Altered Mineral Metabolism in Glucocorticoid-Induced Osteopenia

EFFECT OF 25-HYDROXYVITAMIN D ADMINISTRATION

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A B S T R A C T   Parameters of mineral and bone metabolism were studied in 17 patients treated chronically with supraphysiologic doses of glucocorticoids. When compared to 15 matched normal subjects, the patient group exhibited similar serum 25-hydroxyvitamin D (25-OHD) levels, decreased intestinal ^{47}Ca absorption, increased serum immunoreactive parathyroid hormone, and decreased forearm bone mass. Iliac crest bone biopsies revealed a decreased bone formation rate and increased osteoclast number. Treatment with 25-OHD (mean dose 40.3 μg/d) and calcium (500 mg/d) in nine patients produced a 46% increase in ^{47}Ca absorption ($P < 0.001$) and a 54% decrease in serum immunoreactive parathyroid hormone ($P < 0.001$) by 3 mo. In addition, by 12 mo the treatment group exhibited (*a*) a 13.2±5.1% increase in metaphyseal ($P < 0.001$) and a 2.1±0.4% increase in diaphyseal ($P < 0.05$) forearm bone mass, and (*b*) significant decreases in cortical and endosteal osteoclast number. Biochemical and bone mass changes persisted through 18 mo. No significant changes in any parameter occurred in eight control patients administered calcium 100 mg/d. It is concluded that treatment with 25-OHD and calcium can significantly improve parameters of mineral and bone metabolism in patients with glucocorticoid-induced osteopenia.

INTRODUCTION

Chronic maintenance of supraphysiologic levels of glucocorticoids, whether of enodgenous or exogenous origin, is commonly associated with the development of severe osteopenia in man (1–6). Histologic studies of bone in patients with steroid-induced osteopenia have demonstrated both decreased formation rates and increased numbers of osteoclasts and resorption sites (7, 8). The decrease in formation rate has been attributed to an inhibitory effect on osteoblast function. Moderate doses of glucocorticoids decrease both the synthesis of collagen by preexisting osteoblasts and the recruitment of progenitor cells to functioning osteoblasts (7–10). This reduced function apparently represents a direct cellular effect since the addition of cortisone to osteoblasts in culture markedly reduces protein synthesis (10).

Steroid-induced increases in bone resorption rates have been variously attributed to either direct stimulation of osteoclast activity or increased parathyroid hormone (PTH)[1] secretion. However, glucocorticoids do not directly stimulate bone resorption in vitro (11) and parathyroidectomy abolishes the osteoclastic response in animals (12). Moreover, it has been recently demonstrated that chronic glucocorticoid administration in man is associated with elevated serum immunoreactive PTH (iPTH) concentrations (13–15) which can be suppressed by calcium infusion (14). Thus it appears likely that the increased bone resorptive activity is due to an increase in PTH secretion presumably secondary, at least in part, to inhibition of intestinal calcium absorption (16–19).

The basis for the inhibitory effect of glucocorticoids on intestinal calcium transport remains controversial. Several authors have suggested that glucocorticoids

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[1] *Abbreviations used in this paper:* iPTH, immunoreactive parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; PTH, parathyroid hormone.

may reduce serum 25-hydroxyvitamin D (25-OHD) concentration either by impairing the hepatic conversion of vitamin D to 25-OHD or by altering subsequent 25-OHD metabolism (19–21). In contrast, others have reported normal conversion of [³H]vitamin D_3 to its active metabolites in cortisone-treated rats (17, 22) and normal serum 25-OHD concentrations, measured by competitive-protein binding assay, in steroid-treated patients (23, 24). This question remains unresolved; however, it has been reported that administration of vitamin D in doses of 50,000–100,000 U/wk for 12 wk or 0.4 μg/d of 1,25-$(OH)_2D_3$ for 7 d significantly increases intestinal calcium absorption in steroid-treated patients (18, 19). This being the case, we hypothesized that restoring intestinal calcium absorption to normal levels in such patients by administering 25-OHD and calcium supplements would suppress PTH-induced increases in resorption rates, thereby favorably affecting bone mineral status. The results of our studies indicate that such a regimen does indeed produce a substantial improvement in mineral metabolism parameters.

METHODS

17 patients with glucocorticoid-induced osteopenia were chosen for study. Criteria for the diagnosis included (a) treatment with supraphysiologic doses of glucocorticoids (>7.5 mg prednisone-equivalents/d) for a least 1.5 yr, (b) osteopenia detectable by routine radiographic techniques, and (c) a characteristic disproportionately greater loss of metaphyseal relative to diaphyseal bone mass in the radius as determined by our previously described photon absorption technique (6).

