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Photobiology of Vitamin D

*Tai C. Chen, Zhiren Lu,
and Michael F. Holick*

Abstract The major function of vitamin D (either vitamin D₂ or D₃) is to maintain healthy bone. Most humans obtain their vitamin D requirement through casual exposure of the skin to solar ultraviolet B and from dietary intake. The cutaneous synthesis of vitamin D is a function of 7-dehydrocholesterol concentration in epidermis, melanin pigmentation, and the solar zenith angle which depends on latitude, season, and time of day. Our recent study also indicates that altitude may influence the production of previtamin D₃. One area which has shown more progress during the past decade is the use of simulated sunlamp to improve vitamin D production in patients with intestinal malabsorption and elderly who were infirmed or living in northern latitude. Vitamin D deficiency is common in infants, children, and adults worldwide. The major cause of vitamin D deficiency globally is an underappreciation of the crucial role of sunlight in providing humans with their vitamin D requirement. The association between vitamin D deficiency and the increased risk of cancers, autoimmune diseases, infectious diseases, and cardiovascular disease indicates the importance of sunlight, vitamin D, and overall health and well-being of the general population.

Key Words: Sunlight; vitamin D; previtamin D; latitude; season; sunscreen; melanin; black; tanning; ultraviolet B radiation

1. INTRODUCTION

Vitamin D (represents either D₂ or D₃) is essential for maintaining healthy bone (1, 2). The major source of vitamin D for most humans comes from the exposure of the skin to sunlight (1, 2). During exposure to sunlight, 7-dehydrocholesterol (previtamin D₃) in the skin absorbs high-energy solar ultraviolet B photons (UVB; 290–315 nm) and is photolyzed to previtamin D₃ and then thermoisomerized to vitamin D₃ in the bilayer of the plasma membrane. After vitamin D is made in the skin or ingested in the diet, it is transported to the liver where it is hydroxylated on C-25 to form 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D that has been used as an index to determine vitamin D nutritional status (2). To become active, 25(OH)D is further hydroxylated in the kidney to 1 α ,25-dihydroxyvitamin D [1,25(OH)₂D]. One of the major functions of vitamin D is the stimulation of the small intestine to increase its absorption of dietary calcium and phosphorus to maintain normal levels of calcium and

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phosphorus in the circulation (2). When dietary calcium is inadequate to satisfy the body's calcium requirement, $1,25(\text{OH})_2\text{D}$, in concert with parathyroid hormone (PTH), mobilizes monocytic stem cells in the bone marrow to become mature osteoclasts, which in turn enhances the removal of calcium from the bone into the circulation to maintain normal serum calcium levels (2). In addition, $1,25(\text{OH})_2\text{D}$ has other biologic actions, unrelating to calcium metabolism, in many tissues or cells that possess the $1,25(\text{OH})_2\text{D}$ receptor. These include enhancement of cellular differentiation and/or inhibition of cellular proliferation, maintenance of normal neuromuscular, immunomodulatory, and cardiovascular function (3).

2. HISTORICAL PERSPECTIVE

Rickets, a bone-deforming disease, is synonymous with vitamin D deficiency. It was first described in the mid-seventeenth century in Northern Europe during the industrial revolution as a major health problem for the young children when people began to migrate to city centers and live in an environment that was devoid of direct exposure to sunlight. The first observation into the potential cause of this bone-deforming disease and the implication of exposure to the sun in the prevention and cure of this disease was reported by Sniadecki in 1822 (4). He reported that children who lived in Warsaw, Poland, had a high incidence of rickets, while children who lived on the farms surrounding Warsaw were free of the disease. He concluded that it was the lack of sunlight that was the likely cause of rickets. Later in 1890, Palm investigated factors that might associate with rickets in an epidemiological survey study and concluded that the common denominator for rickets in children was the lack of sunlight exposure (2). Therefore, he suggested a systematic sunbathing as a means for preventing and curing rickets. However, these insightful observations went unnoticed just as was Sniadecki's earlier finding.

Using another approach, Bretonneau in the mid-1800s administered cod liver oil to treat a 15-month-old child with acute rickets and noted an incredible speedy recovery of the patient (5). Later, Trousseau, a student of Bretonneau, used liver oils from a variety of fish and marine animals for the treatment of rickets and osteomalacia. In his monograph on therapeutics he advocated the use of fish liver oil preferably accompanied by exposure to sunlight to rapidly cure both rickets and osteomalacia (5). These clinical observations led many to believe that rickets was caused by some type of nutritional deficiency. Unfortunately, these important observations were also disregarded.

By the turn of the twentieth century rickets had become epidemic in Northern Europe as well as in the industrialized northeastern region of the United States. However, it was not until 1918 when Mellanby fed rachitic beagle puppies cod liver oil and found that their rickets was cured that the scientific community began to consider rickets as a nutritional deficiency disease (6). He concluded that cod liver oil possessed a fat soluble nutritional factor that he called the antirachitic factor. Originally it was thought that the antirachitic factor was the newly discovered vitamin A. However, McCollum et al. (7) found that by heating and aerating cod liver oil with oxygen they were able to destroy vitamin A activity without affecting its antirachitic activity. The finding therefore established a new fat soluble principle in cod liver oil which was subsequently

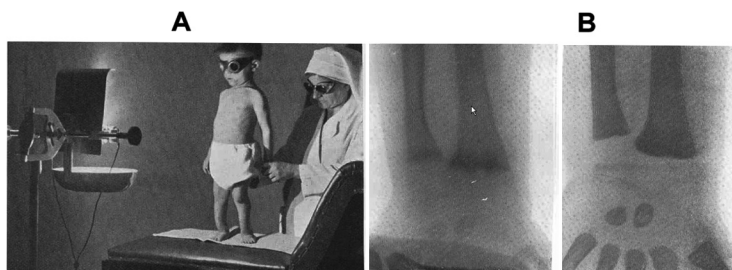


Fig. 1. (a) A rachitic child was irradiated to a mercury vapor arc lamp. (b) A wrist bone X-ray picture before (*left panel*) and after (*right panel*) 4 months of mercury vapor arc lamp therapy. (Reproduced from (89)).

named vitamin D by McCollum. About the same time as Mellanby's observations (6), Huldschinsky exposed four rachitic children to radiation from a mercury vapor arc lamp and demonstrated by X-ray analysis that the rickets was cured after 4 months of therapy (8) (Fig. 1). He further demonstrated that the effect was not localized at the site of irradiation because exposing one arm to the radiation resulted in healing of both arms. In 1921, Hess and Unger exposed seven rachitic children in New York City to sunlight and, based on X-ray examination, reported marked improvement in rickets of each child (9). The finding prompted Goldblatt and Soames (10, 11), Hess and Weinstock (12), and Steenbock and Black (13) to expose a variety of foods, such as wheat, lettuce, olive, linseed oils, and rat chow and other substances, such as human and rat plasma, to ultraviolet radiation. Both groups found that the ultraviolet irradiation imparted antirachitic activity to the substances. This finding led Steenbock to recommend that the irradiation of milk might be an excellent method to provide vitamin D to children and to prevent rickets. This suggestion was followed first by the addition of ergosterol (provitamin D₂) and its subsequent irradiation to impart antirachitic activity (14), and, ultimately, the addition of synthetic vitamin D directly to milk. This simple concept led to the eradication of rickets as a significant health problem in the United States and other countries that used this practice. Thus, nearly one century after Sniadecki had first suggested the importance of sunlight exposure for prevention of rickets it was finally demonstrated that exposure to sunlight alone or ingesting ultraviolet irradiated foods or substances could prevent and cure this crippling bone disease.

3. VITAMIN D FORTIFICATION IN MILK

In the 1950s, there was an outbreak of neonatal hypercalcemia. In Great Britain that was thought to be due to over-fortification of milk of vitamin D. Although there was little evidence that this was the cause, most European countries reacted by banning the fortification of dairy products and other foods with vitamin D. As a result, vitamin D fortification was severely restricted in Europe. Consequently, the incidence of rickets became more frequent and this childhood disease continues to be a health problem in Europe (15, 16). Realizing this problem, many European countries have begun adding vitamin D to various foods, including cereals and margarine. Only recently Sweden

and Finland have permitted milk to be fortified with vitamin D. In the United States, the American Medical Association's Council on Foods and Nutrition recommended in 1957 that milk should contain 400 IU (10 μ g) per quart and that the vitamin D content be measured at least twice yearly by an independent laboratory. Accordingly, the FDA modified its guidelines for the safe levels of vitamin D in milk and stipulated that one quart should contain 400 IU and no more than 600 IU of vitamin D. Fortification above 800 IU/qt might cause a public health threat and should be prohibited. A survey of vitamin D content in fortified milk revealed that 80 and 73% of the milk samples from the United States and Canada, respectively, did not contain 80–120% of label claim (17–19). Some samples even had undetectable amount of vitamin D and some had 2- to 3-fold excess vitamin D.

