

Intestinal Calcium Absorption and Serum Vitamin D Metabolites in Normal Subjects and Osteoporotic Patients

EFFECT OF AGE AND DIETARY CALCIUM

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ABSTRACT Intestinal calcium absorption assessed by a double-isotope method, decreased significantly with aging in 94 normal subjects ($r = -0.22$, $P < 0.025$). In 52 untreated patients with postmenopausal osteoporosis, calcium absorption was significantly lower than normal when either age or habitual calcium intake was used as a covariable ($P < 0.001$). Serum 25-hydroxyvitamin D (25-OH-D) and 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) were measured in 44 normal subjects and 27 osteoporotic patients. For all normals, calcium absorption and serum $1,25(\text{OH})_2\text{D}$ were positively correlated ($r = 0.50$, $P < 0.001$). In nonelderly normal subjects (ages 30–65 yr), dietary calcium intake correlated inversely with both calcium absorption ($r = -0.39$, $P < 0.01$) and with serum $1,25(\text{OH})_2\text{D}$ ($r = -0.50$, $P < 0.01$). Both osteoporotic patients and elderly normal subjects (ages 65–90 yr) differed from nonelderly normals in that these correlations were not present. In addition although serum 25-OH-D was normal, serum $1,25(\text{OH})_2\text{D}$ was significantly decreased in both osteoporotic patients and elderly normals ($P < 0.001$). In osteoporotic patients, calcium absorption increased significantly ($P < 0.001$) after 7 d administration of a small dose ($0.4 \mu\text{g/d}$) of synthetic $1,25(\text{OH})_2\text{D}_3$. In osteoporotics mean serum immunoreactive parathyroid hormone was either normal (COOH-terminal

assay) or low (NH_2 -terminal assay) relative to age-matched controls, and mean serum phosphate was increased.

The data suggest that inadequate metabolism of 25-OH-D to $1,25(\text{OH})_2\text{D}$ contributes significantly to decreased calcium absorption and adaptation in both osteoporotics and elderly normal subjects. In patients with osteoporosis this abnormality could result from a decrease in factors that normally stimulate $1,25(\text{OH})_2\text{D}$ production, such as the decreased parathyroid hormone secretion and increased serum phosphate demonstrated in this group. In elderly subjects a primary abnormality in metabolism of 25-OH-D to $1,25(\text{OH})_2\text{D}$, analogous to that seen in aging rats, cannot be excluded.

INTRODUCTION

Studies using either radiocalcium absorption (1–3) or metabolic balance (4) techniques uniformly have found that intestinal calcium absorption decreases with aging in both sexes, particularly after 70 yr of age (1). With one exception (2), all studies have shown that osteoporotic patients have low calcium absorption when compared with age-matched controls (5–8). Furthermore, in a group of 34 osteoporotic patients who underwent metabolic balance studies, the intestinal absorption of stable calcium was shown to be reduced (8). Available evidence, therefore, implicates a defect in calcium absorption with aging and suggests that this defect is even more pronounced in persons with osteoporosis.

The vitamin D endocrine system now is believed to be the most important regulator of intestinal calcium absorption. Vitamin D undergoes hydroxylation at the

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25-position in the liver and at the 1α -position in the kidney to produce 1,25-dihydroxyvitamin D [$1,25\text{-(OH)}_2\text{D}$],¹ the physiologically important, active form of the vitamin (9). Studies in man have shown that increased intestinal absorption is the principal mechanism that prevents negative calcium balance when dietary calcium intake is decreased (10). Although comparable data are not available in man, Boyle et al. (11) have shown in rats that the adaptive increase in intestinal calcium absorption in response to low calcium intake is mediated by increased production of $1,25\text{-(OH)}_2\text{D}$.

In the present study, we have measured calcium absorption in normal and osteoporotic subjects and related it to serum levels of vitamin D metabolites. We wished to investigate the role of the vitamin D endocrine system in the pathogenesis of impaired calcium absorption in patients with postmenopausal osteoporosis. We also wished to determine whether changes in calcium absorption with aging and in response to differing levels of dietary calcium were related to changes in serum levels of vitamin D metabolites.

METHODS

Normal subjects and patients. 94 normal subjects (77 women and 17 men) whose ages ranged from 30 to 90 yr underwent calcium absorption studies. All normal subjects were volunteers. They were ambulatory, in good health, and not on any medical therapy of taking supplementary vitamins. They had no previous history of back pain, or fractures, and roentgenograms of the spinal column showed no evidence of vertebral fractures. All had normal values for serum calcium, phosphorus, alkaline phosphatase, and glutamic oxaloacetic acid-transaminase. Renal function as assessed by a 24-h urine creatinine clearance was appropriate for their respective age. 44 of them (41 women and 3 men) between ages 30 and 84 yr had fasting blood specimens drawn for determination of vitamin D metabolites on the same day that the calcium absorption studies were performed. All of the studies were performed between March and May.

52 untreated women (ages 54–75 yr) with postmenopausal osteoporosis also underwent calcium absorption studies. Each osteoporotic patient had generalized radiolucency of the spine and one or more vertebral fractures that had developed either spontaneously or after minor incidents such as bending or coughing. They were ambulatory, were in good health except for osteoporosis, and had no recognizable disease or history of use of drugs known to produce osteoporosis. Results of liver function and creatinine clearance tests were within the age-corrected normal range and did not differ from values in the normal subjects. Additional biochemical studies, including measurement of serum vitamin D metabolites were done in 27 osteoporotic women from this group and in 20 of the normal postmenopausal women who were matched as closely as possible for age.