Criteria for inclusion in the study included (a) relatively stable glucocorticoid dose (<25% change in dose over the preceding 6 mo), (b) a creatinine clearance of >80 ml/min per 1.73 m², (c) normal liver function tests, and (d) evidence of normal intestinal fat absorption including absence of symptoms of intestinal malabsorption, a normal serum carotene, a normal D-xylose test, and absence of increased fat on routine stool specimens. Specifically excluded from study were patients who had been maintained on medications known or suspected to alter bone mineral metabolism, such as anticonvulsant drugs, cytotoxic agents, estrogens, androgens, fluoride, phosphate, or vitamin D. 19 age- and sex-matched healthy subjects selected by identical criteria for normal renal, hepatic, and gastrointestinal function were also studied to provide comparative data. Informed consent was obtained from all subjects before entry into the study. Patients were divdied into two groups matched as nearly as possible for age, sex, diagnosis, and dose and duration of steroid therapy. Clinical and dietary data for the 9 25-OHD treatment and 8 control patients, as well as the 19 normal subjects are given in Table I. The distribution of clinical diagnoses in the 25-OHD treatment group was rheumatoid arthritis, six patients; systemic lupus erythematosis, two patients; and chronic obstructive pulmonary disease, one patient. The control group was composed of six patients with rheumatoid arthritis and two patients with systemic lupus erythematosis. All studies were performed on the Clinical Research Center of Barnes Hospital, Washington University School of Medicine. Upon admission to study all patients underwent an extensive interview to determine customary vitamin D and calcium intake and hours of sunlight exposure as previously described (25). Physical functional status was determined by the Steinbrocker criteria (26).

Bone mass was determined by our previously described photon absorption technique (6) in the radius of the lesser-used arm at two locations: a metaphyseal site 2 cm proximal to the distal end of the ulna and a diaphyseal site one-third the distance from the distal to proximal end of the radius. At least four scans were performed at each site. The proximal site is composed primarily of cortical bone whereas the distal site contains a relatively greater proportion of trabecular bone (27). Values were calculated as grams per square centimeter and expressed as a percent of mean age-sex normal values based on our previously established norms (6). The accuracy of this technique is ≅4% and the precision 2–3% (8). Blood samples were obtained at 8 a.m. after an overnight fast. Serum calcium, inorganic phosphate, alkaline phosphatase, albumin, and creatinine concentrations, 24-h urinary calcium and creatinine excretion, and creatinine clearance were determined by Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (25,28). Serum iPTH concentration was determined by the radioimmunoassay technique of Slatopolsky et al. (29) and serum 25-OHD concentration by the competitive protein-binding assay of Haddad and Chyu (30). At least two determinations of each of the above parameters were obtained on all study patients before initiation of the study and then once a month for the first 12 mo of study. Intestinal ⁴⁷Ca absorption was determined by the forearm counting technique of Wills et al. (31) employing a 100-mg ⁴⁰Ca carrier dose given at 8 a.m. after an overnight fast. ⁴⁷Ca absorption was determined before initiation of treatment and at 3-mo intervals thereafter.

Contralateral transiliac needle bone biopsies were performed on 14 patients before and after 12 mo of treatment. Eight patients (four treatment, four control) were administered time-spaced tetracycline labels before each biopsy for morphologic quantitation of the rate of bone mineralization (32). The tetracycline was administered as 250 mg orally at 6-h intervals for 2 d, with a 14-d interval between labels. The clinical characteristics and responses to treatment of the patients in each group who were subjected to bone biopsy were representative of that group as a whole. After biopsy, each specimen was fixed in neutral buffered formalin, embedded in methyl methacrylate, and cut into nondecalcified sections on a Jung model K sledge microtome (American Optical Corp., Scientific Div., Buffalo, N. Y.). An entire section taken from each biopsy, including both cortices and intervening trabeculae, was stained by a modification of the Goldner technique (33) and histologically quantitated using a Zeiss II integrating eyepiece (Carl Zeiss, Inc., New York).

In the pretreatment biopsies, histometric parameters were determined in trabecular bone and the results compared to similar data obtained from 14 individuals of similar age and sex selected from a series of sudden death autopsy cases. Parameters measured were: (a) percent trabecular bone volume, the percent of trabecular space occupied by bone matrix; (b) percent relative osteoid volume, the percent of trabecular bone matrix which is nonmineralized; (c) percent total osteoid surface, the percent of trabecular surface covered by nonmineralized bone matrix (osteoid); (d) percent osteoblastic-osteoid surface, the percent of trabecular bone covered by osteoid lined by characteristic cuboidal osteoblasts; and (e) number of osteoclasts per square millimeter trabecular space.

Biopsies from the steroid-treated patients were then more intensively analyzed for determination of the effects of 25-OHD plus calcium supplement therapy. In addition to the above, the following histometric parameters were quantitated

T. J. Hahn, L. R. Halstead, S. L. Teitelbaum, and B. H. Hahn

in these biopsies: (*f*) percent cortical bone volume, the percent of cortical bone space occupied by bone matrix; (*g*) osteoclasts per square millimeter cortical space; (*h*) osteoclasts per square millimeter cortical-endosteal surface; and (*i*) osteoclasts per millimeter trabecular endosteal surface.

An unstained 10-μm section was then examined by fluorescent microscopy to measure the tetracycline-based parameters in those patients who had been administered the tetracycline labels. The following variables were quantitated as previously described (34): (*a*) cellular rate of mineralization, the mean distance (micrometers) between all double-fluorescent markers within trabecular bone divided by the number of days between administration of the labels (representing the rate at which mineralization occurs at the average bone-forming surface), and (*b*) linear extent of bone mineralization, the absolute extent (micrometers) of bone forming surface (i.e., surface exhibiting a double tetracycline label) per square millimeter trabecular space.

After the completion of base-line studies, patients in the treatment group were begun on a regimen of 25-OHD (40–100 μg/d) plus calcium 500 mg (as calcium carbonate) daily, whereas controls were placed on a minimal calcium supplement (100 mg/d). The dose of 25-OHD was subsequently adjusted at biweekly intervals to maintain urinary calcium excretion below 300 mg/24 h.

