0031-3998/84/1809-0886$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 9, 1984
Printed in U.S.A.

Vitamin D Metabolite Levels in Normal Children

ARLENE F. TAYLOR AND MICHAEL E. NORMAN

The Children's Hospital of Philadelphia and The Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Summary

Vitamin D metabolite levels were measured in 174 normal children throughout the year. 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) levels showed a seasonal variation; both levels were higher in summer than in winter ($p < 0.001$ for both). There was a fall in the 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) level in August and September ($p < 0.001$), which coincided with a rise in mean $25(OH)D_3$ and $24,25(OH)_2D_3$ levels. An inverse correlation was seen between $1,25(OH)_2D$ and $24,25(OH)_2D_3$ levels ($r = -0.233$; $p < 0.01$). $25(OH)D_3$ levels increased with age only for winter values (<3 years, 11.70 ± 3.98 ng/ml; 3–11 years, 18.38 ± 1.65 ng/ml; >11 years, 23.60 ± 4.60 ng/ml) while $1,25(OH)_2D$ and $24,25(OH)_2D_3$ levels did not show an age-related difference. Intake of multivitamins had an interesting effect on $25(OH)D_3$ and $24,25(OH)_2D_3$ levels in the winter but not in the summer; the endogenous metabolite levels were lower in the vitamin supplemented children [$25(OH)D_3$: 23.05 ± 7.35 *versus* 15.77 ± 5.51 ng/ml, $p < 0.001$; $24,25(OH)_2D_3$: 2.30 ± 1.11 *versus* 1.66 ± 0.88 ng/ml, $p < 0.05$]. Children studied in the winter who were not receiving supplemental vitamins were older than those who did receive the vitamins (7.26 ± 2.64 *versus* 5.42 ± 3.17 years; $p < 0.01$). Sixteen of the children had both winter and summer measurements. Their $25(OH)D_3$ and $24,25(OH)_2D_3$ levels showed the same seasonal variation as the overall group data, while their $1,25(OH)_2D$ levels showed no consistent pattern. Our data suggest that establishing normal pediatric values for $25(OH)D_3$, $1,25(OH)_2D$, and $24,25(OH)_2D_3$ requires consideration of season, age, and supplemental vitamin D intake, and that such information be available to investigators in order to make meaningful interpretations of vitamin D metabolite levels in disease states.

Abbreviations

$25(OH)D_3$, 25-hydroxyvitamin D_3
$1,25(OH)_2D$, 1,25-dihydroxyvitamin D
$24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3
HPLC, high pressure liquid chromatography

Major advances in our understanding of vitamin D metabolism over the past 15 years (7, 18) have led to increased interest in the study of vitamin D metabolite levels in a variety of disease states, with or without concomitant vitamin D therapy (3, 10, 11, 19, 20, 21, 23, 25, 28). As with any new laboratory assay, normal values first had to be established, with consideration given to the influence of age and sex on these values. With regard to the major vitamin D metabolites, $25(OH)D_3$, $1,25(OH)_2D$, and $24,25(OH)_2D_3$, additional factors are: 1) methodologic, relating to the several different assays currently employed to measure $25(OH)D$, $24,25(OH)_2D$, and $1,25(OH)_2D$; and 2) geographic, relating to the influence of the duration of sunlight exposure on circulating levels of $25(OH)D_3$ and $24,25(OH)_2D$.

We undertook the following study of vitamin D metabolite levels in a normal pediatric population for several reasons. First, there are no reports of normal values in children living in the densely populated northeastern United States; most of the data are from children living in the midwestern United States or Europe (2, 4, 16, 17, 22). Second, previous studies of normal vitamin D metabolite levels in children did not measure all three major metabolites in the same child but measured only one and in some cases two of these metabolites (2, 4–6, 14, 16, 17, 22, 24, 30). We felt it was important to measure these three metabolites in relation to each other, since their interrelationship may be important in various disease states. Finally, none of the previous studies have measured all three major vitamin D metabolites in the same group of children in both summer and winter. We were interested to learn if seasonal variation [*e.g.*, for $25(OH)D_3$ and possibly for $24,25(OH)_2D_3$] previously reported for group data (2, 5, 13, 14, 22, 29) was also present in individual patients.

MATERIALS AND METHODS

Patients. Heparinized blood samples were obtained from 174 healthy children between the ages of 1.5 and 19 years over a 1.5-year period, after informed consent was given by the parents. Specimens were drawn in the morning. These children were part of a varicella vaccine study. Sixteen of the children had specimens drawn during both the winter and summer seasons within a 1-year period. Plasma was removed and stored at −20°C until analyzed.

