

Intestinal Calcium Absorption in Exogenous Hypercortisonism

ROLE OF 25-HYDROXYVITAMIN D AND CORTICOSTEROID DOSE

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ABSTRACT Pharmacologic doses of corticosteroids impair intestinal calcium absorption and contribute to negative calcium balance. However, the relationship between the impaired calcium absorption and a possible defect in the conversion of vitamin D to its physiologically active form, 1,25-dihydroxyvitamin D, is unknown. We compared fractional calcium absorption (double-isotope method, 100-mg carrier) and serum 25-hydroxyvitamin D (25-OH-D) (Haddad method) in 27 patients receiving pharmacologic doses of prednisone with 27 age-, sex-, and season-matched normal subjects. In patients receiving high daily doses of prednisone (15–100 mg/day), calcium absorption ($P < 0.02$) and serum 25-OH-D ($P < 0.001$) were decreased. However, in patients receiving low doses (8–10 mg/day) or high doses (30–100 mg) of prednisone on an alternate-day schedule, both of these parameters were normal. Calcium absorption in the patients treated with daily prednisone correlated inversely with the dose of corticosteroids ($r = -0.52$, $P < 0.025$) and, in all steroid-treated patients, correlated directly with serum 25-OH-D ($r = 0.58$, $P < 0.01$). In four patients who received high-dose corticosteroid therapy for an average of 4 wk, serum 25-OH-D decreased by 35.5% from pretreatment values. Administration of a physiologic or near-physiologic dose of synthetic 1,25-dihydroxyvitamin D₃ (0.4 μ g daily for 7 days) to patients receiving high-dose corticosteroids led to an increase in calcium absorption in all patients. These results suggest that calcium malabsorption in the cortico-

steroid-treated patients is due to a dose-related abnormality of vitamin D metabolism and not to a direct effect of corticosteroids on depressing transmucosal intestinal absorption of calcium.

INTRODUCTION

Pharmacologic doses of corticosteroids impair intestinal absorption of calcium (1–11) and result in a negative calcium balance (4, 9–11), which may contribute to the development of osteoporosis. It has been postulated that corticosteroids decrease intestinal calcium absorption either by interfering with the metabolic activation of vitamin D or by antagonizing, directly or indirectly, the effects of vitamin D on the gut, or by both mechanisms (12). Conversion of vitamin D to its physiologically active form, 1,25-(OH)₂D₃,¹ requires hepatic 25-hydroxylation followed by renal 1- α -hydroxylation (13). Corticosteroid treatment potentially could interfere with either of the two successive hydroxylations or with other metabolic pathways of vitamin D, or it could antagonize the effect of vitamin D on intestinal calcium transport.

Avioli et al. (14) reported that prednisone administration to normal man reduced the plasma half-life of injected [³H]vitamin D. They found an abnormal silicic acid chromatographic profile of plasma extracts, suggesting decreased conversion of vitamin D to biologically active plasma metabolites. However, Aloia et al. (15), in using a competitive binding assay, found that serum 25-hydroxyvitamin D (25-OH-D) was normal in a group of patients with Cushing's syndrome and with iatrogenic hypercortisonism. Also, studies

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¹ Abbreviations used in this paper: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 25-OH-D, 25-hydroxyvitamin D.

in rats (3, 5, 6, 8, 16) demonstrated a direct effect of very large doses of corticosteroid on transmucosal calcium transport that was independent of vitamin D metabolism. However, other studies in rats by Carré et al. (17) suggested that corticosteroids may affect vitamin D metabolism by inactivating $1,25(\text{OH})_2\text{D}_3$ after intestinal localization. Thus, the relationship of impaired calcium absorption to vitamin D metabolism in hypercortisonism is unclear.

Studies attempting to relate the defect in calcium absorption to vitamin D metabolism have not been reported. Therefore, we evaluated intestinal absorption of radiocalcium, serum 25-OH-D, and corticosteroid dose in 27 patients receiving pharmacologic doses of prednisone for a variety of connective tissue disorders and in 27 age-, sex-, and season-matched normal subjects. In a small number of patients, we determined the effect of small doses of $1,25(\text{OH})_2\text{D}_3$ on calcium absorption to observe the responsiveness of the intestine to the physiologically active metabolite of vitamin D. Our results suggest that calcium malabsorption in the steroid-treated patients is due to abnormality in vitamin D metabolism, the degree of which is proportional to the dose of administered steroid.