The fact that antirachitic activity could be produced by exposing skin or foods to ultraviolet (UV) radiation led scientists to identify the precursor of vitamin D. The first one identified was ergosterol from yeast. Ergosterol is a major sterol in the fungal and plant kingdoms. The product from the irradiated ergosterol was first mistakenly believed to be a single product and was named vitamin D₁. The term vitamin D₁ was dropped after the preparation was found to be a mixture of several compounds. The vitamin D

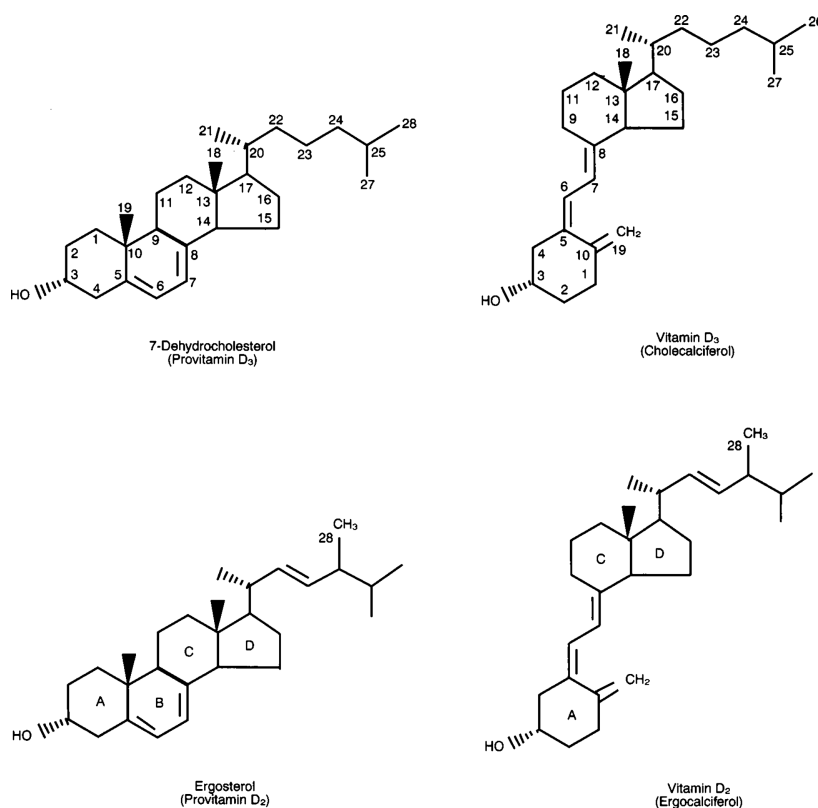


Fig. 2. Chemical structures of vitamin D₂ and vitamin D₃ and their respective precursors, ergosterol, and 7-dehydrocholesterol.

which was isolated in pure form from the irradiated ergosterol was named ergocalciferol or vitamin D₂. Its structure was identified after it was synthesized chemically by Windaus and colleagues (20) (Fig. 2). Originally it was thought that vitamin D₂ was the same product produced in the skin after sunlight exposure. However, it was found that vitamin D₂ was not as effective as the vitamin D from pig skin in curing rickets in chickens. The observations suggested that vitamin D₂ and the vitamin D isolated from the UV irradiated pig skin might not be the same. This suspicion was proven to be correct when Windaus's laboratory isolated a new compound with a side chain structure of cholesterol and showed that this cholesterol-like compound had the same antirachitic potency as those isolated from pig skin (Fig. 2). This finding led Windaus and Bock in 1937 to isolate 7-dehydrocholesterol (7-DHC) from pig skin. They demonstrated that the irradiation of 7-DHC produced a vitamin D which was named vitamin D₃ or cholecalciferol (21). Subsequently, the presence of 7-DHC was demonstrated in human cadaver skin (22), and the skin of fish, amphibians, reptiles, birds, and mammals including rats, hamsters, sheep, and polar bears (23–31).

4. PHOTOSYNTHESIS OF PREVITAMIN D₃ IN THE SKIN

After the discovery of vitamin D₃, it was initially thought that 7-DHC was directly converted to vitamin D₃ after sunlight exposure. However, Velluz et al. were unable to detect any vitamin D₃ from a 7-DHC solution when they exposed the solution to ultraviolet radiation (UVR) at 0°C. Instead, they found an unknown compound and named this compound as previtamin D₃ (32). They also reported that previtamin D₃ was not stable at room temperature and it slowly isomerized to vitamin D₃ (33).

4.1. Photoconversion of 7-DHC to Previtamin D₃

When 7-DHC absorbs UV (290–315 nm) radiation, it causes a bond cleavage between carbon 9 and carbon 10 and an isomerization of 5,7-diene to form the *s-cis*, *s-cis*-previtamin D₃. Because there is a steric interaction between the C ring and the carbon 19 methyl group of *s-cis*, *s-cis*-previtamin D₃, this conformer of previtamin D₃ is energetically unfavorable and therefore is less stable. As a result, a rotation around the carbon 5 and carbon 6 single bond occurs and an energetically more stable *s-trans*, *s-cis*-previtamin D₃ is formed (Fig. 3). Alternately, the unstable *s-cis*, *s-cis*-previtamin D₃ conformer can undergo an intramolecular hydrogen rearrangement to form vitamin D₃.

7-DHC is present in all layers of human skin. Approximately 65% of 7-DHC per unit area is found in the epidermis and the remaining 35% is in the dermis. The highest concentration per unit area is found in the stratum basale and stratum spinosum (34). These epidermal layers, therefore, have the greatest potential for previtamin D₃ synthesis. However, the amount of previtamin D₃ produced also depends on the number and energy of the photons reaching each layer of skin. Bunker and Harris reported in 1937 that the most effective wavelength for curing rickets in rats was 297 nm (35). They and their colleagues fed irradiated 7-DHC to rachitic rats and concluded that 7-DHC

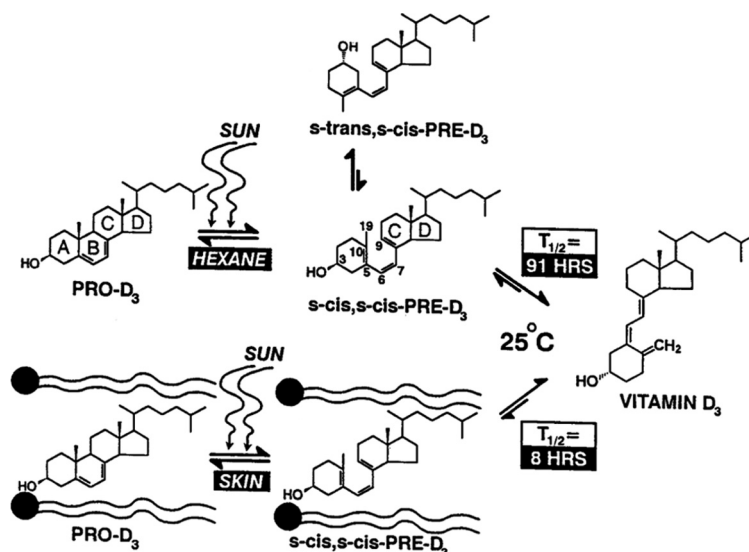


Fig. 3. Photolysis of 7-dehydrocholesterol (pro-D₃ or 7-DHC) to previtamin D₃, its thermal isomerization to vitamin D₃ in *n*-hexane and in the skin, and the stereo-structure of previtamin D₃ in the fatty acid bilayers of skin after exposure to sunlight (Reproduced with permission from (45)).

irradiated at 297 nm had the greatest antirachitic activity, while solutions irradiated at wavelengths longer than 313 nm showed no activity. Later, Kobayashi and Yasumara (36) showed that irradiation of an ergosterol solution with 295 nm radiation gave the maximum yield of previtamin D₂. MacLaughlin et al. examined the photosynthesis of previtamin D₃ from 7-DHC in human skin after exposing the tissue to narrow-band radiation or simulated solar radiation (37). They reported that the optimum wavelengths for the production of previtamin D₃ were between 295 and 300 nm (37). In Caucasians with skin type II, 20–30% of the radiation of 295 nm is transmitted through the epidermis; the majority of the UVB photons (290–320 nm) are absorbed by the stratum spinosum of the epidermis. In Blacks with skin type V only about 2–5% of the UVB photons penetrate through the epidermis (38, 39). As the UVR penetrates the epidermis, it is absorbed by a variety of molecules including DNA, RNA, proteins, as well as 7-DHC. The 5,7-diene of 7-DHC absorbs solar radiation between 290 and 315 nm causing it to isomerize resulting in a bond cleavage between carbon 9 and 10 to form a 9,10-*seco*-sterol previtamin D₃ (24, 34, 37). Because most of the radiation responsible for producing previtamin D₃ is absorbed in the epidermis, greater than 95% of the previtamin D₃ that is produced is in the epidermis (34). Once previtamin D₃ is synthesized in the skin, it can undergo either a photoconversion to lumisterol, tachysterol, and 7-DHC or a heat-induced isomerization to vitamin D₃ (Fig. 4).

