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VITAMIN-D SYNTHESIS AND METABOLISM AFTER ULTRAVIOLET IRRADIATION OF NORMAL AND VITAMIN-D-DEFICIENT SUBJECTS

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THE beneficial effects of sunlight in the treatment and prevention of rickets and osteomalacia have been recognized for over 60 years.[1] However, little is known about the sequential changes in the serum concentrations of vitamin D and its metabolites after a single exposure to sunlight. In an attempt to understand these events, we monitored changes in serum concentrations of vitamin D and its metabolites, 25-hydroxyvitamin D (25-OH-D) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D), over a three-week period in normal subjects and vitamin-D-deficient patients, before and after whole-body exposure to various doses of ultraviolet radiation (UVR). We found that exposure to increasing doses of UVR resulted in a marked, transient increase in the serum vitamin-D concentration within 24 to 48 hours and

that the increase depended on the dose of UVR delivered. We also noted that vitamin-D-deficient patients had a threefold to fourfold increase in serum 1,25-$(OH)_2$-D concentrations in response to a dose of UVR that had no effect on 1,25-$(OH)_2$-D concentrations in normal subjects.

METHODS

Subjects

All UVR was delivered in a walk-in cylindrical irradiation chamber equipped with 16 vertically arranged fluorescent sunlamps (Westinghouse FS40). The irradiance of wavelengths in the 280-to-315-nm band at a distance of 10 cm was 800 μW per square centimeter. The spectral power distribution of these lamps ranged from 280 to 450 nm, with a broad power peak at 313 nm. After informed consent was obtained, eight healthy, white, paid volunteers (mean body-surface area, 1.8 m^2) received whole-body exposure equal to one to four times the minimal erythemal dose of UVR,[2] and three vitamin-D-deficient white women (mean body-surface area, 1.7 m^2) received one minimal erythemal dose. The vitamin-D-deficient patients had longstanding intestinal malabsorption secondary to inflammatory bowel disea e or small-bowel resection and secondary hyperparathyroidism; bone biopsy in two of the three patients showed osteomalacia. The studies were performed in spring or autumn, and the subjects were instructed to avoid prolonged exposure to sunlight for six weeks before and during the course of the study. No subject was receiving exogenous vitamin D, estrogen, or other drugs known to alter calcium homeostasis. All subjects had normal renal and hepatic function.

Assay Procedures

Serial blood samples for determination of serum vitamin D and its metabolites were obtained over a 21-day period after the subjects were exposed to a qua titative dose of whole-body UVR. Lipid extraction of serum samples (3 to 4 ml) was performed in methanol:methylene chloride, and extracts were then subjected to preparative chromatography on silica Sep-Pak cartridges (Waters Associates, Milford, Mass.) for initial separation and purification of vitamin D, 25-OH-D, and 1,25-$(OH)_2$-D fractions.[3] The fraction from Sep-Pak chromatography containing vitamin D was then subjected to a modified two-stage high-performance liquid-chromatography purification procedure.[4] Initial purification was performed on reversed-phase liquid chromatography over a Radial-Pak A column (Waters Associates) in 2 per cent water in methanol at a constant flow rate of 2.0 ml per minute. Final purification was achieved on straight-phase liquid chromatography on a Zorbax-SIL column (25 cm by 6.2 mm, Dupont Instruments, Wilmington, Del.) in 3 per cent isopropanol in n-hexane at a flow rate of 1.5 ml per minute. Quantitation of vitamin D was made by sample ultraviolet-light absorbance at 254 nm in a Waters Model 440 ultraviolet-light detector. The assay's lower detectability limit by this method was 0.5 ng per milliliter. To ensure that increases in the serum vitamin-D concentration after UVR result from an increase in vitamin D_3, and not in vitamin D_2, serum samples containing the highest concentration of vitamin D were studied again on reversed-phase liquid chromatography; they were shown to contain only vitamin D_3. The serum 25-OH-D concentration was determined by a competitive protein-binding assay.[5] The liquid chromatography purification of the Sep-Pak fraction containing 1,25-$(OH)_2$-D was performed on a Radial-Pak B column (Waters Associates) by elution in 10 per cent isopropanol in n-hexane at a flow rate of 2.0 ml per minute, before the competitive protein-binding assay, as described by Eisman et al.,[6] was performed. Tracer quantities of [1,2-^{3}H]vitamin D_3 (1000 cpm; 7.4 Ci per millimole), [26,27-^{3}H]25-OH-D_3 (500 cpm; 22.3 Ci per millimole), and [23,24-^{3}H]1,25-$(OH)_2$-D_3 (2000 cpm; 82 Ci per millimole) (all from Amersham Corporation, Arlington Heights, Ill.) were added to all serum samples, and tracer recovery was determined after extraction and chromatography. Sample recovery for vitamin D, 25-OH-D, and 1,25-$(OH)_2$-D ranged from 40 to 50 per cent, 70