METHODS

Patients. The 27 patients undergoing radiocalcium absorption studies had a variety of connective tissue disorders (Table I), for which they had received pharmacologic doses of corticosteroids for periods ranging from 1 mo to 15 yr (average 3 mo). Three experimental groups (high-dose, alternate-day, and low-dose prednisone) and a control group were studied. 16 patients were receiving high-dose prednisone therapy (mean dose 40 mg/day, range 15–100 mg/day). Six patients were receiving prednisone as a single dose on alternate days (mean dose 68 mg every other day, range 30–100 mg every other day). Five patients were receiving

low-dose prednisone therapy (mean 9.5 mg/day, range 8–10 mg/day). All except one of the patients were studied at a time when their underlying disease was suppressed and when their condition was stable as judged clinically and by laboratory indices. Criteria for inclusion in the study included normal serum glutamic-oxaloacetic transaminase, alkaline phosphatase, and creatinine values, absence of previous stomach or small bowel surgery, and no prior treatment with sex steroids, calcium, or vitamin D. Antacids were stopped at least 24 h before each study. No patient had evidence of osteoporosis at the time of the study. 27 control subjects were selected with the same criteria. Age and sex of these subjects were similar to those of the corticosteroid-treated patients. Patients and controls were studied between the end of April and October to minimize the effects of seasonal variations on serum 25-OH-D and calcium absorption. Complete dietary histories were obtained by a trained dietician from all normal and corticosteroid-treated subjects. Additionally, four patients—two with temporal arteritis, one with rheumatoid arthritis, and one with polymyalgia rheumatica—had serum drawn for 25-OH-D determination before and after 3–7 wk of treatment with high doses of prednisone (mean 45 mg/day, range 15–100 mg/day). Informed consent was obtained in all patients and control subjects before the study.

Laboratory studies. A double-isotope method was used to assess intestinal calcium absorption. The fraction of a radiocalcium dose absorbed at the end of the 6-h test period was calculated by computer with the use of a mathematical deconvolution method as previously described (18, 19). All studies were performed in the fasting state between 8:00 and 9:00 a.m. No food was allowed for the first 4 h of the study, at the end of which time a low-calcium lunch was given. Patients receiving prednisone were not given their usual morning dose on the day of study until the noon meal. Three of the patients receiving alternate-day prednisone were tested on their "on" day and three on their "off" day.

A dose of 10 μCi of high specific activity ^{47}Ca (Atomic Energy Commission, Oak Ridge, Tenn.) was given by mouth with 100 mg of calcium (as calcium chloride) carrier in 200 ml of deionized water. 5 min after the oral calcium drink, 10 μCi of sterile, pyrogen-free ^{45}Ca was injected intravenously. Heparinized blood samples were serially obtained on 10 occasions over the 6-h period of the test. 4 ml of plasma was counted for ^{47}Ca in a standard well-type gamma counter (1185 Searle Automatic, Searle Analytic Inc., Des Plaines, Ill.) 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 scintillation solution (Instagel, Packard Instrument Co., Downers Grove, Ill.), and beta emissions were counted in a scintillation counter (Isocap 300, Searle Analytic Inc.) 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 calcium and magnesium were measured by atomic absorption spectrometry. Serum creatinine, alkaline phosphatase, and phosphorus were measured by standard Auto-Analyzer techniques (Technicon Instruments Corp., Tarrytown, N. Y.).

