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Bone resorption stimulated by elevated serum 1,25-$(OH)_2$-vitamin D concentrations in healthy men

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Bone resorption stimulated by elevated serum 1,25-$(OH)_2$-vitamin D concentrations in healthy men. We evaluated whether calcitriol administration to healthy men stimulates bone resorption. We compared serum 1,25-$(OH)_2$-D concentrations, Ca and PO_4 balances, and urinary hydroxyproline excretion rates in four healthy men adapted to a low Ca diet providing only 4.0 ± 0.2 SD mmoles Ca/day to those in four healthy men eating a comparable diet (4.2 ± 0.9 mmoles Ca/day) during the chronic oral administration of calcitriol, 0.75 μg every 6 hr. Serum 1,25-$(OH)_2$-D levels averaged 94 ± 16 pM during the control studies and 209 ± 35 pM during calcitriol administration. Net intestinal Ca absorption averaged 0.5 ± 0.3 mmoles/day during control and 1.8 ± 0.5 mmoles/day during calcitriol ($P < 0.005$), but urinary Ca excretion averaged 8.7 ± 2.0 mmoles/day during calcitriol as compared to 2.9 ± 1.4 mmoles/day during control ($P < 0.005$). Thus, mean Ca balance, which averaged −2.4 ± 1.2 mmoles/day during control, was more negative during calcitriol at −6.3 ± 2.4 mmoles/day ($P < 0.05$). Average daily PO_4 balances averaged +7.7 ± 1.5 mmoles/day during control but only tended to be negative during calcitriol at −1.1 ± 5.4 mmoles/day, (NS). Urinary hydroxyproline excretion averaged 0.26 ± 0.03 mmoles/day during control and 0.49 ± 0.06 during calcitriol ($P < 0.001$). We conclude that elevated serum 1,25-$(OH)_2$-D concentrations in healthy men eating low Ca diets stimulate bone resorption.

La résorption osseuse stimulé par des concentrations élevées de 1,25-$(OH)_2$-vitamine D chez des hommes normaux. Nous avons recherché si l'administration de calcitriol à des hommes normaux stimule la résorption osseuse. Nous avons comparé les concentrations sériques de 1,25-$(OH)_2$-D, les balances de Ca et PO_4 et l'excrétion urinaire d'hydroxyproline chez quatre hommes normaux adaptés à un régime pauvre en Ca apportant seulement 4,0 ± 0,2 SD mmoles Ca/jour à ceux de quatre hommes normaux consommant un régime comparable (4,2 ± 0,9 mmoles Ca/jour) pendant l'administration orale chronique de calcitriol, 0,75 μg toutes les 6 heures. Les concentrations sériques de 1,25-$(OH)_2$-D étaient en moyenne de 94 ± 16 pM pendant les études contrôles et de 209 ± 35 pM pendant l'administration de calcitriol. L'absorption intestinale nette de Ca était en moyenne de 0,5 ± 0,3 mmoles/jour pendant le contrôle et de 1,8 ± 0,5 mmoles/jour pendant la prise de calcitriol ($P < 0,005$), mais l'excrétion urinaire de Ca était en moyenne de 8,7 ± 2,0 mmoles/jour pendant le calcitriol, par rapport à 2,9 ± 1,4 mmoles/jour pendant le contrôle ($P < 0,005$). Ainsi, la balance moyenne de Ca, qui était en moyenne de −2,4 ± 1,2 mmoles/jour pendant le contrôle, était encore plus négative pendant le calcitriol, de −6,3 ± 2,4 mmoles/jour ($P < 0,05$). Les balances moyennes journalières de PO_4 étaient de +7,7 ± 1,5 mmoles/jour pendant le contrôle, mais n'avaient que simplement tendance à être négatives pendant le calcitriol à −1,1 ± 5,4 mmoles/jour, (NS). L'excrétion urinaire d'hydroxyproline était de 0,26 ± 0,03 mmoles/jour pendant le contrôle et de 0,49 ± 0,06 pendant le calcitriol ($P < 0,001$). Nous concluons que les concentrations élevées de 1,25-$(OH)_2$-D sérique chez des hommes normaux consommant des régimes pauvres en calcium stimulent la résorption osseuse.

