

Influence of Season and Latitude on the Cutaneous Synthesis of Vitamin D₃: Exposure to Winter Sunlight in Boston and Edmonton Will Not Promote Vitamin D₃ Synthesis in Human Skin*

A. R. WEBB, L. KLINE, AND M. F. HOLICK

Vitamin D, Skin, and Bone Research Laboratory, Boston University Medical School, Boston, Massachusetts 02118; and the Department of Physiology, University of Alberta (L.K.), Edmonton, Canada T6G 2R3

ABSTRACT. Sunlight has long been recognized as a major provider of vitamin D for humans; radiation in the UVB (290–315 nm) portion of the solar spectrum photolyzes 7-dehydrocholesterol in the skin to previtamin D₃, which, in turn, is converted by a thermal process to vitamin D₃. Latitude and season affect both the quantity and quality of solar radiation reaching the earth's surface, especially in the UVB region of the spectrum, but little is known about how these influence the ability of sunlight to synthesize vitamin D₃ in skin. A model has been developed to evaluate the effect of seasonal and latitudinal changes on the potential of sunlight to initiate cutaneous production of vitamin D₃. Human skin or [3α -³H]7-dehydrocholes-

terol exposed to sunlight on cloudless days in Boston (42.2°N) from November through February produced no previtamin D₃. In Edmonton (52°N) this ineffective winter period extended from October through March. Further south (34°N and 18°N), sunlight effectively photoconverted 7-dehydrocholesterol to previtamin D₃ in the middle of winter. These results quantify the dramatic influence of changes in solar UVB radiation on cutaneous vitamin D₃ synthesis and indicate the latitudinal increase in the length of the "vitamin D winter" during which dietary supplementation of the vitamin may be advisable. (*J Clin Endocrinol Metab* 67: 373, 1988)

CASUAL exposure to sunlight is responsible for providing adequate amounts of vitamin D for most humans (1). During such exposure the high energy UV photons enter into the epidermis and cause the photochemical transformation of 7-dehydrocholesterol (7-DHC; provitamin D₃) to previtamin D₃ (2). Once formed, previtamin D₃ undergoes a temperature-dependent isomerization to vitamin D₃, which at body temperature takes approximately 2–3 days to reach completion (2). Previtamin D₃ is also photolabile, and excessive sunlight exposure results in the photoisomerization of previtamin D₃ to two biologically inert products, lumisterol and tachysterol (3). The initial step in the production of vitamin D₃, the conversion of 7-DHC to previtamin D₃, has an action spectrum that peaks at 295 nm, dropping to zero at 315 nm (4). Radiation in this waveband (290–315 nm) is at the short wavelength limit of the solar radiation reaching the earth's surface and changes in both absolute and relative magnitude with changing ze-

nith angle (5). Rotation of the earth about the sun (season) and its own axis (day and night) governs periodic changes in the solar zenith angle and, hence, atmospheric attenuation of the solar radiation. When the sun is low in the sky the incoming radiation must travel further and is subject to more scattering and absorption than when the sun is directly overhead. Since Rayleigh scattering and UVB absorption by ozone are strongly wavelength dependent, the ability to synthesize previtamin D₃ in skin is likely to be affected by latitude, season of the year, and time of day.

Children and adults who live at the Northeastern United States and Great Britain and who eat little vitamin D may be at increased risk for developing rickets and osteomalacia, respectively, at the end of the winter months (1, 6–9). The zenith angle of the sun is increased during the winter months, and because of the colder weather more clothing is worn, and less outdoor activities occur. It is not known whether winter sunlight has the ability to produce previtamin D₃ in human skin. To determine the effect of season on the cutaneous production of previtamin D₃ at various latitudes, an *in vitro* model was developed to study the conversion of [³H]7-DHC to [³H]previtamin D₃ and its photoproducts.

Received February 11, 1988.