Serum 25-OH-D was measured by a modification (20) of the competitive protein-binding assay of Haddad and Chyu (21). Preparation of the serum differed. Methanol/chloroform (2:1) was used for extraction of lipids (22). This extract was chromatographed on a Sephadex LH20 column of 0.9×20 cm (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) in chloroform/hexane (1:1), and the 25-OH-D component was isolated (23). Fractions containing 25-

TABLE I
Underlying Connective Tissue Disease in
Prednisone-Treated Patients

Disease	Type of treatment		
	High dose	Alternate day	Low dose
	no. of patients		
Temporal arteritis	6	1	2
Polymyositis	1	2	
Obstructive pulmonary disease	2	2	
Rheumatoid arthritis	1		2
Lupus erythematosus	1		1
Polymyalgia rheumatica	1		
Polyarteritis nodosa	2	1	
Mixed connective tissue disease	2		
Total	16	6	5

OH-D were evaporated to dryness under nitrogen and re-dissolved in 95% ethanol for assay. The competitive binding assay uses a rachitic rat renal cytosol as a binding protein. Competition for binding of labeled 25-OH-D₃ by unlabeled 25-OH-D₂ and 25-OH-D₃ is equivalent in this assay system. The coefficient of interassay variation calculated for the current study was 9.6%.

Administration of 1,25(OH)₂D₃. The short-term effect of a 7-day administration of 0.4 µg/day of synthetic 1,25(OH)₂D₃ (Roche Diagnostics Div., Hoffmann-LaRoche, Inc., Nutley, N. J.) on calcium absorption was studied in five normal subjects and in five of the patients receiving high-dose corticosteroid therapy. The studies were conducted on a metabolic ward. During these studies the patients ingested their usual diets with respect to calories, calcium, phosphorus, and vitamin D. Serum and urine calcium determinations were made daily during 1,25(OH)₂D₃ administration. Calcium absorption tests were performed before and after the 7-day treatment period; no 1,25(OH)₂D₃ was administered the morning of the second test.

RESULTS

The clinical characteristics, results of dietary histories, and biochemical data of the patients and control subjects are summarized in Table II. There were no significant differences in age, sex, or dietary history, or in serum minerals (calcium, phosphorus, magnesium) or alkaline phosphatase.

Serum 25-OH-D. Individual concentrations of serum 25-OH-D were within the range of normal (7–50 ng/ml) in all except one patient, whose value was 6.5 ng/ml. Mean values, however, were significantly ($P < 0.001$) lower in the high-dose corti-

steroid group than in the control group. Mean values for the low-dose and alternate-day groups did not differ from those of the control group (Table II). In both groups of patients receiving daily prednisone, serum 25-OH-D correlated inversely with the dose of the drug ($r = -0.52$, $P < 0.025$) (Fig. 1a) and directly with fractional calcium absorption ($r = 0.58$, $P < 0.01$) (Fig. 2). There was no relationship between calcium absorption and serum 25-OH-D in the 27 normal subjects or between calcium absorption and the estimated intake of vitamin D, serum minerals, or alkaline phosphatase in either normal or corticosteroid-treated patients.

Fig. 1b illustrates the effect of treatment of 15–100 mg of prednisone daily on serum 25-OH-D in four patients treated for an average of 4 wk during the summer. The average decrease in serum 25-OH-D was 37% for this small group. The magnitude of change and the corresponding daily dose of prednisone are as follows: 15 mg was followed by a 24.5% decrease, 30 mg by 39%, 45 mg by 34%, and 100 mg by 44.6%. The proportional decrease in each of these values was significantly more ($P < 0.01$) than changes expected as a result of interassay variation.

Calcium absorption studies. Values for fractional calcium absorption for the corticosteroid-treated and normal subjects are given in Table II, and individual values are given in Fig. 3. In neither the normal subjects nor the corticosteroid-treated patients, was there a significant correlation with fractional calcium

TABLE II
Clinical, Dietary, Biochemical, and Calcium Absorption Data in 27 Patients and 27 Normal Subjects