to 80 per cent, and 50 to 60 per cent, respectively. To minimize interassay variation (12.1 per cent), all samples from each subject were processed in a single assay. In tests conducted throughout the year, serum concentrations of vitamin D ranged from 0.5 to 18 ng per milliliter in 30 normal subjects, 25-OH-D ranged from 10 to 65 ng per milliliter in 32, and 1,25-(OH)$_2$-D ranged from 27 to 61 pg per milliliter in 36. In all subjects, immunoreactive parathyroid hormone concentrations (normal range, <60 μleq per milliliter) were determined by radioimmunoassay[7] before and seven days after exposure to UVR. Serum calcium levels were measured by atomic-absorption photometry before and seven and 14 days after exposure to UVR. Mean data are expressed as means ±S.E.

RESULTS

The changes in serum concentrations of vitamin D, 25-OH-D, and 1,25-(OH)$_2$-D in a representative normal subject exposed to one minimal erythemal dose of UVR and in three normal subjects exposed to three minimal erythemal doses are indicated by the solid lines in Figure 1. In normal subjects there was a dose-related, rapid rise in vitamin D, with peak concentrations one to two days after exposure and a return almost to basal concentrations by seven days. For example, in representative normal subjects exposed in other experiments to one, 1.5, three, and four minimal erythemal doses of UVR (Fig. 2), peak vitamin-D levels were 24, 43, 80, and 138 ng per milliliter, respectively, one to two days after exposure. Thereafter vitamin-D levels fell rapidly but were still slightly above the base line at seven days (Fig. 2).

In contrast to the markedly increased vitamin-D levels after exposure to UVR, the 25-OH-D concentrations in normal subjects increased only gradually, reaching highest concentrations seven to 14 days after exposure to UVR (Fig. 1). Although the 25-OH-D levels rose in all subjects studied, a strictly dose-related response could not be demonstrated. Normal subjects exposed to one minimal erythemal dose of UVR had essentially no change in the serum concentration of 1,25-(OH)$_2$-D; however, those exposed to three minimal erythemal doses had a transient slight increase in the 1,25-(OH)$_2$-D concentration (Fig. 1).