Vitamin D metabolites. [26,27-^{3}H]$25(OH)D_3$ was purchased from Amersham, Arlington Heights, IL (specific activity, 22 Ci/mmol). [26,27-^{3}H]$1\alpha,25(OH)_2D_3$ was purchased from either New England Nuclear, Boston, MA, or Amersham (specific activity, 160 or 158 Ci/mmol). [23,24-^{3}H]$24R,25(OH)_2D_3$ was purchased from Amersham (specific activity, 68 Ci/mmol).

Purified reference standards were gifts of the Upjohn Company, Kalamazoo, MI [$25(OH)D_3$] and Hoffmann-LaRoche, Nutley, NJ [$1,25(OH)_2D_3$ and $24,25(OH)_2D_3$]. The 25-hydroxyvitamin-D_3-26,23-lactone was a gift of Dr. R. Horst, National Animal Disease Center, Ames, IA.

Vitamin D metabolite assays. Plasma $25(OH)D_3$ and $1,25(OH)_2D$ levels were measured by the method of Shepard *et al.* (26). Plasma $24,25(OH)_2D_3$ was measured by the method of Dreyer and Goodman (8). The latter procedure utilizes HPLC and distinguishes between $24,25(OH)_2D_2$ and $24,25(OH)_2D_3$. The common extraction procedure used was that of Dreyer and Goodman (8). A normal plasma pool was run in triplicate with each assay to assess reproducibility. Assay sensitivities were 2 ng for $25(OH)D_3$, 5 pg/tube for $1,25(OH)_2D$, and 1 ng for $24,25(OH)_2D_3$.

Received September 1, 1983; accepted February 10, 1984.
Address correspondence reprint requests to Michael E. Norman, M.D., Department of Pediatrics, Wilmington Medical Center, P.O. Box 1668, Wilmington, DE 19899.
This work was supported by National Institutes of Health Grant AM19278.

Statistical analysis. Statistical analysis employed Student's *t* test, analysis of variance, least squares linear regression, and rank sum methods. Results were expressed as mean ± 1 SD.

RESULTS

The data presented for $25(OH)D_3$ and $24,25(OH)_2D_3$ were separated by season (winter: October–May; summer: July–September). No specimens were obtained in the month of June. This separation was based upon previous reports on seasonal variation and knowledge of maximum sunlight exposure in the Philadelphia area.

25(OH)D_3. The mean value was 17.45 ± 6.57 ng/ml for 128 children studied in the winter and 34.67 ± 10.28 ng/ml for 46 children studied in the summer. The difference between means was statistically significant ($p < 0.001$). Figure 1 shows the mean values for each month. Values were higher in the summer than in the winter, with a statistically significant difference of means ($p < 0.001$).

There were no significant differences between male and female values for either winter or summer. Values grouped by age and season are shown in Table 1. $25(OH)D_3$ levels increased significantly with age only for winter values ($p < 0.001$).

1,25(OH)$_2$D. The mean value was 40.57 ± 12.26 pg/ml (n = 128) for winter specimens and 37.97 ± 11.08 pg/ml (n = 46) for summer specimens; these were not significantly different. Mean values by months are presented in Figure 2. There was a significant fall in $1,25(OH)_2D$ in August and September ($p < 0.001$) which coincided with a rise in mean $25(OH)D_3$ and $24,25(OH)_2D_3$ levels. Furthermore, when all individual plasma determinations were analyzed, there was an inverse correlation between $1,25(OH)_2D$ and $24,25(OH)_2D_3$ ($r = -0.233$; $p < 0.01$) (Fig. 3). There was no difference in means for $1,25(OH)_2D$ when analyzed by age (Table 1) or by sex for each season.

24,25(OH)$_2$D$_3$. The mean value was 1.77 ± 0.92 ng/ml (n = 128) for winter specimens and 3.89 ± 1.48 ng/ml (n = 46) for summer specimens ($p < 0.001$). Presented in Figure 4 are the means by months, which revealed a significant rise in the summer ($p < 0.001$). A direct correlation existed between $25(OH)D_3$ and $24,25(OH)_2D_3$ ($r = 0.773$; $p < 0.001$), which has been reported by others (6, 14, 28, 29).

$24,25(OH)_2D_3$ levels did not vary significantly with age in either summer or winter (Table 1) or with sex. As shown in Figure 5, our method separated $24,25(OH)_2D_3$ from the 25-hydroxyvitamin D_3 26,23-lactone.

