Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects

J R JUTTMANN, T J VISSER, C BUURMAN, E DE KAM, J C BIRKENHÄGER

Abstract

Serum concentrations of 25-hydroxycholecalciferol (25-OHD), 24,25-dihydroxycholecalciferol (24,25-$(OH)_2$D), and 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$D) were measured at monthly intervals throughout the year in eight normal subjects. 25-OHD was measured by competitive protein-binding assay after Sephadex LH 20 chromatography, 24,25-$(OH)_2$D by competitive protein-binding assay after Sephadex LH 20 and high-pressure chromatography, and 1,25-$(OH)_2$D by radioimmunoassay after the same separation procedure as for 24,25-$(OH)_2$D. A seasonal variation, apparently dependent on exposure to ultraviolet light, was found for all three metabolites. A study in six other normal subjects showed that there was no diurnal rhythm in any of the metabolites. Oral administration of 2 μg 1,25-$(OH)_2$D caused a sharp rise in serum concentrations of 1,25-$(OH)_2$D and no change in the concentrations of the two other metabolites, but by 12 hours the 1,25-$(OH)_2$D concentration had returned to the basal value.

The concentrations of all three metabolites studied vary according to the season. Thus to interpret these concentrations in any subject the normal range for the particular season must be referred to.

Introduction

Variations in serum concentrations of 25-hydroxycholecalciferol (25-OHD) and 24,25-dihydroxycholecalciferol (24,25-$(OH)_2$D) dependent on exposure to ultraviolet light have been reported in normal subjects by several workers.[1-3] These findings are more consistent for 25-OHD than for 24,25-$(OH)_2$D,[4] although generally a relation between the serum concentrations of these two metabolites is found.[5] Seasonal variation in the serum concentration of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$D) has not been reported, but we do not know of any studies in which this concentration has been measured in the same normal subjects throughout the year.

Injecting massive quantities of precursors of 1,25-$(OH)_2$D (cholecalciferol or 25-OHD) does not seem to influence the serum concentration of 1,25-$(OH)_2$D in normal people, although such an effect of cholecalciferol has been described in cows.[6] Rosen *et al*[7] reported no difference in mean serum concentrations of 1,25-$(OH)_2$D in 22 children during the winter compared with those in 23 other children during the summer. Some of these children had lead intoxication and some were healthy. Summer and winter concentrations in the same children were not available.

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In our study we aimed at determining the seasonal fluctuation in serum concentrations of 25-OHD, 24-25-$(OH)_2$D, and 1,25-$(OH)_2$D, or the absence of it, in a group of normal subjects, thereby also obtaining normal values for all seasons of the year. We also studied the diurnal rhythm in the concentrations of these vitamin D metabolites.

Subjects and methods

Blood samples were taken monthly over 13 months from eight normal subjects, all healthy members of the hospital staff (five women, three men; mean age 31±3 years). In six other normal subjects, also members of the hospital staff (three women, three men; mean age 29±4 years) blood samples were taken at 0900, 1400, 1700, and 2200 on days 1 and 2 and at 0900 on day 3. At 0900 on day 2, after the blood sample had been taken, 2 μg 1,25-$(OH)_2$D was given by mouth.

The assays of vitamin D metabolites were carried out as described previously[8]—that is, 25-OHD by means of a competitive protein-binding assay after Sephadex LH 20 chromotography using rat serum as a binding protein; 24,25-$(OH)_2$D after Sephadex LH 20 and high-pressure liquid chromatography, also using rat serum as a binding protein; and 1,25-$(OH)_2$D after the same chromatography procedures as for 24,25-$(OH)_2$D with a radioimmunoassay. The antiserum for the assay was kindly donated by Dr R Bouillon, Leuven, Belgium. The assays do not discriminate between vitamin D_3 and D_2 metabolites.