The significance of differences between group means was determined by Dunnett's multiple variable *t* test method (35), correlation coefficients were calculated by Pearson's formula, and reduced major regression axes were determined by the method of Kermack and Haldane (36). All results are reported as mean±standard error of the mean.

RESULTS

Basal biochemical parameters. Clinical characteristics and dietary history data for the patient groups and normal subjects are summarized in Table I. There were no significant differences in age and sex distribution or dietary intake between patients and normal subjects. Moreover, treatment and control patient groups were quite similar with regard to mean daily glucocorticoid dose, duration of therapy, and functional class.

Mean serum concentrations of total and ionized calcium, phosphorus, alkaline phosphatase, and 25-OHD in the steroid-treated patients did not differ significantly from those in normal subjects (Table II). However, mean serum iPTH concentration in the steroid-treated subjects was approximately twice the mean normal value, and serum iPTH exhibited an inverse correlation with serum ionized calcium concentration ($r = -0.40$, $P < 0.05$). 24-h urinary calcium excretion expressed both as mg/g creatinine per 24 h and mg/kg body wt per 24 h was not significantly different between the two groups. However, intestinal ^{47}Ca absorption (percent of 100 mg dose) was significantly reduced in steroid-treated subjects, averaging 59% of the normal value. No significant correlation was observed between serum 25-OHD concentration and percent ^{47}Ca absorption ($r = 0.07$, $P > 0.50$). However, there was a statistically significant inverse correlation of serum iPTH concentration with intestinal ^{47}Ca absorption ($r = -0.52$, $P < 0.02$).

As shown in Table III, bone mass measured by the photon absorption method was reduced by 7.7%±3.2% ($P < 0.05$) in the diaphyseal region of the radius and by 33.0%±3.1% ($P < 0.001$) in the metaphyseal region in glucocorticoid-treated patients relative to normal

TABLE I

Initial Clinical and Dietary Data in Glucocorticoid-Treated Patients and Normal Subjects

	Corticosteroid-treated patients			
	25-OHD treatment group (9)	Control group (8)	Combined patient groups (17)	Normal subjects (19)
Clinical parameters				
Age, *yr*	46.3±4.4	48.1±4.4	47.2±3.0	45.7±3.8
Sex (F/M)	7/2	6/2	13/4	13/6
Glucocorticoid dose, *mg prednisone equivalents/d*	17.5±2.7	17.3±2.1	17.4±1.8	—
Duration of glucocorticoid therapy, *yr*	4.4±1.0	4.2±1.0	4.3±0.7	—
Functional class, *ranking units*	1.9±0.3	2.1±0.2	2.0±0.2	1.0±0.0
Dietary intake				
Vitamin D, *U/d*	259±62	281±77	269±53	276±43
Calcium, *mg/d*	802±193	849±207	824±149	790±133

Values are given as mean±SEM. Numbers of subjects are indicated in parentheses.

TABLE II
Pretreatment Biochemical and Intestinal ^{47}Ca Absorption Data in Patients and Normal Subjects

| | Glucocorticoid-treated patients | | | |
	25-OHD treatment group (9)	Control group (8)	Combined patient group (17)	Normal subjects (19)
Serum biochemical values				
Total calcium, mg/dl	9.26±0.10	9.31±0.09	9.28±0.07	9.48±0.09
Ionized calcium, mg/dl	4.69±0.08	4.54±0.08	4.64±0.06	4.83±0.09
Phosphate, mg/dl	3.82±0.10	3.68±0.16	3.75±0.07	3.96±0.12
Alkaline phosphatase, mIU/ml	80.0±15.4	64.9±7.7	72.9±8.7	81.5±7.6
25-OHD, ng/ml	15.7±1.3	15.1±2.0	15.4±1.1	16.9±1.5
iPTH, ul eq/ml	10.1±0.8*	9.9±0.8*	10.0±0.6*	5.3±0.8
24-h Urinary calcium excretion				
mg/g creatinine/24 h	122±16	134±19	131±13	146±11
mg/kg body wt/24 h	2.01±0.23	2.06±0.28	2.07±0.19	2.24±0.11
Creatinine clearance, ml/min/1.73 m²	89.3±4.3	93.7±7.1	91.4±4.7	98.4±3.1
^{47}Ca absorption % of 100 mg load	40.2±2.7*	41.1±5.4*	40.7±2.8*	63.7±2.6

Values are given as mean±SEM. Numbers of subjects are indicated in parentheses.
* Significantly different from normal subjects at $P < 0.001$.

subjects. The absolute changes observed and the disproportionately greater degree of loss in the metaphyseal region are in accord with previous observations on the pattern of bone loss in steroid-induced osteopenia (6).