¹Abbreviations used in this paper: $1,25\text{-(OH)}_2\text{D}$, 1,25-dihydroxyvitamin D; 25-OH-D, 25-hydroxyvitamin D; iPTH, immunoreactive parathyroid hormone; PTH, parathyroid hormone.

Tests were performed after subjects had been on their usual intake of calcium, phosphorus, and vitamin D. A diet history was obtained by a trained dietitian in 74 of the female subjects. To assess the reproducibility of the diet history, repeat measurements were obtained in 17 nonelderly subjects (12 normal and 5 osteoporotic), ages 21–62 yr, and in 10 elderly subjects (7 normal and 3 osteoporotic), ages 70–85 yr. They were performed by a second dietitian after an interval of 6 mo–3 yr.

Laboratory studies. A double-isotope method was used to assess intestinal calcium absorption. The fraction of radiocalcium dose absorbed at the end of a 6-h test was calculated by computer with the use of a mathematical deconvolution method, as described (7). All studies were initiated in the fasting state between 0800 and 0900 h. No food was allowed during the first 4 h of the study, at the end of which time a low calcium lunch was given.

A dose of $10\ \mu\text{Ci}$ of high specific activity ^{47}Ca (Atomic Energy Commission, Oak Ridge, Tenn.) was given orally with 100 mg of calcium carrier (as calcium chloride) in 200 ml of deionized water. 5 min after the oral calcium drink, $10\ \mu\text{Ci}$ of sterile, pyrogen-free ^{45}Ca was injected intravenously. Heparinized blood samples were serially obtained on 10 occasions during the 6-h period of the test. 4 ml of plasma was counted for ^{47}Ca in a standard well-type γ -counter (1185 Searle Automatic, Searle Analytic Co., Des Plaines, Ill.) by using a single-channel pulse-height analyzer to exclude counts from the daughter isotope, ^{47}Sc . For determination of ^{45}Ca radioactivity, 2 ml of plasma was placed in a scintillation solution (Instagel, Packard Instrument Co., Inc. Downers Grove, Ill.), and β -emissions were counted in a scintillation counter after waiting a minimum of 8 wk for decay of ^{47}Ca and ^{47}Sc . All counts were corrected for quenching and for ^{45}Ca contamination of the oral ^{47}Ca preparation.

Serum 25-hydroxyvitamin D (25-OH-D) was measured by a modification (12) of the radioassay described by Haddad and Chyu (13). The competitive binding assay used rachitic rat renal cytosol for binding. Preparation of the serum differed. Methanol-chloroform (2:1) was used for extraction of lipids (14). This extract was chromatographed on a Sephadex LH-20 column ($0.9 \times 20\ \text{cm}$; Pharmacia Fine Chemicals Inc., Piscataway, N. J.) in chloroform-hexane (1:1), and the fractions containing both 25-OH-D₂ and 25-OH-D₃ were combined (15). Fractions containing 25-OH-D were evaporated to dryness under nitrogen and redissolved in 95% ethanol for assay. Competition for binding of labeled 25-OH-D₃ by unlabeled 25-OH-D₂ and 25-OH-D₃ is equivalent in this assay system, thus enabling us to measure total 25-OH-D. The coefficient of interassay variation calculated for the current study was 9.6%.

Serum $1,25\text{-(OH)}_2\text{D}$ was measured by the method of Eisman et al. (16) in the laboratory of Dr. H. F. DeLuca (Madison, Wis.). 5 ml of plasma was placed in a separatory funnel, to which 900 cpm of [^3H]1,25(OH)₂D₃ (78 Ci/mmol) was added. A methylene chloride extract was chromatographed on a Sephadex LH-20 column ($0.7 \times 9\ \text{cm}$) in hexane-chloroform-methanol (9:1:1). Fractions containing $1,25\text{-(OH)}_2\text{D}$ were evaporated under nitrogen, redissolved in hexane, and then subjected to high-pressure liquid chromatography on a microporasil column ($0.4 \times 30\ \text{cm}$, Waters Associates, Inc., Milford, Mass.) run in 9:1 hexane-isopropanol at a flow rate of 2 ml/min and 700 lb/in². The overall recovery during extraction and chromatography was $74 \pm 5.2\%$ (SD). The fraction containing $1,25\text{-(OH)}_2\text{D}$ was collected and measured by a competitive binding assay using a rachitic chick intestinal cytosol preparation. [^3H]1,25(OH)₂D₃, 3,000 cpm, was added to each tube. A standard curve from 3 to 150 pg was constructed, and $1,25\text{-(OH)}_2\text{D}$ in each sample, measured in quadruplicate, was calculated from the ^3H counts per minute bound in the pellet and cor-

rected for recovery. Crystalline $1,25(\text{OH})_2\text{D}_3$ and $1,25(\text{OH})_2\text{D}_2$ gave identical competition curves, and the assay measured both $1,25(\text{OH})_2\text{D}_3$ and $1,25(\text{OH})_2\text{D}_2$ as total serum $1,25(\text{OH})_2\text{D}$. For the current study, the interassay variation was 16%.

Serum samples for all normal and osteoporotic subjects were coded in Rochester, Minn. in such a way that the identity of the individual sample was unknown in the Madison laboratory. Also, coding arranged for equal numbers of normal and osteoporotic samples to be present at all stages of the extraction process and assay. Samples were transferred in a frozen state on solid CO_2 to the Madison laboratory. Analysis were performed and the results returned to Rochester.

Serum immunoreactive parathyroid hormone (iPTH) was measured by two radioimmunoassay systems, as described by Arnaud et al. (17, 18): antiserum GP-1M reacts primarily to determinants on the COOH-terminal assay and CH-14M reacts primarily to determinants on the NH_2 -terminal assay portion of the parathyroid hormone (PTH) molecule. Serum calcium was measured by atomic absorption spectrophotometry on samples made up on 0.2% lanthanum chloride. Serum creatinine and phosphorus was measured by standard AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) techniques. All biochemical measurements were performed in research laboratories.