Determination of the subcellular localization of 7-DHC and previtamin D₃ in human epidermal tissue revealed that most 7-DHC and previtamin D₃ were in the membrane fraction, while only 20% was in the cytosol. Based on this finding, it has been postulated that most 7-DHC is entrapped in membrane. It is likely that the 3β-hydroxyl group of the

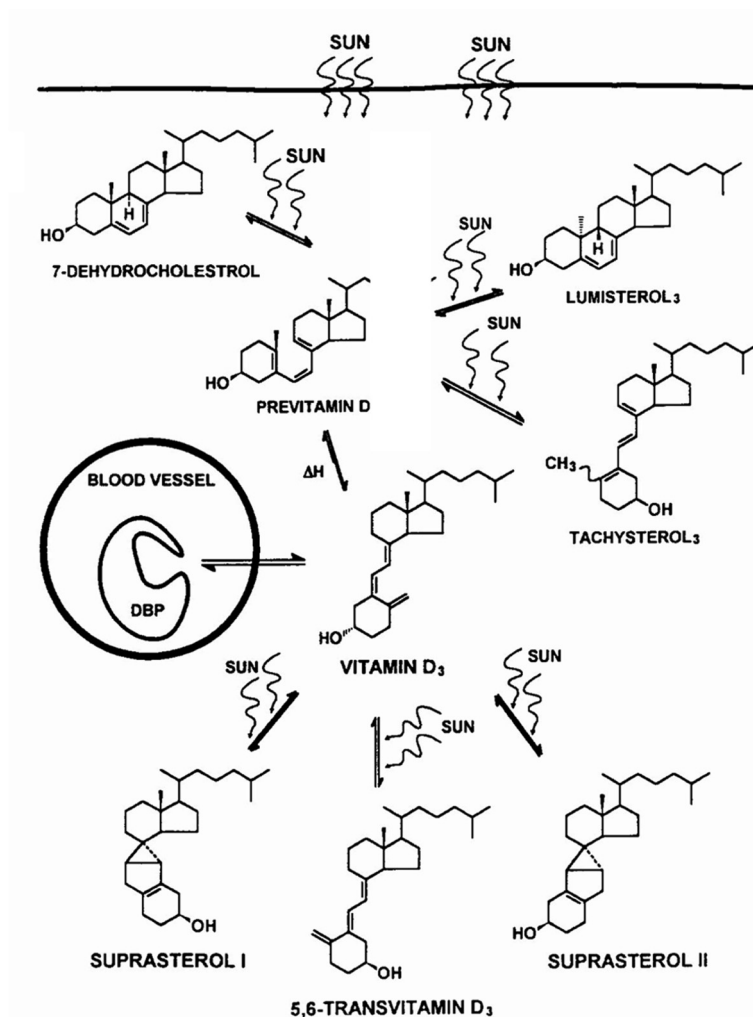


Fig. 4. Photosynthesis of vitamin D₃ from 7-dehydrocholesterol (pro-D₃ or 7-DHC), its photoisomerization and photodegradation, and the photoisomers of previtamin D₃ (Holick copyright 2006 with permission).

7-DHC molecule is near the polar head group of the membrane fatty acids and interacts with it through hydrophilic forces, while the nonpolar rings and side chain are associated with the nonpolar tail of the fatty acid by hydrophobic van der Waals interactions. Thus, when 7-DHC in the skin's plasma membrane is exposed to UVB radiation the thermodynamically less favorable conformation of the *s-cis*, *s-cis*-previtamin D₃ is preserved through hydrophobic and hydrophilic interactions with the bilayer lipid membrane fatty acids. Thus, a rotation around carbon 5 and carbon 6 to form the thermodynamically more stable *s-trans*, *c-cis* conformer is presented in the membrane and is only permissible in an organic solvent, such as hexane.

4.2. Conversion of Previtamin D₃ to Vitamin D₃

The isomerization of previtamin D₃ to vitamin D₃ is the last step in the synthesis of vitamin D₃ in human skin. The reaction rate of this isomerization is temperature dependent and is enhanced by raising the temperature. Earlier studies found that this process was not affected by acids, bases, catalysts, or inhibitors of radical chain processes (40). Furthermore, no intermediate was detected during the isomerization. This led to the conclusion that the reaction was an intramolecular concerted process involving a [1,7]-sigmatropic hydrogen rearrangement (40), which is an antarafacially (opposite side of a plane) allowed and a suprafacially (same side of a plane) forbidden process (41). Much of the information about the previtamin D₃ isomerization has been obtained from experiments using organic solvents for the conversion (42, 43) and assumed to be the same in human skin. There is no evidence for the existence of an enzymatic process in the skin that can convert previtamin D₃ to vitamin D₃. It is postulated that in an organic solvent, such as hexane, previtamin D₃ preferentially exists in a *s-trans*, *s-cis* conformation, which is thermodynamically more stable and cannot be easily converted to vitamin D₃. Therefore, it takes several days for the isomerization reaction between previtamin D₃ and vitamin D₃ to reach equilibrium at 37°C. However, previtamin D₃ in the skin is maintained in the *s-cis*, *s-cis* conformation, a conformation which greatly facilitates its conversion to vitamin D₃. Thus, instead of taking 30 h for 50% of previtamin D₃ to convert to vitamin D₃ at 37°C in hexane, it took only 2.5 h in the human skin at the same temperature. The results suggest that the interaction of previtamin D₃ with membrane fatty acids in skin is responsible for the efficient formation of vitamin D₃ in the skin. During the formation of vitamin D₃, the hydrophilic and hydrophobic interactions of the *s-cis*, *s-cis*-previtamin D₃ with the membrane fatty acids are disrupted, thereby facilitating the ejection of vitamin D₃ from the skin cell membrane into the extracellular space. By diffusion it enters the circulation by binding to the serum vitamin D binding protein (DBP). The removal of vitamin D₃ from the skin as it is being produced, thereby, changes the isomerization reaction from a reversible process to an irreversible process (34, 44). This would explain the relatively rapid rise in the serum vitamin D₃ concentration after UVB exposure.

A comparative study of the kinetic and thermodynamic properties of the isomerization reaction in human skin and in an organic solvent revealed that not only the equilibrium of the reaction was shifted in favor of vitamin D₃ synthesis in human skin (equilibrium constant K at 37°C = 11.44) compared to hexane (K = 6.15) but also the rate of the reaction was increased by more than 10-fold in human skin ($T_{1/2}$ at 37°C = 2.5 h) when compared to hexane ($T_{1/2}$ = 30 h) (44). This accelerated rate of isomerization was also observed in chicken, frog, and lizard skin (44, 45). The enthalpy (H°) change for the reaction was -21.58 and -15.60 kJ mol⁻¹ in human skin and in hexane, respectively. The activation energy for both the forward and the reverse reactions was lower in human skin than in hexane. Thus, human skin profoundly changed the rate constant and equilibrium constant in favor of vitamin D₃ formation.

The importance of membrane microenvironments on previtamin D₃ \rightleftharpoons vitamin D₃ isomerization received further support from a kinetic study in an aqueous solution of β -cyclodextrin (46). Cyclodextrins, a group of naturally occurring, truncated cone-shaped oligosaccharides, have a unique ability to complex a variety of foreign

molecules including steroids into their hydrophobic cavities in aqueous solution (47) and catalyze reactions of a wide variety of guest molecules (48). Among the various cyclodextrins, β -cyclodextrin has been shown to form 2:1 (host/guest) inclusion complexes with vitamin D₃ (49). Using this model, Tian and Holick demonstrated that, at 5°C, the forward (κ_1) and reversed (κ_2) rate constants for previtamin D₃ \rightleftharpoons vitamin D₃ isomerization were increased by more than 40 and 600 times, respectively, compared with those in *n*-hexane (46). The equilibrium constant of the reaction was significantly reduced by more than 12-fold when compared to that in *n*-hexane at 5°C, and the percentage of vitamin D₃ at equilibrium was increased as the temperature was increased. When complexed with β -cyclodextrin, the previtamin D₃ \rightleftharpoons vitamin D₃ isomerization became endothermic ($H^\circ = 13.05 \text{ kJ mol}^{-1}$) in contrast to being exothermic in *n*-hexane, suggesting that the thermodynamically unfavorable *s-cis*, *s-cis*-previtamin D₃ conformers are stabilized by β -cyclodextrin; and therefore, the rate of the isomerization is increased (46).

4.3. Translocation of Vitamin D₃ from the Skin into the Circulation

After vitamin D₃ is formed from the thermally induced isomerization of previtamin D₃ in the epidermis, it is transported into the dermal capillary bed beneath the dermoepidermal junction. Little is known about the mechanism of this translocation process. In an attempt to understand this event, Tian et al. studied the kinetics of vitamin D₃ formation and the time course of appearance of vitamin D₃ in the circulation after exposure of chicken to UVB radiation. Their data indicate a much faster rate of formation of vitamin D₃ from previtamin D₃ than the reverse reaction (return back to previtamin D₃ from vitamin D₃) and a relatively fast rate of translocation from skin to circulation (42). By examining the time course of appearance of vitamin D₃ in circulation, they found a rapid phase of vitamin D₃ appearance from 8 to about 30 h post-irradiation and a relatively slower phase of its disappearance after the circulating concentration of vitamin D₃ reached its peak. No previtamin D₃ could be detected 1 h after UVB irradiation. Thus, only vitamin D₃ is preferentially removed from skin into circulation, leaving behind previtamin D₃ in the epidermis for the continued thermoisomerization to vitamin D₃.

The role of DBP in the translocation of vitamin D₃ into circulation is implicated from the work of Haddad et al. (50). They investigated the transport of cutaneously synthesized vitamin D₃ into circulation in seven healthy volunteers who received whole-body irradiation with 27 mJ/cm² dosage of UVB light (290–320 nm) by comparing the time course distribution of plasma protein-bound vitamin D₃ in high- (>1.3 g/ml) and low-density (<1.3 g/ml) fractions after UVB irradiation. They found that plasma vitamin D concentration began to increase 10 h after irradiation, peaked at 24 h, and lasted for a week in the high-density layer where all the hDBP was present. When actin affinity chromatography was used to specifically bind DBP, it also removed vitamin D₃ from the plasma of irradiated subjects. These observations indicate that the endogenously photosynthesized vitamin D₃ circulates in serum almost exclusively on DBP, which differs from the orally administered vitamin D₂ (50), which is evenly distributed between the high- and low-density layers at 4, 8, and 24 h after the ingestion. Thus, DBP is important in the translocation of vitamin D from skin into the circulation.