Response to 25-OHD and calcium administation. All treatment patients were maintained on 500 mg/d calcium given in divided doses at 8 a.m. and 4 p.m., throughout the study period. The first four patients

were initially started on 100 µg/d 25-OHD, given orally as a single dose at 8 a.m. However, rapid elevation of urinary calcium to >300 mg/24 h in all four subjects by 2 wk of therapy (base line 117±9; 2-wk value 356±25 mg/24 h, $P < 0.001$) necessitated a rapid reduction in dose to levels of 40–60 µg/d. By 4 wk after reduction in dose, 24-h urine calcium had returned to acceptable levels (248±19 mg/24 h; mean 25-OHD dose, 50 µg/d). Hypercalcemia (serum calcium > 11.0 mg/dl) was not

TABLE III
Initial Forearm Bone Mass Data in Patients and Normal Subjects

| | Corticosteroid-treated patients | | | |
	25-OHD treatment group (9)	Control group (8)	Combined patient group (17)	Normal subjects (19)
Metaphyseal				
g/cm²	0.356±0.029‡	0.397±0.016‡	0.375±0.020‡	0.560±0.018
% of age-sex norm	64.9±5.1‡	72.4±2.9‡	68.4±3.1‡	102.1±3.7
Diaphyseal				
g/cm²	0.729±0.021	0.687±0.034*	0.709±0.027*	0.768±0.017
% of age-sex norm	96.7±3.2	91.1±5.3*	94.1±3.3*	101.9±2.3

Bone mass was determined in the radius of the lesser-used arm by the photon absorption technique (Methods). Values are given as mean±SEM. Numbers of subjects are indicated in parentheses.
* Significantly different from normal subjects at $P < 0.05$.
‡ Significantly different from normal subjects at $P < 0.001$.

observed. All five subsequent patients were started at doses of 40–60 μg/d and hypercalciuria (24-h urine calcium >300 mg) was not observed (base line 130±19; 2 wk of treatment 208±29, $P < 0.05$ vs. base line; 4-wk value 231±28 mg/24 h. $P < 0.001$ vs. baseline, ns vs. 2 wk value). Mean 25-OHD dose at 2 and 4 wk was 58.0 and 56.0 μg/d, respectively. The 25-OHD dose was subsequently adjusted in increments of 10 μg/d biweekly to maintain 24-h urine calcium excretion below 300 mg. The mean daily 25-OHD dose over the first 12 mo of treatment was 42.3 μg (0.68 μg/kg body wt per d).

Mean 24-h urinary calcium excretion in the 25-OHD-treated patients remained significantly elevated over base-line values and normal control levels throughout the study period (Fig. 1). Concurrently, mean serum total calcium concentration showed a slight but significant increase (Fig. 1). However, hypercalcemia was not observed at any point during 25-OHD treatment. 24-h creatinine clearance was not significantly altered in the treated patients, averaging 89.3±4.3, 94.1±5.7, 93.6±4.1, 90.9±4.4, and 92.3±4.0 ml/min per 1.73 m²

FIGURE 2 Intestinal ⁴⁷Ca absorption values (A) and serum iPTH concentration (B) in 25-OHD-treated and control patients over the initial 12 mo of treatment. Comparable values for normal subjects are indicated at the right side of each panel. Vertical bars represent 1 SEM. (*)significantly different from original value at $P < 0.001$ by paired data analysis.
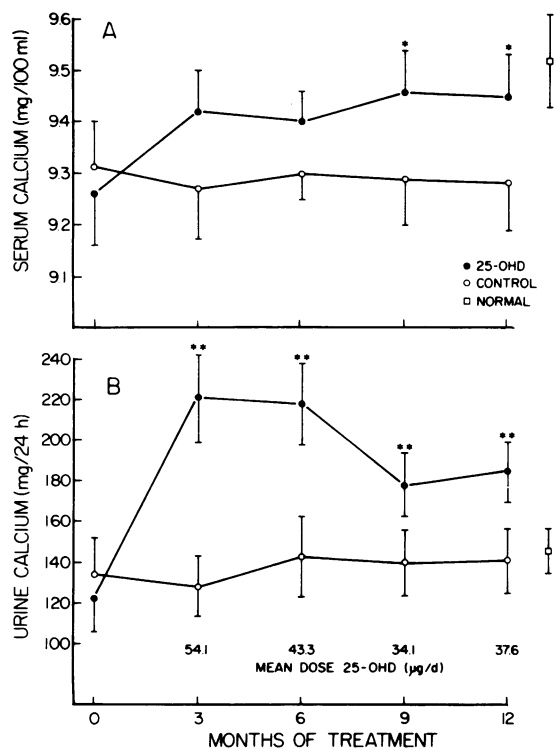
FIGURE 1 Serum calcium concentration (A) and 24-h urinary calcium excretion (B) in 25-OHD-treated and control patients over the initial 12 mo of treatment. Comparable values for normal subjects are indicated at the right side of each panel. Vertical bars represent 1 SEM. (**)significantly different from original value at $P < 0.01$ by paired data analysis, (*)significantly different from original value at $P < 0.05$ by paired data analysis.
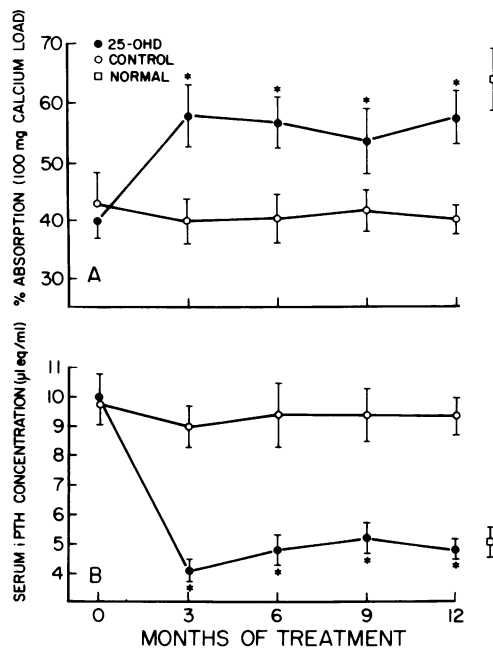
at 0, 3, 6, 9, and 12 mo, respectively. Neither serum calcium concentration nor 24-h urinary calcium excretion changed significantly in control patients.