Vitamin intake. We reanalyzed our data by comparing vitamin D metabolite levels in children who were or were not receiving supplemental standard multivitamins (including vitamin D) in the winter and summer months (Table 2). Both $25(OH)D_3$ and $24,25(OH)_2D_3$ were higher in children not receiving vitamin supplements, but only in the winter ($p < 0.001$, $p < 0.05$, respectively). Children studied in the winter who were not receiving supplemental vitamins were older than those who did receive the vitamins. That the observed age-related difference in $25(OH)D_3$ levels for all winter specimens was in fact due to age and not to vitamin D intake was confirmed by analysis of only those winter specimens from children who took multivitamins regularly (<3 years, 10.48 ± 3.10 ng/ml; 3–11 years, 16.59 ± 5.12 ng/ml; >11 years, 24.14 ± 0.57 ng/ml; $p < 0.001$). No

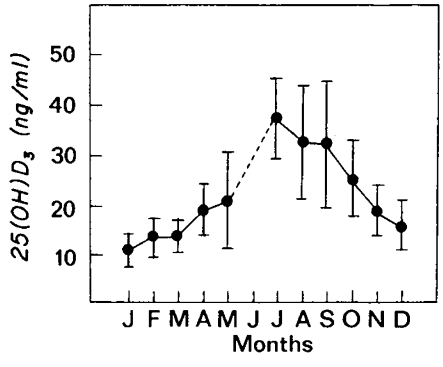

Fig. 1. Mean $25(OH)D_3$ levels by months. Results are ±1 SD. ---, no June specimens. The number of samples in each month were: January, 10; February, 10; March, 11; April, 24; May, 19; July, 20; August, 14; September, 12; October 7; November, 4; and December, 43.

Table 1. *Metabolite levels by age group and season**

Season and age group	n	$25(OH)D_3$ (ng/ml)	$1,25(OH)_2D$ (pg/ml)	$24,25(OH)_2D_3$ (ng/ml)
Winter				
1.5–2.5	18	11.70 ± 3.98	39.81 ± 10.59	1.67 ± 0.80
3.0–4.5	33	16.34 ± 5.14	41.48 ± 10.74	1.54 ± 0.59
5.0–6.5	31	17.76 ± 5.45	40.17 ± 12.93	1.80 ± 1.05
7.0–8.5	20	19.52 ± 6.81	40.44 ± 14.41	1.81 ± 1.04
9.0–11.0	19	19.90 ± 8.74	40.90 ± 14.65	1.94 ± 1.11
12.0–19.0	7	23.60 ± 4.60	39.56 ± 10.02	2.45 ± 0.79
		$p < 0.001$	NS	NS
Summer				
1.5–2.5	4	35.45 ± 3.61	39.60 ± 12.38	3.12 ± 0.66
3.0–4.5	18	32.75 ± 9.75	34.76 ± 11.12	3.71 ± 1.38
5.0–6.5	12	37.50 ± 13.99	41.69 ± 9.01	4.37 ± 2.14
7.0–8.5	7	37.04 ± 9.55	38.08 ± 11.06	4.21 ± 0.95
9.0–11.0	4	28.90 ± 3.87	38.30 ± 17.77	3.30 ± 0.58
12.0–19.0	1	38.39	42.70	4.55
		NS	NS	NS

* All values are expressed as mean ± 1 SD. NS, not significant.

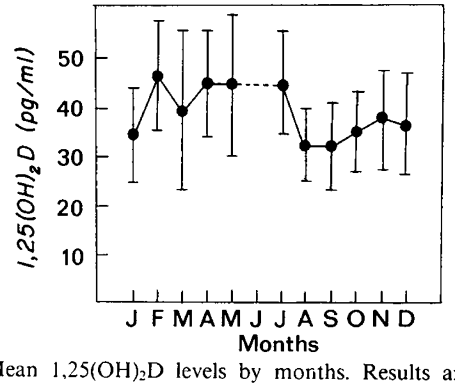

Fig. 2. Mean $1,25(OH)_2D$ levels by months. Results are ±1 SD. ---, no June specimens. The numbers of samples in each month were the same as in Figure 1.

