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# Reversal of Vitamin-$D_2$-Induced Hypercalciuria by Chlorothiazide

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## Summary

**To test the effects of chlorothiazide on vitamin-$D_2$-induced hypercalciuria, we carried out 17 metabolic studies lasting 12 days each in adult Sprague-Dawley male rats. Three groups were studied: (A) control rats receiving only the vitamin-$D_2$ vehicle; (B) vitamin-$D_2$-treated rats receiving 50 IU/day; and (C) rats treated in the same manner as group B with the addition of chlorothiazide 20 mg/day for the last 6 days of the study. Urine was collected during the last 3 days, and a blood sample was obtained at the end of each study period. Analysis of the data showed that there were no significant differences between the groups in changes of serum calcium concentration (A, 6.1 ± 0.1 mg/dl; B, 6.1 ± 0.2 mg/dl; C, 6.0 ± 0.2 mg/dl), serum creatinine concentration (A, 0.5 ± 0.07 mg/dl; B, 0.52 ± 0.08 mg/dl; C, 0.48 ± 0.04 mg/dl), and creatinine clearance (A, 4.8 ± 0.7 ml/min/kg; B, 5.2 ± 1.2 ml/min/kg; C, 4.9 ± 0.5 ml/min/kg). The administration of vitamin-$D_2$ significantly increased the urinary calcium excretion from 6.7 ± 1.0 mg/kg/day to 19.5 ± 9.7 mg/kg/day ($p < 0.02$), but the calciuria was inhibited in group C rats by the addition of chlorothiazide, which restored urinary calcium excretion to 6.8 ± 2.5 mg/kg/day ($p < 0.02$). Evaluation of the ratio of calcium/creatinine excretion (A, 0.19 ± 0.03; B, 0.53 ± 0.25; C, 0.20 ± 0.07) and calcium/sodium excretion (A, 0.22 ± 0.05; B, 0.48 ± 0.25; C, 0.19 ± 0.04) further confirmed these effects of vitamin-$D_2$ and chlorothiazide on urine calcium excretion. We conclude that in rats conventional doses of vitamin-$D_2$ consistently induce marked hypercalciuria, even without hypercalcemia, and that this hypercalciuria can be effectively prevented by chlorothiazide.**

A major untoward effect of the administration of vitamin-D metabolites to human subjects is the marked elevation in urinary calcium excretion (4, 11, 14, 15). Chronic hypercalciuria can eventually lead to nephrocalcinosis, nephrolithiasis, and deterioration in kidney functions (11). The thiazides, a group of diuretic agents, have been shown to be effective in preventing excessive renal calcium excretion in both humans and research animals (7, 13, 16, 23). They have been widely used for more than 15 years to treat hypercalciuric patients (5, 24). Previous attempts to evaluate the effect of thiazides on vitamin-D-induced hypercalciuria have produced the conflicting claims that urine calcium excretion is reduced in some patients (12, 18) but that it is not reduced in others (3, 19, 24). It has, therefore, been recommended that further studies are needed (11).

In the controlled study presented below, we tested the effects of chlorothiazide on vitamin-$D_2$-induced hypercalciuria in rats.

## MATERIALS AND METHODS

Seventeen metabolic studies were conducted in adult Sprague-Dawley male rats, weighing 465 ± 30 g, which were maintained in metabolic cages with free access to water, on a standard commercial Purina chow diet. After 1 wk of equilibration, the animals were randomly assigned to one of three groups for a 12-day study as follows:

Group A, the control group, comprised five rats that received daily subcutaneous injection of 0.2 ml of the vitamin-$D_2$ vehicle only;

Group B, the vitamin-$D_2$ group was composed of six rats that were injected daily subcutaneously with 50 IU of vitamin-$D_2$ (Drisdol, Winthrop Laboratories, New York, NY); and

Group C, the vitamin-$D_2$ and chlorothiazide group included six rats that were treated in the same manner as those in Group B but additionally with chlorothiazide 20 mg/day injected intraperitoneally (Diuril, Merck Sharp and Dohme, West Point, PA) for the last six days.

During the last 3 days of the study all urine was collected, mixed, and measured. At the end of the study a blood sample was obtained from each rat by aortic puncture.

The urine was analyzed for calcium, creatinine, and sodium, and the serum for calcium and creatinine. Calcium was determined by atomic absorption spectrophotometry, nonchromogen creatinine, and sodium by standard methods. Student's *t* test was used to determine the significance of the difference between the groups.