Address requests for reprints to: M. F. Holick, Boston University School of Medicine, Building M, 1013, 80 East Concord Street, Boston, Massachusetts 02111.

* This work was supported by NIH Grants AM-32324, AG-02918, and AG-04390.

Materials and Methods

$[^3\text{H}]7\text{-DHC}$ in methanol model

Quartz vessels containing 25 μg 7-DHC (Aldrich Chemical Co., Milwaukee, WI) and 500,000 cpm $[3\alpha\text{-}^3\text{H}]7\text{-DHC}$ (SA, 14 Ci/mmol) (10) in a total volume of 2.5 mL methanol were exposed to sunlight at various locations in the northern hemisphere. Irradiation was carried out on clear cloudless days as close to the 15th of each month as possible. In each study solutions were exposed on the roof of south-facing balconies for up to 3 h from 1130–1430 h local standard time, giving maximum radiation intensity during the first hour (around solar noon). Aliquots (250 μL) were removed from each vessel at 0, 1, 2, and 3 h; during the summer months additional aliquots were removed during the first hour of exposure. These aliquots were dried under nitrogen and analyzed by high performance liquid chromatography (HPLC) on a Waters Radial Pak 5- μm silica column (Waters Associates, Inc., Milford, MA) with 8% ethyl acetate in hexane at a flow rate of 1.5 mL/min. (2), using, in series, an UV detector at 254 nm and a Radiomatic Flow One in-line tritium detector (Radiomatic Instruments and Chemical Co., Inc., Tampa, FL). The sensitivity of the HPLC system was 5, 2, and 1 ng for 7-DHC, previtamin D_3 , and vitamin D_3 , respectively. During the early months of the study a single aliquot was assayed at each time point. Later, triplicate samples were analyzed, and the reproducibility of the system was found to be $\pm 1\%$ photoproduct. Polysulphone film badges (11) were exposed for the same periods adjacent to the vessels in order to measure incident broadband UVB radiation. Each batch of film was calibrated under natural solar irradiation at or near the time of use, thus avoiding discrepancies of calibration due to a changing solar spectrum. Calibration was made by comparing the change in absorbance of the film badges at a wavelength of 330 nm (11) with the accumulated incident broadband UVB radiation measured with an International Light Newburyport, MA) 700A Research Radiometer. In addition, at various times throughout the year an Optronics 742 spectroradiometer (Optronic Laboratories Inc., Orlando, FL) was used to measure the incident radiation in Boston.

Human skin samples

Neonatal human (caucasian) foreskin samples obtained at the time of circumcision were cleaned of sc fat and divided into two pieces. Each piece was exposed to warm water at 60 C for 30 s to split the epidermis at the stratum basale, as previously described (2). The skin samples were placed in quartz petri dishes on gauze moistened with 150 mmol/L saline. The number of skins available at any one time varied from two to five. Half of each freshly excised skin sample was exposed to sunlight on a cloudless day in Boston from 1130–1430 h Eastern Standard Time (EST) in the months of October, November, February, March, April, May, June, July, and August. Skin was not available in the remaining months of the year. The other half of each skin sample was kept in the dark. After exposure, the epidermis and stratum basale were separated from the dermis and extracted with 8% ethyl acetate in hexane (2). The extraction solvent was collected, dried under nitrogen, and redissolved in 8% ethyl acetate in hexane for HPLC analysis, as previously

described (2). The photoproducts were identified based on straight phase and reverse phase cochromatography and UV absorption spectrophotometry, as previously described (2). Further proof of the identification of previtamin D_3 was obtained by demonstration of its conversion to vitamin D_3 at 37 C (12).