Administration of $1,25(\text{OH})_2\text{D}_3$. The short-term effect of a 7-d administration of synthetic $1,25(\text{OH})_2\text{D}_3$ (Roche Diagnostics Div., Hoffman-LaRoche Inc., Nutley, N. J.) on calcium absorption was studied in 24 patients with postmenopausal osteoporosis and 13 normal subjects. $1,25(\text{OH})_2\text{D}_3$ was administered in 20 ml of milk each day, 1 h before breakfast. The studies were conducted on a metabolic ward while the patients were ingesting a diet calculated by a dietitian to approximate the usual diet with respect to calories, calcium, phosphorus, and vitamin D. Serum and urine calcium determinations were made daily during $1,25(\text{OH})_2\text{D}_3$ administration. Calcium absorption tests were performed before and after the 7-d treatment period: no $1,25(\text{OH})_2\text{D}_3$ was administered on the morning of the second test. Reproducibility of the calcium absorption test was assessed by repeat measurements after no treatment in 17 normal subjects (after 7 d–6 mo) and in 14 osteoporotic subjects (after 1–6 mo).

RESULTS

In the 94 normal subjects, fractional calcium absorption decreased with age ($r = -0.22$, $P < 0.025$). Individual values in 52 osteoporotics are shown in relationship to the 95% confidence limits about the normal regression of calcium absorption on age for normal subjects in Fig. 1. The mean for osteoporotic subjects was significantly lower than normal ($P < 0.001$, analysis of covariance). In 74 normal subjects in whom diet histories were obtained, fractional calcium absorption was correlated inversely with the dietary calcium intake ($r = -0.29$, $P < 0.05$). When these subjects were divided into nonelderly (<65 yr) and elderly (>65 yr) groups, however, the correlation between fractional calcium absorption and dietary calcium intake was improved in the 54 subjects in the nonelderly group ($r = -0.39$, $P < 0.02$), whereas in the 20 elderly subjects there was no significant correlation ($r = 0.03$). Individual values for osteoporotic patients and elderly normal subjects are shown in relationship to the 95%

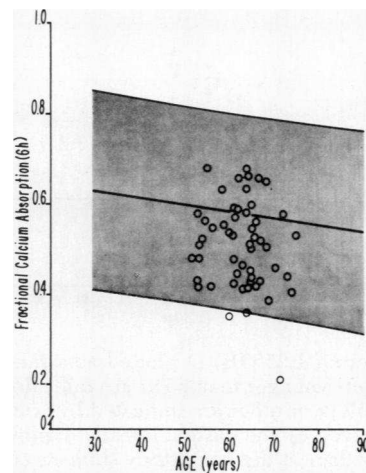


FIGURE 1 Calcium absorption plotted as a function of age. Shaded area represents the mean and 95% confidence limits for normal subjects. Individual values for 52 patients with postmenopausal osteoporosis are given by (○).

confidence limits about the normal regression of calcium absorption on dietary calcium intake for non-elderly normal subjects in Fig. 2. In 17 out of 20 elderly normal subjects values for absorption on intake were below the mean line. The mean for the osteoporotic patients was significantly lower than normal ($P < 0.001$, analysis of covariance) and 15 of the 52 values were below the 95% confidence limits. In the osteoporotic patients, there was no correlation between intestinal calcium absorption and dietary calcium intake.

In the 44 normal subjects who had serum vitamin D metabolites measured, 34 were nonelderly (<65 yr) and 10 were elderly (>65 yr). Mean \pm SE for serum 25-OH-D was 18.6 ± 1.2 ng/ml in the elderly group and 17.2 ± 1.2

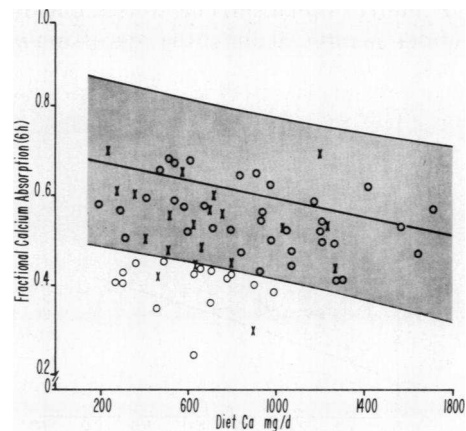


FIGURE 2 Calcium absorption plotted as a function of dietary calcium intake. Shaded area represents the mean and 95% confidence limits for nonelderly normal subjects. Individual values for osteoporotic patients are given by (○). Elderly subjects (>65 yr) are denoted by the symbol (x).

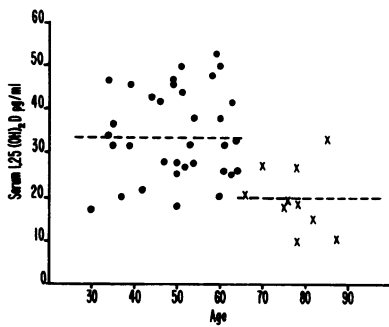


FIGURE 3 Serum 1,25(OH)₂D plotted as a function of age. Normal subjects younger than 65 yr are indicated by (●) and patients age 65 yr or older are indicated by (x). Mean serum 1,25(OH)₂D given by the dashed lines, is significantly lower in the elderly than in the nonelderly subjects ($P < 0.001$).