Intestinal ⁴⁷Ca absorption in 25-OHD-treated patients was increased by a mean of 45.5% ($P < 0.001$) above base-line values at 3 mo, the earliest time point examined (Fig. 2), and was maintained at similar levels during the remainder of the initial 12 mo of treatment. Mean percent intestinal ⁴⁷Ca absorption in treated patients at the end of 12 mo was 57.1±2.3%, slightly but not significantly below that of the mean value for normal subjects (63.7±4.6%, $P > 0.10$); mean percent ⁴⁷Ca absorption in untreated patients remained significantly reduced at 43.0±4.6% ($P < 0.001$ compared to normals).

Concurrent with the increase in intestinal ⁴⁷Ca absorption, serum iPTH concentration fell significantly after 25-OHD treatment, reaching a mean value not significantly different from normal by 3 mo and remaining essentially unchanged during the remainder of the study (Fig. 2). Values for serum iPTH concentration during the period of 25-OHD treatment showed a strong inverse correlation with ⁴⁷Ca absorption ($r = -0.71$, $P < 0.001$). In control subjects the serum iPTH concentration remained elevated.

As shown in Fig. 3, serum 25-OHD concentration showed a strong positive correlation with 25-OHD dose
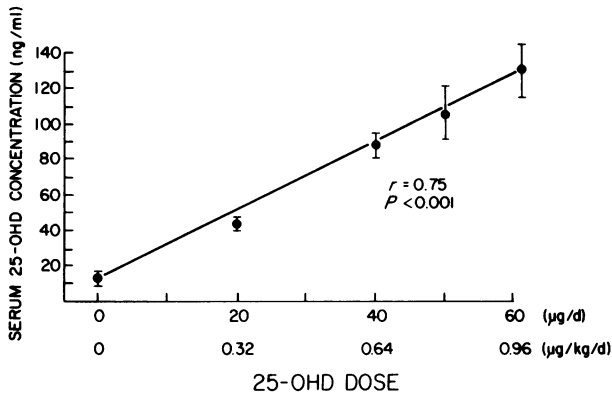
FIGURE 3 Correlation of percent ^{47}Ca absorption with serum 25-OHD concentration in 25-OHD-treated patients. The points represent the results of determinations in all nine patients after 3, 6, 9, and 12 mo of 25-OHD administration in addition to determinations at 6 wk in three of the nine patients.

over the range of 20–60 μg/d ($r = 0.75$, $P < 0.001$). Moreover, although there was no demonstrable correlation between pretreatment ^{47}Ca absorption and serum 25-OHD values, as indicated in Fig. 4 there was a definite positive correlation of percent ^{47}Ca absorption with serum 25-OHD concentration ($r = 0.67, P < 0.001$) after initiation of 25-OHD treatment.

Bone mass in the radius measured by photon absorption densitometry was significantly increased over initial values at both the diaphyseal and metaphyseal sites in treated patients by the end of the first 12 mo (Fig. 5). Diaphyseal mass in the 25-OHD-treated subjects rose slightly, with a mean 2.1±0.4% increase over initial values occurring by 12 mo ($P < 0.05$ by
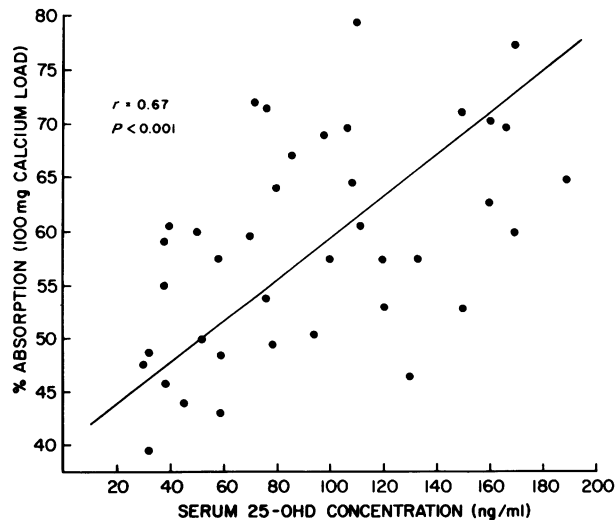

FIGURE 4 Correlation of serum 25-OHD concentration with daily 25-OHD dose in treated patients. Each point represents the mean±SEM for 3–30 determinations.

paired data analysis). Diaphyseal mass in control subjects exhibited a slight but not statistically significant decline of -1.8±0.3% of initial values by 12 mo. However the difference in mean changes observed at 12 mo in the 25-OHD-treated and control groups was highly significant ($P < 0.001$).