4.4. Photodegradation of Vitamin D₃

Once vitamin D is formed in the skin from previtamin D₃, it is transported into the circulation by a diffusion process from the epidermis into the dermal capillary bed. It is believed that this process is prompted by its attraction to the DBP (34). If vitamin D₃ in the skin is exposed to sunlight prior to its transfer into the circulation, the triene system of vitamin D₃ structure will absorb solar UVR and photolyze to three major photoproducts, 5,6-*trans*-vitamin D₃, suprasterol 1, and suprasterol 2 (51). Exposure to as little as 10 min of sunlight in Boston in the summer resulted in the photodegradation of 30% of [³H]-vitamin D₃ in a test-tube model system (51). Likewise, exposure to 0.5, 1, and 3 h of the summer sunlight caused 50, 75, and 95% degradation of the original [³H]-vitamin D₃, respectively. Although winter sunlight in Boston does not promote vitamin D₃ synthesis, the longer wavelength UVR such as UVA present in the winter sunlight could potentially photodegrade vitamin D stores in the skin and in the circulation.

4.5. Photoisomers of Vitamin D₃

Previtamin D₃ photosynthesized in the skin can isomerize either to vitamin D₃ or to a variety of products, including tachysterol₃ and lumisterol₃. For example, during the first 10 min of simulated equatorial solar radiation, about 10–15% of the epidermal 7-DHC in Caucasian skin was converted to previtamin D₃ without any detectable amounts of tachysterol₃ or lumisterol₃ (52). After 1 h of exposure, 5 and 30% of the original 7-DHC was converted to tachysterol₃ and lumisterol₃, respectively, whereas the amount of previtamin D₃ remained at about 15%. The concentrations of lumisterol increased with increasing exposure times, reaching 60% by 8 h. When black skin was irradiated under the same conditions, longer exposure times were required to reach the maximal previtamin D₃ formation (52). Thus, it is the photochemical degradation of previtamin D₃ rather than melanin pigmentation that is most responsible for limiting the production of previtamin D₃ in human skin.

5. REGULATION OF THE CUTANEOUS SYNTHESIS OF PREVITAMIN D₃

5.1. Role of Melanin Pigmentation

In 1967, Loomis (53) suggested that melanin pigmentation evolved for protection from vitamin intoxication because of excessive exposure to sunlight. He further promoted the concept that as people migrated away from the equator, they gradually lost their skin pigmentation in order to synthesize adequate amounts of vitamin D₃ in their skin to maintain a healthy skeleton. However, it is unlikely that this is the major mechanism that prevents excessive vitamin D₃ formation in the skin. It is now recognized that sunlight itself is the most important factor for regulating the total production of vitamin D₃ in the skin. Sunlight can destroy any excess previtamin D₃ and vitamin D₃ in the skin (51).

It is well known that melanin effectively absorbs solar radiation from 290 to 700 nm, including the portion that is responsible for previtamin D₃ synthesis (290–315 nm).

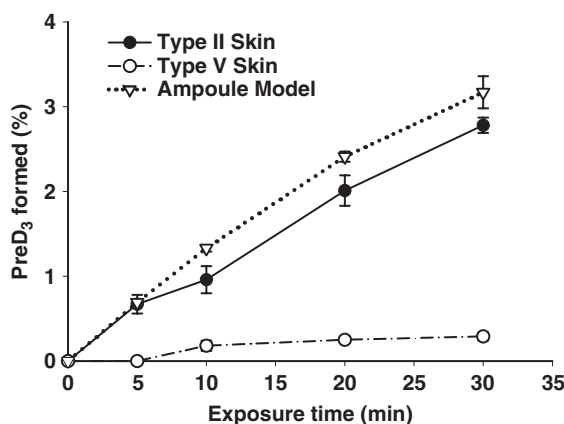


Fig. 5. The conversion of 7-dehydrocholesterol (pro-D₃ or 7-DHC) to previtamin D₃ in an ampoule model, type II and type V skin after exposing to noon sunlight in June at Boston (42°N), Massachusetts. The data represent the mean \pm SEM of duplicate determinations (Reproduced with permission from (56)).

Thus, melanin pigmentation can diminish the production of previtamin D₃ in the skin. The effects of skin pigmentation on previtamin D₃ synthesis in humans were studied in two in vitro models by exposing type II and type V skin along with ampoules containing 7-DHC solution to noon sunlight in June on a cloudless day in Boston, Massachusetts. It was observed that in June, $0.67 \pm 0.11\%$ of 7-DHC in epidermis was converted to previtamin D₃ in type II skin, but no detectable amount was found in type V skin samples after 5 min of sunlight exposure (Fig. 5). A small amount ($0.18 \pm 0.06\%$) of epidermal 7-DHC was converted to previtamin D₃ in type V skin after 10 min of sun exposure, whereas in the same time period, 0.95% of 7-DHC was converted to previtamin D₃ in type II skin. The synthesis of previtamin D₃ in epidermis continued to increase to 2.01 ± 0.18 and $2.78 \pm 0.09\%$ after 20 and 30 min sunlight exposure, respectively, in type II skin. Unlike type II skin, the conversion of epidermal 7-DHC to previtamin D₃ in type V skin samples only increased modestly to 0.25 ± 0.04 and $0.29 \pm 0.05\%$ after 20 and 30 min of exposure, respectively. The production of previtamin D₃ in the ampoule model (14) was about 0.4% more than that found in type II skin between 10 and 30 min of exposure.

Studies were performed to determine the effect of increased skin pigmentation on the cutaneous production of vitamin D₃ by measuring the circulating concentrations of vitamin D₃ in Caucasian, Indian, Pakistani, and Black volunteers (54, 55). Exposure of Caucasian subjects to 1.5 MED (0.054 J/cm^2) of UVR greatly increased serum vitamin D₃ concentration by more than 50-fold 24–28 h after the exposure, whereas the same dose had no effect on Black volunteers (Fig. 6) (54). However, when a 6-fold greater dose of UV radiation (0.32 J/cm^2) was applied to one of the Black volunteers, there was an increase in serum vitamin D₃. In this study, the time course of appearance and disappearance of vitamin D₃ was similar to that found in Caucasian volunteers, but that the peak concentration was still lower than those seen in Caucasian volunteers exposed to 0.054 J/cm^2 UV radiation by 40%. These results indicate that increased

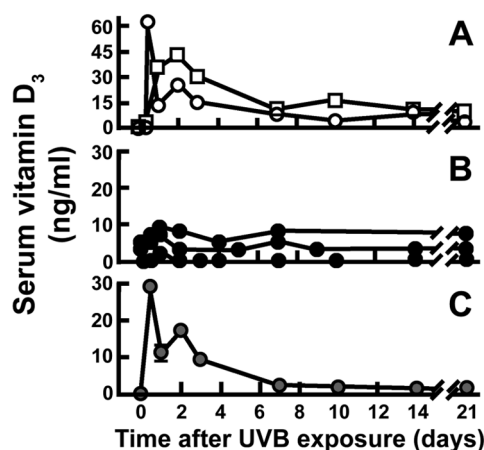


Fig. 6. Increase in serum vitamin D₃ after total-body exposure to 0.054 J/cm² of UVB of (a) two lightly pigmented Caucasians and (b) three heavily pigmented Blacks. (c) Serum level of vitamin D₃ in one black subject in panel b after exposure to a 0.32 J/cm² dose of UV radiation (Reproduced with permission from (54)).

skin pigmentation can greatly reduce the UVR-mediated synthesis of vitamin D₃. When people with intermediate skin color, such as Indians and Pakistanis, were exposed to 1.5 MED (0.073–0.2 J/cm²) of whole-body UVR, there was a similar time course of appearance and disappearance of serum vitamin D₃ as that seen in Caucasian controls exposed to 1.5 MED (0.046–0.072 J/cm²) of UVR (55). Thus, people with darker skin need longer exposure to UVR than fair-skinned people to photosynthesize the same amount of vitamin D. However, the capacity to produce vitamin D is no different among the groups.

The effect of skin pigmentation on the serum levels of 25(OH)D after UVB irradiation was investigated in volunteers with different skin types (56). Healthy volunteers with different skin types (II, III, IV, and V) were recruited in the beginning of winter. Based on the manufacturer recommendation each volunteer received a total of 0.75 MED (minimal erythema dose) in each session. To achieve his goal, the skin types II, III, IV, and V received an average of 6, 8, 11, and 12 min of UV irradiation during the first session, respectively. The sessions were held three times a week for 12 weeks for a total of 36 sessions. The blood was drawn for 25(OH)D measurement at the baseline visit before UVB exposure, during and at the conclusion of the study. Figure 7 demonstrates that UVB irradiation greatly increased the serum 25(OH)D in all skin types. The percentage increase in the 25(OH)D level at the end of the study for skin type II, III, IV, and V were 210 ± 53 , 187 ± 64 , 125 ± 55 , and 40%, respectively.