ng/ml in the nonelderly group, and were not significantly different. All individual values for serum 25-OH-D were within the normal range of 7–50 ng/ml. There was no correlation between fractional calcium absorption and serum 25-OH-D. Mean serum 1,25-(OH)₂D however, was significantly lower ($P < 0.001$) in the elderly (20.2 ± 2.4 pg/ml) than in the nonelderly group (34.0 ± 1.9 pg/ml) as shown in Fig. 3. For all subjects, fractional calcium absorption and serum 1,25-(OH)₂D were positively correlated ($r = 0.50$, $P < 0.001$) and the results are shown in Fig. 4. The correlation between the estimated dietary calcium intake and serum 1,25(OH)₂D for the total group was not quite significant ($r = -0.29$). There was however, a significant inverse correlation between calcium intake and serum 1,25(OH)₂D for the 34 subjects younger than 65 yr of age ($r = -0.50$, $P < 0.01$), (Fig. 5), whereas in subjects 65 yr of age or older there was no significant correlation ($r = 0.03$). Individual values for osteoporotic and elderly normal subjects in relationship to the 95% confidence interval about the regression of serum

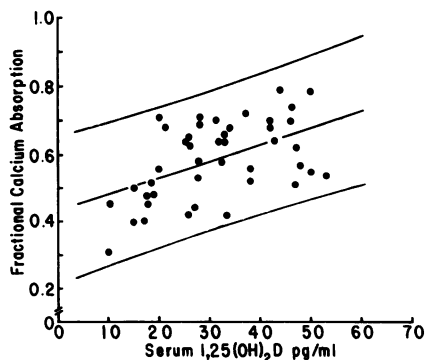


FIGURE 4 Fractional calcium absorption in normal subjects plotted as a function of serum 1,25(OH)₂D. Solid lines represent the mean regression line and 95% confidence limits for all normal subjects.

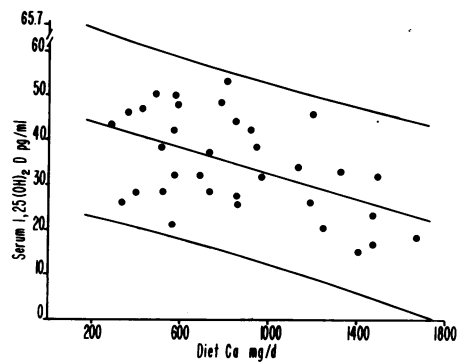


FIGURE 5 Serum 1,25(OH)₂D in normal subjects plotted as a function of dietary calcium intake. Solid lines represent mean regression line and 95% confidence limits for young normal subjects.

1,25(OH)₂D on dietary calcium intake for nonelderly normal subjects are shown in Fig. 6. Adjusted serum 1,25(OH)₂D values were calculated from the regression of serum 1,25(OH)₂D on calcium intake. In the normal nonelderly subjects the adjusted serum 1,25(OH)₂D values showed a much narrower normal range of 23–43 pg/ml compared to an unadjusted range of 15–53 pg/ml.

The results of the substudy evaluating the relationship between calcium absorption and serum vitamin D metabolites in 27 osteoporotics and 20 normals are given in Table I. Mean \pm SE for age of the osteoporotic patients was 61 ± 1 yr, (range, 53–66 yr), compared with 57 ± 1.3 yr for the normal subjects (range, 50–65 yr). Mean fractional calcium absorption was significantly lower in osteoporotic patients than in normal subjects ($P < 0.001$). The slightly younger age in the normal subjects could not account for the difference in absorption between the two groups. The regression equation for fractional calcium absorption on age in normal subjects shows that only 0.02 (17%) of the difference in ab-

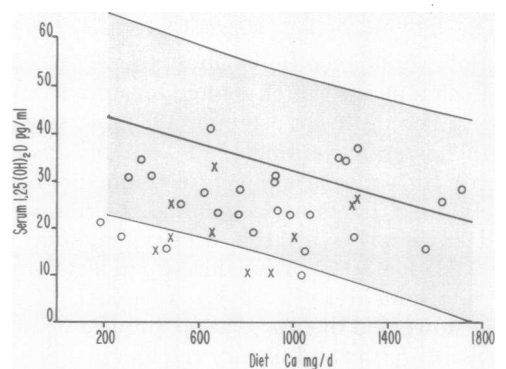


FIGURE 6 Serum 1,25(OH)₂D plotted as a function of dietary calcium intake. Shaded area represents mean and 95% confidence limits for normal subjects. Individual values for patients with osteoporosis are given by (○). Elderly subjects (>65 yr) are denoted by (x).

TABLE I

Comparison between Age-Matched Postmenopausal Osteoporotic Patients and Normal Subjects for Calcium Absorption, Serum iPTH, and Vitamin D Metabolites

	n	Normals*	n	Osteoporotics*	
Serum values					
Calcium, mg/dl	20	9.27±0.07	27	9.34±0.06	NS
Phosphate, mg/dl	20	3.54±0.08	27	3.78±0.07	<0.025
Creatinine, mg/dl	20	0.83±0.02	27	0.82±0.02	NS
iPTH					
Antiserum GP-1M, μ l eq/ml	20	26.3±1.5	12	23.7±1.3	NS
Antiserum CH-14M, ng eqH(1-84)/ml	10	526±51	12	358±43	<0.02
25-OH-D, ng/ml	20	15.9±1.3	27	19.5±1.2	NS
1,25(OH) ₂ D, pg/ml	20	33.2±2.3	27	25.9±1.5	<0.005
Dietary calcium intake, mg/d	20	844±84	27	833±83	NS
Calcium absorption, fraction absorbed/6 h	20	0.61±0.02	27	0.49±0.02	<0.001

* Values are mean±SE.

sorption could possibly be accounted for by the 4-yr difference in age. Both normal and osteoporotic subjects had values for serum 25-OH-D within the normal range (7–50 ng/ml). There was no significant difference between mean serum 25-OH-D levels of the normal and osteoporotic groups. By contrast, the mean serum 1,25(OH)₂D measurement was significantly lower in the osteoporotic group ($P < 0.005$). Individual values are shown in Fig. 7. All but two of the osteoporotic patients had serum 1,25(OH)₂D levels that were at or below the normal mean. In the osteoporotic subjects, there was no correlation between calcium absorption and serum 1,25(OH)₂D ($r = 0.01$) (Fig. 8).