Mean data from the three vitamin-D-deficient patients are also shown in Figure 1. Basal vitamin-D concentrations were lower in the three patients (4.0±3.0 ng per milliliter) than in eight normal subjects (5.9±6.1 ng per milliliter), but were clearly detectable in all vitamin-D-deficient patients studied. In response to one minimal erythemal dose of UVR, the magnitude and the time course of the rise in vitamin-D levels in the patients were comparable to those in their normal counterparts exposed to the same dose of UVR. Basal 25-OH-D concentrations were below 4 ng per milliliter in all three patients; after the patients were exposed to UVR the 25-OH-D concentrations rose slightly, but remained below 10 ng per milliliter for the duration of the study. The mean basal 1,25-(OH)$_2$-D concentrations were similar in the vitamin-D-deficient patients (43.3±12.6 pg per milliliter) and the eight normal subjects (45.1±9.0 pg per milliliter). However, the 1,25-(OH)$_2$-D levels rose dramatically in the patients, reaching a peak
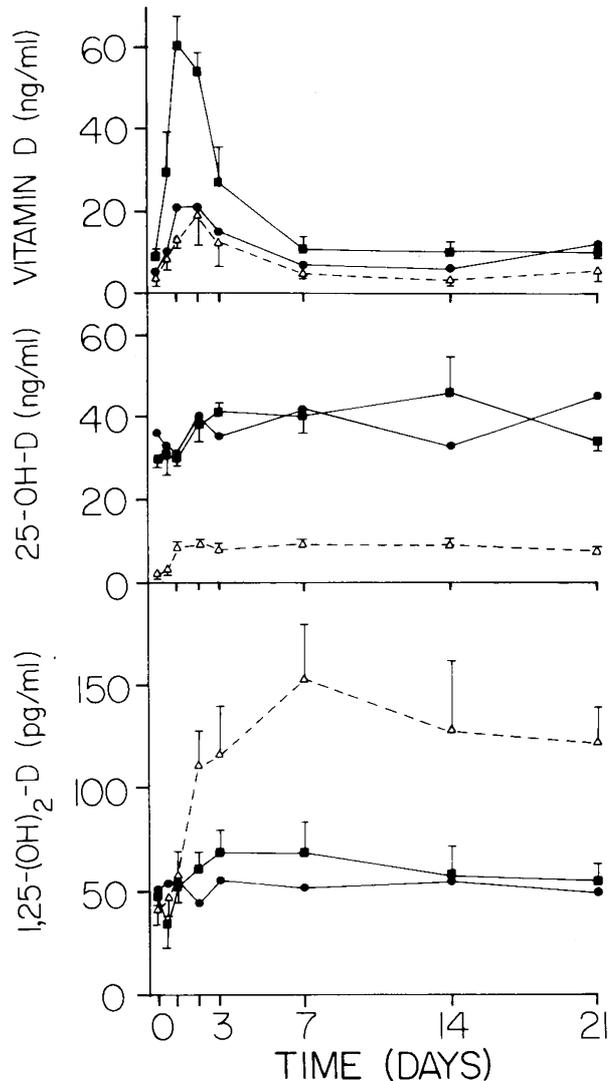

Figure 1. Changes in Serum Concentrations of Vitamin D and Its Metabolites after Exposure to Ultraviolet Radiation.

Concentrations of vitamin D, 25-OH-D, and 1,25-(OH)$_2$-D were measured in three normal subjects exposed to three minimal erythemal doses of ultraviolet radiation (UVR) (solid squares, solid lines), in a representative normal subject exposed to one minimal erythemal dose of UVR (solid circles, solid lines), and in three vitamin-D-deficient patients exposed to one minimal erythemal dose of UVR (open triangles, dashed lines). To convert nanograms of vitamin D per milliliter to nanomoles per liter, nanograms of 25-OH-D per milliliter to nanomoles per liter, and picograms of 1,25-(OH)$_2$-D per milliliter to picomoles per liter, multiply by 2.58, 2.50, and 2.40, respectively. Bars denote S.E.M.

