

## Demonstration that circulating 1 alpha, 25-dihydroxyvitamin D is loosely regulated in normal children.

P H Stern, ... , N H Bell, S Epstein

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### Research Article

The effects of vitamin D, 2.5 mg (100,000 U)/d for 4 d, on serum calcium, serum 25-hydroxyvitamin D (25-OHD) and serum 1 alpha, 25-dihydroxyvitamin D (1 alpha, 25(OH)2D) were compared in 24 normal adults and 12 normal children. The daily dose of vitamin D was 1,500 U/kg body wt in children weighing less than 45 kg. Vitamin D increased mean serum calcium from 9.5 +/- 0.1 to 9.8 +/- 0.1 mg/dl (P less than 0.05), increased mean serum phosphorus from 4.6 +/- 0.1 to 5.0 +/- 0.1 mg/dl (P less than 0.01), increased mean serum 25-OHD from 25 +/- 3 to 34 +/- 4 ng/ml (P less than 0.001), and increased mean serum 1 alpha, 25(OH)2D from 34 +/- 3 to 42 +/- 4 pg/ml (P less than 0.02) in children. In contrast, vitamin D increased mean serum 25-OHD from 18 +/- 2 to 39 +/- 6 ng/ml (P less than 0.001) and did not change mean serum calcium (9.4 +/- 0.1 vs. 9.5 +/- 0.1 mg/dl), mean serum phosphorus (4.0 +/- 0.1 vs. 4.1 +/- 0.1 mg/dl), or mean serum 1 alpha, 25(OH)2D (31 +/- 2 vs. 29 +/- 3 pg/ml) in adults. Mean serum 1 alpha, 25(OH)2D was significantly higher after vitamin D in children than in adults (P less than 0.02). These results provide evidence that circulating [...]

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# Demonstration That Circulating $1\alpha,25$ -Dihydroxyvitamin D Is Loosely Regulated in Normal Children

PAULA H. STERN, ASHBY B. TAYLOR, NORMAN H. BELL, and SOL EPSTEIN,  
*Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611; Veterans Administration Medical Center and Departments of Pediatrics, Medicine and Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29403; Department of Medicine, Indiana University Medical School, Indianapolis, Indiana 46202*

**ABSTRACT** The effects of vitamin D, 2.5 mg (100,000 U)/d for 4 d, on serum calcium, serum 25-hydroxyvitamin D (25-OHD) and serum  $1\alpha,25$ -dihydroxyvitamin D ( $1\alpha,25(\text{OH})_2\text{D}$ ) were compared in 24 normal adults and 12 normal children. The daily dose of vitamin D was 1,500 U/kg body wt in children weighing <45 kg. Vitamin D increased mean serum calcium from  $9.5\pm 0.1$  to  $9.8\pm 0.1$  mg/dl ( $P < 0.05$ ), increased mean serum phosphorus from  $4.6\pm 0.1$  to  $5.0\pm 0.1$  mg/dl ( $P < 0.01$ ), increased mean serum 25-OHD from  $25\pm 3$  to  $34\pm 4$  ng/ml ( $P < 0.001$ ), and increased mean serum  $1\alpha,25(\text{OH})_2\text{D}$  from  $34\pm 3$  to  $42\pm 4$  pg/ml ( $P < 0.02$ ) in children. In contrast, vitamin D increased mean serum 25-OHD from  $18\pm 2$  to  $39\pm 6$  ng/ml ( $P < 0.001$ ) and did not change mean serum calcium ( $9.4\pm 0.1$  vs.  $9.5\pm 0.1$  mg/dl), mean serum phosphorus ( $4.0\pm 0.1$  vs.  $4.1\pm 0.1$  mg/dl), or mean serum  $1\alpha,25(\text{OH})_2\text{D}$  ( $31\pm 2$  vs.  $29\pm 3$  pg/ml) in adults. Mean serum  $1\alpha,25(\text{OH})_2\text{D}$  was significantly higher after vitamin D in children than in adults ( $P < 0.02$ ). These results provide evidence that circulating  $1\alpha,25(\text{OH})_2\text{D}$  is not as tightly regulated in children as it is in adults. This difference in regulation could account in part for the higher values for serum  $1\alpha,25(\text{OH})_2\text{D}$  observed in children.

## INTRODUCTION

Previous findings indicate that serum values for  $1\alpha,25$ -dihydroxyvitamin D ( $1\alpha,25(\text{OH})_2\text{D}$ )<sup>1</sup> are con-

Dr. Stern is the recipient of a Career Development Award from the U. S. Public Health Service. Dr. Bell is a Veterans Administration Medical Investigator. Address reprint requests to Dr. Bell, Veterans Administration Medical Center, 109 Bee St., Charleston, S. C. 29403.