Changes in metaphyseal mass were more striking. By 5 mo of treatment a significant increase in metaphyseal mass in the 25-OHD-treated subjects was observed, with the 10.3±5.7% increase being statistically significant at $P < 0.01$ by paired data analysis. No further statistically significant increase in metaphyseal mass was observed after this time. The 12-mo mean increase over base line averaged 13.2±5.1%. Again, no significant change in metaphyseal bone mass was observed in control subjects although there appeared to be a slight downward trend with time; 12-mo values in control subjects declined -2.8±1.2% from base line. The difference in percent change in metaphyseal mass at 12 mo in treated and control subjects was again highly significant ($P < 0.001$). Although in the 25-OHD-treated patients mean glucocorticoid dose decreased slightly (initial, 17.5±2.7; 12 mo, 14.9±2.0 mg prednisone equivalents/d) and mean functional class improved slightly (initial, 1.9±0.3; 12 mo, 1.6±0.3 ranking units) over the study period, neither change
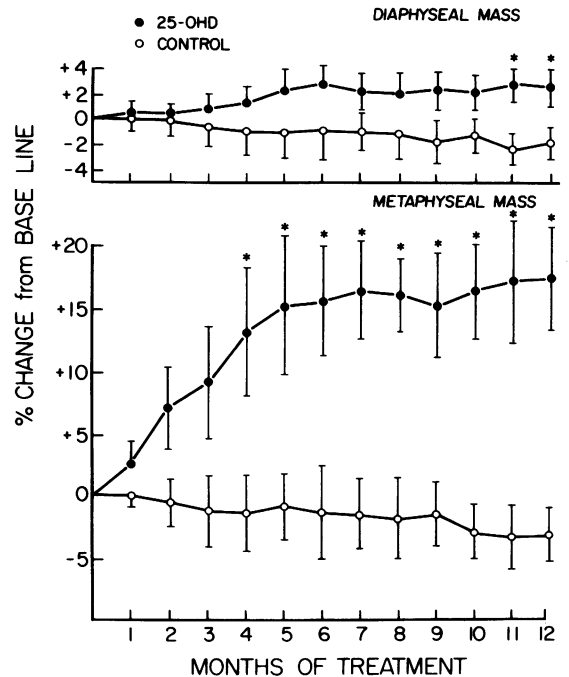

FIGURE 5 Monthly percentage change in diaphyseal and metaphyseal bone mass in the radius determined by photon absorption densitometry in nine 25-OHD-treated and eight control patients. Vertical bars represent 1 SEM. (*)significantly different from original value at $P < 0.01$ by paired data analysis.

TABLE IV
18-mo Follow-Up Data in 25-OHD-Treated and Control Subjects

	25-OHD treatment group (9)	Control group (8)
Treatment interval, *mo*	18.5±1.5	17.9±1.4
25-OHD dose, *μg/d*	40.0±0.0	—
Serum biochemical data		
Total calcium, *mg/dl*	9.48±0.10	9.37±0.08
Phosphate, *mg/dl*	3.94±0.13	3.60±0.15
Alkaline phosphatase, *mIU/ml*	76.5±9.9	68.3±7.6
25-OHD, *ng/ml*	82.1±5.1‡	17.5±2.2
iPTH, *ul eq/ml*	4.6±0.7‡	10.7±0.8
24-hour urine data		
Calcium, *mg/g creatinine/24 h*	178±16*	123±22
Creatinine clearance, *ml/min/1.73 m²*	92.0±4.9	88.9±6.3
Intestinal ⁴⁷Ca absorption, *% of 100 mg load*	57.9±5.1*	43.0±4.6
Forearm bone mass, *% change from base line*		
Metaphyseal	+14.7±5.1‡	−4.3±2.4
Diaphyseal	+3.8±1.4‡	−2.0±1.1

Values are given as mean±SEM. Number of patients is indicated in parentheses.
* Significantly different from controls at $P < 0.05$.
‡ Significantly different from controls at $P < 0.001$.

was statistically significant. Moreover, corresponding 12-mo values in control patients were not statistically different from 25-OHD-treated patients: glucocorticoid dose, 14±0.9 mg prednisone equivalent/d, functional class 2.0±0.2 ranking units. Therefore it is unlikely that the observed increase in bone mass in the 25-OHD-treated subjects was due to either decreased steroid dose or increased physical activity.

The results of more prolonged follow-up of 25-OHD-treated and control subjects are presented in Table IV. After a mean period of 18 mo on treatment, the 25-OHD-treated patients maintained the improved in-

testinal ⁴⁷Ca absorption, serum iPTH, and bone mass status observed after 12 mo of treatment. There were no statistically significant differences between 12- and 18-mo values for either the treated or control groups.

Bone histology. When compared to the normal subjects, the glucocorticoid-treated patients were osteopenic and had an excess of osteoid (Table V). The mean trabecular bone volume was 61.5% of normal and the average relative osteoid volume in patient biopsies was more than twice that of controls. However, since the percent total osteoid surface in the steroid-treated patients was almost three times that observed in con-

TABLE V
Bone Histometric Measurements in Glucocorticoid-Treated Patients and Normal Subjects

	Trabecular bone volume	Relative osteoid volume	Total osteoid surface	Osteoblastic osteoid surface	Osteoclasts/mm²
	%	%	%	%	
Patients (14)	12.9±1.8‡	2.98±0.47‡	25.0±4.4§	4.0±1.25*	0.34±0.12
Normals (14)	20.5±2.4	1.47±0.37	8.81±2.4	0.96±0.35	0.12±0.03

Values represent mean±SEM. Numbers of subjects are indicated in parentheses.
* Significantly different from normals at $P < 0.05$.
‡ Significantly different from normals at $P < 0.02$.
§ Significantly different from normals at $P < 0.005$.

