

Rapid Publication

Evidence that 1,25-Dihydroxyvitamin D₃ Inhibits the Hepatic Production of 25-Hydroxyvitamin D in Man

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Abstract. Previous in vitro studies in rachitic rat liver suggested that 1,25-dihydroxyvitamin D inhibits the hepatic production of 25-hydroxyvitamin D (25-OHD). An investigation therefore was carried out in eight normal subjects to determine whether concomitant administration of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] would alter the response of serum 25-OHD to challenge with vitamin D. In control studies, vitamin D, 100,000 U/d for 4 d, significantly increased mean serum 25-OHD, from 26.3±2.9 to 66.7±12.6 ng/ml ($P < 0.01$). In contrast, 1,25(OH)₂D₃, 2 µg/d for 4 d, completely prevented an increase in serum 25-OHD in response to the same dose of vitamin D in the same individuals (25.1±2.2 vs. 27.4±5.3 ng/ml, NS). In a post-control study in seven of the normal subjects, vitamin D again significantly increased mean serum 25-OHD, from 18.2±3.1 to 42.8±4.7 ng/ml ($P < 0.001$). In each of the three studies, mean serum calcium, phosphorus, and creatinine did not change and remained within the normal range. Whereas mean urinary calcium did not change in response to vitamin D alone during the 4 d of the two control studies, it increased significantly in the study in which vitamin D and 1,25(OH)₂D₃ were given together. A dose-response inhibition of the response of serum 25-OHD to vitamin D by 1,25(OH)₂D₃ was demonstrated in two of the normal subjects. The results provide evidence that 1,25(OH)₂D₃ inhibits the hepatic synthesis of its precursor 25-OHD in man.

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Introduction

Available evidence indicates that 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]¹ inhibits the production of 25-hydroxyvitamin D (25-OHD) by the liver. Thus, 1,25(OH)₂D₃ inhibited the in vitro synthesis of 25-OHD by liver homogenates and perfused liver from rachitic rats at a concentration of 100 pg/ml (1). A dose-response inhibition of synthesis of 25-OHD at this and higher concentrations of 1,25(OH)₂D₃ was also demonstrated. Accordingly, the present studies were carried out to determine whether concomitant administration of 1,25(OH)₂D₃ alters the increase in serum 25-OHD that occurs after vitamin D challenge in normal adult human subjects.

Methods

Eight normal adult subjects, four men and four women, were studied by methods previously described (2). They ranged in age from 24 to 35 yr. All of them were hospitalized at the General Clinical Research Center of the Medical University of South Carolina. Each gave informed consent. They were given a constant daily diet estimated to contain 400 mg/d of calcium and 900 mg/d of phosphorus, and a constant fluid intake. Three studies were conducted in sequence. In the first study, all subjects were given vitamin D₂, 2.5 mg (100,000 U) per day for 4 d, as a single morning dose. In the second study, all of them were given 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] in divided doses at intervals of 12 h for the 4-d period together with the same dose of vitamin D. In the third study, vitamin D was given again by itself for 4 d to seven of the subjects. Fasting blood samples were obtained on the first day, before vitamin D either with or without 1,25(OH)₂D₃, and again on the fifth day, 24 h after the last dose of the vitamin and, when 1,25(OH)₂D₃ was given, 12 h after the last dose for determination of serum calcium (3), phosphorus (4), and creatinine (5) by automated methods and for measurement of serum 25-OHD. Serum 1,25(OH)₂D also was measured in the study in which 1,25(OH)₂D₃ was administered. 24-h urines were collected and analyzed for calcium (3).

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

Serum 25-OHD was measured in duplicate at two concentrations by the competitive protein binding method with vitamin D-deficient rat serum (6) after extraction with methanol/methylene chloride 2:1 (vol/vol), alkaline backwash, chromatography of samples on Lipidex-5000 (Packard Instrument Co., Downers Grove, IL), and elution with hexane/chloroform 85:15 (vol/vol) as previously reported (7, 8). The mean \pm SD in normal subjects is 23.3 \pm 11.4 ng/ml ($n = 42$). Serum 1,25(OH) $_2$ D was measured in duplicate by the chick intestinal receptor method (7) after extraction without high performance liquid chromatography by the procedure of Reinhardt et al. (9). The mean \pm SD in normal subjects is 25.9 \pm 12.1 pg/ml ($n = 27$). The t test was used to determine the statistical significance of differences between paired and unpaired samples. Statistical analyses were conducted with a calculator (model 9815A; Hewlett-Packard Co., Palo Alto, CA).