Results

Figure 1 shows the serum concentrations of 25-OHD, 24,25-$(OH)_2$D, and 1,25-$(OH)_2$D in eight normal subjects throughout the year. In all eight subjects the three concentration curves have similar shapes. One subject (case 1) visited Canada in July. In the North American countries some foodstuffs are fortified with vitamin D_2 (ergocalciferol), which may explain the sudden rise in the 25-OHD concentration. Another subject (case 5) visited Australia in January of the second year, which explains the rise in the concentrations of all three vitamin D metabolites in this subject in the middle of winter. Figures 2-4 show the monthly mean concentrations of, respectively, 25-OHD, 24,25-$(OH)_2$D, and 1,25-$(OH)_2$D in the same eight subjects. Serum concentrations of all three metabolites in case 5 in February and of 25-OHD in case 1 in July were not included for the reasons mentioned above.

Table I shows the mean concentrations of all three metabolites of vitamin D corresponding to the four seasons together with the results of statistical analysis (Student's *t* test).

Because of the unexpected finding of a yearly rhythm in the concentrations of 1,25-$(OH)_2$D we carried out some extra studies to

TABLE I—*Mean (± 1 SD) concentrations of vitamin D metabolites in serum of normal subjects through seasons*

	25-OHD (nmol/l)	24,25-$(OH)_2$D (nmol/l)	1,25-$(OH)_2$D (pmol/l)
Winter (Jan-March)	37±12	3·6±1·3	131±39
Spring (April-June)	54±14*	4·5±1·3*	162±40†
Summer (July-Sept)	78±21*	7·0±1·6†	190±51†
Autumn (Oct-Dec)	50±15*	4·5±1·5*	160±42*

Significance of difference from winter value: *$p<0.05$; †$p<0.01$.
Conversion: SI to traditional units—25-OHD, 24,25-$(OH)_2$D: 1 nmol/l ≈ 0·4 ng/ml. 1,25-$(OH)_2$D: 1 pmol/l ≈ 0·4 pg/ml.

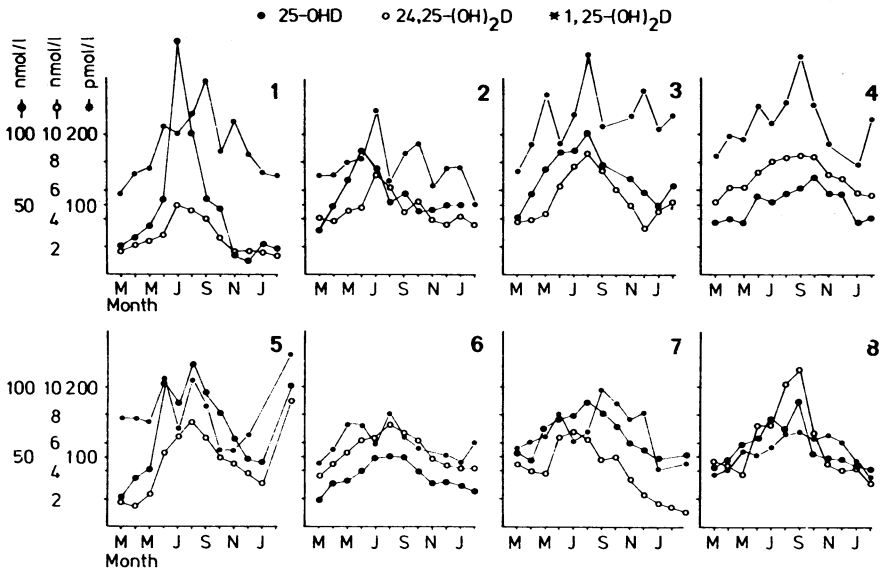

FIG 1—Individual curves of serum concentrations of 25-OHD, 24,25-$(OH)_2D$, and 1,25-$(OH)_2D$ during year in eight normal subjects.
Conversion: SI to traditional units—25-OHD, 24,25-$(OH)_2D$: 1 nmol/l≈0·4 ng/ml. 1,25-$(OH)_2D$: 1 pmol/l≈0·4 pg/ml.