Currently available data indicate that 1,25-$(OH)_2$-D is the major, if not the sole, hormonal stimulus for intestinal calcium (Ca) absorption in animals [1, 2] and in humans [3–5]. 1,25-$(OH)_2$-D is also capable of stimulating bone resorption in animals both in vivo and in vitro [6, 7]. We have observed that the administration of 1,25-$(OH)_2$-D to healthy men eating a low Ca diet produced average daily urinary Ca excretion rates in excess of dietary Ca intake [8]. This observation is consistent with the view that 1,25-$(OH)_2$-D can stimulate bone resorption in humans. However, the brevity of those studies did not permit measurements of intestinal Ca absorption. Therefore, those studies did not completely exclude the possibility that the extra urinary Ca might simply represent enhanced intestinal Ca absorption. We have now observed Ca and phosphate (PO_4) balances in healthy men adapted to a low Ca diet to minimize expression of the effect of 1,25-$(OH)_2$-D to augment intestinal Ca absorption. These data have been compared to studies in healthy men eating the same diet while chronically receiving calcitriol. The elevated serum 1,25-$(OH)_2$-D concentrations achieved during chronic calcitriol administration were associated with hypercalciuria, more negative Ca balance, and increased urinary hydroxyproline excretion. These results provide further evidence to support the view that 1,25-$(OH)_2$-D can stimulate bone resorption in humans.

Methods

Metabolic balance studies were carried out in eight healthy men in the Medical College of Wisconsin Clinical Research Center, Milwaukee, Wisconsin, with their consent and the approval of the Medical College of Wisconsin Human Research Review Committee. All studies were performed while the subjects ate constant whole food diets low in Ca. Because of the length of the studies, two diets of nearly identical composition were alternated daily. The average daily diet provided: Ca 4.1 ± 0.8 mmoles/day, PO_4 52.8 ± 5.2 mmoles/day, Mg 17.4 ± 1.4 mmoles/day, K 75 ± 5 mmoles/day, Na 138 ± 14 mmoles/day, and Cl 119 ± 9 mmoles/day. The diets provided an average of 2840 ± 270 calories, 54 ± 3% as carbohydrate, 13 ± 1% as protein, and the remainder as fat. The diets did not contain gelatin. Four subjects were adapted to the low Ca diet for 4 days and then observed during two 6-day control balance periods.

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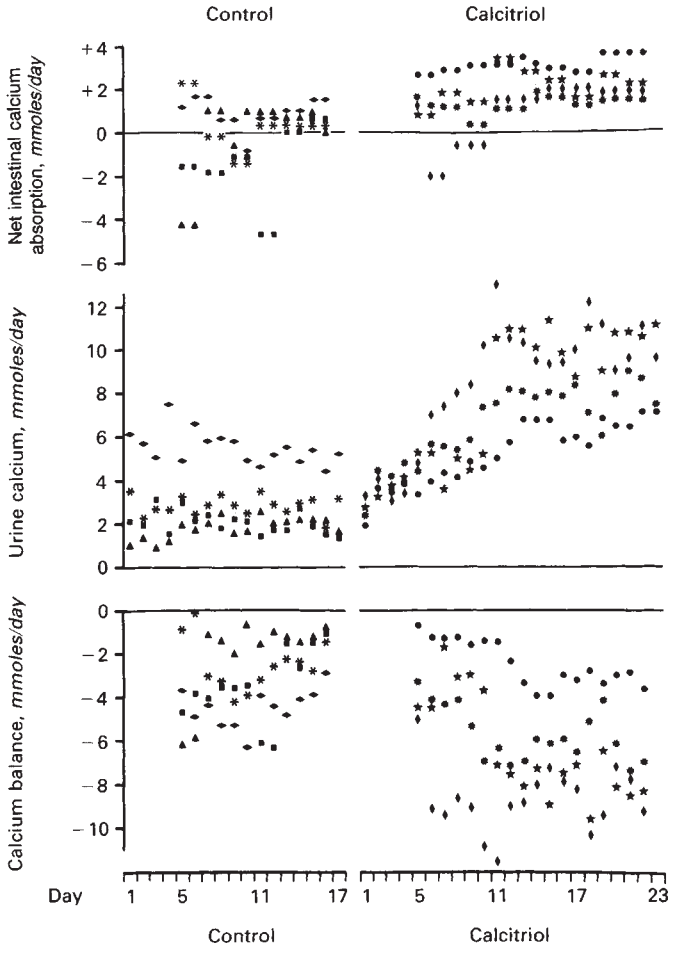