Results

Synthesis of previtamin D_3 in human skin and $[^3\text{H}]$ previtamin D_3 in methanol

Previtamin D_3 was detected in the irradiated skin samples exposed to sunlight in Boston from April through October. However, skin exposed in November and February did not convert 7-DHC to previtamin D_3 . Figure 1 shows the photoconversion of 7-DHC to previtamin D_3 in skin in June and February compared with the conversion of $[3\alpha\text{-}^3\text{H}]7\text{-DHC}$ to $[3\alpha\text{-}^3\text{H}]$ previtamin D_3 in methanol. Chromatograms A–C in Fig. 1 show the results of the 7-DHC in methanol studies analyzed using the in-line ^3H detector. The results of analysis of corresponding skin extracts are shown in Fig. 1, D–F, with detection by UV absorbance. Figure 1, A and D, show the results in samples kept in the dark for 3 h. Exposure of skin or $[3\alpha\text{-}^3\text{H}]7\text{-DHC}$ in methanol to 3 h of sunlight

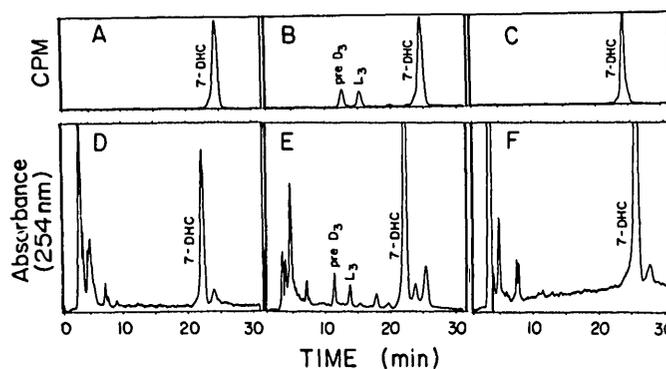


FIG. 1. HPLC profiles of aliquots of solutions of $[3\alpha\text{-}^3\text{H}]7\text{-DHC}$ or lipid extracts of pieces of excised human skin that were exposed to sunlight in Boston at different times of the year. The aliquots were dried under nitrogen, redissolved in 8% ethyl acetate in hexane and applied to a 5- μm silica column and developed with 8% (vol/vol) ethyl acetate in hexane at a flow rate of 1.5 mL/min. 7-DHC and its products in the skin extracts were detected with a 254-nm UV detector (full scale represents 0.02 absorbance units), while $[3\alpha\text{-}^3\text{H}]7\text{-DHC}$ and its photoproducts were detected by a HPLC tritium detector (full scale represents 3000 cpm). Chromatograms of the $[^3\text{H}]7\text{-DHC}$ -methanol solutions are shown before exposure (A) and after a 3-h exposure between 1130 and 1430 h standard time in June (B) and February (C). The remaining chromatograms are from lipid extracts of skin before exposure (D) and after 3 h of exposure between 1130 and 1430 h standard time in June (E) and February (F). The skin extracts and aliquots from the $[^3\text{H}]7\text{-DHC}$ -methanol solutions were analyzed separately. The migration times differ for previtamin D_3 (pre D_3), lumisterol (L_3), and 7-DHC in the two types of experiments because the two detection systems are in series, the sample passing first through the UV detector and then through the tritium detector. The skin sample analyzed in February (F) was chromatographed on a different 5- μm silica column.

in June resulted in conversion of 7-DHC to previtamin D₃ and lumisterol (Fig. 1, B and E). The conversion, expressed as the percentage of 7-DHC, was 12% and 11% for the 7-DHC-methanol solution and 7% and 5% for the skin, respectively. Exposure to 3 h of sunlight in February did not result in any photosynthesis of previtamin D₃ or lumisterol (Fig. 1, C and F). It was expected that the 7-DHC-methanol solutions would show the maximum possible conversion of 7-DHC to previtamin D₃, because, unlike human skin which contains a variety of UVB-absorbing substances (such as DNA, RNA, proteins, and urocanic acid) (13), the former did not contain any UVB-absorbing substances other than [³H]7-DHC. This expectation was confirmed when [³H]7-DHC and human skin were exposed simultaneously to sunlight during the last days of February and the first 3 weeks of March. [³H]Previtamin D₃ production was first identified on February 28th in methanol solution, while at the same time previtamin D₃ was not apparent in skin. It was not until March 17th that previtamin D₃ was detectable in skin. Upon further evaluation with both natural and simulated sunlight the conversion of 7-DHC to previtamin D₃ in skin was between 40–70% of the total amount of [³H]previtamin D₃ that was generated from [³H]7-DHC in methanol, depending on the pigmentation of the skin sample. Since the results using skin and 7-DHC-methanol were comparable, and solar UVB absorbance by 7-DHC was optimized in the latter, we concluded that the 7-DHC-methanol solution was a satisfactory model for evaluating how latitude, season, and time of day affected the conversion of 7-DHC to previtamin D₃ in skin.