TABLE VI
Bone Histometric Parameters in 25-OHD₃-Treated and Control Patients

	Initial		12 mo	
	25-OHD treated (7)	Control (7)	25-OHD treated (7)	Control (7)
Cortical bone volume, %	86.6±3.4	84.6±5.7	94.1±1.4	93.2±1.9
Trabecular bone volume, %	12.6±2.6	13.0±2.6	9.47±0.87	12.3±3.6
Relative osteoid volume, %	3.58±0.89	2.48±0.61	2.36±0.83	1.31±0.50
Osteoblastic osteoid surface, %	2.64±1.01	5.43±2.22	1.62±0.87	1.60±1.48
Total osteoid surface, %	26.5±6.0	19.8±5.3	15.5±4.2	11.2±2.9
Osteoclasts/mm² cortex	1.06±0.37	0.63±0.40	0.26±0.09‡	1.48±0.39
Osteoclasts/mm² trabeculum	0.23±0.12	0.47±0.21	0.20±0.09	0.45±0.19
Osteoclasts/mm cortical endosteum	0.23±0.09*	0±0	0.19±0.10*	1.74±0.38
Osteoclasts/mm trabecular endosteum	0.23±0.11	0.30±0.09	0.17±0.05	0.41±0.20
Cellular rate of mineralization, $\mu M/d$	0.127±0.127	0.642±0.221	0.592±0.207	0.555±0.189
Linear extent of bone mineralization, $\mu m/mm^2$	2.85±2.85	13.2±9.5	8.37±3.39	4.50±2.39

Values represent mean±SEM of determination in 25-OHD₃-treated and control subjects at the indicated times. Numbers of subjects are indicated in parentheses.
* Significantly different from controls at $P < 0.05$.
‡ Significantly different from controls at $P < 0.025$.

trol biopsies, the osteoid seams tended to be thinner than normal. Additionally, there were abundant skeletal cells in the bones of the glucocorticoid-treated patients. The percent osteoblastic-osteoid surface was increased and the number of osteoclasts within trabecular bone averaged three times normal, although this latter difference was not statistically significant.

The only significant histologic difference between the control and 25-OHD treatment patient groups observed on the *initial* biopsies related to osteoclasts on the cortical-endosteal surface (Table VI). The number of these cells was significantly less ($P < 0.05$) in the control group than in the 25-OHD treatment group. On the other hand, after therapy the number of osteoclasts per square millimeter of cortical endosteal bone surface was greater in the control than in the 25-OHD-treated patients ($P < 0.05$). Furthermore, posttreatment biopsies in the 25-OHD group contained fewer osteoclasts per square millimeter of cortical bone than did those of control patients ($P < 0.05$). No other significant differences existed between the posttreatment biopsies of 25-OHD-treated and control subjects.

As compared to published normal values (37), the cellular rate of mineralization was suppressed in four pretreatment biopsies of patients in the 25-OHD treatment group and in one of four control subjects in whom this parameter was examined (Table VII). Indeed, three of the four initial biopsies of the 25-OHD group and one initial biopsy of the control group contained no double-fluorescent tetracycline labels. Three of the four second biopsies in the 25-OHD-treated group exhibited normal cellular mineralization rates. On the

other hand, the second biopsy taken from the control patient whose initial biopsy contained no fluorescent labels again exhibited no evidence of bone mineralization. However, these differences between groups were not statistically significant. There were also no significant changes in either group in the linear extent of bone mineralization, which reflects the extent of bone surface involved in active bone formation. However, although the mean linear extent of bone mineralization decreased in the control patients, after 12 mo there was an increase in the mean linear extent of bone mineralization in 25-OHD-treated patients.

DISCUSSION

In the present study decreased intestinal calcium absorption, increased serum iPTH concentrations,

TABLE VII
Initial and Posttreatment Cellular Mineralization Rates in 25-OHD₃-Treated and Control Patients

25-OHD₃ treated			Control		
Patient No.	Initial	12 mo	Patient No.	Initial	12 mo
1	0	0.93	2	1.01	0.63
3	0.51	0	4	0.76	0.71
5	0	0.81	6	0.80	0.82
7	0	0.62	8	0	0

Normal mineralization rate: 0.70±0.03 μm/d (37).
Values represent determinations for individual patients at the times indicated.

T. J. Hahn, L. R. Halstead, S. L. Teitelbaum, and B. H. Hahn

decreased metaphyseal bone mass, and bone histomorphometric evidence of decreased bone formation and a tendency toward increased numbers of osteoclasts were demonstrated in a group of patients receiving chronic moderate-dose glucocorticoid therapy. These findings are generally in accord with those previously reported in patients with glucocorticoid-induced osteopenia (7, 8, 13–19).

Despite evidence of markedly reduced calcium absorption, the mean serum total and ionized calcium concentration in the patient group was not significantly different from normal values, presumably reflecting the effects of secondarily increased PTH secretion with maintenance of serum calcium concentration partially at the expense of mobilization of calcium from bone (38). In addition, the slight decrease in mean urinary calcium excretion in our patients, relative to normal subjects, may in part reflect the renal tubular calcium-retaining effects of increased circulating PTH concentrations (39).