Serum iPTH in the osteoporotic group was not significantly lower when assessed by the COOH-terminal assay but was significantly lower than normal ($P < 0.02$) when assessed by the NH₂-terminal assay. Mean serum calcium was similar in the two groups, but mean serum phosphate was significantly higher in the osteoporotic group ($P < 0.025$). The correlation of serum 1,25(OH)₂D with serum phosphate in the normal subjects was not significant ($r = -0.26$). When the values for serum

phosphate in osteoporotic patients and all normal subjects were merged, however, there was a significant inverse correlation between serum phosphate and serum 1,24(OH)₂D ($r = -0.35$, $P < 0.01$). In comparison, serum calcium in the normal subjects, the osteoporotic patients, or in the combined group showed no correlation with serum 1,25(OH)₂D ($r = 0.08$).

Administration of 1,25(OH)₂D₃. For 31 subjects who had replicate calcium absorption studies made without intervening treatment, the mean±SE change (Δ) in fractional absorption was -0.0012 ± 0.01 , which was not significantly different from zero. In 20 of the 24 osteoporotic patients who received 0.4 μ g/d, calcium absorption increased. For this group, the mean increase in fractional absorption of 0.12 ± 0.02 was highly significant ($P < 0.001$). Three osteoporotic patients who failed to respond to the lower test dose were retested while taking 0.8 μ g/d, and all three had increased fractional absorption with this larger dose. In the 13 normal subjects, the mean±SE increase in fractional calcium

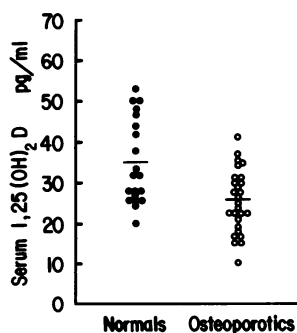


FIGURE 7 Serum 1,25(OH)₂D levels in normal and osteoporotic subjects. The mean level in osteoporotics is significantly lower than in normals ($P < 0.005$).

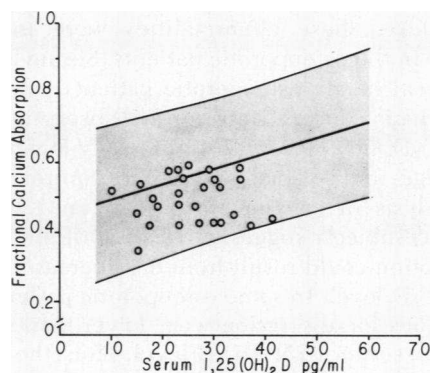


FIGURE 8 Calcium absorption vs. serum 1,25(OH)₂D levels. Shaded area represents mean and 95% confidence limits for normal subjects. Values for osteoporotics are represented by (O).

absorption was 0.10 ± 0.02 after the administration of $0.4 \mu\text{g/d}$ of $1,25(\text{OH})_2\text{D}_3$, and this was significant ($P < 0.001$). The mean increase in 24-h urinary calcium excretion on the 7th d was 63 mg/24 h in the osteoporotic subjects and 78 mg/24 h in the normals subjects; in both groups the increase in urinary calcium was significant ($P < 0.01$).

Reproducibility of diet history. In the 27 subjects the correlation between the first and second diet history was 0.87. The percent difference between the two diet histories was 11% in the elderly groups and 15% in the younger group, indicating good reproducibility of the method.

DISCUSSION

Our finding that serum $1,25(\text{OH})_2\text{D}$ levels are positively correlated with fractional calcium absorption in normal humans is consistent with the belief that $1,25(\text{OH})_2\text{D}$ is an important physiologic regulator of calcium absorption. Although a correlation between absorption and serum $1,25(\text{OH})_2\text{D}$ has previously been reported in humans for merged data of normals and patients with idiopathic hypercalciuria and primary hyperparathyroidism (19), our study is the first demonstration of a significant correlation within the normal range. Also, it is the first demonstration in normal humans that serum $1,25(\text{OH})_2\text{D}$ correlates negatively with dietary calcium intake and, thus, probably is the mediator of intestinal adaptation to changes in dietary calcium intake.

In osteoporotic women, calcium absorption assessed by an accurate double-isotope technique was lower than normal when either age or dietary calcium intake was used as a covariable. The abnormality in absorption was greatest in those osteoporotic patients who had relatively low dietary calcium intakes, which suggests impaired intestinal adaptation. Although elderly nonosteoporotic subjects also had decreased calcium absorption and impaired adaptation to low dietary calcium intake, these abnormalities were more pronounced in the osteoporotic patients. Serum 25-OH-D was normal in the osteoporotic patients, which indicates normal vitamin D nutrition and absorption. Serum $1,25(\text{OH})_2\text{D}$, however, was significantly lower in them than in age- and sex-matched normal controls. Regression analysis of calcium absorption on $1,25(\text{OH})_2\text{D}$ in normal subjects suggests that most of the decrease in absorption could result from the decrease in serum $1,25(\text{OH})_2\text{D}$ level. In some osteoporotic patients, however, values for absorption were lower than expected for a given serum $1,25(\text{OH})_2\text{D}$ level. Thus, the possibility remains that an additional factor may contribute to impaired calcium absorption in some osteoporotic patients. The impaired calcium absorption and adaptation do not appear to be caused by a primary abnormality of intestinal calcium transport or by structural

disease of the small intestine because in most osteoporotic patients calcium absorption was increased after 7 d of treatment with a small dose of either 0.4 or $0.8 \mu\text{g/d}$ of synthetic $1,25(\text{OH})_2\text{D}_3$ (a physiologic dose of $1,25(\text{OH})_2\text{D}_3$ has been estimated to be between 0.2 and $0.8 \mu\text{g/d}$ [20, 21]).

