine clearance,[c] mL/s/1.73 m^2	1.37 ± 0.31	1.36 (0.98,1.82)	−0.56	<0.001
Urine calcium,[c] mmol/day	3.33 ± 1.57	2.97 (1.50,5.54)	−0.21	0.18
Urine hydroxyproline/GFR,[c] μmol/dL GF	133 ± 32	128 (99,178)	−0.03	≥0.20
Lumbar spine bone mineral density, g/cm^2	1.17 ± 0.19	1.17 (0.88,1.43)	−0.74	<0.001
Dietary calcium, mmol/day	21.6 ± 9.6	20.8 (11.4,33.8)	−0.08	≥0.20

[a] Ranges from 10th–90th percentile.
[b] n = 34.
[c] n = 42.

TABLE 2. Relationships between age and other variables involved in bone and calcium metabolism in 44 women aged 20–87 yr (note: includes all measurements)

Dependent variable	Estimates from linear regressions on age				*P*	Estimated difference per decade of age (95% confidence interval)
	Intercept	β	SE (β)	R^2 (%)		
Intestinal VDR,[a] fmol/mg protein	394	−1.40	0.60	14	0.03	−14 (−26,−2)
Serum 1,25-$(OH)_2D_3$, pmol/L	51.0	0.25	0.12	10	0.048	2.5 (0.1,5.0)
Serum 25(OH)D, pmol/L	90.2	−0.35	0.17	9	0.047	−3.5 (−6.9,0.2)
Serum PTH, pmol/L	1.57	$2.3 \cdot 10^{-2}$	$0.7 \cdot 10^{-2}$	23	0.001	0.23 (0.11,0.37)
Serum calcium, mmol/L	2.27	$9 \cdot 10^{-4}$	$5 \cdot 10^{-4}$	7	0.08	0.009 (−0.001,0.018)
Creatinine clearance,[b] mL/s/1.73 m^2	1.88	$-1.0 \cdot 10^{-2}$	$0.2 \cdot 10^{-2}$	32	<0.001	−0.10 (−0.14,−0.05)
Urine calcium,[b] mmol/day	4.26	$-1.8 \cdot 10^{-2}$	$1.4 \cdot 10^{-2}$	4	≥0.20	
Urine hydroxyproline GFR,[b] nmol/dL GF	138	$-9 \cdot 10^{-2}$	$29 \cdot 10^{-2}$	<1	≥0.20	
Lumbar spine bone mineral density, (g/cm^2)	1.57	$-8 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	56	<0.001	−0.08 (−0.10,−0.06)
Dependent variable is natural log						Estimated % difference per decade of age (95% confidence interval)
FCA, %/h	−0.401	$-3.3 \cdot 10^{-3}$	$3.7 \cdot 10^{-3}$	2	≥0.20	
Intestinal binding of 1,25-$(OH)_2D_3$,[b] fmol/mg protein	4.72	$-8.9 \cdot 10^{-3}$	$8.2 \cdot 10^{-3}$	4	≥0.20	
Serum osteocalcin, ng/mL	1.85	$4.1 \cdot 10^{-3}$	$2.1 \cdot 10^{-3}$	8	0.06	4.2% (0.1%,8.7%)
Serum bone alkaline phosphatase, μkat/L	−1.47	$4.9 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$	11	0.03	5.0% (0.6%,9.6%)
Dietary calcium, mmol/day	3.08	$-1.8 \cdot 10^{-3}$	$3.5 \cdot 10^{-3}$	<1	≥0.20	

[a] n = 34.
[b] n = 42.

dependent variable, the best-fitting multiple regression equation involved age and urinary hydroxyproline corrected for glomerular filtration rate (GFR) (Table 3). The positive univariate correlation between intestinal VDR and creatinine clearance ceased to be significant after age entered the multiple regression equation. Intestinal VDR binding activity by hydroxyapatite binding did not correlate with age (Table 1) or serum 1,25-$(OH)_2D_3$ concentration.