Results

The results are summarized in Tables I and II. In the first study, mean serum 25-OHD increased significantly in response to vitamin D, 100,000 U/d for 4 d, and mean serum calcium, phosphorus, and creatinine did not change in the eight normal subjects (Table I). In contrast, in the second study, mean serum 25-OHD, calcium, phosphorus, and creatinine did not change in the same eight individuals in response to the same dose of vitamin D when 1,25(OH) $_2$ D $_3$, 2 μ g/d, was given concomitantly in divided doses for the 4 d. In the third study in seven of the subjects, mean serum 25-OHD again increased significantly in response to vitamin D by itself, and mean serum calcium, phosphorus, and creatinine did not change. Mean urinary calcium increased in response to 1,25(OH) $_2$ D $_3$ and vitamin D during the 4 d of that study but was not altered by vitamin D alone in the other two studies (Table II). Thus,

simultaneously administered 1,25(OH) $_2$ D $_3$ completely prevented an increase in serum 25-OHD produced by vitamin D and increased urinary calcium significantly. Individual values for serum 25-OHD in the normal subjects during the first two studies are depicted in Fig. 1. Dose-response inhibition by 1,25(OH) $_2$ D $_3$ of the increases in serum 25-OHD in response to vitamin D in two of the normal subjects was also observed (Fig. 2).

In the second study, there was only a modest increase in mean serum 1,25(OH) $_2$ D in response to 1,25(OH) $_2$ D $_3$, but the increment was not statistically significant. This small change is attributed to the rapid metabolism of the metabolite after its oral administration (10). Nevertheless, two biologic effects of 1,25(OH) $_2$ D $_3$ were demonstrated, inhibition of hepatic production of 25-OHD and increase in urinary calcium.

Discussion

Previous findings in normal adult subjects indicate that 25-hydroxyvitamin D-1 α -hydroxylase in the kidney is much more tightly regulated than vitamin D-25-hydroxylase in the liver (11, 12). When vitamin D in pharmacologic doses (100,000 U/d for 4 d) is administered to normal subjects, mean serum 25-OHD increases significantly, but mean serum 1,25(OH) $_2$ D does not change (11, 12). Indeed, vitamin D intoxication is characterized by and results from abnormal elevation of serum 25-OHD, and serum 1,25(OH) $_2$ D is either normal or only minimally elevated (13). Parathyroid hormone is the major regulator of the renal production of 1,25(OH) $_2$ D. Values are low in hypoparathyroidism, may be increased in primary

Table I. Effects of Vitamin D and 1,25(OH) $_2$ D $_3$ on Serum Calcium, Phosphorus, Creatinine, 25-OHD, and 1,25(OH) $_2$ D in Normal Subjects

Treatment	Serum				
	Calcium	Phosphorus	Creatinine	25-OHD	1,25(OH) $_2$ D
	mg/dl	mg/dl	mg/dl	ng/ml	pg/ml
Study I (8)					
Control	9.1 \pm 0.1	4.0 \pm 0.2	0.9 \pm 0.1	26.3 \pm 2.9	—
Vitamin D	9.2 \pm 0.2	4.1 \pm 0.1	0.9 \pm 0.1	66.7 \pm 12.6	—
P value	NS	NS	NS	<0.01	
Study II (8)					
Control	9.2 \pm 0.2	4.0 \pm 0.2	1.0 \pm 0.1	25.1 \pm 2.2	23.6 \pm 1.3
Vitamin D + 1,25(OH) $_2$ D $_3$	9.2 \pm 0.2	4.4 \pm 0.2	1.0 \pm 0.1	27.4 \pm 5.3	32.2 \pm 3.4
P value	NS	NS	NS	NS	NS
Study III (7)					
Control	8.8 \pm 0.2	4.1 \pm 0.3	1.0 \pm 0.1	18.2 \pm 3.1	—
Vitamin D	9.0 \pm 0.1	4.0 \pm 0.2	1.0 \pm 0.1	42.8 \pm 4.7	—
P value	NS	NS	NS	<0.001	

Results are presented as mean \pm SE. Figures in parentheses are the number of subjects.