Figure 2 shows the formation of [³H]previtamin D₃, [³H]lumisterol, and [³H]tachysterol after exposure of [³H]7-DHC to sunlight in Boston in June beginning at

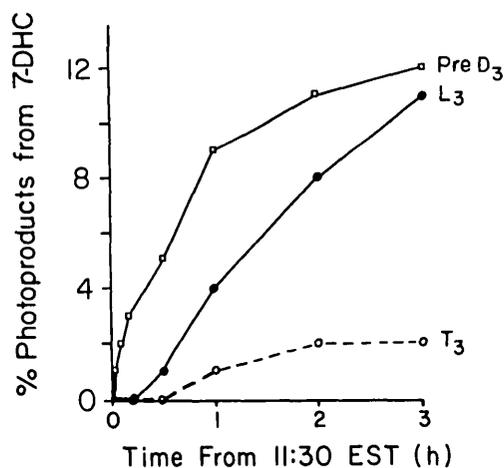


FIG. 2. [³H]7-DHC was exposed to sunlight on June 17th, 1986, in Boston. There was an increase in the percentage of photoproducts with time from the start of the exposure at 1130 h EST. The precision of the measurements was $\pm 1\%$ photoproduct for a single sample. Pre D₃, Previtamin D₃; L₃, lumisterol; T₃, tachysterol.

1130 h EST. The amount of each ³H-labeled photoproduct in the irradiated solution is expressed as a percentage of the tritium recovered after HPLC. The assay was able to detect changes of as little as $1.0 \pm 0.1\%$ (\pm SE) when run in triplicate. After only 2 min of exposure to noon-time sunlight previtamin D₃ was detected. After 5 min 2% of the original [³H]7-DHC was converted to [³H]previtamin D₃. The production of [³H]previtamin D₃ steadily increased during the first hour of exposure to about 9% of the original [³H]7-DHC level. During the next 2 h [³H]previtamin D₃ production slowed, and the amount of previtamin D₃ approached a plateau level of $12.3 \pm 0.3\%$ after 3 h. [³H]Lumisterol and [³H]tachysterol were first detected after 30 min and 1 h, respectively. While [³H]lumisterol continued to accumulate during the next 2 h to $10.8 \pm 0.3\%$, [³H]tachysterol remained relatively constant at about 3% after the first hour (Fig. 2).

Figure 3 represents the conversion of [³H]7-DHC to [³H]previtamin D₃ and its photoproducts throughout the year in Boston beginning at 1130 EST for exposure times of 1 and 3 h: 1 h as the end of the period with maximum irradiation intensity (and, hence, photolysis potential) around noon, and 3 h as the time when the production of previtamin D₃ was maximal. There was no detectable