Fig. 1. *Individual measurements of net intestinal Ca absorption, daily urinary Ca excretion and Ca balance in the four subjects studied while eating the low Ca diet alone* (*, ■, ▲, ◆) *and in the four subjects also receiving calcitriol* (★, ●, ♦, *). *See* **Results.**

Four subjects were begun on the low Ca diet together with oral calcitriol, 0.75 μg every 6 hr (6 A.M., 12 P.M., 6 P.M., 12 A.M.). After 4 days of adaptation to this regimen, three 6-day balance periods were observed. Carmine markers were used to separate the stool periods.

Duplicate diets were analyzed for mineral content. Although carmine markers were used to separate 6-day stool periods, feces were pooled for analysis every 1 to 3 days to permit more frequent estimation of mineral excretion in the feces and net intestinal mineral absorption. Twenty-four-hour urine samples were collected daily and, in addition, 6-day urine pools were prepared for some measurements to minimize the number of analyses. Morning fasting urine samples were obtained on 2 days during control and on 2 days during calcitriol administration to estimate fasting urinary Ca excretion and fasting $TmPO_4/GFR$. Blood specimens were drawn after fasting at 6 A.M. on 5 to 7 control days beginning at least 4 days after the subjects started to eat the low Ca diet alone and on 6 to 10 days beginning at least 4 days after calcitriol administration was begun. The time of blood sampling was 6 hr after the last dose and just before the next dose of calcitriol.

The analytical methods used for estimation of mineral and acid balances [9], iPTH [8], urinary hydroxyproline, and oxalate [8] have been previously reported. Urinary cAMP was measured using a kit from Becton-Dickinson. Fasting $TmPO_4/GFR$ was estimated from the Walton-Bijovet nomogram [11]. Vitamin D metabolites were measured by competitive protein binding assay as previously reported [10]. For the measurement of $1,25\text{-}(OH)_2\text{-D}$, the interassay coefficient of variation averaged 18% in a plasma pool measured in 36 assays over the period during which the present studies were performed. Normal values in 97 healthy adults eating ad lib average 89 ± 25 SD pM.

Results are presented as group means ± SD. Comparison of the control subjects and the subjects given calcitriol were made by analysis of variance for repeated measurements with time [12]. Linear regressions were calculated by least squares.

Table 1. Fasting blood composition

	Control	Calcitriol	P^a
Number of subjects	4	4	
$1,25\text{-}(OH)_2\text{-D}$, pM	94 ± 16[b]	209 ± 35	<0.001
25-OH-D, nM	43 ± 14	66 ± 18	NS
Ca, mM	2.45 ± 0.07	2.44 ± 0.05	NS
PO_4, mM	1.18 ± 0.17	1.41 ± 0.20	NS
iPTH, μlEq/ml	8.0 ± 1.9	6.8 ± 2.2	NS
Mg, mM	0.93 ± 0.06	0.88 ± 0.05	NS
Creatinine, μM	105 ± 11	110 ± 15	NS

[a] These values reflect the probability that observations during calcitriol do not differ from control observations based on *t* test for unpaired groups.

[b] Values throughout the table are group means ± SD.

Results

An overview: The problem of the steady state

Figure 1 shows the individual daily net intestinal Ca absorption, urinary Ca excretion, and Ca balance for each of the eight subjects (the four subjects while eating the low Ca diet alone and the four subjects studied while also taking calcitriol). It is evident (Fig. 1) that average daily net intestinal Ca absorption rose progressively during the first 6-day control period, reaching an approximately stable level only during the second 6-day control period. Similarly, daily urinary Ca excretion rose progressively during the first 10 days of calcitriol administration, reaching a stable high rate only during the second and third 6-day calcitriol balance periods. Because of these delays in attaining the steady state during both the control and calcitriol phases, we limited further analysis of the results to comparison of the second 6-day control period to the second and third 6-day calcitriol periods.