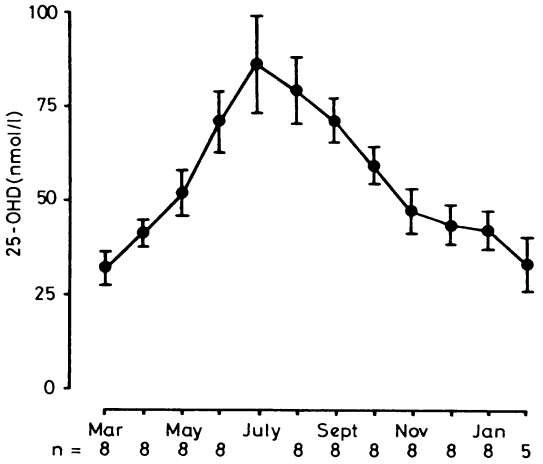

FIG 2—Mean±SEM serum 25-OHD concentrations in eight normal subjects throughout year.
Conversion: SI to traditional units—25-OHD: 1 nmol/l≈0·4 ng/ml.

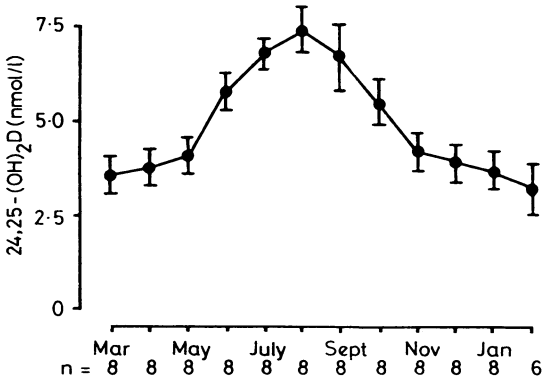

FIG 3—Mean±SEM serum 24,25-$(OH)_2D$ concentrations in eight normal subjects throughout year.
Conversion: SI to traditional units—24,25-$(OH)_2D$: 1 nmol/l≈0·4 ng/ml.

investigate the possible influence of increased serum concentrations of 25-OHD and 24,25-$(OH)_2D$ on the determination of 1,25-$(OH)_2D$ in the same serum by radioimmunoassay. Table II shows the effects on the determinations of the three metabolites of adding vitamin D derivatives to a standard serum. From these results we may conclude that adding "cold" 25-OHD or 24,25-$(OH)_2D$ to a standard serum has no influence on the determination of 1,25-$(OH)_2D$ in that serum, and by extrapolation we may conclude that a normal (ultraviolet-dependent) increase in 25-OHD or 24,25-$(OH)_2D$, or both, had no effect on the concentration of 1,25-$(OH)_2D$ in our eight subjects during the summer.

Figure 5 shows the lack of diurnal variation in the concentrations of the three metabolites and the effect on them of administering 2 μg 1,25-$(OH)_2D$ by mouth.

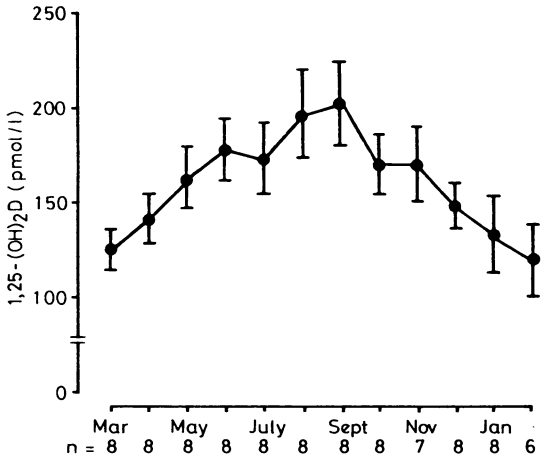

FIG 4—Mean±SEM serum 1,25-$(OH)_2D$ concentrations in eight normal subjects throughout year.
Conversion: SI to traditional units—1,25-$(OH)_2D$: 1 pmol/l≈0·4 pg/ml.

Discussion

Seasonal variation in concentrations of 25-OHD and 24,25-$(OH)_2D$ in normal subjects and patients with disease has been reported. Until now exposure to ultraviolet light has not been observed to influence the formation of 1,25-$(OH)_2D$ in man.