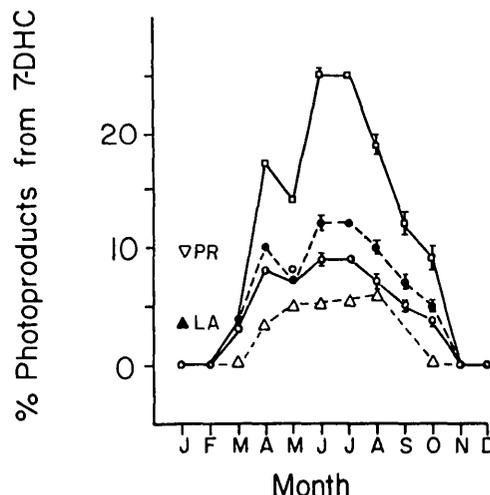


FIG. 3. [³H]7-DHC in methanol was exposed to sunlight at different seasons and latitudes. Shown are the mean ± 2 SD ($n = 3$) annual change in percent conversion of 7-DHC to previtamin D₃ after sunlight exposure for 1 h (\circ — \circ) and 3 h (\bullet — \bullet), and total photoproducts (previtamin D₃, lumisterol, and tachysterol) after 3 h (\square — \square) in Boston. The data were collected from November 1985 through 1986 to May 1987, and the figure shows compiled data for the 12 calendar months. For months where data was available for more than 1 yr the results were the same within the uncertainty of a single point measurement, except for the photosynthesis of previtamin D₃ in May when exposure for 1 and 3 h gave the same result in 1986 (\bullet), and only a 1 h value is available for 1987 (\circ). Also shown is the conversion of 7-DHC to previtamin D₃ throughout the year after exposure to 1 h of sunlight in Edmonton (Δ — Δ) and in January in Los Angeles (LA) and Puerto Rico (PR; single samples accurate to $\pm 1\%$ photoproduct).

photolysis of [^3H]7-DHC from November through February. The formation of [^3H]previtamin D_3 was first evident after exposure to sunlight in March, when 1 and 3 h of exposure resulted in conversion of 3% and 4% of [^3H]7-DHC to [^3H]previtamin D_3 , respectively. As single measurements these values are not significantly different, and at this time of the year it is probable that only the most intense radiation around noontime was effective for producing previtamin D_3 . The photolysis of [^3H]7-DHC to [^3H]previtamin D_3 was greatest in June and July [$9.1 \pm 0.5\%$ ($\pm\text{SE}$; $n = 3$) after 1 h; $11.7 \pm 0.3\%$ ($n = 3$) after 3 h in June]. Once formed, previtamin D_3 was available for further photolysis; during the summer months its photoisomers were found in both systems studied. [^3H]Lumisterol was photosynthesized between the months of April and October. [^3H]Tachysterol was found only after the periods of most intense solar irradiation in June and July. Thereafter, decreasing photolysis of [^3H]7-DHC to [^3H]previtamin D_3 and its photoisomers paralleled declining solar altitude through the fall months. In October approximately 4% of [^3H]7-DHC was converted to [^3H]previtamin D_3 after 3 h, and its production decreased to undetectable levels in November (Fig. 3).

Effect of latitude and season on the photolysis of [^3H]7-DHC

To determine the effect of a change in latitude on the production of previtamin D_3 , the conversion of [^3H]7-DHC to [^3H]previtamin D_3 was determined throughout the year in Edmonton, Canada (52°N), and at other locations in the middle of winter. In Edmonton on a cloudless day the synthesis of [^3H]previtamin D_3 was not initiated until the beginning of April and ceased after October (Fig. 3). When similar studies were conducted in January in Los Angeles (34°N) and Puerto Rico (18°N) [^3H]7-DHC was photolyzed to [^3H]previtamin D_3 (Fig. 3). However, in Los Angeles, which is 16° farther north than Puerto Rico, the efficiency of conversion was less. During the month of January exposure of [^3H]7-DHC to noontime sunlight for 1 h on a cloudless day resulted in 10% conversion of 7-DHC to previtamin D_3 in Puerto Rico and only 3% in Los Angeles.