Steady state comparisons

Serum composition. Table 1 presents the measurements of serum composition. During control, serum $1,25\text{-}(OH)_2\text{-D}$ levels averaged 94 ± 16 pM in the four subjects adapted to the low calcium diet and averaged 209 ± 35 pM in the four subjects eating the low calcium diet while receiving calcitriol. The mean of the individual coefficients of variation for this measurement averaged 25% for the subjects eating the low Ca diet alone and 16% for the subjects also receiving calcitriol. Serum $1,25\text{-}(OH)_2\text{-D}$ levels were stable in individual subjects as we have observed previously [8]. There were no significant differences

Table 2. Body weights, daily dietary intake, fecal excretion, net intestinal absorption, urinary excretion, and balances of Ca, PO_4, and Mg

	Control	Calcitriol	P[a]
Number of subjects	4	4	
Body weight, *kg*	71 ± 11[b]	69 ± 9	NS
Calcium diet, *mmoles/day*	4.0 ± 0.2	4.2 ± 0.9	NS
Feces	3.5 ± 0.4	1.8 ± 0.5	<0.001
Net intestinal absorption	0.5 ± 0.3	2.4 ± 0.7	<0.005
Urine	2.9 ± 1.4	8.7 ± 2.0	<0.005
Balance	−2.4 ± 1.2	−6.3 ± 2.4	<0.05
PO_4 diet, *mmoles/day*	56.0 ± 4.1	49.5 ± 7.5	NS
Feces	14.1 ± 2.0	11.7 ± 2.1	NS
Net intestinal absorption	41.9 ± 2.5	37.8 ± 6.5	NS
Urine	34.2 ± 2.7	38.9 ± 6.1	NS
Balance	+7.7 ± 1.5	−1.1 ± 5.4	NS
Mg diet, *mmoles/day*	17.8 ± 0.6	17.1 ± 1.8	NS
Feces	9.8 ± 1.0	11.1 ± 2.2	NS
Net intestinal absorption	8.0 ± 0.5	6.0 ± 1.8	NS
Urine	5.4 ± 1.0	5.7 ± 0.1	NS
Balance	+2.6 ± 0.7	+0.4 ± 1.9	NS
Fecal wet weight, *g/day*	83 ± 16	100 ± 7	NS

[a] These values reflect the probability that observations during calcitriol do not differ from control observations based on the analysis of variance of repeated measurements with time.

[b] Values throughout the table are group means ± SD.

in measured serum constituents although serum iPTH levels tended to fall and serum PO_4 levels tended to be higher among the subjects taking calcitriol.

Mineral balances

Table 2 presents mean body weight and the components of the mineral balances for the subjects adapted to the low calcium diet alone and the subjects observed during chronic calcitriol administration.

Fecal calcium excretion was significantly lower in the group receiving calcitriol, so that net intestinal calcium absorption was significantly higher, averaging +0.5 ± 0.3 mmoles/day or 12% of dietary calcium intake in the control studies and +2.4 ± 0.7 mmoles/day or 57% of dietary calcium intake during calcitriol. However, calcitriol administration increased urinary calcium excretion strikingly to 8.7 mmoles/day. This very high urinary calcium excretion rate during calcitriol administration was 200% of dietary calcium intake. Thus, during calcitriol administration, mean daily calcium balances were significantly more negative than during the control studies ($P < 0.05$).

Neither fecal nor urinary phosphate excretion rates differed significantly during calcitriol. Moreover mean daily PO_4 balances showed only an insignificant trend to become negative. However, when the data for the subjects adapted to the low Ca diet alone and the data for the subjects given calcitriol as well were considered together, daily PO_4 balances were directly correlated with daily calcium balances: PO_4 balance, mmoles/day = 12.9 + 2.06 × Ca balance, mmoles/day; r = 0.84; $P < 0.01$.

Dietary Mg intake was very slightly lower for the subjects given calcitriol, yet fecal Mg excretion tended to be higher during calcitriol administration. However, neither mean net intestinal Mg absorption, urinary Mg excretion nor daily Mg balance differed during calcitriol as compared to control.