TABLE II—*Effect of adding different vitamin D derivatives on determination of concentrations of those derivatives in standard serum*

Amount of vitamin D derivative added/ml	25-OHD (nmol/l)		24,25-$(OH)_2$D (nmol/l)		1,25-$(OH)_2$D (pmol/l)	
	Concentration	Difference from control	Concentration	Difference from control	Concentration	Difference from control
None (control value)	66·3±3·3		3·5±0·5		98·3±11·5	
50 ng 25-OHD	177·5±9·0	111·3	4·0±0·3		94·5± 8·4	
2·5 ng 24,25-$(OH)_2$D*	61·5±7·0		8·0±0·5	4·5	89·5± 5·0	
50 pg 1,25-$(OH)_2$D	64·5±4·8		3·5±0·8		205·5±28·8	107·3

*Non-radioactive ("cold") 24,25-$(OH)_2$D had to be purified by high-pressure liquid chromatography because it appeared to contain a certain amount of 1,25-$(OH)_2$D. The amount of 24,25-$(OH)_2$D indicated as having been added is the amount before purification; the amount of 24,25-$(OH)_2$D finally added was less than 2·5 ng/ml.
Conversion: SI to traditional units—25-OHD, 24,25-$(OH)_2$D: 1 nmol/l ≈ 0·4 ng/ml. 1,25-$(OH)_2$D: 1 pmol/l ≈ 0·4 pg/ml.

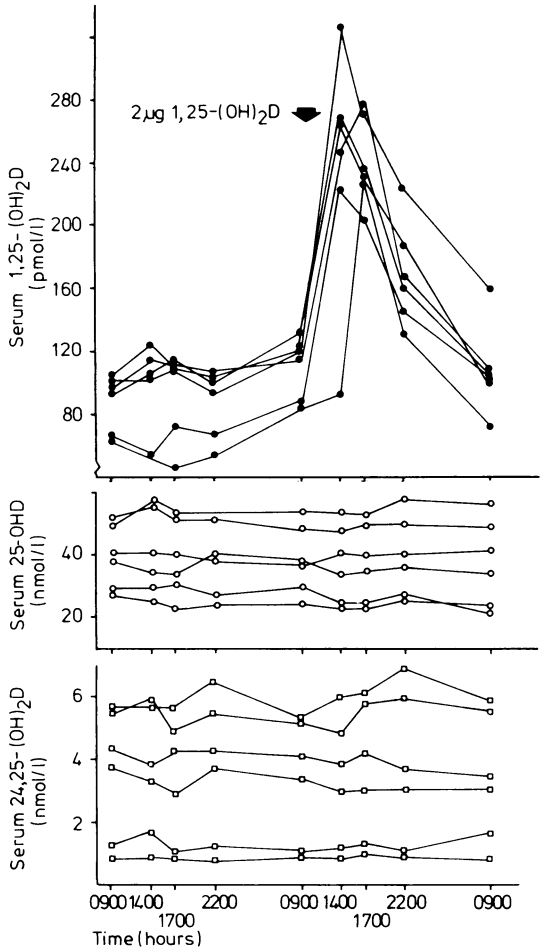

FIG 5—Diurnal course of serum concentrations of 25-OHD, 24,25-$(OH)_2$D, and 1,25-$(OH)_2$D in six normal subjects, and course in same subjects after oral dose of 1,25-$(OH)_2$D. (For conversion factors see legend to figure 1.)

Serum concentrations of 1,25-$(OH)_2$D during the summer and winter have been reported, but in general they have been measured in different subjects.[7 9] Although the highest values of 1,25-$(OH)_2$D in cases 1, 3, and 4 (fig 1) were well over 200 pmol/l (80 pg/ml), the fluctuations in the other subjects were generally within the normal range of 50-180 pmol/l (20-72 pg/ml) that has been reported.[10] The normal range of 1,25-$(OH)_2$D concentrations is wide,[11 12] leaving room for individual fluctuations over the year. The fluctuations in serum 1,25-$(OH)_2$D concentrations through the year reported here agree with the findings of Malm,[13] who found the same seasonal dependence for intestinal calcium absorption as measured by studies of calcium balance.