Seasonal changes in incident radiation

To obtain better insight as to how fluctuations in the incident solar radiation affected the cutaneous photosynthesis of previtamin D_3 , polysulfone film badges, and a spectroradiometer were used to monitor incident UV radiation in Boston. The polysulfone film badges gave an estimate of the effective radiation that is responsible for converting 7-DHC to previtamin D_3 (14). As shown in Fig. 4 very little solar UVB radiation was detected

between the months of November and February, and the broadband polysulfone film badge measurements result from the longer wavelength components of the UV irradiation by which the badges, but not 7-DHC, are affected ($315 < \lambda < 330 \text{ nm}$). This finding is consistent with the observation that in the months November through February no significant previtamin D_3 synthesis was found (Fig. 2). To quantitate this phenomenon a spectroradiometer was used to evaluate the changing intensities at four wavelengths (296, 300, 306, and 316 nm) whose relative efficiencies for converting 7-DHC to previtamin D_3 are 1, 0.92, 0.45, and 0, respectively. The values for each day were determined from the spectrum measured within 0.5 h of solar noon and represent the maximum intensity for that day. As shown in Fig. 5 there were dramatic changes in the amount of photons at all wavelengths throughout the year. However, the most dramatic changes occurred at the wavelengths (296 and 300 nm) that were most responsible for the conversion of 7-DHC to previtamin D_3 (4). Between February and March there was a steep increase in the intensity at all wavelengths, more than an order of magnitude in each instance. In March when previtamin D_3 photosynthesis was first detected, the radiation at 296 nm also became measurable with the spectroradiometer. All wavelengths measured continued to increase in intensity through July and then declined. At 296 nm the detection limit ($10^{-9} \text{ W cm}^{-2} \text{ nm}^{-1}$) of the spectroradiometer was reached between September and October, but in October there was still 10 times the radiation measured in February at 300 and 306 nm. It is radiation of these latter wavelengths that was most likely to be responsible for the conversion of

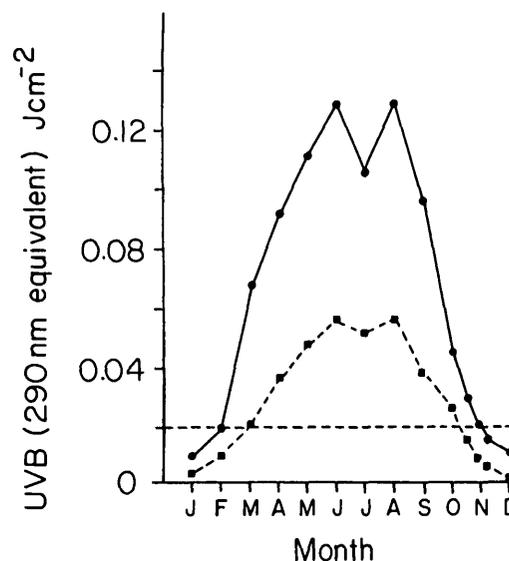


FIG. 4. Annual change in broadband UVB radiation measured in Boston using polysulfone film badges for periods of 1 h (■—■) and 3 h (●—●) from 1130 h EST. Each point is the mean of two or three badges. — — —, The level of broadband UV radiation below which no conversion of 7-DHC occurred.

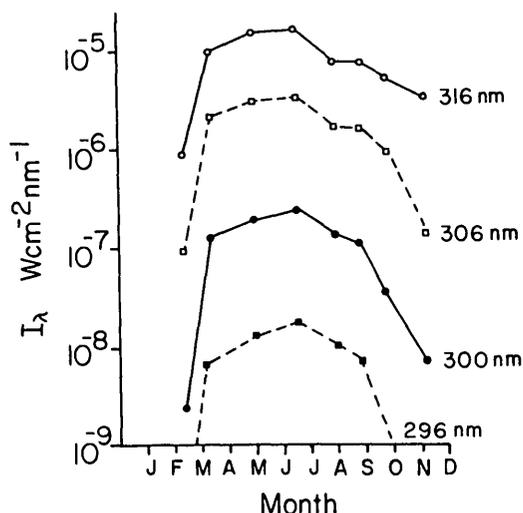


FIG. 5. Annual change in incident solar radiation at four UVB wavelengths in Boston: 296 nm (■—■), 300 nm (●—●), 306 nm (□—□), and 316 nm (○—○).