5.2. Influence of Altitude, Latitude, Time of Day, and Weather Conditions on Previtamin D₃ Production

It was first observed in 1897 by Kassowitz that incidence of rickets was markedly increased during the winter months and decreased during summer and fall (57). Later, Schmorl (58) demonstrated from his autopsy studies that the highest incidence of rickets

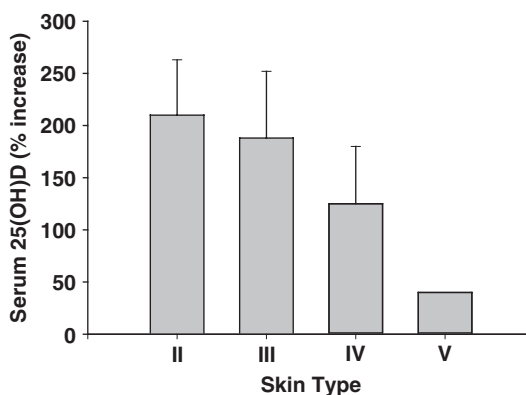


Fig. 7. The serum 25(OH)D levels in volunteers with different skin types after weekly exposure to simulated sunlight for 12 weeks (0.75 MED per session). The data represent the mean \pm SEM of 4–5 volunteers for skin type II, III, and IV. A single determination was obtained for skin type IV (Reproduced with permission from (56)).

occurred between November and May. Hanseman (59) also noted that almost all children who were born in the autumn and died in the spring showed marked manifestations of rickets while those born in the spring and during the summer were essentially free of the disease. During the winter months in the Northern hemisphere, people wear more clothing and go outdoors less often that prevents their skin from direct exposure to sunlight (60). Furthermore, the zenith angle of the sun is increased in the winter, and the amount of UVB radiation reaching the earth's surface is significantly diminished because most of it is absorbed by the stratospheric ozone layer. Therefore, the amount of solar UVB radiation reaching the biosphere is a function of the solar zenith angle and depends on latitude, season, and time of day (61, 62). Several studies were conducted to determine the effect of latitude, season, and time of day on the cutaneous production of previtamin D₃ (61–63). A more comprehensive study was conducted utilizing a model consisting of 7-DHC solution (50 μ g/ml ethanol) sealed in borosilicated glass ampoules. On a cloudless day, the ampoules were exposed to sunlight on an hourly basis throughout the day once a month in seven cities located in both the Northern and Southern hemispheres. Because of the absorption properties of human skin and the effect of skin pigmentation on the absorption of solar UVB radiation, the results obtained from the ampoules represented the maximal conversion of 7-DHC to previtamin D₃. In order to correlate the synthesis of previtamin D₃ in ampoules with human skin, 1 cm² of Caucasian skin samples with skin type III (64), which sometimes burns and always tans, were exposed to simulated sunlight along with the ampoules containing 50 μ g 7-DHC in 1 ml of absolute alcohol. It was found that 0.8% of 7-DHC was converted to previtamin D₃ in the ampoules before any previtamin D₃ appeared in the skin samples. Based on this correlation, we have estimated the capacity of type III skin to synthesize vitamin D₃ from natural sunlight each month at seven locations with different latitudes in both the Northern and Southern hemispheres. It was found that following exposure to noon sunlight for 1 h, previtamin D₃ formation was negligible during the months of December through February in Boston, USA (42°N); from November through March in Edmonton,

Canada (52°N); and from October through March in Bergen, Norway (61°N). Similarly, people with the same skin type cannot synthesize previtamin D₃ from April through September in Ushuaia, Argentina (55°S). However, individuals with this skin type can synthesize previtamin D₃ year round in Cape Town, South Africa (35°S); Johannesburg, South Africa (26°S); and Buenos Aires, Argentina (34°S) (Fig. 8). Thus, previtamin D₃ synthesis increased from the spring to summer months and decreased thereafter. These changes are reflected in the seasonal variation of serum 25(OH)D₃ concentrations in children and adults (65–68). Correlation studies between the synthesis of previtamin D₃ in ampoules and in skin type V samples indicated that 1.8% of 7-DHC was converted to previtamin D₃ in ampoules before any previtamin D₃ could be detected in the skin samples. Therefore, individuals with skin type V would require a considerably longer period of sun exposure to make the same amount of vitamin D in their skin compared to those with skin type III.

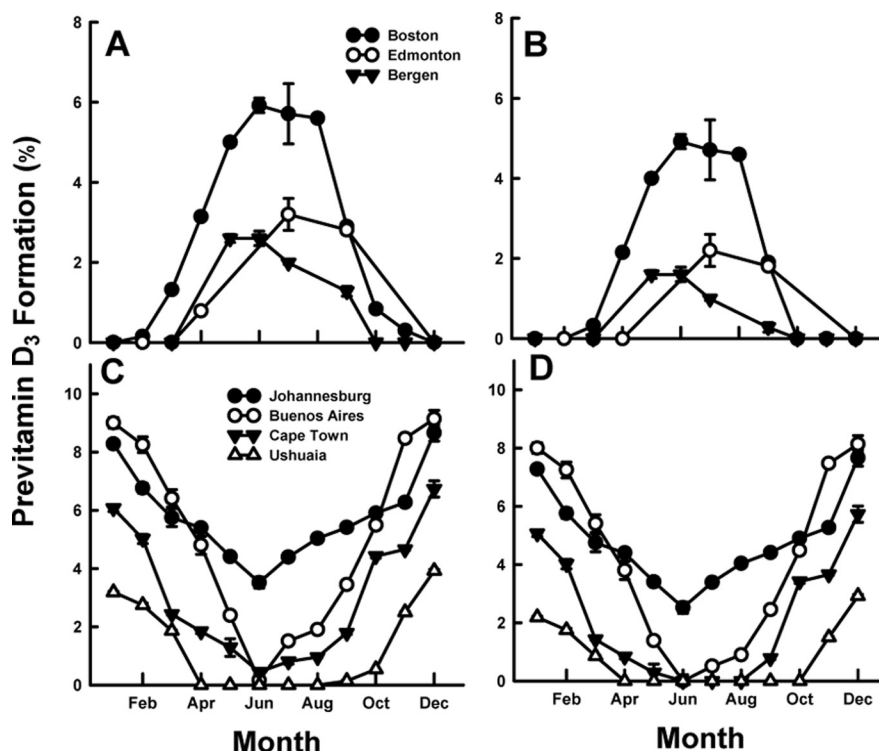


Fig. 8. Influence of season and latitude on the synthesis of previtamin D₃ in Blacks (skin type V) and Caucasians (skin type II). **Panels a** and **c** represent the calculated formation of previtamin D₃ in the Northern and Southern hemispheres in the skin of Caucasians and **panels b** and **d** represent the calculated formation of previtamin D₃ in the Northern and Southern hemispheres in the skin of Blacks. The cities in the Northern hemisphere include Boston (42°N), Edmonton (52°N), and Bergen (61°N), and the cities in the Southern hemisphere are Johannesburg (26°S), Buenos Aires (34°S), Cape Town (35°S), and Ushuaia (55°S). The data represent the means ± SEM of duplicate determinations. It should be noted that the data for May and June in Edmonton are missing (Copyright Chen and Holick, 2009 with permission).

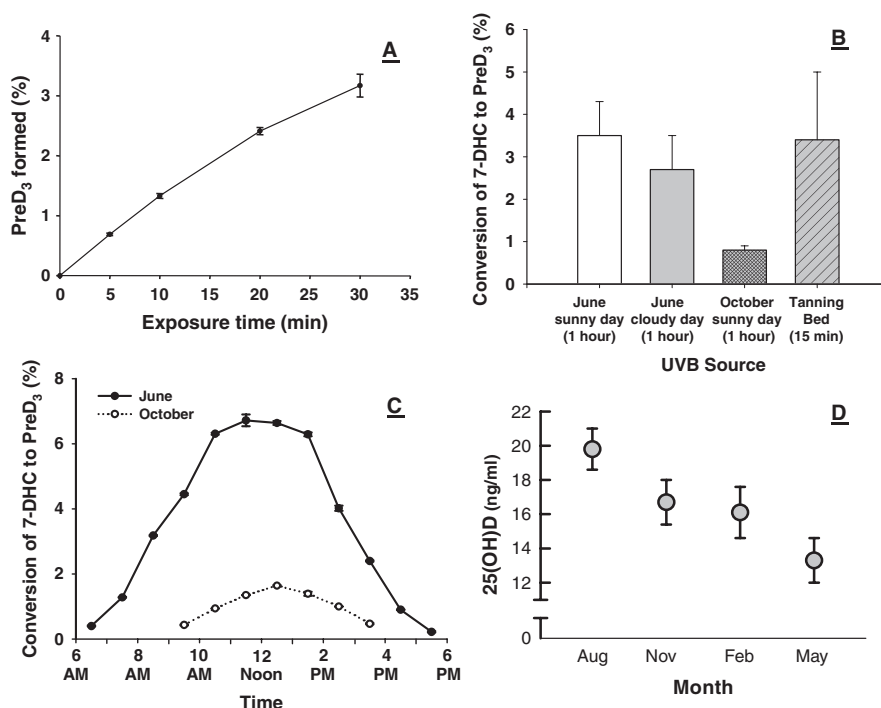


Fig. 9. (a) Time-dependent formation of previtamin D₃ in ampoules containing 7-DHC in ethanol after exposure to sunlight at noon in June in Boston. (b) A comparison of previtamin D₃ production after exposure to sunlight in June on a sunny day and cloudy day for 1 h, on a sunny day in October for 1 h, and tanning bed for 15 min. (c) Conversion of 7-DHC to previtamin D₃ at various times throughout the day in June and in October on a sunny day in Boston. (d) Circulating levels of 25(OH)D in healthy free-living nursing home residents at various seasons of the year (Reproduced with permission from (69)).