seven days after exposure to UVR. The increase in 1,25-(OH)$_2$-D from basal to peak concentrations in the three patients was 160, 90, and 83 pg per milliliter (data not shown) and correlated with the basal concentrations of immunoreactive parathyroid hormone (210, 99, and 87 μleq per milliliter, respectively). Seven days after exposure to UVR, serum concentrations of immunoreactive parathyroid hormone fell in two of the vitamin-D-deficient patients but did
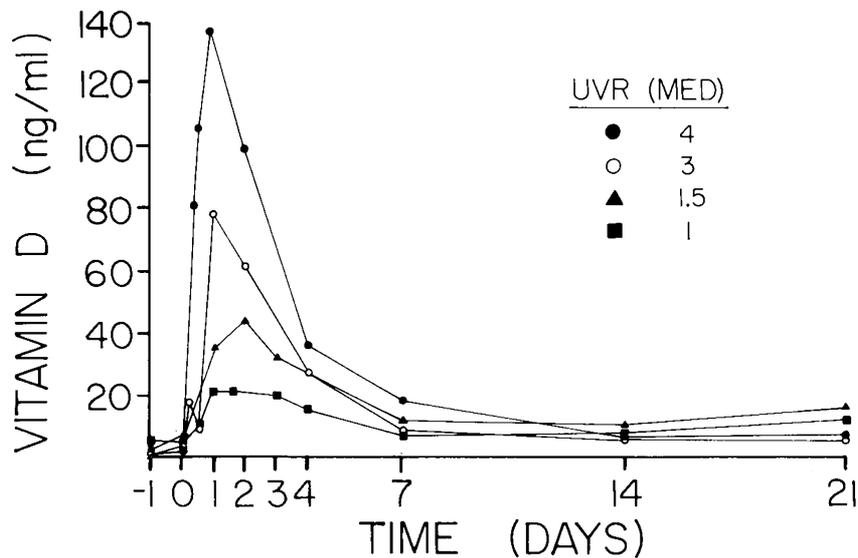
Figure 2. Appearance of Vitamin D in Serum after Exposure of Normal Subjects to One, 1.5, Three, and Four Minimal Erythemal Doses (MED) of Whole-Body Ultraviolet Radiation.
To convert nanograms of vitamin D per milliliter to nanomoles per liter, multiply by 2.58.

not change in the third patient or in the normal subjects. The mean basal serum calcium concentration in the normal subjects (9.1±0.2 mg per deciliter; 2.3±0.1 mmol per liter) was higher than it was in the vitamin-D-deficient patients (8.1±0.4 mg per deciliter; 2.0±0.1 mmol per liter), but there was no change in calcium concentration in either group at seven and 14 days after exposure to UVR.

DISCUSSION

Investigators have demonstrated a seasonal variation in 25-OH-D and 1,25-(OH)$_2$-D concentrations in persons living in temperate climates, with increases late in summer and declines late in winter.[8-10] In addition, increases in vitamin D and in 25-OH-D have been documented in healthy adults returning from a tropical vacation,[11,12] and increases in plasma 25-OH-D have been reported after repeated exposure to UVR.[13,14] In this report we describe the appearance of vitamin D and its metabolites in serum after exposure to a single quantitative dose of UVR.

Exposure of normal subjects and vitamin-D-deficient patients to increasing doses of UVR (Fig. 1) resulted in marked, transient increases in the serum vitamin-D concentration, which peaked within two days of irradiation. Vitamin D then began to disappear from the serum, with an apparent half-life of 48 hours, returning almost to basal levels by seven days. Moreover, the increase in the vitamin-D concentration was dependent on the dose of UVR (Fig. 2). Assuming a plasma volume of 5 per cent of mean body weight, the net increase in vitamin-D concentration in normal subjects after exposure to three minimal erythemal doses of UVR indicates the release into the plasma pool of at least 0.03 mg of vitamin D$_3$ per

square meter of body-surface area for each minimal erythemal dose after synthesis of the vitamin in the skin. Although 25-OH-D concentrations increased in the normal and in the vitamin-D-deficient subjents in this study, the small increases in this metabolite did not reflect the large increments in the serum vitamin-D concentration observed in subjects receiving a "sunburning dose" (three minimal erythemal doses) of UVR. This observation may be explained by the rapid deposition of vitamin D in the very large tissue reservoir for the compound,[15] dilution of newly synthesized 25-OH-D in a relatively large and stable serum pool for the metabolite, or by vitamin-D-mediated changes in the rate of output of 25-OH-D from the liver.[16]