7-DHC to previtamin D₃ (Fig. 3). By November the power of the sun at shorter wavelengths had decreased by almost an order of magnitude to 10⁻⁹ W cm⁻² nm⁻¹ at 300 nm and 10⁻⁷ W cm⁻² nm⁻¹ at 306 nm, and 7-DHC could no longer be converted by sunlight to previtamin D₃.

Discussion

Exposure to sunlight is a very important factor for maintaining calcium and bone metabolism because of the unique photochemistry that gives rise to previtamin D₃ in skin, but little is known about how season and latitude affect the cutaneous production of previtamin D₃. We developed a model system whereby we could determine the impact of environment on this vital cutaneous process. It is known that increased skin pigmentation (3), aging (15), and the use of sunscreens (16) all limit the amount of previtamin D₃ that is generated in the skin. Our results provide unequivocal evidence that changes in the season and latitude also have a dramatic influence on the capacity of the skin to produce previtamin D₃.

As the zenith angle of the sun increases during the winter months the sunlight is filtered at a more oblique angle through the stratospheric ozone layer, decreasing the UVB radiation (responsible for previtamin D₃ production) that reaches the earth's surface. In Boston (42°N) this filtering effect is sufficient to prevent previtamin D₃ production from November through February. The zenith angle also systematically increases with latitude, so that 10° north of Boston, in Edmonton, the photosynthesis of previtamin D₃ ceases in October and does not occur again until April. *In vivo*, further attenuation of UVB radiation by the UVB-absorbing sub-

stances in the outer layers of skin probably extends the period when little previtamin D₃ production occurs. This supposition is borne out by our observation that sunlight in late February converted 2% of the [³H]7-DHC to [³H] previtamin D₃, whereas no conversion occurred in the skin; cutaneous previtamin D₃ was first detected in mid-March. Additional factors that could also affect cutaneous previtamin D₃ production include melanin (3) and aging (15). However, while the absolute concentration of 7-DHC in the skin decreases with age, aging *per se* does not appear to significantly alter the efficiency of conversion (15). Thus, neonatal skin is likely to be a good predictor of what occurs in the skin of older individuals. These observations are especially important for those individuals, especially the elderly, who rely on exposure to sunlight for their vitamin D requirement (17). If sufficient stores of vitamin D₃ are not built up in fat (18) during the summer months because of limited outdoor activity, it is likely that without vitamin D supplementation these people will be at risk for developing vitamin D deficiency because exposure to sunlight during winter is ineffective for producing vitamin D₃ in the skin.

It is estimated that 30–40% of elderly individuals living in Great Britain are vitamin D deficient at the time of their first hip fracture (19–21). Similar observations have been made in Boston (8). Safeguarding against vitamin D deficiency is one step that may be taken to help prevent these debilitating fractures. Our *in vitro* system can identify times and places at which cutaneous vitamin D₃ synthesis does not occur during exposure to sunlight. Individuals potentially at risk may then be advised to supplement their vitamin D intake if casual exposure to sunlight throughout the year is insufficient to meet their needs.

Acknowledgments

We thank Drs. D. Endres (Los Angeles) and F. Diaz (Puerto Rico) for their help in exposing solutions of 7-DHC to sunlight in their respective locations. Our thanks also to Dr. B. De Costa for [³H]7-DHC, Mr. D. Maytum for use of an Optronics 742 spectroradiometer, and Miss N. Hanafin for comments on the manuscript. Part of this work was conducted at Tufts University Human Nutrition Research Center. We are most grateful to the USDA and Dr. I. Rosenberg for the use of their Optronics 742 spectroradiometer.