The decreased ratio of serum $1,25(\text{OH})_2\text{D}$:25-OH-D in the osteoporotic patients indicates inadequate metabolism of 25-OH-D to $1,25(\text{OH})_2\text{D}$. Probably this is because of decreased production of $1,25(\text{OH})_2\text{D}$, a process mediated by the renal enzyme, 25-OH-D 1α -hydroxylase. Nonetheless, the possibility of accelerated metabolic clearance of serum $1,25(\text{OH})_2\text{D}$ cannot be excluded.

Decreased production of $1,25(\text{OH})_2\text{D}$ could be caused either by a primary (intrinsic) defect of 1α -hydroxylation in the kidney or by a secondary decrease of one or more factors that normally stimulate this enzyme. In the first instance, serum PTH should be increased; in the second, it should be decreased. As we and others have previously reported (22–24),² and as we show again here, serum iPTH is low or normal (22, 25) in most patients with osteoporosis. In two studies higher serum iPTH levels have been reported in some osteoporotic subjects (26, 27) and in a further study of a small but selected population, serum iPTH was elevated in 38% of their sixteen patients (28). In our own combined series of 87 osteoporotics this subset with high serum iPTH represents only about 10% of the total (22, 24) and may represent etiologically a subgroup (29). Thus, we believe that in the majority of osteoporotic subjects in this study, decreased conversion of 25-OH-D to $1,25(\text{OH})_2\text{D}$ is more likely to be a secondary rather than a primary event. The secondary factors that could decrease 25-OH-D 1α -hydroxylase activity (9) and that were either observed or inferred to be present in our study are decreased mean serum PTH and increased mean serum phosphate levels. Also, there is indirect evidence in birds (30, 31) and in humans (32) that estrogen deficiency per se decreases 25-OH-D 1α -hydroxylase activity. The menopause would be an incomplete explanation however, because postmenopausal women both with and without osteoporosis are estrogen-deficient. It has been suggested that when estrogen is deficient, bone resorbing cells have increased sensitivity to circulating endogenous PTH, whereas the kidney retains its normal sensitivity (22, 33, 34). In patients with postmenopausal osteoporosis, bone cells might be even more sensitive to endogenous PTH. Alternatively, the degree of postmenopausal estrogen deficiency could be greater in the osteoporotic patients, a finding observed by some (8, 35) but not other (36) investigators.

It is not clear to what extent, if any, impaired intestinal calcium absorption exacerbates negative calcium bal-

² D. Baylink. Personal communication.

ance. Impaired absorption would most likely be harmful in those osteoporotic patients with a low intake of dietary calcium. Heaney et al. (37) have reported that calcium intake and balance are positively related in a group of normal perimenopausal women. Moreover, they found that the requirement for zero balance in premenopausal women was 0.987 g of Ca/d, whereas in postmenopausal women it was 1.451 g/d. Both requirements were well above the average intake of postmenopausal American women, which is about 0.7 g/d. They further showed that the higher dietary calcium requirement for zero balance in the postmenopausal women could be largely accounted for by a decrease in the efficiency of calcium absorption. Osteoporotic patients may require an even higher intake of dietary calcium to prevent negative calcium balance. Using the metabolic balance technique, Gallagher et al. (8) found that the amount of calcium in the feces equaled or exceeded dietary calcium intake in 15 of 34 patients with postmenopausal osteoporosis. At least in these patients, impaired gut absorption must have aggravated negative calcium balance. Resolution of this question will require long-term metabolic balance studies before and after treatment with a physiologic dose of 1,25-(OH)₂D₃. Our preliminary results of a double-blind control study in osteoporotic subjects have shown that after 6 mo of treatment with 0.5 μg daily of synthetic 1,25-(OH)₂D₃, calcium absorption was increased and negative calcium balance reduced in every patient, whereas calcium balance was unchanged in those patients treated with placebo (38).

In summary, our studies are consistent with the hypothesis that increased bone loss in postmenopausal osteoporotic women may be the indirect cause of impaired calcium absorption. A possible sequence of events that could account for our findings are shown in Fig. 9. In normal male and female subjects, calcium absorption continues to decrease into the 10th decade of life (1–3), and the effect of aging on calcium absorption may be additive to the effects of the menopause. Also, there is evidence (summarized by Gallagher and Riggs [39]) that the amount of dietary calcium required to prevent negative calcium balance increases with age and is much higher than that consumed by most elderly persons. Both Ireland and Fordtran (40), who studied calcium absorption with a triple-lumen jejunal perfusion system after 4 to 8 wk of adaptation to first a 300 mg, and then a 2000 mg calcium diet, and ourselves in the present study found that elderly subjects had poor adaptation to lower dietary calcium intakes. Thus, impaired intestinal calcium absorption and adaptation, especially at low calcium intakes, might contribute to age-related bone loss.