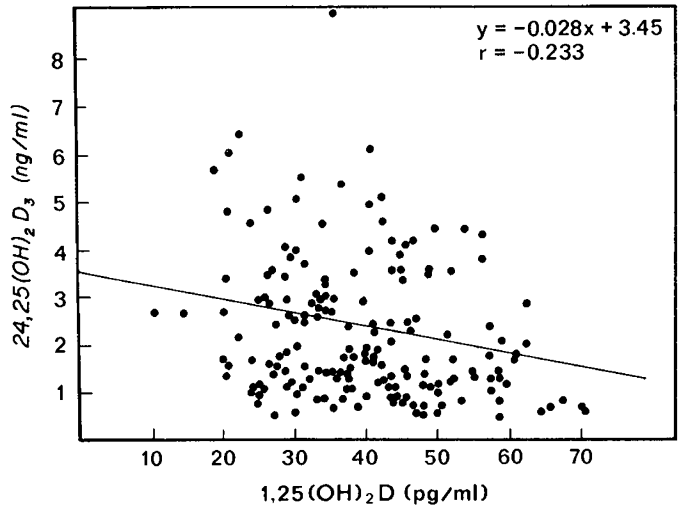

Fig. 3. Correlation between $1,25(OH)_2D$ and $24,25(OH)_2D_3$.

significant differences were noted for $1,25(OH)_2D$ in either season, either taking or not taking multivitamins.

Children studied in both seasons. Figure 6 shows the vitamin D metabolite values for the 16 children from whom we obtained both winter and summer specimens. In all cases, the summer values were higher than the winter values for both $25(OH)D_3$ and $24,25(OH)_2D_3$. When the 16 values were grouped by season, the means for both $25(OH)D_3$ and $24,25(OH)_2D_3$ were statistically significantly different [$p < 0.001$ for both $25(OH)D_3$ and $24,25(OH)_2D_3$]. Mean values were as follows: $25(OH)D_3$: winter, 15.87 ± 5.04 ng/ml; summer, 30.16 ± 7.66 ng/ml; $24,25(OH)_2D_3$: winter, 1.78 ± 0.58 ng/ml; summer, 3.94 ± 1.11 ng/ml. The values for $1,25(OH)_2D$ were not consistently higher or lower in relation to season and the mean difference was nonsignificant. The winter mean was 32.97 ± 9.50 pg/ml and the summer mean was 31.55 ± 7.81 pg/ml.

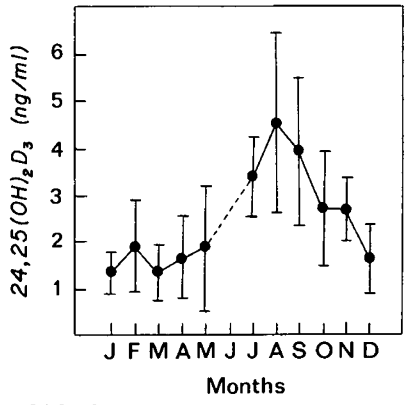

Fig. 4. Mean $24,25(OH)_2D_3$ levels by months. Results are ±1 SD. ---, no June specimens. The numbers of samples in each month were the same as in Figure 1.

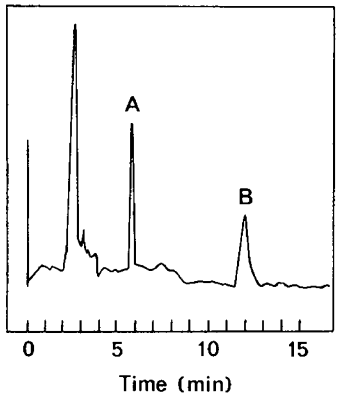

Fig. 5. HPLC chromatographic separation of $25(OH)D_3$ 26,23-lactone (*A*) and $24,25(OH)_2D_3$ (*B*) with a solvent system of 2.5% methanol in methylene chloride at a flow rate of 1.2 ml/min.

DISCUSSION

This study revealed a seasonal variation in mean levels of $24,25(OH)_2D_3$ in normal children. Some previous reports have shown this variation (13, 14, 29) while others have not (6, 17). For example, Weisman *et al.* (30) studied children in Florida where sunlight exposure is high throughout the year, and his data are comparable to our summer but not to our winter values. Chesney *et al.* (6) on the other hand did not show a seasonal variation, which might relate to one of two factors: 1) a different amount of sunlight exposure in the summer in Wisconsin *versus* Philadelphia; or 2) a different assay. Other assays do not separate $24,25(OH)_2D_2$ from $24,25(OH)_2D_3$ whereas our HPLC method does. This could be important in comparing patient populations to so-called "normal values" because it has been shown that increases in total 25(OH)D are primarily due to increases in the $25(OH)D_3$ moiety (2, 5) and it is probable that $24,25(OH)_2D$ and 25(OH)D behave similarly in this regard. In addition, our method separated $24,25(OH)_2D_3$ from the 25-hydroxyvitamin D_3-26,23-lactone. Therefore, our results reported $24,25(OH)_2D_3$ and not a combination of this metabolite plus the lactone, as has been commented upon in a previous report (6).