References

- Holick MF 1986 Vitamin D requirements for the elderly. *Clin Nutr* 5:121
- Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts Jr JT, Anderson RR, Blank IH, Parrish JA, Elias P 1980 Photosynthesis of previtamin D₃ in human skin and the physiologic consequences. *Science* 210:203
- Holick MF, MacLaughlin JA, Doppelt SH 1981 Regulation of cutaneous previtamin D₃ photosynthesis in man: skin pigment is not an essential regulator. *Science* 211:590
- MacLaughlin JA, Anderson RR, Holick MF 1982 Spectral character of sunlight modulates photosynthesis of previtamin D₃ and

- its isomers in human skin. *Science* 216:1001
5. Bener P 1969 Spectral intensity of natural UVR and its dependence on various parameters. In Urbach F (ed) *The Biologic Effects of Ultraviolet Radiation*. Pergamon Press, Oxford and New York, pp 351-358
 6. Stamp TCB, Round JM 1974 Seasonal changes in human plasma levels of 25-hydroxyvitamin D. *Nature* 247:563
 7. Clemens TL, Zhou X-Y, Myles M, Endres D, Lindsay R 1986 Serum vitamin D₂ and vitamin D₃ metabolite concentrations and absorption of vitamin D₂ in elderly subjects. *J Clin Endocrinol Metab* 63:656
 8. Doppelt SH, Neer RM, Daly M, Bourvet L, Schiller A, Holick MF, Mankin H 1983 Vitamin D deficiency and osteomalacia in patients with hip fractures. *Orthop Trans* 7:512
 9. Toss G, Almqvist B, Larrson L, Zetterqvist H 1980 Vitamin D deficiency in welfare institutions for the aged. *Acta Med Scand* 208:87
 10. Holick MF, Frommer JE, McNeill SC, Richtand NM, Henley JW, Potts Jr JT 1977 Photometabolism of 7-dehydrocholesterol to previtamin D₃ in skin. *Biochem Biophys Res Commun* 76:107
 11. Davis A, Deane GHW, Diffey BL 1976 Possible dosimeter for ultraviolet radiation. *Nature* 261:169
 12. Holick MF, Richtand NM, McNeill SC, Holick SA, Frommer JE, Henley JE, Potts Jr JT 1979 Isolation and identification of previtamin D₃ from the skin of rats exposed to ultraviolet radiation. *Biochemistry* 18:1003
 13. Parrish JA, Anderson RR, Urbach F, Pitts D 1978 UV-A Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet. Plenum Press, New York and London, pp 59-77
 14. Webb AR 1985 Solar Ultraviolet Radiation and Vitamin D Synthesis in Man. PhD Thesis, Nottingham University, England, pp 175-186
 15. MacLaughlin JA, Holick MF 1985 Aging decreases the capacity of skin to produce vitamin D₃. *J Clin Invest* 76:1536
 16. Matsuoko LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF 1987 Sunscreens suppress cutaneous vitamin D₃ synthesis. *J Clin Endocrinol Metab* 64:1165
 17. Omdahl JL, Garry PJ, Hunsaker LA, Hunt WC, Goodwin JS 1982 Nutritional status in a healthy elderly population: vitamin D. *Am J Clin Nutr* 36:1225
 18. Lawson DEM 1980 Metabolism of vitamin D. In Norman AW (ed) *Vitamin D Molecular Biology and Clinical Nutrition*. Marcel Dekker, New York and Basel, p 103
 19. Aron JE, Gallagher JC, Anderson J, Stasiak L, Longton EB, Nordin BEC, Nicholson M 1974 Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. *Lancet* 1:229
 20. Chalmers J, Conacher WDH, Gardner DL, Scott PJ 1967 Osteomalacia-a common disease in elderly women. *J Bone Joint Surg [Br]* 49B:403
 21. Jenkins DH, Roberts JG, Webster D, Williams EO 1974 Osteomalacia in elderly patients with fractures of the femoral neck. *J Bone Joint Surg [Br]* 55B:575