The time-dependent conversion of 7-DHC to previtamin D₃ was also studied at noon time in June in Boston (Fig. 9). The figure indicates that after 5 min, ~0.8% of 7-DHC was converted to previtamin D₃, and by 35 min, ~3.3% of 7-DHC was photolyzed to previtamin D₃ and its photoproducts (Fig. 9a). This showed that previtamin D₃ production occurred when 7-DHC was exposed to sunlight and that the efficiency of conversion was almost linear as a function of time over a period of 30 min. Because the zenith angle during a day is dependent on the time of a day and is much more oblique in the early morning and late afternoon that will result in a longer path length for the solar UVB photons to pass through, it is important to evaluate the effect of time of day on previtamin D₃ synthesis. As shown in Fig. 9c, greater than 6% of 7-DHC was converted to previtamin D₃ in June in Boston, whereas no previtamin D₃ production was observed before 7:00 a.m. or after 6:00 p.m. on the same day. The previtamin D₃ production between the hours of 8:00 and 10:00 a.m. and 4:00 and 6:00 p.m. was <20% of that produced at noon time. The figure also shows that much less previtamin D₃ was produced in October even at noon time; there was an 80% reduction in the efficiency of conversion of 7-DHC

to previtamin D₃ at noon time compared to noon time in June. Likewise, the length of no-conversion periods was found to be longer in the morning and in the afternoon in October comparing to June. The length of these periods will also increase in people with higher degrees of skin pigmentation (53).

When the efficiency of 7-DHC conversion to previtamin D₃ on a cloudy day was compared to a cloudless day, the efficiency was reduced by about 20%. Furthermore, the conversion efficiency on a cloudless day in June for 1 h is equivalent to that exposed to tanning bed radiation for 15 min (~ 30 mJ/cm²) (Fig. 9b).

Recently, we investigated the effects of altitude on the production of previtamin D₃ in Nepal, India, and Mount Everest using an ampoule model (69). The ampoules containing 7-DHC in ethanol and sealed under argon were exposed to sunlight on cloudless days at 27°N in Nepal and India during the last week of October and during the first 2 weeks of November, 2006. The lowest altitude was in Agra at 169 m and the highest altitude was at Mount Everest base camp at 5,350 m. After exposure for 1 h between 11:30 a.m. and 12:30 p.m., the samples were stored in the dark before they were evaluated by high-performance liquid chromatography for the conversion of 7-DHC to previtamin D₃ and its other photoproducts, including lumisterol and tachysterol. A dramatic influence of altitude on the synthesis of previtamin D₃ and its photoproducts at the same latitude of 27°N during the period of October (last 2 weeks) and November (first 2 weeks) was observed (Fig. 10). In Agra (169 m) and Katmandu (1,400 m), about 0.5% of 7-DHC was converted to previtamin D₃ and its photoproducts. There was an almost linear increase in the production of previtamin D₃ and its photoproducts with increasing altitude that was about 400% higher at the base camp of Everest at 5,350 m compared to Agra at 169 m.

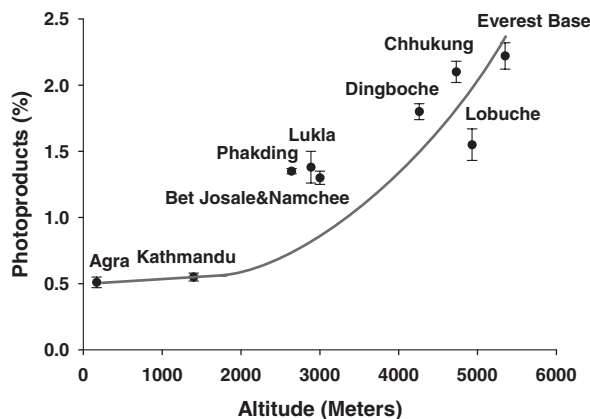


Fig. 10. Effect of altitudes on the production of previtamin D₃. Ampoules containing 7-DHC in ethanol were exposed for 1 h between 11:30 a.m. and 12:30 p.m. at 27°N in India and Nepal at various altitudes (Reproduced with permission from (69)).

5.3. Effect of Aging on the Cutaneous Production of Previtamin D₃

Abundant studies have suggested that osteomalacia caused by vitamin D deficiency is becoming an epidemic in Asia, Europe, and the United States (70–76). It has been

estimated that about 30–40% of elderly people with hip fracture in the United States and Great Britain are vitamin D deficient. Lamberg-Allard reported that circulating concentrations of 25(OH)D were low in long-stay geriatric patients and in residents at the old peoples home due to lack of exposure to sunlight and low vitamin D intake (76). Similarly, a study conducted at a nursing home in the Boston area demonstrated that approximately 60% of the nursing home residents were vitamin D deficient during the winter months (77). Even near the end of the summer we found that in the Boston area 30, 43, and 80% of the free-living elderly Caucasian, Hispanic, and Black subjects, respectively, had 25(OH)D levels of less than 20 ng/ml.

Furthermore, Lester et al. (68) reported that elderly subjects had lower circulating levels of 25(OH)D than healthy young adults. These data suggested that aging decreased the capacity of human skin to produce vitamin D₃. It has been known that skin thickness decreases linearly with age after the age of 20 years (78). This is correlated with an age-dependent decrease in the epidermal concentrations of 7-DHC (79). Exposure of human skin samples from various age groups to simulated sunlight showed that the skin from 8- and 18-year-old subjects produced 2- to 3-fold greater amounts of previtamin D₃ than the skin from 77- and 82-year-old subjects. Whole-body exposure of healthy young and older adults to the same amount of UV radiation confirmed the *in vitro* finding. When the circulating concentrations of vitamin D were determined before and at various times after the exposure, the young volunteers (age range 22–30 years) raised their circulating concentrations of vitamin D₃ to a maximum of 30 ng/ml within 24 h while the elderly subjects (62–80 years) were able to reach a maximum concentration of only 8 ng/ml (80). In one study, we exposed 7-DHC in ethanol in an ampoule to tanning bed irradiation (MedSun by Wolff System Technology Corporation, Atlanta, GA.) for 10 min. We observed a linear increase of 7-DHC conversion to previtamin D₃ from about 1% at 1 min to about 10% at 10 min (Fig. 11 a). We also determined the circulating concentrations of 25(OH)D in 15 healthy young adults (skin types II and III, 20–53 year) after most of their body were exposed to the same tanning bed three times a week (0.75 MED per week) for 7 weeks (total radiation was approximately 4 MED for the period). After 1 week, there was a 50% increase in 25(OH)D that continued to increase for 5 weeks before reaching a plateau of about 150% above baseline values (Fig. 11b). Using the same tanning bed, we also exposed a 76-year-old male volunteer to 0.75 MED three times a week for 7 weeks. His serum 25(OH)D level increased from 29 to 47 ng/ml after 7 weeks of exposure. However, unlike the young adults (Fig. 11b), his serum 25(OH)D reached plateau with about 60% increase after 2 weeks of exposure (Fig. 11c). Because there was no significant increase in this subject's skin pigment throughout the study, it is likely that the photochemical synthesis and degradation of vitamin D₃ might have reached equilibrium after 2 weeks.

5.4. Effect of Sunscreen Use and Clothing on Previtamin D₃ Formation

There is a great concern about the damaging effects of chronic exposure to sunlight on the skin. Long-term exposure to sunlight can cause dry wrinkled skin as well as increase risk of non-melanoma skin cancer such as squamous cell and basal cell carcinoma. As a result of this concern, there has been a major effort by dermatologists to encourage

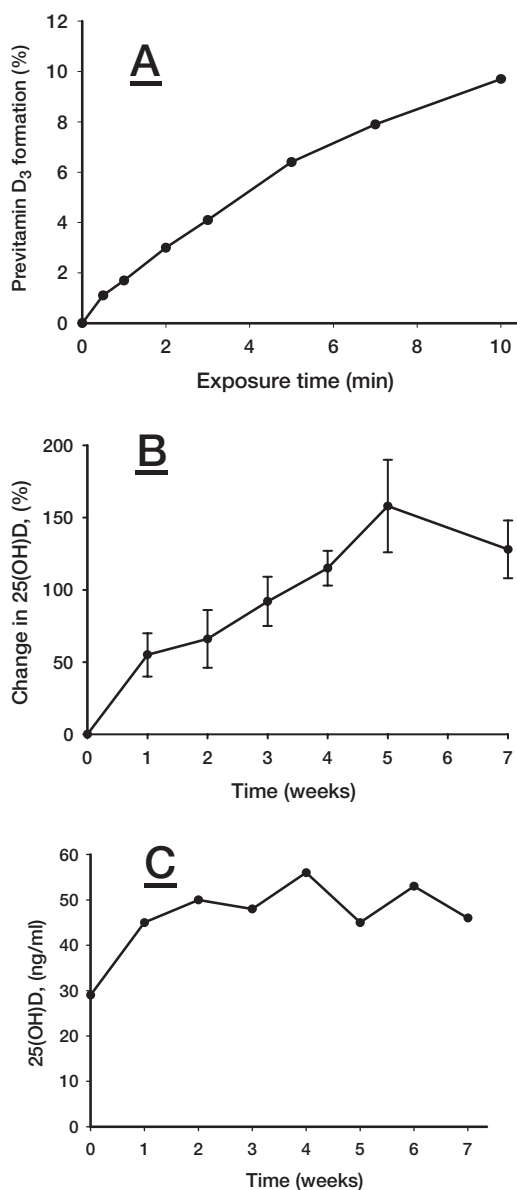


Fig. 11. Production of previtamin D₃ and serum level of 25(OH)D after the exposure of 7-DHC solution in ampoules and human volunteers to a tanning bed lamp. **(a)** Ampoules containing 7-DHC were placed and exposed to a tanning bed lamp. At various times, an ampoule was removed and the conversion of 7-DHC to previtamin D₃ was measured by HPLC. **(b)** Healthy adults were exposed to 0.75 MED in a tanning bed three times a week for 7 weeks. Circulating concentrations of 25(OH)D were determined at baseline and once a week thereafter. **(c)** A healthy 76-year-old man was exposed to tanning bed radiation equivalent to 0.75 MED three times a week for 7 weeks. His circulating concentrations of 25(OH)D were obtained at weekly intervals (Reproduced with permission from (69)).