Table II. Effects of Vitamin D and 1,25(OH)₂D₃ on Urinary Calcium in Normal Subjects

Treatment	Urinary calcium (mg/d)			
	Days			
	1	2	3	4
Study I (8)				
Vitamin D	174±24	179±19	189±20	205±20
Study II (8)				
Vitamin D				
+1,25(OH) ₂ D ₃	190±18	252±27*	296±40*	310±47‡
Study III (7)				
Vitamin D	208±25	206±25	204±20	203±17

Results are presented as mean±SE. Figures in parentheses are the number of subjects.

* $P < 0.01$ vs. day 1 of vitamin D and 1,25(OH)₂D₃.

‡ $P < 0.02$ vs. day 1 of vitamin D and 1,25(OH)₂D₃.

hyperparathyroidism, and are increased by the administration of parathyroid extract in patients with hypoparathyroidism (14–16). Hypercalcemia caused by vitamin D intoxication is associated with suppression of circulating immunoreactive parathyroid hormone and urinary cyclic 3'5'-adenosine monophosphate (17). Also, adaptation from a high to a low calcium diet is mediated by increases in circulating parathyroid hormone and 1,25(OH)₂D (18).

Our results show that 25 hydroxylation of vitamin D is impaired by exogenously administered 1,25(OH)₂D₃. Thus, there appears to be feedback regulation of hepatic synthesis of 25-OHD by 1,25(OH)₂D₃ in human subjects in vivo as there is in rat liver in vitro (1). A number of clinical observations support this concept. First, we described two patients with sarcoidosis, hypercalcemia, and increased circulating

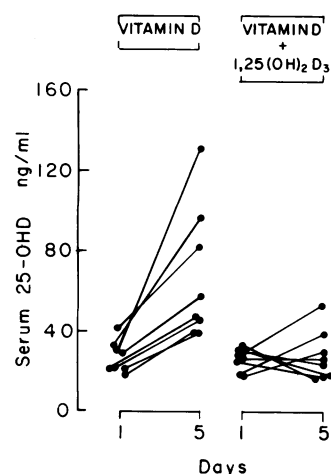


Figure 1. The effects of vitamin D, 100,000 U/d for 4 d, by itself and together with 1,25(OH)₂D₃, 2 µg/d for 4 d, on serum 25-OHD in eight normal adult subjects. The vitamin D was given as a single morning dose, and the 1,25(OH)₂D₃ was given in divided doses, 1 µg every 12 h for 4 d.

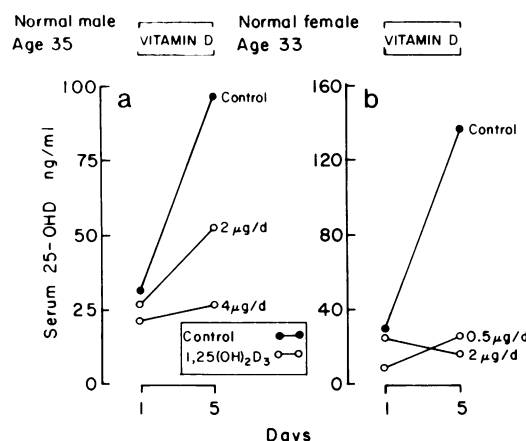


Figure 2. The effects of vitamin D, 100,000 U/d for 4 d, by itself and together with 1,25(OH)₂D₃, on serum 25-OHD in two normal subjects. The vitamin D was given as a single morning dose, and the 1,25(OH)₂D₃ was given in divided doses every 12 h for 4 d. Note that one of the subjects required 4 µg/d of 1,25(OH)₂D₃ for maximum suppression (a) and that the other suppressed with smaller doses of 1,25(OH)₂D₃ (b).