For $25(OH)D_3$, we showed a seasonal variation, confirming the findings of Arnaud *et al.* (2) for $25(OH)D_3$ and others (2, 5, 13, 14, 29) for 25(OH)D. Our levels of $25(OH)D_3$ were comparable to those reported by others (2, 5). This variation in $25(OH)D_3$ most likely results from the degree of sunlight exposure (22).

With regard to $1,25(OH)_2D$, our levels agreed with some previous reports (4, 24) and were higher than reported for normal adults (5). There was no overall seasonal variation (5, 29) but in contrast to the findings of Juttmann *et al.* (13), we noted a fall in $1,25(OH)_2D$ for August and September which coincided with the higher levels for $25(OH)D_3$ and $24,25(OH)_2D_3$. Tjellesen and Christiansen (29), while not commenting on this finding, showed a decrease in $1,25(OH)_2D$ in August in one figure from a recent publication. The explanation for this observation is uncertain. We did show an inverse correlation between $1,25(OH)_2D$ and $24,25(OH)_2D_3$, which has not been reported previously. These findings could reflect an effect of increased $25(OH)D_3$ on the reciprocal activities of the 1α-hydroxylase and 24*R*-hydroxylase, since it has been demonstrated in rats and chicks that exogenously administered vitamin D caused a decrease in 1α-hydroxylase activity and a reciprocal increase in 24*R*-hydroxylase activity (9, 27).

An additional new finding in this study was an age-related increase in $25(OH)D_3$ levels. This was in contrast to previous reports (5, 17). This difference was only apparent when the values were broken down by season, and then only for the winter. It may be that true age-related variations are obscured when there is increased sunlight exposure. It appeared that the other authors did not break their age data down by season. Because of the seasonal variation, it is necessary to do this, unless all specimens were collected in one season. Since older children may be less likely to take multivitamins regularly and the observed age-

Table 2. *Metabolite levels by vitamin intake and season**

Season and vitamin supplement	*n*	$25(OH)D_3$ (ng/ml)	$1,25(OH)_2D$ (pg/ml)	$24,25(OH)_2D_3$ (ng/ml)	Age (yr)
Winter					
No	22	23.05 ± 7.35	38.23 ± 9.82	2.30 ± 1.11	7.26 ± 2.64
Yes	77	15.77 ± 5.51	40.27 ± 11.57	1.66 ± 0.88	5.42 ± 3.17
		$p < 0.001$	NS	$p < 0.05$	$p < 0.01$
Summer					
No	17	36.49 ± 9.49†	39.71 ± 14.68	3.78 ± 1.25	6.26 ± 2.99
Yes	23	33.29 ± 11.46‡	35.70 ± 7.95	4.14 ± 1.69	5.00 ± 2.08
		NS	NS	NS	NS

* All values are expressed as mean ± 1 SD. NS, not significant.

† No vitamin supplements (summer) *versus* no vitamin supplements (winter): $p < 0.001$.

‡ Vitamin supplements (summer) *versus* vitamin supplements (winter): $p < 0.001$.

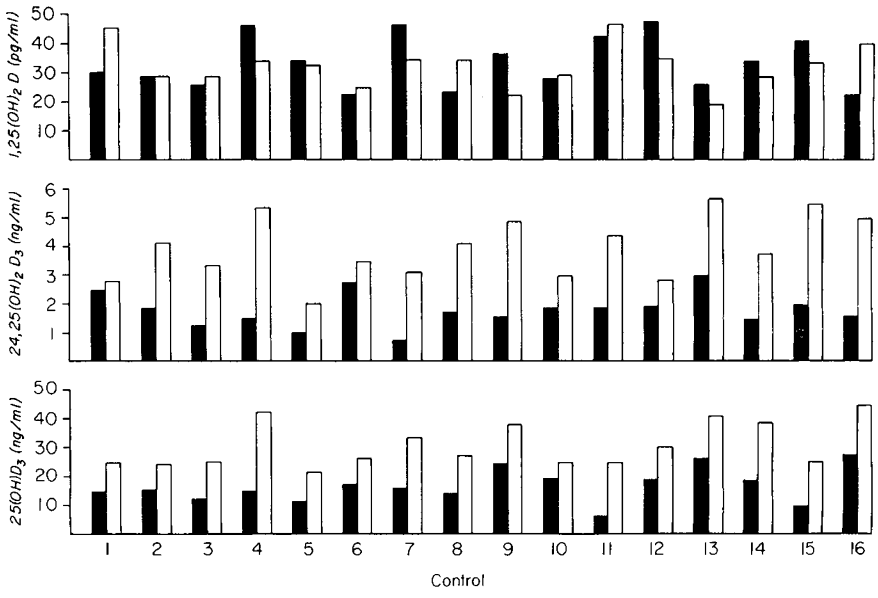

Fig. 6. Vitamin D metabolite levels for 16 children studied in both winter (■) and summer (□).