Serum 1,25-$(OH)_2D_3$ concentration increased with age (Fig. 2, *lower*). Serum 1,25-$(OH)_2D_3$ concentration correlated positively with serum intact PTH concentration ($r = 0.44$; $P < 0.005$) but not with creatinine clearance. Serum 25OHD concentration decreased significantly with age, but was not related to serum PTH level or FCA.

Serum intact PTH concentration increased with age ($r = 0.48$, $P = 0.001$). There was a trend for serum osteocalcin concentration to increase with age ($r = 0.29$, $P = 0.05$); it did not correlate significantly with serum intact PTH concentration ($r = 0.24$; $P = 0.12$). Serum bone alkaline phosphatase activity also increased with age ($r = 0.34$, $P = 0.02$). Urinary hydroxyproline excretion did not increase with age. Creatinine clearance (adjusted for body surface area) decreased significantly with age. All creatinine clearance values (range, 0.79–1.91 mL/s/1.73 m^2) were within the age-specific, pop-

ulation-based normal range as previously reported (25). There was a highly significant decrease in lumbar spine bone mineral density with age.

Discussion

Despite the significant age-related increase in serum 1,25-$(OH)_2D_3$, FCA did not increase with age, and there was, in fact, a negative correlation with age which did not reach statistical significance perhaps because of the relatively small number of experimental subjects, particularly in the later decades of life. Moreover, multiple regression analysis predicted that women aged 80 should have FCA values 38% lower than women aged 20 if adjusted for differences in serum 1,25-$(OH)_2D_3$ and dietary calcium intake. These findings are evidence for an age-related intestinal resistance to 1,25-$(OH)_2D_3$ action.

Our findings agree with those of Eastell *et al.* (8), who determined true calcium absorption by tracing all three meals, and found no change in calcium absorption with age in normal women. These findings differ from other studies showing age-related decreases in calcium absorption (2–6). The reason for these differences among studies is unclear. In both this study and that of Eastell *et al.* (8), we carefully screened our experimental subjects and excluded those who were hospitalized or housebound or who had secondary diseases, such as abnormal glomerular filtration rates and vitamin D deficiency. Such rigorous screening was not made in the other studies. Based on previous reports of a high incidence of subclinical vitamin D deficiency in elderly persons residing in northern England (26), studies from that area (4, 6) are particularly suspect in view of the demonstrated association between FCA and serum 1,25-$(OH)_2D_3$ concentration. Another difference may be the lack in our studies of large numbers of subjects in the eighth and ninth decades. The lack of a decrease in intestinal calcium absorption with aging suggests that the tendency for FCA to decrease was overcome by the demonstrated compensatory increases in PTH secretion and in 1,25-$(OH)_2D_3$ production. This is also supported by the positive correlation between serum 1,25-$(OH)_2D_3$ concentration and calcium absorption shown in this study and a previous study (5).

The increase in parathyroid function with aging has been unequivocally established using assays detecting the biologically active intact molecule (8, 25, 27) and is demonstrated again here. This secondary hyperparathyroidism is responsible for the age-related increase in serum 1,25-$(OH)_2D_3$ concentration and also is the probable cause of the previously demonstrated increase in bone turnover with aging (19). In earlier studies we demonstrated that serum osteocalcin (8, 19, 20, 28), serum bone alkaline phosphatase isoenzyme (20, 28), and urinary hydroxyproline (19, 28) increase with age in women. However, although trends for increases in biochemical markers of bone turnover were also found in the present study, they were not significant because of the relatively small numbers of experimental subjects.