1,25(OH)₂D who had values for serum 25(OH)D that were low or low-normal and ranged from 5 to 9 ng/ml (9). Second, one patient with vitamin D-dependent rickets type II and elevated serum 1,25(OH)₂D was reported to have "apparent defective synthesis" of 25-OHD (19). In this individual, serum 25-OHD was in the lower range of normal, 18.6 ng/ml, despite earlier administration of large doses of vitamin D. Third, serum 25-OHD was reported to fall progressively and significantly in a group of 25 postmenopausal women who were being treated chronically with 1,25(OH)₂D₃ (20). Fourth, mean serum 25-OHD was significantly decreased and mean serum 1,25(OH)₂D was significantly increased in a group of patients with hyperphosphatemic tumoral calcinosis as compared with values in normal subjects and nonaffected family members (21). Finally, we conducted studies in a patient who had had hypercalcemia and elevated serum 1,25(OH)₂D and did not have sarcoidosis while he was normocalcemic after treatment with prednisone (Bell, N. H., unpublished observations). Whereas his serum 1,25(OH)₂D, serum calcium, and urinary calcium increased abnormally in response to vitamin D, his serum 25-OHD, which initially was abnormally low, never rose above 10 ng/ml. The findings indicate that circulating 25-OHD may be reduced in patients with abnormal elevation of serum 1,25(OH)₂D or by administration of 1,25(OH)₂D₃.

There is also evidence that feedback inhibition of hepatic production of 25-OHD may be of physiologic importance in children. We reported previously that whereas in adults 25-OHD production is loosely regulated and 1,25(OH)₂D production is tightly regulated in response to vitamin D challenge, the opposite is true in children (12). Vitamin D, 1,500 U/kg body wt per d for 4 d, produced modest but significant

increases in mean serum calcium, from 9.5 to 9.8 mg/dl; in mean serum 25-OHD, from 25 to 34 ng/ml (an average of 36%); and in mean serum 1,25(OH)₂D, from 34 to 42 pg/ml (an average of 24%) in a group of 12 normal children. Serum calcium, serum 25-OHD, and serum 1,25(OH)₂D remained within the normal range in all subjects. The lack of a more substantial increase in serum 25-OHD probably resulted from the increases in serum 1,25(OH)₂D and consequent inhibition of 25 hydroxylation of the vitamin. Thus, it appears that feedback regulation of synthesis of the precursor 25-OHD by 1,25(OH)₂D protects against abnormal increases in serum 1,25(OH)₂D of normal children in whom 1,25(OH)₂D production is loosely regulated.

These findings contrast with those we observed in six children with the Williams syndrome. In those individuals, vitamin D, 1,500 U/kg body wt per d for 4 d, produced a marked increase in mean serum 25-OHD, from 16.7 to 66.8 ng/ml (an average of 298%) (2). Mean serum calcium and 1,25(OH)₂D did not change. The lack of tight regulation of serum 25-OHD in these children probably resulted from lack of an increase in serum 1,25(OH)₂D, which would diminish hydroxylation of vitamin D. The exaggerated response of serum 25-OHD in the children with the syndrome therefore appears to be secondarily related to tight regulation of circulating 1,25(OH)₂D in response to vitamin D and not to any intrinsic abnormality in 25-hydroxylation of the vitamin.

In summary, the present and previous observations provide strong evidence that 25 hydroxylation of vitamin D is regulated by circulating 1,25(OH)₂D. Thus, 1,25(OH)₂D acts to limit production of its precursor. In adults this feedback regulation of synthesis of the precursor, together with tight regulation of renal production of the most active metabolite of vitamin D, provides mechanisms that protect against an abnormal elevation of circulating 1,25(OH)₂D. The inadequacy of feedback regulation of 25-OHD production by itself to prevent abnormal increases in circulating 1,25(OH)₂D in diseases in which 1,25(OH)₂D production is unregulated, however, is attested to by the hypercalcemia and abnormal calcium metabolism that may spontaneously occur in sarcoid, (9, 22, 23), disseminated candidiasis (24), and lymphoma (25). The mechanism by which the feedback regulation of 25-OHD production occurs has not been established. In view of the almost 1,000-fold difference in concentration of the two metabolites in the circulation, it is not likely that 1,25(OH)₂D₃ acts to competitively inhibit 25 hydroxylation of the vitamin. Further studies are needed to determine how regulation is mediated.

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