Table 1. *Effects of vitamin-$D_2$ and chlorothiazides on serum calcium and creatinine concentrations and creatinine clearance*

| | Serum creatinine (mg/dl) | | | Serum calcium (mg/dl) | | | Creatinine clearance (ml/min/kg) | | |
|---|---|---|---|---|---|---|---|---|---|
| | A[1] | B[2] | C[3] | A | B | C | A | B | C |
| 1 | 0.5 | 0.6 | 0.5 | 6.2 | 6.1 | 5.8 | 4.83 | 3.17 | 4.44 |
| 2 | 0.6 | 0.5 | 0.5 | 6.1 | 6.2 | 5.8 | 4.05 | 5.47 | 4.38 |
| 3 | 0.5 | 0.6 | 0.5 | 6.2 | 6.2 | 6.1 | 4.40 | 4.51 | 5.26 |
| 4 | 0.5 | 0.5 | 0.5 | 6.1 | 5.8 | 6.1 | 4.79 | 5.97 | 5.35 |
| 5 | 0.4 | 0.4 | 0.5 | 6.0 | 6.2 | 6.1 | 5.95 | 6.70 | 4.63 |
| 6 | | 0.5 | 0.4 | | 5.8 | 5.9 | | 5.18 | 5.44 |
| Mean | 0.50 | 0.52 | 0.48 | 6.1 | 6.1 | 6.0 | 4.80 | 5.16 | 4.91 |
| S.D. | 0.07 | 0.08 | 0.04 | 0.1 | 0.2 | 0.2 | 0.71 | 1.22 | 0.48 |

[1] A, Control.
[2] B, Vitamin-$D_2$.
[3] C, Vitamin-$D_2$ + cholorothiazide.

## RESULTS

All rats consumed 16 ± 2 g of the Purina chow and lost 2.1 ± 1.2% of their weight during the 12-day study, with no difference between the groups. As shown in Table 1, no significant differences appeared between the groups also for serum calcium and creatinine concentrations or for glomerular filtration rates as expressed by creatinine clearance. Administration of vitamin-$D_2$, however, affected a 3-fold increase in the mean rate of daily urine calcium excretion (Table 2); an increase that remained significant when the calcium excretion was also considered relative to urinary creatinine and urinary sodium excretion rates (Table 2). Chlorothiazide, administered to group C rats during the last 6 days, inhibited the calciuric effects of vitamin-$D_2$ and returned the calcium excretion rate to its control values. No significant differences in calcium excretion were found between the control group and the thiazide-treated group at the end of the study.

Table 2. *Effects of vitamin-$D_2$ and chlorothiazide on urine calcium excretion*

| | Urinary calcium (mg/kg/24 h) | | | Urinary calcium/urinary creatinine | | | Urinary calcium/urinary sodium | | |
|---|---|---|---|---|---|---|---|---|---|
| | A[1] | B[2] | C[3] | A | B | C | A | B | C |
| 1 | 7.6 | 15.1 | 3.1 | 0.22 | 0.58 | 0.09 | 0.27 | 0.74 | 0.17 |
| 2 | 6.3 | 12.3 | 6.5 | 0.17 | 0.31 | 0.20 | 0.19 | 0.25 | 0.23 |
| 3 | 7.5 | 37.3 | 5.5 | 0.23 | 0.95 | 0.14 | 0.21 | 0.79 | 0.12 |
| 4 | 5.2 | 15.6 | 10.2 | 0.15 | 0.36 | 0.27 | 0.27 | 0.27 | 0.20 |
| 5 | 6.9 | 24.3 | 6.7 | 0.20 | 0.62 | 0.19 | 0.16 | 0.26 | 0.18 |
| 6 | | 12.6 | 8.8 | | 0.34 | 0.28 | | 0.59 | 0.21 |
| Mean | 6.7 | 19.5 | 6.8 | 0.19 | 0.53 | 0.20 | 0.22 | 0.48 | 0.19 |
| S.D. | 1.0 | 9.7 | 2.5 | 0.03 | 0.25 | 0.07 | 0.05 | 0.25 | 0.04 |
| B *vs* A | | $P < 0.02$ | | | $P < 0.02$ | | | $P < 0.05$ | |
| C *vs* B | | | $P < 0.02$ | | | $P < 0.01$ | | | $P < 0.02$ |
| C *vs* A | | | N.S. | | | N.S. | | | N.S. |

[1] A, Control.
[2] B, Vitamin-$D_2$.
[3] C, Vitamin-$D_2$ + chlorothiazide.