In the elderly subjects, the decreased serum 1,25-(OH)₂D values and decreased 1,25-(OH)₂D:25-OH-D ratio suggests inadequate metabolism of 25-OH-D to 1,25-(OH)₂D. However, serum iPTH increases with

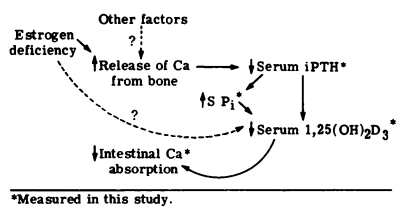


FIGURE 9 Model for pathogenesis of impaired calcium absorption in postmenopausal osteoporosis, which is consistent with the data observed in this study.

aging both in normal subjects (24, 41) and in osteoporotic patients (24) although at any given age the osteoporotic values are always lower than age-matched normal subjects. These results might suggest that in osteoporotics and normal elderly subjects two different mechanisms are occurring. In osteoporotics, the low PTH leads to reduced serum 1,25(OH)₂D levels and impaired calcium absorption, whereas in normal elderly subjects there is an age-related decline in the activity of the 1 α -hydroxylase enzyme in the kidney leading to low serum 1,25(OH)₂D levels, low calcium absorption, and subsequent stimulation of PTH. Recently, Horst et al. (42) demonstrated that calcium absorption, serum 1,25(OH)₂D, and 25-OH-D 1 α -hydroxylase activity were decreased in aging rats. Thus, the possibility remains that an analogous mechanism exists in elderly persons, who may have a primary decrease in the conversion of 25-OH-D to 1,25(OH)₂D. The resultant decrease in serum 1,25(OH)₂D may contribute to their impairment in calcium absorption and adaptation. Further studies, however, are needed to establish this hypothesis.

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REFERENCES

1. Bullamore, J. R., R. Wilkinson, J. C. Gallagher, B. E. C. Nordin, and D. H. Marshall. 1970. Effects of age on calcium absorption. *Lancet*. II: 535–537.
2. Avioli, L. V., J. E. McDonald, and S. W. Lee. 1965. The influence of age on the intestinal absorption of ⁴⁵Ca in women and its relation to ⁴⁵Ca absorption in postmenopausal osteoporosis. *J. Clin. Invest.* 44: 1960–1967.
3. Alevizaki, C. C., D. C. Ikkos, and P. Singhelakis. 1973. Progressive decrease of true intestinal calcium absorption with age in normal man. *J. Nucl. Med.* 14: 760–762.
4. Nordin, B. E. C., R. Williams, D. H. Marshall, J. C. Gallagher, A. Williams, and M. Peacock. 1976. Calcium absorption in the elderly. 11th European Symposium on Calcified Tissues. *Calcif. Tissue Res.* 215: 422–451.
5. Caniggia, A., C. Gennari, V. Bianchi, and R. Guideri. 1963.