An explanation for the season-dependent behaviour of serum 1,25-$(OH)_2$D concentrations is hard to find. We excluded the possibility of interference of 25-OHD and 24,25-$(OH)_2$D concentrations with the assay of 1,25-$(OH)_2$D. Apart from placental tissue,[14] no extrarenal site for the 1α-hydroxylation of 25-OHD has been found. Thus the increase in serum 1,25-$(OH)_2$D concentrations in our subjects is unlikely to have been caused by an increase in 1α-hydroxylase activity other than in the kidney. Our own experience with anephric patients and patients with severe chronic renal failure (creatinine clearance <20 ml/min) is that serum 1,25-$(OH)_2$D concentrations do not increase during the summer (unpublished results). Although the mechanism by which serum concentrations of 1,25-$(OH)_2$D in normal subjects increase during exposure to ultraviolet light remains obscure, the increase in this metabolite's precursor, 25-OHD, seems to be a possible cause; an increase in the concentration of 25-OHD is caused by an increase in the concentration of its precursor, cholecalciferol. In general, however, serum concentrations of 1,25-$(OH)_2$D and 25-OHD have not been found to be related like those of 24,25-$(OH)_2$D and 25-OHD. In this respect the finding of normal serum concentrations of 1,25-$(OH)_2$D (with very low to undetectable concentrations of 25-OHD and 24,25-$(OH)_2$D) in nutritional osteomalacia must be mentioned.[15] Certainly the season-dependent increase in the concentration of 24,25-$(OH)_2$D is considered to depend on the increased availability of the precursor 25-OHD.

Giving 1,25-$(OH)_2$D by mouth had no influence on concentrations of 25-OHD and 24,25-$(OH)_2$D in the same sera (fig 5). With regard to 24,25-$(OH)_2$D this finding appears to contradict the results of Tanaka and Deluca[16] and Taylor *et al*,[17] who obtained in-vitro and in-vivo evidence, respectively, that 1,25-$(OH)_2$D may stimulate formation of 24,25-$(OH)_2$D. In our subjects the increase in the 1,25-$(OH)_2$D concentration may have lasted for too short a time for any measurable reaction of the 24,25-$(OH)_2$D concentration to occur. From the course of the 1,25-$(OH)_2$D concentration after one oral dose of this metabolite we may conclude that the plasma half life is probably less than half a day (fig 5). Finally we conclude that to interpret serum concentrations of 25-OHD, 24-25-$(OH)_2$D, and 1,25-$(OH)_2$D normal ranges for the different seasons have to be used (table I).

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ONE HUNDRED YEARS AGO SIR,—The recent discussion in the House of Commons on the opium traffic makes it desirable that we should endeavour to arrive at some more definite opinion as to the effects on the system of the habitual use of opium. Is opium-eating or opium-smoking necessarily and universally pernicious? It has been too much the custom of writers on this subject to content themselves with drawing a doleful picture of a confirmed opium-debauchee, plunged in the lowest depths of moral and physical exhaustion; and, having formed the premisses of their argument from this exception, to proceed at once to involve the whole practice in the sweeping condemnation. We are told that the habitual opium-eater can be "recognised at a glance"; that there is a "characteristic attenuation," a "withered countenance," a "halting gait," and a glassy deep-sunk eye." His digestive organs are "in the highest degree disturbed"; he eats nothing, has hardly an evacuation a week, and is a "perfect wreck" both mentally and bodily. By degrees, as the habit becomes more confirmed, his strength fails, so that at last he can hardly crawl from place to place. After a long indulgence, he suffers from "nervous or neuralgic pains," from which even his beloved opium affords no relief. His agony when the drug fails to produce its accustomed effect is terrible, and he suffers "the torments of the damned." Such are the accounts given by most of the older writers on the subject. These symptoms may possibly have been observed in exceptional cases, but they are not met with when the drug is taken in anything like moderation. Much of our knowledge of the effects of opium on the system is derived from the confessions of De Quincey, the English opium-eater; and he tells us that, from 1804 to 1812, a period of eight years, during which he was a dilettante eater of opium, he enjoyed perfect health, and was never better in his life. It was only later, when he took the drug in enormous quantities, that his health suffered.