It has been postulated that the synthesis of 1,25-(OH)$_2$-D is tightly regulated and that increases in 25-OH-D concentrations due to exposure to sunlight have no effect on serum 1,25-(OH)$_2$-D levels.[10] Although our data suggesting a small transient increase in 1,25-(OH)$_2$-D concentrations in normal subjects after exposure to three minimal erythemal doses of UVR must be confirmed in more extensive investigations, there was an unequivocal increase in the 1,25-(OH)$_2$-D concentrations after UVR in our three vitamin-D-deficient patients. Inasmuch as parathyroid hormone is a known stimulator of 1,25-(OH)$_2$-D synthesis,[17] the persistently elevated 1,25-(OH)$_2$-D concentration in vitamin-D-deficient patients after only one minimal erythemal dose of UVR was probably the result of secondary hyperparathyroidism and chronic stimulation of the renal 25-OH-D-1α-hydroxylase. Similar increases in 1,25-(OH)$_2$-D concentrations were recently demonstrated by Papapoulos et al.[18] and by Mawer,[19] after administration of

small daily oral doses of vitamin D_3 to vegetarians with nutritional vitamin-D deficiency. Finally, the presence of normal 1,25-$(OH)_2$-D and measurable vitamin-D concentrations in the vitamin-D-deficient patients in our study strongly suggests that unlike levels of 25-OH-D, levels of vitamin D and 1,25-$(OH)_2$-D in serum are not a reliable gauge of a person's "vitamin-D status." The vitamin-D level is dependent primarily on the amount of exposure to UVR and the interval between exposures, whereas the 1,25-$(OH)_2$-D concentration may be influenced by the prevailing concentration of parathyroid hormone.

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RAPID DIAGNOSIS OF AMNIOTIC-FLUID INFECTION BY GAS-LIQUID CHROMATOGRAPHY

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AMNIOTIC-FLUID infection, a common event during pregnancy, occurs most frequently in association with rupture of membranes, particularly prolonged rupture, and with premature delivery. It has been an important cause of perinatal mortality.[1,2] Only a portion of amniotic-fluid infections are clinically apparent intrapartum[3]; they are usually recognized only on the basis of maternal leukocytosis, maternal or fetal tachycardia, uterine tenderness and irritability, or foul-smelling amniotic fluid.[4] These signs represent late findings of amniotic-fluid infections; contamination of the fluid by pathogenic bacteria probably occurs several hours before the appearance of clinical signs of infection.[5,6] Because of the difficulty of applying clinical criteria in order to establish an early diagnosis, investigators have sought other diagnostic tests for amniotic-fluid infection. Results of such traditional tests as amniotic-fluid leukocyte count and Gram stain,[5-9] quantitative microbial cultures of amniotic fluid,[5,9] and measurement of C-reactive protein in maternal serum,[10] are often insensitive, nonspecific, or not immediately available.

Recent data underscore the importance of pathogenic bacteria indigenous to the female lower genital tract, such as Group B and D streptococci, and of vaginal anaerobes as causes of amniotic-fluid infection.[7-9,11,12] Organic acid metabolites of these bacteria are detectable by gas-liquid chromatography (GLC).[13] Elevated levels of bacterial metabolic products have been detected by GLC analysis in the vaginal secretions of women with nonspecific vaginitis,[14] in purulent-abscess material,[15] in peritoneal fluid,[16] and in blood.[17] We studied the bacteriology of acute amniotic-fluid infection and determined the ability of GLC to detect bacterial metabolites in amniotic fluid as an aid in the diagnosis of amniotic-fluid infection. We found an abnormal chromatographic pattern in 15 of 16 patients with clinical signs of infection, but in only one of 22 patients without them.

METHODS

Study Population

During a 12-month period ending in June 1981, 36 (1.8 per cent) of 1988 patients who delivered at University Hospital in Seattle had clinical signs of acute amniotic-fluid infection, according to criteria