Table 3. Renal function, fasting urine composition, oxalate, hydroxyproline excretion, and cAMP

	Control	Calcitriol	P[a]
Number of subjects	4[b]	4	
Creatinine clearance, *ml/min/1.73 m²*	109 ± 7	106 ± 11	NS
Fasting urine Ca/creatinine, *mmoles/mmole*	0.17 ± 0.05	0.48 ± 0.19	<0.025
Fasting $TmPO_4/GFR$, *mM*	1.24 ± 0.22	1.32 ± 0.19	NS
Fasting urine oxalate/creatinine, *mmoles/mmole*	21 ± 7	20 ± 7	NS
Urine oxalate, *mmoles/day*	0.44 ± 0.04	0.41 ± 0.17	NS
Urine hydroxyproline, *mmoles/day*	0.26 ± 0.03	0.49 ± 0.06	<0.001
Urine cAMP, *mmoles/day*	5.7 ± 1.6	2.9 ± 0.9	<0.025

[a] These values reflect the probability that observations during calcitriol do not differ from control observations based on *t* test for unpaired groups.

[b] Values throughout the table are group means ± SD.

Sodium, potassium, chloride, and acid balances

During the control studies the daily balances of Na, K, and Cl averaged +10 ± 11, +6 ± 6, and +5 ± 13 mmoles/day, respectively, and were not significantly different for the subjects observed during calcitriol administration, averaging +13 ± 5, +12 ± 6, and +12 ± 13 mmoles/day, respectively. For the control studies blood pH, serum Na, K, Cl, and HCO_3 averaged 7.40 ± 0.02, 142 ± 1, 4.2 ± 0.1, 104 ± 2, and 27.4 ± 0.6, respectively. These values were not different for the subjects given calcitriol. Moreover endogenous fixed acid production, renal net acid excretion and acid balance, which averaged 76 ± 8, 68 ± 7, and +8 ± 4 mEq/day, respectively, during the control studies were similar during calcitriol, averaging 69 ± 16, 66 ± 11, and +3 ± 12 mEq/day, respectively.

Renal function, fasting urine composition, urinary oxalate, and hydroxyproline excretion. As shown in Table 3, calcitriol administration did not alter endogenous creatinine clearance, taking into account the small differences in body size (Table 2) and thus surface area of the two groups of subjects. Fasting urinary calcium/creatinine and daily urinary hydroxyproline excretion rates were strikingly higher among the subjects receiving calcitriol. Neither mean fasting $TmPO_4/GFR$, fasting urine oxalate/creatinine, or daily urinary oxalate excretion were detectably altered by calcitriol administration. Daily urinary cAMP excretion was significantly lower during calcitriol administration.

Relationships between Ca and PO_4 metabolism and hydroxyproline excretion and prevailing serum 1,25-$(OH)_2$-D concentrations. As shown in Figure 2, individual net intestinal Ca absorption and urinary Ca excretion rates were positively correlated to prevailing serum 1,25-$(OH)_2$-D levels when the data for the four subjects adapted to the low calcium diet alone and the four subjects also given calcitriol were considered together. Since urinary Ca excretion rose to a greater extent than did net intestinal Ca absorption in the subjects given calcitriol, Ca balance was inversely correlated to prevailing serum 1,25-$(OH)_2$-D levels. Daily PO_4 balances during control and during calcitriol were also negatively correlated to serum 1,25-$(OH)_2$-D levels: PO_4 balance, mmoles/day = 17.7 − 0.0907 × serum 1,25-$(OH)_2$-D, pM; r = −0.89; $P < 0.005$.