Marsden, in his well-known work on Sumatra, says that, among the Malays, the practice of opium-smoking is all but universal. He thinks that possibly the custom may be injurious, but that the effects attributed to it have been greatly exaggerated. The *bugis* soldiers who are most attached to it, and who use it in excess, are commonly emaciated; but they are abandoned to a degree, and addicted to debauchery of all kinds. The gold traders, on the other hand, who indulge freely in opium, but are an active, steady, and laborious class, are amongst the most healthy and vigorous in the island, and rarely suffer in any way. It is well known that the Chinese are a nation of opium-eaters, and yet they are a muscular and well-formed race, the labouring classes being capable of great and prolonged exertion under a fierce sun and in an unhealthy climate. Their disposition is cheerful and peaceable, and, in general intelligence, they rank deservedly high among Orientals. Many people have attained the age of sixty or seventy who have been habitual opium-eaters for thirty years or more. Surgeon Smith, a most determined opponent of opium as a luxury, says that such cases have come under his own observation, and adds that it does not appear that the Chinese in easy circumstances, who can obtain the ordinary comforts of life, are materially affected as regards longevity by this practice. Again, Dr Eatwell, who for three years was resident in China, states, as the result of his experience, that the effects of the abuse of the drug do not often come under observation; and that, when such cases do occur, the habit is frequently found to have been induced by some painful chronic disorder, to escape from the sufferings of which the patient has fled to this resource. He believes that, as regards the effects of the habitual use of the drug on the mass of the people, there is no evidence whatever.

It is often supposed that the excitement produced by opium is similar to that resulting from indulgence in alcohol; but such is not the case. De Quincey is most explicit on this point, and says: "Opium, I affirm peremptorily, is incapable of producing any state of body at all resembling that which is produced by alcohol, and not in *degree* only incapable, but even in *kind*; it is not in the quantity of its effects merely, but in the quality, that it differs altogether. The pleasure given by wine is always mounting and tending to a crisis, after which it declines; that from opium, when once generated, is stationary for eight or ten hours: the first, to borrow a technical distinction from medicine, is a case of acute, the second of chronic pleasure; the one is a flame, the other a steady and equable glow. But the main distinction lies in this: that whereas wine disorders the mental faculties, opium, on the contrary (if taken in a proper manner), introduces among them exquisite order, legislation, and harmony. Wine robs a man of his self-possession; opium greatly invigorates it. Wine unsettles and clouds the judgment, and gives a preternatural brightness and a vivid exaltation to the contempts and admirations, to the loves and hatreds, of the drinker; opium, on the contrary, communicates serenity and equipose to all the faculties active and passive, and, with respect to the temper and moral feelings in general, it gives simply that sort of vital warmth which is approved by the judgment, and which would probably always accompany a bodily constitution of primeval or antediluvian health." If we contrast the furious madman, the subject of delirium tremens, with the prostrate debauchee, the victim of opium; or the violent drunkard with the dreamy sensualist, intoxicated with his favourite drug, it must be admitted that the comparison is not in favour of the former. The opium-eater is at least harmless to everyone except himself; whilst the drunkard is not only a dangerous nuisance, but an enemy to the community at large.

Lord Hartington, in his recent speech, stated, on the authority of Sir Rutherford Alcock, that opium in moderation was probably a tonic and prophylactic against fever, especially in the marshy districts where it is most used; but of this there is very little evidence, if we except a pamphlet or opuscule on the subject, published by Schärtlich about a century ago. Attempts have of late years been made to introduce opium-smoking as a remedy for neuralgia, asthma, and phthisis, and as a palliative in cancer, but with comparatively little success.

The whole subject is replete with interest; and it is desirable to elicit further information, in order that we may be afforded an opportunity of arriving at a definite opinion on this vexed question.—I am, sir, your obedient servant, WILLIAM MURRELL, MD. (*British Medical Journal*, 1881.)

ONE HUNDRED YEARS AGO Sir,—An intimate friend of mine, who is, I think, inclined to take more alcohol than he is entitled to, alleges, as a reason, the amount of midwifery he attends. He is of opinion that the quantity of beer, etc, consumed, should be regulated by the amount of work performed: the more midwifery the more beer, etc. My friend is, moreover, of opinion that beer, as it contains comparatively little alcohol, may be consumed in considerable quantity, even by middle-aged persons, without doing harm. I should be glad to know your opinion on the above points, and whether the more hard-worked general practitioners among your readers find it beneficial to take stimulants, except as an accompaniment to a substantial meal, or before retiring to rest.—Yours truly, CERES. (*British Medical Journal*, 1881.)