Although our data implicate intestinal resistance to 1,25-$(OH)_2D_3$ action as the main abnormality in the subjects studied here, they do not exclude a superimposition of impaired renal 25(OH)D 1α-hydroxylase activity occurring in the general population late in life. This has been demonstrated directly in experimental animals (29, 30) and indirectly in elderly humans (31, 32). This additional abnormality would impair metabolic conversion of 25OHD to 1,25-$(OH)_2D_3$ and lead to a blunting or reversal of the increase in serum 1,25-$(OH)_2D_3$ that had previously occurred with age. Although some studies have shown decreases in serum 1,25-$(OH)_2D_3$ with aging (33–36), others have found no change (37) or increases in serum 1,25-$(OH)_2D_3$ with aging (8, 38). Moreover, the largest study showed an increase until age 65 and then a small decrease thereafter (38). In elderly women, the severity of the 1α-hydroxylase defect was found to be inversely proportional to creatinine clearance with a threshold value of 0.83 mL/s/1.73 m^2 below which serum 1,25-$(OH)_2D_3$ decreased (35). Whereas the inclusion of such patients may have contributed to the age-related decrease in serum 1,25-$(OH)_2D_3$ in other reported studies, only 1 of the 44 subjects reported here had a creatinine clearance below that threshold value.

Based on the findings reported here and those reported in the literature, the following model can be proposed for the pathophysiology of bone loss with aging (Fig. 4). Beginning in early adulthood, an age-related decrease in intestinal VDR leads to intestinal resistance to 1,25-$(OH)_2D_3$ action and impaired calcium absorption. This results in secondary hyperparathyroidism which increases 1,25-$(OH)_2D_3$ production and normalizes calcium absorption, but at the cost of increasing bone turnover (28). Because of the well-documented age-

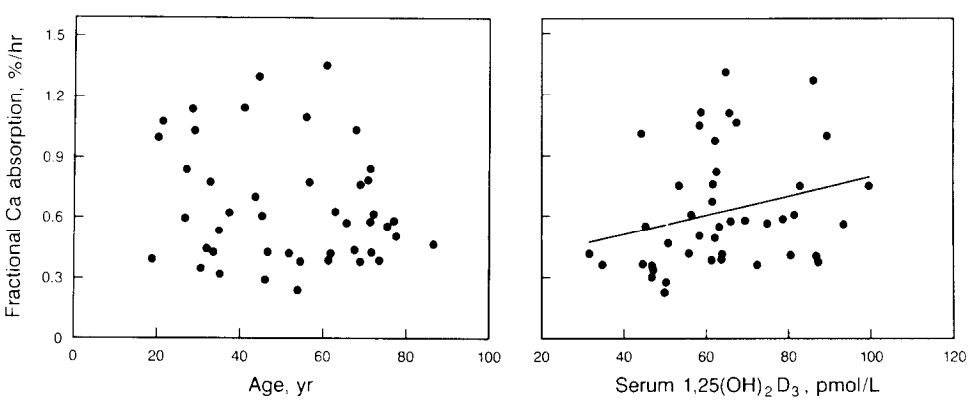

FIG. 1. Relationships with FCA in 44 normal women. *Left*, with age; *right*, with serum 1,25-$(OH)_2D_3$ ($r = 0.36$; $P < 0.05$). FCA = 0.12 + 0.021 × serum 1,25-$(OH)_2D_3$.

TABLE 3. Multiple regression equations for the dependent variables log FCA and intestinal VDR concentration

Dependent variable	Independent variable	β	Individual P value	Overall R^2 (%)	P Value
Log FCA (n = 44)	Intercept	0.86			
	1/serum 1,25-$(OH)_2D_3$	−7.4	0.004		
	Dietary calcium	$-1.5 \cdot 10^{-2}$	0.021		
	Age	$-7.6 \cdot 10^{-3}$	0.031		
				29	0.003
Intestinal VDR (n = 32)	Intercept	504			
	Age	−1.8	0.008		
	Urinary hydroxyproline/GFR	−0.66	0.047		
				29	0.007