	Study group			
	Normal subjects (n = 27)	Low-dose therapy (n = 5)	High-dose therapy (n = 16)	Alternate-day therapy (n = 6)
Prednisone dose, mg (mean and range)	None	9.5 (8–10)	40 (15–100)	68 (30–100)
Age, yr	55.5±3.2	48.0±8.1	57.1±3.2	52.3±7.9
Sex, M/F	9/19	0/5	4/13	4/2
Diet				
Vitamin D, U/day	215±35	142±62	189±31	303±76
Calcium, mg/day	994±116	652±216	839±109	1,154±224
Serum biochemical data				
Calcium, mg/dl	9.31±0.09	9.50±0.21	9.31±0.07	9.43±0.13
Phosphorus, mg/dl	3.48±0.04	3.52±0.15	3.68±0.18	3.20±0.14
Magnesium, mg/dl	1.98±0.01	2.00±0.05	2.00±0.05	2.03±0.03
Alkaline phosphatase, IU/liter	133±5	149±23	148±13	124±19
Serum 25-OH-D, ng/ml	21.4 (12.4–38.8)	19.4 (10.2–22.0)	13.9 (6.5–24.8)*	17.9 (15.4–27.3)
Calcium absorption (fraction of dose absorbed)	0.616±0.018	0.690±0.035	0.537±0.027‡	0.650±0.230

All values are mean±SE except for serum 25-OH-D, for which mean and range are given.

* Significance of difference from normal group: $P < 0.001$.

‡ Significance of difference from normal group: $P < 0.02$.

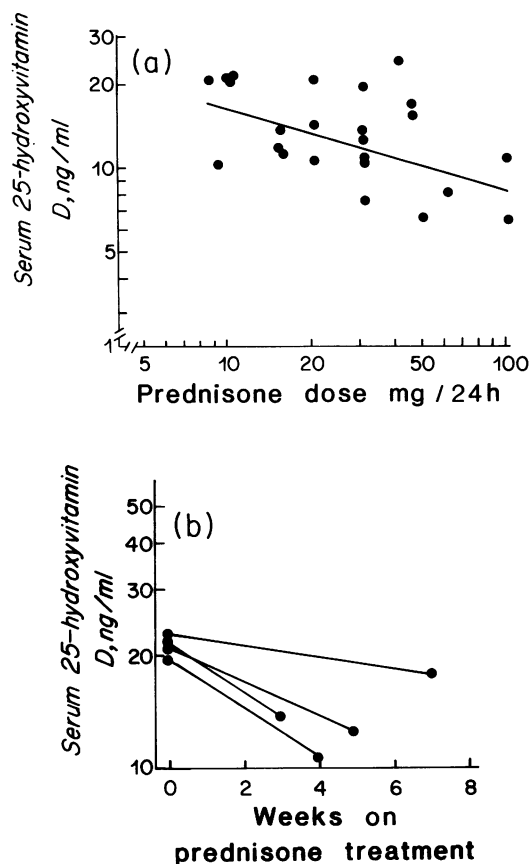


FIGURE 1 (a) Relationship of daily dose of prednisone (8–100 mg) to concentration of serum 25-OH-D. Regression is logarithmic and significant ($r = -0.52$, $P < 0.025$). (b) Effect of daily doses of 15–100 mg prednisone on serum 25-OH-D measured before and 3–7 wk after initiation of treatment.

absorption and estimated habitual dietary intake of calcium. Mean values for calcium absorption were within the normal range in the low-dose and alternate-day groups but were significantly lower ($P < 0.02$) in the high-dose group of corticosteroid patients than in the control group or other treatment groups. In four of the patients who were receiving high-dose, daily prednisone, values for fractional calcium absorption were in the high-normal range. The diets of these four patients did not differ from those of other high-dose, steroid-treated patients or normal controls. However, they were suntanned and had been exposed to 2 or 3 h of midafternoon sunshine daily. An additional three suntanned patients in the high-dose group had significantly decreased fractional calcium absorption values and correspondingly lower concentrations of serum 25-OH-D.

Effect of 1,25(OH)₂D₃ administration. The mean variation of calcium absorption in replicate weekly studies in five normal subjects was 4.8%. As shown in

Fig. 4, administration of 0.4 $\mu\text{g/day}$ of synthetic 1,25-(OH)₂D₃ resulted in an increased calcium absorption in four of five normal individuals and in all five of the corticosteroid-treated patients. Both the difference in calcium absorption before and after treatment and the posttreatment values were greater in the corticosteroid-treated group (Fig. 4) than in controls.