- Intestinal absorption of ^{47}Ca in senile osteoporosis. *Acta Med. Scand.* **173**: 613–617.
6. Kinney, V. R., W. N. Tauxe, and W. H. Dearing. 1966. Isotopic tracer studies of intestinal calcium absorption. *J. Lab. Clin. Med.* **66**: 187–203.
 7. Szymendera, J., R. P. Heaney, and P. D. Saville. 1972. Intestinal calcium absorption: concurrent use of oral and intravenous tracers and calculation by the inverse convolution method. *J. Lab. Clin. Med.* **79**: 570–578.
 8. Gallagher, J. C., J. Aaron, A. Horsman, D. H. Marshall, R. Wilkinson, and B. E. C. Nordin. 1973. The crush fracture syndrome in postmenopausal women. *Clinic Endocrinol. Metabol.* **2**: 293.
 9. DeLuca, H. F. 1976. Recent Advances in our understanding of the vitamin D endocrine system. *J. Lab. Clin. Med.* **87**: 7–26.
 10. Malm, O. J. 1958. Calcium requirement and adaptation in adult men. *Scand. J. Clin. Lab. Invest.* **10**(Suppl 36): 1–289.
 11. Boyle, I. T., R. W. Gray, and H. F. DeLuca. 1971. Regulation by calcium of in vivo synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 2131–2134.
 12. Arnaud, S. B., G. B. Stickler, and J. C. Haworth. 1976. Serum 25-hydroxyvitamin D in infantile rickets. *Pediatrics.* **57**: 221–225.
 13. Haddad, J. G., and K. J. Chyu. 1971. Competitive protein-binding radioassay for 25-hydroxycholecalciferol. *J. Clin. Endocrinol. Metab.* **33**: 992–995.
 14. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* **37**: 911–917.
 15. Holick, M. F., and H. F. DeLuca. 1971. A new chromatographic system for vitamin D_3 and its metabolites; resolution of a new vitamin D_3 metabolites. *J. Lipid Res.* **12**: 460–465.
 16. Eisman, J. A., A. J. Hamstra, B. E. Kream, and H. F. DeLuca. 1975. 1,25-dihydroxyvitamin D in biological fluids: a simplified and sensitive assay. *Science (Wash. D. C.)*. **193**: 1021–1023.
 17. Arnaud, C. D., H. S. Tsao, and E. T. Littledike. 1971. Radioimmunoassay of human parathyroid hormone in serum. *J. Clin. Invest.* **50**: 21–34.
 18. Arnaud, C. D., G. W. Sizemore, S. B. Oldham, J. A. Fischer, H. S. Tsao, and E. T. Littledike. 1971. Human parathyroid hormone: Glandular and secreted species. *Am. J. Med.* **50**: 360.
 19. Kaplan, R. A., M. R. Haussler, L. J. Deftos, H. Bone, and C. Y. C. Pak. 1977. The role of $1\alpha,25$ -dihydroxyvitamin D in the mediation of intestinal hyperabsorption of calcium in primary hyperparathyroidism and absorptive hypercalciuria. *J. Clin. Invest.* **59**: 756–760.
 20. Brickman, A. S., J. W. Coburn, and S. G. Massry. 1974. 1,25-dihydroxyvitamin D_3 in normal man and patients with renal failure. *Ann. Intern. Med.* **80**: 161–168.
 21. Gray, R. W., D. R. Wilz, A. E. Caldas, and J. Lemann, Jr. 1977. The importance of phosphate in regulating plasma $1,25(\text{OH})_2\text{D}$ levels in humans: studies in healthy adults, in calcium-stone formers and in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **45**: 299.
 22. Riggs, B. L., C. D. Arnaud, J. Jowsey, R. S. Goldsmith, and P. J. Kelly. 1973. Parathyroid function in primary osteoporosis. *J. Clin. Invest.* **52**: 181–184.
 23. Franchimont, P., and G. Heynen. 1976. Parathormone and Calcitonine Radioimmunoassay in Various Medical and Osteoarticular Disorders. J. B. Lippincott Company, Philadelphia, Pa. 101–107.
 24. Gallagher, J. C., B. L. Riggs, C. M. Jerpak, and C. D. Arnaud. 1977. Aging and immunoreactive parathyroid hormone in normal and osteoporotic subjects. *Clin. Res.* **25**: 623A. (Abstr.)
 25. Dequeker, J., and R. Bouillon. 1977. Parathyroid hormone secretion and 25-hydroxyvitamin D levels in primary osteoporosis. *Calcif. Tissue Res.* **22**(Suppl.): 495–496.
 26. Fujita, T., H. Orimo, K. Okano, and M. Yoskhikawa. 1973. Clinical application of parathyroid hormone radioimmunoassay. *Excerpta Med. Int. Congr. Ser.* **270**: 274–279.
 27. Jowsey, J., and K. P. Offord. Osteoporosis: Juvenile, Idiopathic and Postmenopausal Mechanisms of Localized Bone Loss. Information Retrieval Inc., Washington, D. C. and London.
 28. Teitelbaum, S. L., E. M. Rosenberg, C. A. Richardson, and L. V. Avioli. 1976. Histological studies of bone from normocalcemic postmenopausal osteoporotic patients with increased circulating parathyroid hormone. *J. Clin. Endocrinol. Metab.* **42**: 537–543.
 29. Riggs, B. L., J. C. Gallagher, H. F. DeLuca, A. J. Edis, P. W. Lambert, and C. D. Arnaud. 1978. A syndrome of osteoporosis, increased serum immunoreactive parathyroid hormone, and inappropriately low serum 1,25-dihydroxyvitamin D. *Mayo Clin. Proc.* **53**: 701–706.
 30. Tanaka, Y., L. Castillo, and H. F. DeLuca. 1976. Control of renal vitamin D hydroxylases in birds by sex hormones. *Proc. Natl. Acad. Sci. U. S. A.* **73**: 2701–2705.
 31. Kenny, A. D. 1976. Physiological regulation in egg-laying Japanese quail. *Am. J. Physiol.* **23**: 1609–1615.
 32. Gallagher, J. C., B. L. Riggs, A. Hamstra, and H. F. DeLuca. 1978. Effect of estrogen therapy on calcium absorption and vitamin D metabolism in postmenopausal osteoporosis. *Clin. Res.* **25**: 415A. (Abstr.)
 33. Heaney, R. P. 1965. A unified concept of osteoporosis. (editorial) *Am. J. Med.* **39**: 877–880.
 34. Jasani, C., B. E. C. Nordin, D. A. Smith, and I. Swanson. 1965. Spinal osteoporosis and the menopause. *Proc. R. Soc. Med.* **58**: 441–444.
 35. Marshall, D. H., R. G. Crilly, and B. E. C. Nordin. 1977. Plasma androstenedione and oestrone levels in normal and osteoporotic postmenopausal women. *Br. Med. J.* **2**: 1177–1179.
 36. Riggs, B. L., R. J. Ryan, H. W. Wahner, N. S. Jiang, and V. R. Mattox. 1973. Serum concentrations of estrogen, testosterone and gonadotropins in osteoporotic and nonosteoporotic postmenopausal women. *J. Clin. Endocrinol. Metab.* **36**: 1097–1099.
 37. Heaney, R. P., R. R. Recker, and P. D. Saville. 1978. Menopausal changes in calcium balance performance. *J. Lab. Clin. Med.* **92**: 953–963.
 38. Gallagher, J. C., B. L. Riggs, K. A. Johnson, and H. F. DeLuca. 1978. Effect of treatment with synthetic 1,25-dihydroxyvitamin D in postmenopausal osteoporosis. Proceedings of the 50th Annual Meeting of the Endocrine Society, Miami, Fla., June 1978.
 39. Gallagher, J. C., and B. L. Riggs. 1978. Current concepts in nutrition. Nutrition and bone disease. *N. Engl. J. Med.* **298**: 193–195.
 40. Ireland, P., and J. S. Fordtran. 1973. Effect of dietary calcium and age on jejunal calcium absorption in humans studied by intestinal perfusion. *J. Clin. Invest.* **52**: 2672–2681.
 41. Berlyne, G. M., J. Ben-Ari, A. Kushelevsky, A. Idelman, D. Galinsky, M. Hirsch, R. Shainkin, R. Yagil, and M. Zlotnik. 1975. The aetiology of senile osteoporosis: secondary hyperparathyroidism due to renal failure. *Q. J. Med.* **44**: 501–525.
 42. Horst, R. W., H. F. DeLuca, and N. Jorgenson. 1978. The effect of age on calcium absorption and accumulation of $1,25(\text{OH})_2\text{D}_3$ in intestinal mucosa of rats. *Metab. Bone Dis. Rel. Res.* **1**: 29–33.