related difference could be a result of this, we looked at the winter specimens from only those children who took multivitamins regularly. We still found a significant age-related difference between the younger children and the older children, suggesting that age exerts a real influence on $25(OH)D_3$ levels. Children with metabolic bone disease studied in the winter should perhaps have their $25(OH)D_3$ levels compared to age-matched normal values, a caution not needed in the summer.

We, as others (6, 17), did not note a significant age-related difference for $24,25(OH)_2D_3$ in general, or when factored for season. We were also unable to show age-related differences for $1,25(OH)_2D$, as shown by others (4, 16). This may have been due to the small number of older children we had in our study, who in previous reports (4, 16) have been shown to have higher values of $1,25(OH)_2D$. Aksnes and Aarskog (1) also reported that $1,25(OH)_2D$ levels were directly related to pubertal stages. We did not know the pubertal stages of our adolescent subjects so we cannot comment upon the possible influence of this factor on our results.

Intake of multivitamins had an interesting effect on $25(OH)D_3$ and $24,25(OH)_2D_3$ levels in the winter but not in the summer; the endogenous metabolite levels were lower in the vitamin-supplemented children. Others have examined the effect of vitamin D intake on total 25(OH)D but not on $25(OH)D_3$ (15, 22). Poskitt *et al.* (22) showed no difference in 25(OH)D levels between those with and those without regular vitamin intake. Lund and Sørensen (15) were able to show a seasonal variation for 25(OH)D only in those without regular vitamin intake. We were able to show a seasonal variation for both those with and those without regular vitamin intake (Table 2). Since both D_2 and D_3 have the same effect on 25-hydroxylase activity (31), the possibility exists that in those children on regular vitamin D_2 intake there is a certain amount of competitiveness between D_2 and D_3 for the 25-hydroxylase system which could result in a lower $25(OH)D_3$ level. Another explanation for these results might be that the children not receiving multivitamins in the winter happened to be older than those who did receive vitamins. These metabolite levels might thereby reflect the effect of age and not supplemental vitamin intake. Caution should be exercised in evaluating the vitamin D status of patients, especially those receiving any form of vitamin D_2 supplementation. In these cases, measurement of total 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] and $24,25(OH)_2D$ [$24,25(OH)_2D_2$ and $24,25(OH)_2D_3$] could prove more beneficial. The mean values for $1,25(OH)_2D$ were no different for those with or without regular vitamin intake. $1,25(OH)_2D_2$ has a lower affinity than does $1,25(OH)_2D_3$ for the chick intestinal cytosol receptor so the $1,25(OH)_2D_2$ level is probably underestimated (12). Therefore, the true contribution of multivitamins to the $1,25(OH)_2D$ level is unknown.

An additional purpose of this study was aimed at determining whether or not seasonal variations in vitamin D metabolites would exist for individual children studied serially as well as for groups of children studied once. Our data revealed that this was in fact the case for $25(OH)D_3$ and $24,25(OH)_2D_3$. The mean values for the 16 children so studied were quite similar for $25(OH)D_3$ and $24,25(OH)_2D_3$ when compared to the overall group data. The mean values for $1,25(OH)_2D$ were slightly lower than the overall group data. These data provide evidence that the seasonal variation or lack of it is real and individual and not just a phenomenon of grouped data.

In summary, our data suggest that establishing normal pediatric values for the major vitamin D metabolites, $25(OH)D_3$, $1,25(OH)_2D$, and $24,25(OH)_2D_3$, requires consideration of three major modifying factors: season, age, and supplemental vitamin D intake. These factors individually or in combination exert different effects on each metabolite. Such information should be available in each investigator's laboratory in order to make meaningful interpretations of vitamin D metabolite levels in disease states.

Acknowledgements. The authors are grateful to Dr. Allan Arbeter and Patricia Paciorek who obtained the blood specimens used in this study, and to Bea McCray for secretarial assistance.