Treatment with 1,25(OH)₂D₃ resulted in hypercalciuria, i.e. more than 300 mg calcium/day, in one normal subject, who excreted 277 mg/24 h before 1,25-(OH)₂D₃ treatment and 345 mg/24 h after. After 1,25-(OH)₂D₃ administration to the five normal subjects, the mean urine calcium increased from 209 ± 20.6 to 234 ± 32.6 mg/24 h; this increase was not significant. In the corticosteroid-treated patients, the mean urine calcium increased significantly after 1,25(OH)₂D₃ administration, from 163 ± 40 to 197 ± 32 mg/24 h ($P < 0.05$). Serum calcium did not change significantly in either group.

DISCUSSION

Our data show that the decrease in intestinal absorption of calcium associated with high-dose corticosteroid administration is accompanied by decreased concentration of serum 25-OH-D. The latter could be due to nutritional deficiency of vitamin D or to an effect of corticosteroid therapy on vitamin D metabolism, by decreasing either the absorption of vitamin D or the reabsorption of metabolites involved in a recently described enterohepatic circulation of 25-OH-D (24). However, these explanations are not supported by clinical or biochemical findings. All patients had adequate amounts of vitamin D in their diets, and they were studied during the summer months when nutritional status of vitamin D generally is best (25). None of the patients had evidence of steatorrhea or the concentrations of blood minerals or alkaline phosphatase that are found in nutritional deficiency disease. In the four patients studied before and

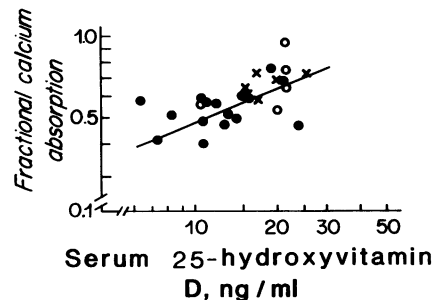


FIGURE 2 Relationship between fractional intestinal absorption of calcium, calculated at 6 h, and serum 25-OH-D. Regression is logarithmic and significant ($r = 0.58$, $P < 0.01$). ●, high-dose corticosteroid patients; ○, low-dose patients; ×, alternate-day patients.

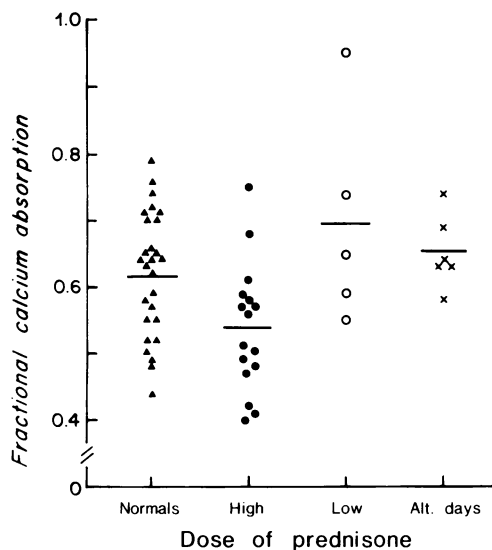


FIGURE 3 Individual values for fractional intestinal absorption of calcium, calculated at 6 h. ▲, normal individuals; ●, high-dose corticosteroid patients; ○, low-dose patients; ×, alternate-day patients. Mean for high-dose group is significantly lower than that for normals.

after 3–7 wk of therapy with high doses of corticosteroids, serum 25-OH-D decreased by 35.5%. It is unlikely that depletion of vitamin D stores could have occurred in such a short time. Also, a specific effect on vitamin D metabolism is suggested by the inverse correlation between corticosteroid dose and serum 25-OH-D.

Gallagher et al. (4) have reported that small doses of vitamin D were ineffective and that large pharmacologic doses (mean dose 250 $\mu\text{g}/\text{day}$)² were required to correct calcium malabsorption in hypercortisonism. By contrast, we found that calcium malabsorption was reversed by 0.4 $\mu\text{g}/\text{day}$ of synthetic 1,25(OH)₂D₃, an extremely small dose. Based on information presently available (26–28), this probably is a physiologic or near-physiologic dose. Thus, impaired conversion of vitamin D to 1,25(OH)₂D₃ is suggested.