Pretreatment serum 25-OHD levels in our patients were normal and showed no significant correlation with intestinal ^{47}Ca absorption. These data are in concert with previous reports from this laboratory as well as others (23, 24). However, they are somewhat in contrast to the observations of Klein et al. (19) who reported significantly decreased serum 25-OHD concentration correlating with decreased intestinal calcium absorption in subjects treated chronically with higher doses of glucocorticoids (equivalent to 40 mg prednisone/d). The explanation for this difference is not readily apparent, although it could be postulated that at the higher doses employed in the patients studied by Klein et al. secondary alterations in 25-OHD metabolism, such as steroid-induced increases in hepatic mixed oxidase catabolism of vitamin D metabolites (40, 41) or accelerated conversion of 25-OHD to dihydroxy metabolites secondary to increased PTH activity (42), could result in lowering of serum 25-OHD levels. Whatever the case, our present findings demonstrate that marked reductions in intestinal calcium absorption can occur in the presence of normal concentrations of circulating 25-OHD in patients treated with moderate doses of glucocorticoids.

In response to supplementation with 25-OHD at a mean dose of 40 μg/d, intestinal calcium absorption rose to approximately normal levels and serum iPTH was reduced. Stimulation of calcium absorption may have been the result of both the effects of an increased circulating 25-OHD concentration and the marked increase in local 25-OHD concentration at the intestinal mucosal cell as a result of oral 25-OHD administration. At the point at which ^{47}Ca absorption was normalized, serum 25-OHD concentrations were in the range of 100 ng/ml, approximately five times normal levels. This disparity suggests a relative resistance to the calcium absorption-stimulating effects of 25-OHD.

It could be proposed that this apparent resistance was the result of glucocorticoid-induced alterations in the conversion of 25-OHD to 1,25-(OH)$_2$D, the apparent final and most biologically potent vitamin D metabolite (43). This viewpoint is supported by the observations of (a) Carre et al. (21) who have suggested that glucocorticoids may accelerate the metabolism of 1,25-(OH)$_2$D$_3$ to an inactive product, and (b) Klein et al. (19) who have reported that 1,25-(OH)$_2$D$_3$ in near-physiologic doses can restore intestinal calcium absorption to approximately normal levels in glucocorticoid-treated individuals. On the other hand, it has been reported that conversion of vitamin D$_3$ to 1,25-(OH)$_2$D$_3$ and localization of 1,25-(OH)$_2$D$_3$ in intestinal mucosal cell nuclei proceeds normally in cortisone-treated rats (22). However, because serum 1,25-(OH)$_2$D levels were not measured in our patients, our present data do not allow us to choose between hypotheses regarding the mechanism of resistance to 25-OHD.

At the bone level, treatment with 25-OHD and calcium was accompanied by a significant decrease in osteoclast number. This decrease can apparently be attributed to suppression of PTH secretion, since it has been demonstrated that the osteoclastic response to glucocorticoids is mediated through increased PTH activity, whereas the suppression of bone formation is apparently the result of a direct effect on osteoblast function (10). It is of interest that double fluorescent labels were present in the post-25-OHD treatment biopsies of three individuals whose initial biopsies showed no evidence of bone formation. However, the number of patients receiving time-spaced tetracycline labels is too few to draw definite conclusions regarding effects on bone accretion rates. Thus, the histomorphometric changes suggest that the net increase in bone mass demonstrated by photon absorption densitometry resulted at least in part from decreased osteoclastic resorptive activity, with the possibility existing that the bone formation rate may have been increased concurrently. Since bone mass measured by photon absorption was not increasing detectably at 12 mo in the 25-OHD-treated group, the bone histomorphometric parameters at that point may reflect a new steady state of formation and resorption under the influence of 25-OHD administration.

It is not surprising that the most marked increment in bone mass was observed at the metaphyseal site, since this region contains a larger proportion of more metabolically active bone and exhibits the greatest degree of change in states of increased parathyroid activity (6, 9, 27).

Although our results indicate that restoration of intestinal calcium absorption and serum iPTH values to normal levels with 25-OHD and calcium supplementation is accompanied by improvement in several parameters of mineral metabolism in glucocorticoid-treated patients, several questions remain to be

answered. First, the normalization of serum iPTH concentration produced by increasing intestinal calcium absorption was accompanied by an increase in urinary calcium excretion to levels significantly above base line. This increase was apparently the result of a stimulation of intestinal calcium absorption to a level greater than that which could be readily assimilated by bone. Although prolonged hypercalciuria can ultimately lead to renal calculus formation and a deterioration of renal function (44), in our patients urinary calcium levels were generally only slightly above normal and creatinine clearance was not altered over the 18-mo study. It is possible that a more precise adjustment of 25-OHD and calcium dose could produce a normalization of PTH activity with only minimal changes in urinary calcium excretion.

Secondly, since bone formation and resorption rates frequently change in parallel fashion (45), a reduction in bone resorption rate might ultimately be accompanied by a concomitant decline in bone formation. If this were to occur, a later decrease in bone mass might follow with formation and resorption restored to their previous equilibrium, albeit at a lower level of bone turnover. However, no evidence of a secondary decrement in bone mass was observed in our patients.

Finally, since the resistance of bone to stress fracture correlates positively with bone mass as determined by photon absorption densitometry (46), the observed increase in bone mass might ultimately lead to a reduced propensity to bone fractures in glucocorticoid-treated patients receiving 25-OHD chronically. However, the clinical efficacy of this mode of treatment remains to be determined.

ACKNOWLEDGMENTS

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