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<sup>1</sup>Abbreviations used in this paper:  $1\alpha,25(\text{OH})_2\text{D}$ ,  $1\alpha,25$ -dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D; PTH, parathyroid hormone.

sistently higher in normal children and adolescents than in normal adults (1-6). It is known that growth hormone secretion is increased in children and adolescents (7) and that intestinal absorption of calcium increases in response to administration of growth hormone (5, 8). Thus, one explanation could be that the production of  $1\alpha,25(\text{OH})_2\text{D}$  is regulated by growth hormone. Short-term studies do not support this possibility (3, 5). In one investigation, for example, administration of growth hormone to children with deficiency of the hormone increased intestinal absorption of calcium and reduced serum  $1\alpha,25(\text{OH})_2\text{D}$  (5). On the other hand, preliminary long-term studies provide evidence that growth hormone may increase the sensitivity of the kidney to parathyroid hormone and thereby augment circulating  $1\alpha,25(\text{OH})_2\text{D}$  (9).

We previously demonstrated tight regulation of circulating  $1\alpha,25(\text{OH})_2\text{D}$  in normal adults given pharmacologic doses of vitamin D (10, 11). One mechanism which could account for higher values for serum  $1\alpha,25(\text{OH})_2\text{D}$  in children is that production of the metabolite is not closely regulated. The present studies were carried out to examine this hypothesis. The response of circulating  $1\alpha,25(\text{OH})_2\text{D}$  to challenge with vitamin D was compared in children and adults.

## METHODS

24 normal adult subjects, 10 men and 14 women, ranging in age from 21 to 56 yr, and 12 normal children, 5 boys and 7 girls, ranging in age from 21 mo to 17 yr, were studied. All except one of the adults were between 21 and 49 yr of age. They were hospitalized in the Clinical Research Center of the Indiana University Medical School or of the Medical University of South Carolina. Informed consent was obtained from each of the adults and from one or both parents of each of the children. Each of them was given a constant daily diet estimated to contain 400 mg/d of calcium and 900 mg/d of phosphorus. Daily fluid intake was constant. Vitamin D, 2.5 mg (100,000 U)/d for 4 d, was given as a single morning dose. The daily dose of vitamin D was 1,500 U/kg in children who weighed <45 kg. Fasting blood samples were obtained on the 1st d before vitamin D and again on the 5th d,

TABLE I  
Effects of Vitamin D<sub>2</sub> on Serum Calcium, Phosphorus, Creatinine, 25-OHD,  
and 1 $\alpha$ ,25(OH)<sub>2</sub>D in Normal Children and Adults

	Serum				
	Calcium	Phosphorus	Creatinine	25-OHD*	1 $\alpha$ ,25(OH) <sub>2</sub> D
	mg/dl	mg/dl	mg/dl	ng/ml	pg/ml
Children (12)					
Control	9.5±0.1	4.6±0.1	0.7±0.1	25±3	34±3
Vitamin D	9.8±0.1	5.0±0.1	0.7±0.1	34±4	42±4
P value	<0.05	<0.01	NS	<0.001	<0.02
Adults (24)					
Control	9.4±0.1	4.0±0.1	0.9±0.1	18±2	31±2
Vitamin D	9.5±0.1	4.1±0.1	0.9±0.1	39±6	29±3
P value	NS	NS	NS	<0.001	NS

Results are given as mean±SE.

\* Serum 25-OHD was measured in 21 normal adults before vitamin D and in 17 normal adults after vitamin D.

24 h after the last dose of the vitamin, for determination of calcium, phosphorus, creatinine, 25-hydroxyvitamin D (25-OHD), and 1 $\alpha$ ,25(OH)<sub>2</sub>D. Serum calcium (12, 13), phosphorus (14), and creatinine (15, 16) were determined by automated methods. 24-h urines were collected from the children and analyzed for calcium (13).

Serum 25-OHD was measured in triplicate at two dilutions by the protein-binding method, with sera from vitamin D-deficient rats (17) after extraction and LH-20 chromatography (10). The normal range in our laboratory is from 8 to 61 ng/ml.

Serum 1 $\alpha$ ,25(OH)<sub>2</sub>D was measured by bioassay (10, 11). With few exceptions, the two samples from each subject were measured in the same assay. The mean value in normal adult subjects is 32±2 pg/ml and the normal range is from 12 to 55 pg/ml (*n* = 33). The 95% confidence values (mean±2 SD) are 13–52 pg/ml. Interassay variation is 9.1% (*n* = 17).

Serum samples were obtained from other normal outpatient subjects, both children and adults, after overnight fast for determination of parathyroid hormone (PTH). Serum immunoreactive PTH was measured by radioimmunoassay with antiserum from chicken 77125 at a final concentration of 1:10,000 (10, 18).

Student's *t* test was used to determine the significance of differences between paired and unpaired samples (19). A Hewlett-Packard calculator (model 9815 A, Hewlett Packard Co., Palo Alto, Calif.) was used to make these calculations and to determine the correlation coefficient.