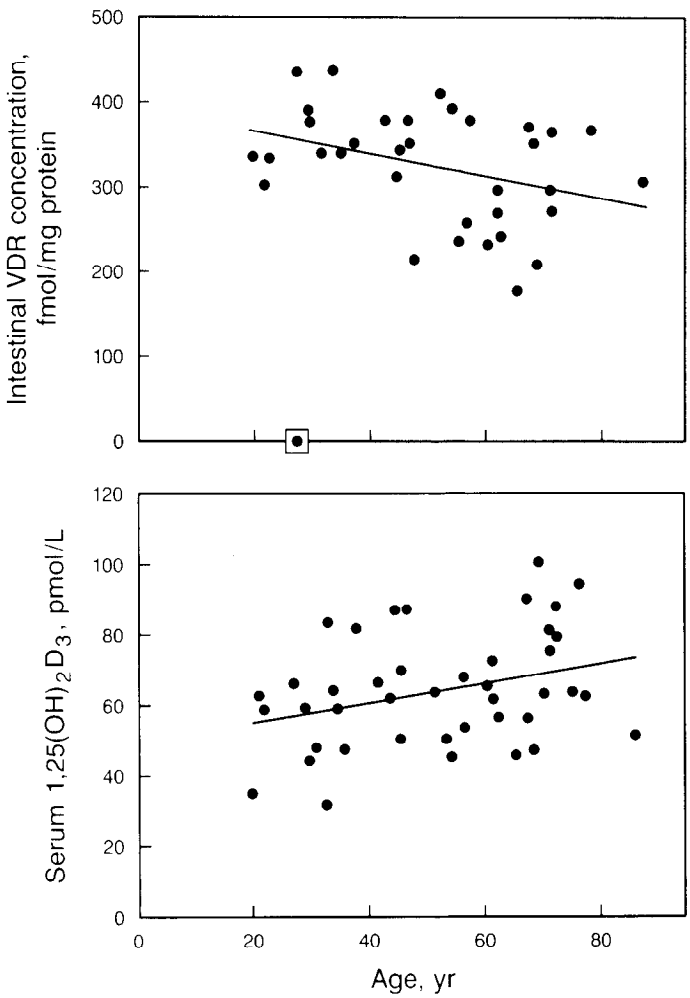

FIG. 2. Relationships with age. *Upper,* With intestinal VDR concentration in 35 normal women (r = 0.38; $P = 0.03$; VDR = 394 − 1.4 × age). The indicated outlier was excluded from the analysis. *Lower,* With serum 1,25-$(OH)_2D_3$ concentration in 43 normal women (r = 0.32; $P < 0.05$; serum 1,25-$(OH)_2D_3$ = 20.6 + 0.11 × age).

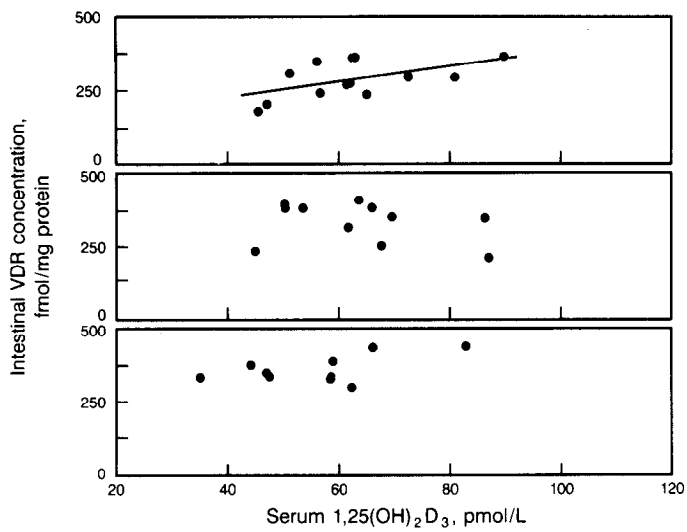

FIG. 3. Relationships with serum 1,25-$(OH)_2D_3$. *Upper,* With intestinal VDR in 13 normal women, ages >60 yr (r = 0.54, $P = 0.06$; VDR = 123 + 2.6 × serum 1,25-$(OH)_2D_3$); *middle,* with intestinal VDR in 11 normal women, ages 40 to 60 yr (r = −0.28, $P = 0.40$); *lower,* with intestinal VDR in 10 normal women, ages 20 to 39 yr (r = 0.57, $P = 0.09$).