Additionally, correction of calcium malabsorption in our patients with a physiologic or near-physiologic dose of synthetic 1,25(OH)₂D₃ is strong evidence against a specific corticosteroid-induced defect in trans-mucosal calcium transport that is unrelated to vitamin D metabolism. Such an abnormality has been reported to be present in corticosteroid-treated rats (3, 5, 6, 8, 16). However, this species is particularly resistant to developing corticosteroid-induced osteoporosis (29), and doses of corticosteroids required to demonstrate this effect were in the order of 5 mg/kg larger than those encountered under clinical circumstances in man.

The metabolic pathway of vitamin D metabolism which is impaired concerns the intermediary metabolite of vitamin D, 25-OH-D. It is not clear whether the decrease in serum 25-OH-D which we have observed reflects decreased hepatic 25-hydroxylation of vitamin D or, as has been shown to occur after chronic therapy with anticonvulsant drugs (30, 31), a more rapid degradation and metabolism of 25-OH-D to biologically inactive forms. It is of interest that both glucocorticoids and anticonvulsant drugs are known to induce drug- and steroid-metabolizing microsomal enzymes (32, 33). However, the data do not exclude the possibility of an additional abnormality in subsequent metabolism of 25-OH-D such as decreased renal 1- α -hydroxylation or accelerated inactivation of 1,25(OH)₂D₃ (17, 34).

Previously, it has been reported in renal transplant recipients that calcium absorption was inversely related to corticosteroid dose, independent of the degree of renal function (35). Our observation that neither small daily doses of prednisone (<15 mg/day) nor large doses given on an alternate-day schedule decreased calcium absorption or lowered serum 25-OH-D is of practical clinical importance. The latter observation may explain in part why less negative calcium balance data were reported on an alternate-day schedule of medication (36). Also, the normal concentrations of serum 25-OH-D reported by Aloia et al. (15) could be reconciled with our findings if some of their patients were receiving the equivalent of <15 mg/day of prednisone or were receiving corticosteroids on an alternate-day schedule.

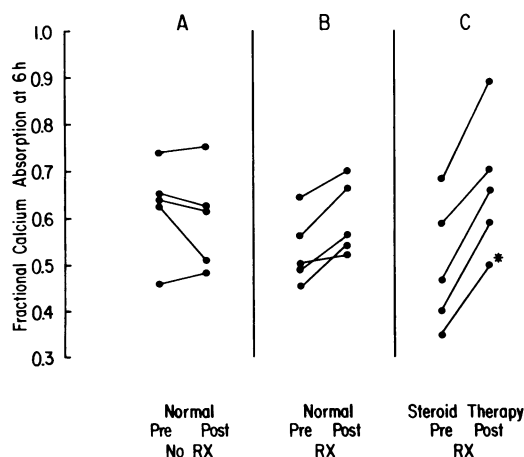


FIGURE 4 Fractional intestinal absorption of calcium before and after 7 days of no therapy or oral administration of 1,25(OH)₂D₃. (A) No therapy in normal volunteers; (B) 0.4 μg daily for 7 days to normal volunteers; (C) 0.4 μg daily for 7 days to high-dose steroid patients. One patient (*) on high-dose steroid treatment represents the only patient in the study whose underlying collagen disease was clinically active.

² Equivalent to 20,000 IU.

The cause of bone loss in corticosteroid-induced osteoporosis is undoubtedly complex. The several factors that many contribute to the bone disease include decreased collagen synthesis, decreased renal conservation of calcium, and intestinal calcium malabsorption (12). Our data document the relationship between steroid dose and abnormal vitamin D metabolism and calcium malabsorption. Thus, they suggest that it may be possible to control the defect in calcium transport by specific therapy with synthetic metabolites of vitamin D. In this regard, it is of interest that Hahn et al. (37) have recently reported that therapy with moderately large doses of synthetic 25-OH-D increased intestinal calcium absorption and peripheral bone density in patients with exogenous hypercortisolemia.

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