## RESULTS

Vitamin D<sub>2</sub> was given for 4 d to each subject. The daily dose averaged 1,482±48 U/kg body wt/d in the children and 1,549±56 U/kg/d in the adults. The results are summarized in Table I. In children, vitamin D produced significant increases in mean serum calcium, serum phosphorus, serum 25-OHD, and serum 1 $\alpha$ ,25(OH)<sub>2</sub>D. The increases in mean serum 25-OHD and serum 1 $\alpha$ ,25(OH)<sub>2</sub>D averaged 36 and 24%, respectively. In contrast, vitamin D significantly increased mean serum 25-OHD and did not change mean serum

calcium, serum phosphorus, or serum 1 $\alpha$ ,25(OH)<sub>2</sub>D in the adults. The increase in mean serum 25-OHD averaged 116% and was greater in adults than in children. Despite the smaller increment in mean serum 25-OHD in children, mean serum 1 $\alpha$ ,25(OH)<sub>2</sub>D was significantly higher after vitamin D in them than in the adults (*P* < 0.02). In children the correlation between serum 25-OHD and serum 1 $\alpha$ ,25(OH)<sub>2</sub>D was not significant. After vitamin D, serum 1 $\alpha$ ,25(OH)<sub>2</sub>D either increased or did not change (±10%) in 10 of the 12 children (84%) whereas it either decreased or did not change in 16 of the 24 adults (75%). In children the increase in serum 1 $\alpha$ ,25(OH)<sub>2</sub>D was not related to age, sex, or age at onset of puberty.

Serum immunoreactive PTH averaged 314±157 pg/ml (mean±SD) in a group of 28 normal children (age range: 8–19 yr) and 281±119 pg/ml in a group of 85 normal adults (age range: 21–49 yr). These values are not significantly different (*P* > 0.2).

Urinary calcium did not change in any of the children during the 4 d of vitamin D administration (data not shown).

## DISCUSSION

Our findings show an unequivocal "impairment" in regulation of circulating 1 $\alpha$ ,25(OH)<sub>2</sub>D in a group of normal children in response to a challenge with pharmacologic doses of vitamin D. There were proportional increases in the concentrations of 1 $\alpha$ ,25(OH)<sub>2</sub>D and of the precursor, 25-OHD, which were associated with significant increases in serum calcium and phosphorus. The results are in clear contrast to those obtained in a group of adults who demonstrated no change in mean serum 1 $\alpha$ ,25(OH)<sub>2</sub>D, mean serum calcium, or

mean serum phosphorus, in response to challenge with vitamin D (10, 11).

It is unlikely that the enhanced response of serum  $1\alpha,25(\text{OH})_2\text{D}$  in children is related to an increase in the concentration of circulating PTH. Whereas mean serum immunoreactive PTH was higher in a group of normal children than in a group of normal adults, the difference was not statistically significant. As noted already, however, preliminary evidence in long-term studies in children suggests that growth hormone may increase serum  $1\alpha,25(\text{OH})_2\text{D}$  by enhancing the renal response to PTH (9).

The findings in children are qualitatively similar to those we reported previously in patients with sarcoidosis and normal calcium metabolism. When challenged with the same dose of vitamin D, these individuals exhibited a mean twofold increase in serum  $1\alpha,25(\text{OH})_2\text{D}$  (11). Also, glucocorticoids decreased serum  $1\alpha,25(\text{OH})_2\text{D}$  in children (20) and, when given for 8 d, lowered serum  $1\alpha,25(\text{OH})_2\text{D}$  in patients with sarcoid who were normocalcemic (21), but did not alter serum  $1\alpha,25(\text{OH})_2\text{D}$  in normal adults (21). When given for 14 d to adults, glucocorticoids increased serum  $1\alpha,25(\text{OH})_2\text{D}$  and lowered the fractional intestinal absorption of calcium (22).

The question of extrarenal production of  $1\alpha,25(\text{OH})_2\text{D}$  was raised in sarcoid because of the demonstration of increased serum  $1\alpha,25(\text{OH})_2\text{D}$  (which correlated with the serum calcium) and suppressed serum immunoreactive PTH in a patient with the disorder who had undergone bilateral nephrectomy (23). In this regard, recent preliminary studies showed that a positive correlation existed between serum 25-OHD and serum  $1\alpha,25(\text{OH})_2\text{D}$  in children with chronic renal disease who were undergoing treatment with 25-OHD<sub>3</sub> only when the glomerular filtration rate was  $\geq 25$  ml/min (24). The findings in these patients could be interpreted to mean that there is extrarenal synthesis of  $1\alpha,25(\text{OH})_2\text{D}$  that is not closely regulated. These and the present observations suggest that children may have either a different control mechanism for regulation of renal synthesis of the metabolite as compared to adults, extrarenal production of  $1\alpha,25(\text{OH})_2\text{D}$ , or some combination of these possibilities. Our findings do not allow us to distinguish among these alternatives.

Whatever the mechanism, our results showing lack of tight regulation of circulating  $1\alpha,25(\text{OH})_2\text{D}$  provide a possible explanation for the higher serum values of this metabolite in normal children and adolescents as compared to adults (1-6).

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