## DISCUSSION

Vitamin-D metabolites enhance intestinal calcium absorption and promote a positive calcium balance (1, 4, 10, 17). Vitamin-D, however, can lead to increased urine calcium excretion (4, 11, 15), and thus may sometimes promote nephrolithiasis, nephrocalcinosis, and deterioration in kidney functions (11). The present study demonstrates that vitamin-$D_2$ administered to rats for 12 days at a dose similar to that in clinical use for human beings, results in a 3-fold increase in the mean daily urinary calcium excretion (Table 2). When we tested the influences of other factors, such as body mass, fluid-electrolyte balance, and diet on calcium excretion, by evaluating the interrelationships between urine calcium and creatinine or sodium excretion (11, 22), the relative increases in calcium excretion remained consistently significant (Table 2).

We also confirmed (Table 1) the earlier observations of Litvak *et al.* (14) that vitamin-D-induced hypercalciuria can occur without concomitant hypercalcemia, an effect we have recently demonstrated (4) in children with sex-linked dominant hypophosphatemic rickets treated by 1,25-dihydroxyvitamin-$D_3$. The hypercalciuria brought about by vitamin-D metabolites administration is believed to be secondary to enhanced intestinal calcium absorption, an elevated filtered load of ionized calcium, and depressed serum parathyroid hormone (20). The stability of glomerular filtration rates in our study (Table 1) is important, because other parameters such as phosphaturia and acid-base disorders that might be implicated in the calciuria are dependent on changes in glomerular filtration rates. Furthermore, because hypercalcemia can cause kidney function deterioration with concomitant elevation in serum creatinine concentration (11), the maintenance of normal serum calcium concentration in our rats is reflected in the stability of their serum creatinine and creatinine clearances.

The administration of thiazides, well-known as hypocalciuric agents (5, 23, 24), to patients with vitamin-D-induced hypercalciuria is a matter of some controversy. Parfitt (18) and Higgins *et al.* (12) reported that thiazides were effective in decreasing the amount of calcium in the urine of vitamin-D-treated patients. Yendt and Cohanim (24), Parfitt (19), and Brickman *et al.* (3), who studied patients with hypoparathyroidism treated by vitamin-D, found that thiazide had no influence on the excretion of calcium; on the other hand, normal controls receiving vitamin-D studied by the last group (3) experienced reduction in calcium excretion rates under treatment. Harrison and Harrison (11) pressed for further controlled studies of thiazide therapy for vitamin-D-induced hypercalciuria before conclusions should be drawn about their applicability in man.

Previous studies in the dog (7) and the rat (13, 16) have demonstrated the effectiveness of thiazides in decreasing urinary calcium excretion, but did not study the specific action of the thiazides in hypercalciuria caused by vitamin-D. The ability of chlorothiazide to reverse vitamin-$D_2$-induced hypercalciuria and effect a decrease in calcium excretion to control values in rats is clearly demonstrated in the present study (Table 2).

An absence of hypercalcemia induction and a persistence of stable serum creatinine and creatinine clearance accompanied the treatment (Table 1). A mild but still insignificant increase in serum calcium concentration was also observed by Jorgensen (13) when he treated normal Wistar rats with bendroflumethiazide.

As has been domonstrated previously, there are two mechanisms by which the thiazides reduce calcium excretion: (1) by the decrease in extracellular fluid volume and hence by nonspecific stimulation of the sodium-coupled calcium reabsorption in the proximal tubule (2, 3, 21) and (2) by specific dissociative action on the distal tubule, which results in enhanced reabsorption of calcium in contrast to decreased reabsorption of sodium (6, 8, 9). This dissociation of calcium/sodium reabsorptions is also evident in the present study (Table 2), as the ratio of urine calcium excretion to urine sodium excretion decreased with chlorothiazide administration.

In summary, we conclude that in rats (1), vitamin-$D_2$ administration in conventional doses induces marked hypercalciuria that is independent of serum calcium concentration, creatinine clearance, or the urine excretion of sodium and (2) that administration of chlorothiazide effectively prevents and precludes hypercalciuria presumably by acting directly to augment tubular calcium reabsorption, without changes in the glomerular filtration rates or in serum calcium concentration.

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