REFERENCES

1. Aksnes L, Aarskog D 1982 Plasma concentrations of vitamin D metabolites in puberty: effect of sexual maturation and implications for growth. J Clin Endocrinol Metab 55:94
2. Arnaud SB, Matthusen M, Gilkinson JB, Goldsmith RS 1977 Components of 25-hydroxyvitamin D in serum of young children in upper midwestern United States. Am J Clin Nutr 30:1082
3. Bell NH, Stern PH, Pantzer E, Sinha TK, DeLuca HF 1979 Evidence that increased circulating 1,25-dihydroxyvitamin D is the probable cause for abnormal calcium metabolism in sarcoidosis. J Clin Invest 64:218
4. Chesney RW, Rosen JF, Hamstra AJ, DeLuca HF 1980 Serum 1,25-dihydroxyvitamin D levels in normal children and in vitamin D disorders. Am J Dis Child 134:135
5. Chesney RW, Rosen JF, Hamstra AJ, Smith C, Mahaffey K, DeLuca HF 1981 Absence of seasonal variation in serum concentrations of 1,25-dihydroxyvitamin D despite a rise in 25-hydroxyvitamin D in summer. J Clin Endocrinol Metab 53:139
6. Chesney RW, Hamstra AJ, DeLuca HF 1982 Absence of seasonal fluctuation

in serum concentration of 24,25(OH)$_2$-vitamin D in childhood. Calcif Tissue Int 34:527
7. DeLuca HF, Paaren HE, Schnoes HK 1979 Vitamin D and calcium metabolism. Top Curr Chem Biochem 83:1
8. Dreyer BE, Goodman DBP 1981 A simple direct spectrophotometric assay for 24,25-dihydroxyvitamin D_3. Anal Biochem 114:37
9. Evans IMA, Colston KW, Galante L, MacIntyre I 1975 Feedback regulation of 25-hydroxycholecalciferol metabolism by vitamin D_3. Clin Sci Mol Med 48:227
10. Frazer TE, White NH, Hough S, Santiago JV, McGee BR, Bryce G, Mallon J, Avioli LV 1981 Alterations in circulating vitamin D metabolites in the young insulin-dependent diabetic. J Clin Endocrinol Metab 53:1154
11. Hahn TJ, Halstead LR, Haddad JG Jr 1977 Serum 25-hydroxyvitamin D concentrations in patients receiving chronic corticosteroid therapy. J Lab Clin Med 90:399
12. Jones G, Byrnes B, Palma F, Segev D, Mazur Y 1980 Displacement potency of vitamin D_2 analogs in competitive protein-binding assays for 25-hydroxyvitamin D_3, 24,25-dihydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3. J Clin Endocrinol Metab 50:773
13. Juttmann JR, Visser TJ, Buurman C, DeKam E, Birkenhäger JC 1981 Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects. Br Med J 282:1349
14. Kano K, Yoshida H, Yata J, Suda T 1980 Age and seasonal variations in the serum levels of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D in normal humans. Endocrinol Jpn 27:215
15. Lund B, Sørensen OH 1979 Measurement of 25-hydroxyvitamin D in serum and its relation to sunshine, age and vitamin D intake in the Danish population. Scand J Clin Lab Invest 39:23
16. Lund B, Clausen N, Lund B, Andersen E, Sørensen OH 1980 Age-dependent variations in serum 1,25-dihydroxyvitamin D in childhood. Acta Endocrinol 94:426
17. Nguyen TM, Guillozo H, Garabedian M, Mallet E, Balsan S 1979 Serum concentration of 24,25-dihydroxyvitamin D in normal children and in children with rickets. Pediatr Res 13:973
18. Norman AW, Roth J, Orci L 1982 The vitamin D endocrine system: steroid metabolism, hormone receptors, and biological response (calcium binding proteins). Endocr Rev 3:331
19. Norman ME 1982 Vitamin D in bone disease. Ped Clin N Am 29:947
20. O'Regan S, Chesney RW, Hamstra A, Eisman JA, O'Gorman AM, DeLuca HF 1979 Reduced serum 1,25(OH)$_2$ vitamin D_3 levels in prednisone-treated adolescents with systemic lupus erythematosus. Acta Pediatr Scand 68:109
21. Pitt MJ, Haussler MR 1977 Vitamin D: biochemistry and clinical applications. Skeletal Radiol 1:191
22. Poskitt ME, Cole TJ, Lawson DEM 1979 Diet, sunlight, and 25-hydroxyvitamin D in healthy children and adults. Br Med J 1:221
23. Rosen JF, Chesney RW, Hamstra A, DeLuca HF, Mahaffey KR 1980 Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. N Engl J Med 302:1128
24. Scriver CR, Reade TM, DeLuca HF, Hamstra AJ 1978 Serum 1,25-dihydroxyvitamin D levels in normal subjects and in patients with hereditary rickets or bone disease. N Engl J Med 299:976
25. Shen FH, Baylink DJ, Nielsen RL, Sherrard DJ, Ivey JL, Haussler MR 1977 Increased serum 1,25-dihydroxyvitamin D in idiopathic hypercalciuria. J Lab Clin Med 90:955
26. Shepard RM, Horst RL, Hamstra AJ, DeLuca HF 1979 Determination of vitamin D and its metabolites in plasma from normal and anephric man. Biochem J 182:55
27. Tanaka Y, DeLuca HF 1981 Measurement of mammalian 25-hydroxyvitamin D_3 24R- and 1α-hydroxylase. Proc Natl Acad Sci USA 78:196
28. Teitelbaum SL, Halverson JD, Bates M, Wise L, Haddad JG 1977 Abnormalities of circulating 25-OH vitamin D after jejunal-ileal bypass for obesity. Evidence of an adaptive response. Ann Intern Med 86:289
29. Tjellesen L, Christiansen C 1982 Seasonal variation of vitamin D metabolites in normal adults. A longitudinal study. In: Norman AW, Schaefer K, Herrath DV, Grigoleit H-G (eds): Vitamin D, Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism. Proceedings of the Fifth Workshop in Vitamin D. Walter de Gruyter, Berlin, p 49
30. Weisman Y, Reiter E, Root A 1977 Measurement of 24,25-dihydroxyvitamin D in sera of neonates and children. J Pediatr 91:904
31. Whyte MP, Haddad JG Jr, Walters DD, Stamp TCB 1979 Vitamin D bioavailability: serum 25-hydroxyvitamin D levels in man after oral, subcutaneous, intramuscular, and intravenous vitamin D administration. J Clin Endocrinol Metab 48:906