people to apply sunscreens on their body before going outdoors (81). There is no question of the benefit of sunscreen use for the protection of the skin from the damaging effects of sunlight. However, the radiation that is responsible for causing sunburn, skin wrinkling, and skin cancer is the same radiation that is responsible for producing previtamin D₃. Thus, the sunscreen use can also prevent the beneficial effect of sunlight, the photoconversion of 7-DHC to previtamin D₃ (82, 83). Matsuoka et al. studied the effects of *para*-aminobenzoic acid (PABA) on previtamin D₃ formation in skin samples in vitro and on the cutaneous vitamin D synthesis in vivo. They found that 5% PABA solution totally blocked the photoisomerization of 7-DHC to previtamin D₃ in human skin specimens. When the volunteers who were applied with 5% PABA, which had a sun protection factor of 8, did not raise their circulating concentrations of vitamin D₃ after exposure to 1 MED UVR. The same dose of UVR increased the serum level of vitamin D₃ 10-fold in control subjects (without PABA) 24 h after exposure. Therefore, any UVB blocking agent that prevents the damaging effects of sunlight will also prevent the cutaneous production of previtamin D₃. The impact of chronic sunscreen use on the vitamin D status of the elderly has been evaluated (83). It was found that the 25(OH)D concentrations in serum of long-term PABA users were significantly lower than the non-users. Among the 19 long-term PABA users, 4 had borderline to overt vitamin D deficiency based on low circulating concentrations of 25(OH)D.

Clothing also blocks transmission of nonvisible UV radiation (60, 84). Robson and Diffey (84) tested 60 different fabrics and observed that the nature and type of the textures and the structure of the fabric affect the sun protection factor. For example, a polyester blouse would have a SPF-2, while cotton twill jeans would have a SPF of 1,000 or complete protection against extreme sunlight exposures. Matsuoka and her colleagues examined the effect of the commonly used fabrics including cotton, wool, and polyester in black and white colors, on UVB transmission properties; the photo-production of previtamin D₃ from 7-DHC; and the elevation of serum vitamin D₃ after irradiation with 1 MED of UVB in volunteers wearing jogging garments made of these fabrics (60). They found that direct transmission of UVB was attenuated the most by black wool and the least by white cotton. None of the fabrics allowed the conversion of 7-DHC to previtamin D₃ after irradiation with up to 40 min of simulated sunlight or the increase in serum vitamin D₃ in volunteers. Increasing the whole-body irradiation dose to 6 MEDs still failed to elevate their serum vitamin D₃ levels in garment-clad subjects. It was concluded that clothing significantly impairs the formation of vitamin D₃ after 6 MEDs of UVB photostimulation. They also studied the effect of regular (seasonal) street clothing on serum vitamin D₃ response to whole-body irradiation with suberythematous dose of UVB (27 mJ/cm²) radiation in seven healthy subjects. They found that summer clothing partially and autumn clothing totally prevented an elevation of the vitamin D₃ in response to UVB radiation 24 h later (60).

5.5. Influence of Season on 25(OH)D Levels in Nursing Home and Home Care Elderly

The influence of season on vitamin D production in ampoule model was confirmed with a study conducted in a nursing home in Boston (69, 85). Forty-five residents in this

nursing home, who were taking a multivitamin that contained 400 IU of vitamin D₂, showed a dramatic decline in their 25(OH)D levels from the end of the summer to the beginning of the following summer (Fig. 9d). Based on the new definition of vitamin D deficiency [25(OH)D <20 ng/ml], 49, 67, 74, and 78% of the nursing home residents were vitamin D deficient in August, November, February, and May, respectively, as the mean serum 25(OH)D levels declined. Results obtained from free-living White elders cared for by Boston Medical Center also showed similar seasonal effects. Among the 69 White patients, 62 and 40% had serum 25(OH)D concentrations less than 20 ng/ml during February/March and September/October, respectively. In the same study, no significant difference in vitamin D deficiency was observed between February/March and September/October in 46 Black patients.

5.6. Tanning Bed Irradiation Enhances Vitamin D Status and Bone Mineral Density

In patients with intestinal disease, resection, or bypass, there is impaired absorption of both vitamin D and calcium. Therefore, patients with short-bowel syndrome and malabsorption are prone to vitamin D deficiency and nutritional osteomalacia. One alternative to improve their vitamin D nutrition is through cutaneous synthesis of vitamin D. We reported that a 57-year-old woman with a long history of Crohn's disease and short-bowel syndrome who had only 2 ft of small intestine remaining after three bowel resections remained to be vitamin D deficient (serum 25(OH)D, <20 ng/ml) after 36 months of treatment with a daily 400 IU of oral vitamin D supplement and 200 IU of vitamin D through TPN infusion (86). She was then exposed to UVB radiation in a tanning bed wearing a one-piece bathing suit for 10 min, three times a week for 6 months. After 4 weeks of exposure, her serum 25(OH)D level increased by 357% from 7 to 32 ng/ml, parathyroid hormone level decreased by 52% from 92 to 44 pg/ml, and the serum calcium level increased from 7.8 to 8.5 mg/dl (Fig. 12). After 6 months of UVB, her serum 25(OH)D level was maintained above 30 ng/ml and was free of muscle weakness and bone and muscle pain, those are common symptoms due to vitamin D deficiency. In another study, two short-bowel syndrome patients were irradiated twice a week for 8 weeks (6 min each session) with a commercial portable UV indoor tanning lamp that has a spectral output that mimics natural sunlight (87). These two patients were able to maintain their serum 25(OH)D levels during the winter month. In a follow-up study, an increased frequency of UV lamp exposure (five times per week for 8 weeks, 5–10 min each session depending on the skin type) was applied to five cystic fibrosis patients at their lower backs in a seated position (87). Their serum 25(OH)D levels increased from 21 ± 3 ng/ml at the baseline to 27 ± 4 ng/ml at the end of 8 weeks of exposure ($p = 0.05$).

Tangpricha et al. (88) investigated the serum levels of 25(OH)D and bone mineral density of subjects who regularly used a tanning bed and compared those biomarkers to subjects who did not use a tanning bed. They found that the subjects who used a tanning bed had serum 25(OH)D concentrations 90% higher than those of non-user group (46.2 ± 3.2 and 24.12 ± 1.2 ng/ml, respectively; $p < 0.001$). In addition, tanning bed users had lower PTH levels compared to the tanning bed non-users (21.4 ± 1.0 and

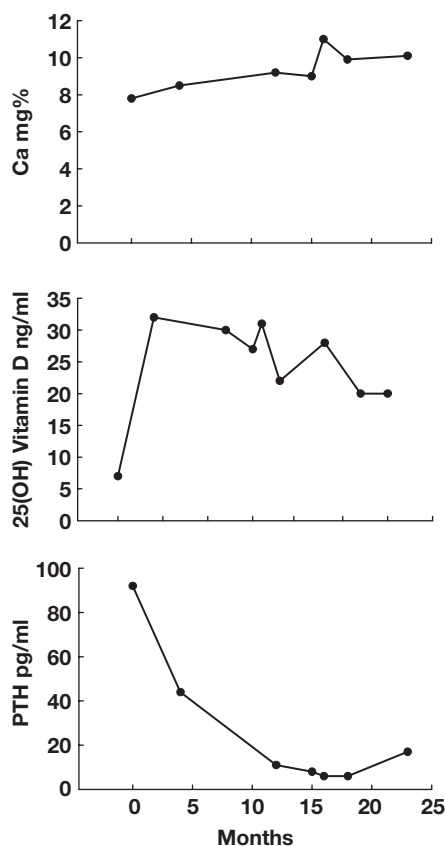


Fig. 12. Serum calcium, 25(OH)D, and PTH levels in a patient with Crohn's disease who had whole-body UVB exposure for 10 min, three times a week for 6 months (Reproduced with permission from (86)).

25.3 ± 0.8 pg/ml, respectively; $p = 0.01$). Consequently, tanners had significantly higher bone mineral density and z scores at the total hip than did nontanners.

6. SUMMARY

Vitamin D deficiency is synonymous with rickets. The initial description of the bone-deforming disease, rickets, in the mid-seventeenth century by two English physicians, Francis Glisson and Daniel Whistler, led to the observation by Sniadecki in 1822 that lack of sunlight exposure was likely a cause of rickets and the discovery of vitamin D a century later by Elmer McCollum in 1922. It is well established that most vertebrates, including amphibians, birds, reptiles, and primates, depend on sun exposure for their vitamin D requirement, because very few foods naturally contain vitamin D.

The first step leading to the cutaneous synthesis of vitamin D₃ is the absorption of solar UVB photons with energies 290–315 nm by 7-DHC in the skin that leads to the opening of its C-9 and C-10 bond and the formation of previtamin D₃. Previtamin D₃

then undergoes a rapid thermoisomerization within the plasma membrane to form vitamin D₃. However, excessive exposure to sunlight will not result in vitamin D intoxication, because both previtamin D₃ and vitamin D₃ can be photolyzed to noncalcemic or inactive photoproducts. During the winter, there is a minimal previtamin D₃ production in the skin at latitudes above 35°N. Altitude also significantly influences vitamin D₃ production. For example, in November at 27°N, very little previtamin D₃ synthesis was detected in Agra (169 m) and Katmandu (1,400 m). Production of previtamin D₃ increased 2- and 4-fold at 3,400 m and at Everest base camp (5,300 m), respectively. In addition, increased skin pigmentation, application of a sunscreen, aging, and clothing have a dramatic effect on previtamin D₃ production in the skin. It is estimated that exposure in one-piece bathing suit to 1 MED is equivalent to ingesting between 10,000 and 25,000 IU of vitamin D₂. The importance of sunlight exposure for providing most humans with their vitamin D requirement is well documented by the seasonal variation in circulating levels of 25(OH)D.