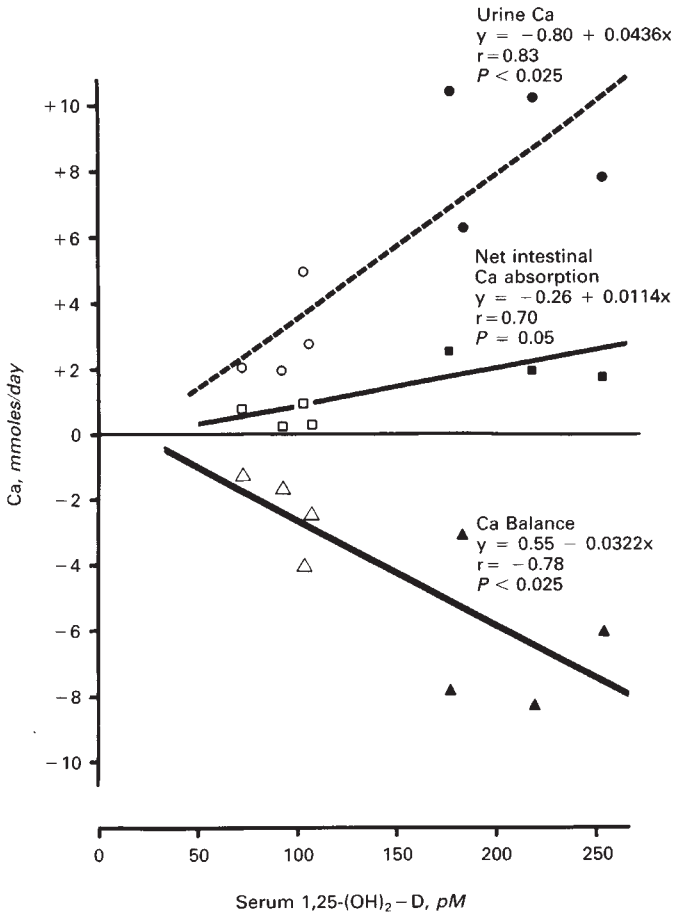

Fig. 2. *Relationship between individual average daily net intestinal Ca absorption* (squares), *average daily urinary Ca excretion* (circles), *and average daily Ca balance* (triangles) *and individual serum 1,25-$(OH)_2$-D concentrations for the four subjects studied eating the low Ca diet alone* (open symbols) *and the four subjects also receiving calcitriol* (closed symbols).

Figure 3 shows that urinary hydroxyproline excretion was also directly correlated to prevailing serum 1,25-$(OH)_2$-D levels and was inversely correlated to daily Ca balances.

Fasting urine Ca/creatinine was also directly related to prevailing serum 1,25-$(OH)_2$-D levels, as we have observed previously [8]: fasting urinary Ca/creatinine, mmoles/mmole = $-0.10 + 0.00283 \times$ serum 1,25-$(OH)_2$-D, pM; $r = 0.89$; $P < 0.005$.

Discussion

Control conditions

Previous Ca balance studies have emphasized the time required to reach a steady state when healthy adults begin to eat a lower Ca diet because of the delay in achieving maximally efficient intestinal Ca absorption, that is, intestinal adaptation [13]. To be sure that the control Ca balance data for our four subjects indicated that they were adequately adapted to the low Ca diet, we have compared our results to previously published balance studies in healthy adults eating diets providing less than 6 mmoles Ca/day. We have selected studies of 25 healthy adults (3 women and 22 men), older than 21 years to be sure skeletal growth was complete, from eight publications [14–21]. Those subjects were observed during balance periods beginning at least 9 days after they had begun to eat their low Ca diet (range, 9 to 89 days). For these 25 subjects the components of Ca balance averaged: diet 4.5 ± 1.2, feces 5.2 ± 1.6, net intestinal absorption −0.7 ± 1.6, urine 2.6 ± 1.4, and balance −3.3 ± 1.1 mmoles day, respectively. When these means are compared to the corresponding means for the control observations in our four subjects (Table 2), only fecal Ca excretion was significantly different in our subjects, being lower ($P < 0.05$). This difference was probably the result of a slightly lower average dietary Ca intake and a slightly greater average net intestinal Ca absorption among our volunteers. Thus, our subjects were at least as well adapted to the low Ca diet as were the previously studied subjects.

The effects of experimental elevation of serum 1,25-$(OH)_2$-D concentrations

Bone resorption. Since experimentally elevated serum 1,25-$(OH)_2$-D levels were accompanied by more negative Ca balances and a trend toward more negative PO_4 balances, augmentation of daily urinary hydroxyproline excretion and fasting hypercalciuria, we conclude that net bone resorption was increased. Only the skeleton could provide a source for the cumulative loss of an average of about 40 mmoles or 1.6 g of Ca during the final 10 days of calcitriol administration (the difference in mean daily Ca balance between control and calcitriol groups × 10 days, Table 2). These observations confirm in humans the potent effect of 1,25-$(OH)_2$-D to stimulate bone resorption previously observed in animals and in cultured fetal bone [6, 7].