0031-3998/84/1809-0890$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 9, 1984
Printed in U.S.A.

Calcium-ATPase Activity in Cystic Fibrosis Erythrocyte Membranes: Decreased Activity in Patients with Pancreatic Insufficiency

DORR G. DEARBORN, ROBERT J. WITYK, LYNELLE R. JOHNSON, LOUIS PONCZ, AND ROBERT C. STERN

Cystic Fibrosis Center, Departments of Pediatrics and Biochemistry, Case Western Reserve University, Rainbow Babies and Childrens Hospital, Cleveland, Ohio 44106

Summary

The activity of Ca-ATPase (Ca^{2+},Mg^{2+}-ATPase, ATP phosphohydrolase, EC 3.6.1.3) was measured in erythrocyte membrane preparations from 37 cystic fibrosis patients, 27 with pancreatic insufficiency and 10 with pancreatic sufficiency, and from 24 healthy controls. The mean maximal calcium-stimulated specific activities, in the absence and presence of purified calmodulin, of the pancreatic sufficient patients (34.3 ± 4.2 and 75.9 ± 6.9 nmol/min/mg) was indistinguishable from that of controls (35.8 ± 2.6 and 84.3 ± 4.7 nmol/min/mg), while both activities of patients with pancreatic insufficiency were significantly decreased (28.9 ± 1.3, $p < 0.02$; 65.2 ± 3.0, $p < 0.001$) compared to the control group. Similarly, the mean erythrocyte membrane (Na + K)ATPase activity was decreased only for those patients with a history of steatorrhea and who clinically required pancreatic enzyme therapy and had low immunoreactive trypsin levels (10.6 ± 0.8 *versus* control, 13.4 ± 1.1, and pancreatic sufficient patients, 13.3 ± 1.4 nmol/min/mg; $p < 0.025$). No correlation was found between any of the ATPase activities and the clinical scores of the patients, suggesting the lack of significant contribution of general clinical status to the activities of those cation transporters.

Received August 18, 1983; accepted March 12, 1984.
Requests for reprints should be addressed to: Dorr G. Dearborn, Department of Pediatrics, Case Western Reserve University, 2101 Adelbert Road, Cleveland, OH 44106.
This research was supported by Grants AM-08305 and P30-27651 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. R. J. W. was a recipient of a student fellowship of the Cystic Fibrosis Foundation. L. P. was a postdoctoral fellow on Training Grant HL-07415.