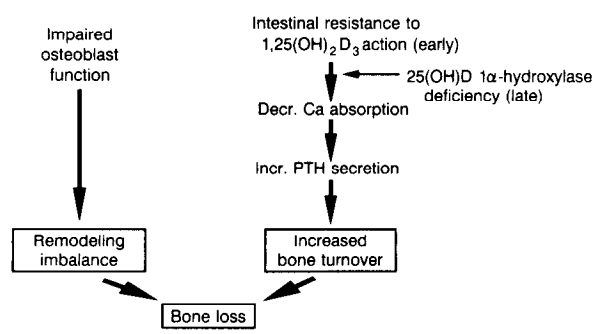

FIG. 4. Pathophysiological model of age-related bone loss.

related defect in osteoblast function at the cellular level (39), however, increased bone turnover leads to increased bone loss. Later in life, impaired 25OHD 1α-hydroxylase activity also contributes to the impairment of calcium absorption. This model has important theoretical and practical implications for the pathogenesis and treatment of type II (age-related) osteoporosis (1).

The mechanism of the apparent intestinal resistance to 1,25-$(OH)_2D_3$ action is not established. However, the age-related decrease in intestinal VDR despite significant increases in serum 1,25-$(OH)_2D_3$ is consistent with the hypothesis that this is caused, at least in part, by a receptor-mediated process. Exposure to exogenous 1,25-$(OH)_2D_3$ normally results in homologous up-regulation of VDR number *in vitro* (40) and *in vivo* in the rat intestine and rat kidney (41, 42). Homologous up-regulation of intestinal VDR in the subgroup older than 60 yr was inadequate to increase the VDR concentrations to within the range of those for the younger

women. We cannot exclude the possibility that local changes in duodenal villi with age could contribute to our findings, although we know of no data from other studies to support this. Although age was the best predictor for VDR in multiple regression analysis, urinary hydroxyproline also was inversely correlated, consistent with a role of the abnormality in the pathogenesis of bone loss. The failure of intestinal VDR to correlate with FCA may have been related to the high variability of the latter technique. Peacock *et al.* (43) have reported that it has a coefficient of variation of 19%.

The reason for the age-related decrease in intestinal VDR is unclear. We favor an age-related process as has been demonstrated in rats (9). Postmenopausal estrogen deficiency may have a direct inhibitory effect on VDR synthesis, independent of vitamin D status, as previously demonstrated in the oophorectomized rat jejunum (44). As assessed by changes in fractional absorption of ^{47}Ca in women 6 months postoophorectomy, only estrogen replacement therapy preserved normal intestinal responsiveness to exogenous 1,25-$(OH)_2D_3$, despite similar increases in serum 1,25-$(OH)_2D_3$ in the placebo group (45). In our study, the correlation between intestinal VDR concentration and serum 1,25-$(OH)_2D_3$ was weakest in perimenopausal women, also suggesting an effect of the menopause on VDR regulation. Our ability to detect decreased intestinal VDR concentration in elderly women was dependent upon use of the newly developed immunoradiometric assay (IRMA) for VDR. Although the results of the IRMA and of the traditional hydroxyapatite binding assay correlated significantly with each other, only the IRMA had the sensitivity and specificity to demonstrate decreased intestinal VDR concentrations with aging.

There are at least three forms of the VDR detectable by immunoblot analysis (46). The IRMA assay measures all forms of the VDR that retain the epitope to which the antibody binds. It will include the form of the receptor that stimulates gene transcription, and it will also measure other forms as well. Since the functional form of the receptor is unknown, the IRMA assay represents a valid total receptor protein measurement, whereas the hydroxyapatite binding assay measures only one form of the receptor.

In conclusion, despite the increase in serum PTH and plasma 1,25-$(OH)_2D_3$ concentrations with age, FCA and intestinal VDR concentration did not increase and, in fact, intestinal VDR decreased significantly with age. These findings are consistent with impaired regulation of intestinal VDR by 1,25-$(OH)_2D_3$. Whether this abnormality is responsible, at least in part, for the intestinal resistance to 1,25-$(OH)_2D_3$ action should now be assessed by studies of direct intervention.

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