Whether a higher dietary Ca intake, in the face of elevated serum 1,25-$(OH)_2$-D levels, would prevent or reduce this effect of 1,25-$(OH)_2$-D to stimulate net bone resorption in healthy subjects has not been studied. Speculatively, a higher dietary Ca intake might not prevent such negative Ca balances. Support for this view is derived from studies in patients with nephrolithiasis and hypercalciuria, many of whom exhibit high serum 1,25-$(OH)_2$-D levels [3, 22, 23]. Balance studies in these patients, while they are eating diets providing 20 to 30 mmoles Ca/day, show that they exhibit slightly but significantly negative Ca balances, while healthy subjects with normal serum 1,25-$(OH)_2$-D levels eating comparable diets exhibit slightly but significantly positive Ca balances [24, 25].

Mechanism of hypercalciuria. When serum 1,25-$(OH)_2$-D concentrations were experimentally elevated, the resulting daily hypercalciuria (Fig. 2) and fasting hypercalciuria occurred despite serum total Ca and iPTH concentrations which were within the normal range (Table 1). It seems likely, however, that subtle elevations of serum Ca concentrations and thus glomerular filtration of Ca, as well as subtle reductions in iPTH levels (as evidenced by the fall in urinary cAMP excretion) and reduced renal tubular reabsorption of Ca, were both responsible for the hypercalciuria. This explanation is based on our previous observations of a reciprocal rise in serum Ca and a fall in iPTH concentrations when each subject given calcitriol served as his own control and underwent multiple measurements of serum Ca and iPTH throughout the day [8].

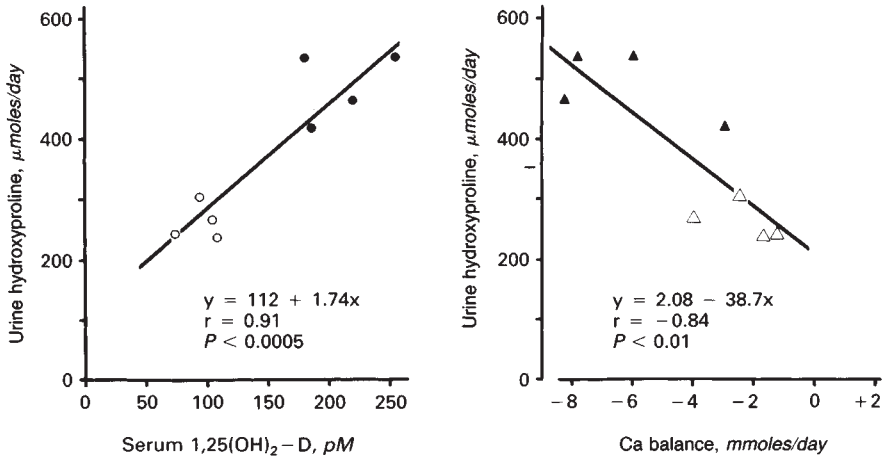

Fig. 3. *Relationships between individual average daily urinary hydroxyproline excretion and individual serum 1,25-$(OH)_2$-D concentrations* (left panel) *and individual average daily Ca balances* (right panel) *for the four subjects studied while eating the low Ca diet alone* (open symbols), *and the four subjects also receiving calcitriol* (closed symbols).

These findings also confirm that neither elevated fasting urine Ca/creatinine nor increased daily urine calcium excretion rates provide evidence in themselves for a renal Ca leak during prolonged dietary Ca deprivation when serum 1,25-$(OH)_2$-D levels are elevated. Documentation of a renal Ca leak would require the additional demonstration of hypocalcemia and evidence of secondary hyperparathyroidism.

In summary, these studies document that elevated serum 1,25-$(OH)_2$-D levels in healthy men eating low Ca diets result in net loss of bone. These observations may have relevance for the care of patients with nephrolithiasis. Such patients may exhibit elevated serum 1,25-$(OH)_2$-D levels [3, 5, 26] and also may exhibit reduced bone density [22, 24, 26, 27]. Speculatively, therefore, the common recommendation that these patients limit their dietary Ca intake may increase their long-term risk for significant bone disease.

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