Vitamin D deficiency [serum 25(OH)D < 20 ng/ml] is common in infant, children, and adults worldwide. Exposure to simulated sunlamps that produce UVB radiation is an excellent source for producing vitamin D₃ in the skin and is especially efficacious in patients with fat malabsorption syndromes. The major cause of vitamin D deficiency globally is an underappreciation of the crucial role of sunlight in providing humans with their vitamin D requirement. The association regarding increased risk of common deadly cancers, autoimmune diseases, infectious diseases, and cardiovascular disease with living at higher latitudes and being prone to vitamin D deficiency should alert all health-care professionals about the importance of vitamin D for overall health and well-being.

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REFERENCES

1. DeLuca HF (2008) Evolution of our understanding of vitamin D. *Nutr Rev* 66(10 Suppl 2):S73–S87
2. Holick MF (2008) Vitamin D: a D-Lightful health perspective. *Nutr Rev* 66(10 Suppl 2):S182–S194
3. Holick MF (2008) The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. *Mol Aspects Med* 29(6):361–368
4. Sniadecki J (1939) Cited by W Mozolowski: “Jedrzej Sniadecki (1768–1883) on the cure of rickets.”. *Nature* 143:121
5. Holick MF (1990) Vitamin D and the skin: photobiology, physiology and therapeutic efficacy for psoriasis. In: Heersche JNM and Kanis JA (eds) *Bone and mineral research*, vol 7. Elsevier, Amsterdam, pp 313–366
6. Mellanby T (1918) The part played by an ‘accessory factor’ in the production of experimental rickets. *J Physiol* 52:11–14
7. McCollum EF, Simmonds N, Becker JE, Shipley PG (1922) Studies on experimental rickets and experimental demonstration of the existence of a vitamin which promotes calcium deposition. *J Biol Chem* 53:293–312

8. Huldschinsky K (1919) Heilung von Rachitis durch kunstliche Honensonne. Dtsch Med Wochenschr 45:712–713
9. Hess AF, Unger LJ (1921) Cure of infantile rickets by sunlight. JAMA 77:39
10. Goldblatt H, Soames KN (1924) A study of rats on a normal diet irradiated daily by the mercury vapor quartz lamp or kept in darkness. Biochem J 17:294–297
11. Goldblatt H (1924) A study of the relation of the quantity of fat-soluble organic factor in the diet to the degree of calcification of the bones and the development of experimental rickets in rats. Biochem J 17:298–326
12. Hess AF, Weinstock M (1924) Antirachitic properties imparted to inert fluids and green vegetables by ultraviolet irradiation. J Biol Chem 62:301–313
13. Steenbock H, Black A (1924) The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. J Biol Chem 61:408–422
14. Steenbock H (1924) The induction of growth-promoting and calcifying properties in a ration by exposure to light. Science 60:224–225
15. Stamp TC, Walker PG, Perry W, Jenkins MV (1980) Nutritional osteomalacia and late rickets in greater London, 1974–1979: clinical and metabolic studies in 45 patients. Clin Endocrinol Metab 9: 81–105
16. Marksted T, Halvorsen S, Halvorsen KS et al (1984) Plasma concentrations of vitamin D metabolites before and during treatment of vitamin D deficiency rickets in children. Acta Paediatr Scand 73: 225–231
17. Tanner JT, Smith J, Defibaugh P et al (1988) Survey of vitamin content of fortified milk. J Assoc Off Anal Chem 71:607–610
18. Holick MF, Shao Q, Liu WW, Chen TC (1992) The vitamin D content of fortified milk and infant formula. N Engl J Med 326:1178–1181
19. Chen TC, Shao Q, Heath H, Holick MF (1993) An update on the vitamin D content of fortified milk from the United states and Canada. N Eng J Med 329:1507
20. Fieser LD, Fieser M (1959) Vitamin D. In: Steroids. Reinhold, New York, pp 90–168
21. Windaus A, Bock F (1937) Uber das provitamin aus dem sterin der schweineschwarte. Hoppe-Seylers Z Physiol Chem 245:168
22. Rauschkolb EW, Winston D, Fenimore DC, Black HS, Fabre LF (1969) Identification of vitamin D₃ in human skin. J Invest Dermatol 53:289–293
23. Okano T, Yasumura M, Mizuno K, Kobayashi T (1977) Photochemical conversion of 7-dehydrocholesterol into vitamin D₃ in rat skins. J Nutr Sci Vitaminol (Tokyo) 23:165–168
24. Esvelt RR, Schnoes HK, DeLuca HF (1978) Vitamin D₃ from rat skins irradiated in vitro with ultraviolet light. Arch Biochem Biophys 188:282–286
25. Pask-Hughes PA, Calam DH (1982) Determination of vitamin D₃ in cod-liver oil by high performance liquid chromatography. J Chromatogr 246:95–104
26. Muller-Mulot VW, Rohrer G, Schwarzbauer K (1979) Zur auffindung naturilich vorkommender vitamin D₃-ester im lebertran chemische bestimmung des freien, verester-ten und gesamt-vitamin D₃. Fette Seifen Anstrichm 81:38–40
27. St Lezin MA. Phylogenetic occurrence of vitamin D and provitamin D sterols. M.S. Dissertation, MIT Press, Cambridge, MA, 1983.
28. Koch EM, Koch FC (1941) The provitamin D of the covering tissues of chickens. J Poult Sci 20:33
29. Wheatley RH, Sher DW (1969) Studies of the lipids of dog skin. J Invest Dermatol 36:169–170
30. Kenny DE, Irlbeck NA, Chen TC, Lu Z, Holick MF (1998) Determination of vitamins D, A and E in sera and vitamin D in milk from captive and free-ranging polar bears (*Ursus maritimus*) and 7-dehydrocholesterol levels in skin from captive polar bears. Zoo Biol 17:285–293.
31. Morris JG (1997) Ineffective vitamin D synthesis of vitamin D in kittens exposed to sun and ultraviolet light is reversed by an inhibitor of 7-dehydrocholesterol- Δ^7 -reductase. In: Norman AW, Bouillon R, Thomasset M (eds) Proceedings of the tenth workshop on vitamin D, Strasbourg, France, 24–29 May, 1997, 721–722.
32. Velluz L, Petit A, Amiard G (1948) Sur un stage non photochimique dans la formation des calciferols: essais d'interpretation. Bull Soc Chim Fr 15:1115–1120

33. Velluz L, Amiard G, Petit A (1949) Le precalciferol-ses relations d'équilibre avec le calciferol. *Bull Soc Chim Fr* 16:501–508
34. Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts JT Jr, Anderson RR, Blank IH, Parrish JA, Elias P (1980) Photosynthesis of vitamin D₃ in human skin and its physiologic consequences. *Science* 210:203–205
35. Bunker JWM, Harris RS, Mosher ML (1940) Relative efficiency of active wavelengths of ultraviolet light in activation of 7-dehydrocholesterol. *J Am Chem Soc* 62:508–511
36. Kobayashi T, Yasumara M (1973) Studies on the ultraviolet irradiation of previtamin D and its related compounds effect of wavelength on the formation of potential vitamin D₂ in the irradiation of ergosterol by monochromatic ultraviolet rays. *J Nutr Sci Vitamino* 119:123–128
37. MacLaughlin JA, Anderson RR, Holick MF (1982) Spectral character of sunlight modulates photosynthesis of previtamin D₃ and its photoisomers in human skin. *Science* 216:1001–1003
38. Anderson RR, Parrish JA (1982) Optical properties of human skin. In: Regan JD, Parrish JA (eds) *The science of photomedicine*. Plenum Press, New York, pp 147–194
39. MacLaughlin JA, Holick MF (1983) Photobiology of vitamin D in the skin. In: Goldsmith LA (ed) *Biochemistry and physiology of the skin*. Oxford University Press, New York, pp 734–754.
40. Havinga E (1973) Vitamin D, example and challenge. *Experientia* 29:1181–1193
41. Woodward RB, Hoffmann R (1965) Selection rules for sigmatropic reactions. *J Am Chem Soc* 87:2511–2513
42. Buisman JA, Hanewald KH, Mulder FJ, Roborgh JR, Keuning KJ (1968) Evaluation of the effect of isomerization on the chemical and biological assay of vitamin D. *Analysis of fat-soluble vitamins X. J Pharm Sci* 57:1326–1329.
43. Tian XQ, Chen TC, Matsuoka LY, Wortsman J, Holick MF (1993) Kinetic and thermodynamic studies of the conversion of previtamin D₃ to vitamin D₃ in human skin. *J Biol Chem* 268: 14888–14892
44. Tian XQ, Chen TC, Lu Z, Shao Q, Holick MF (1994) Characterization of the translocation process of vitamin D₃ from the skin into the circulation. *Endocrinology* 135:655–661
45. Holick MF, Tian XQ, Allen M (1995) Evolutionary importance for the membrane enhancement of the production of vitamin D₃ in the skin of poikilothermic animals. *Proc Natl Acad Sci USA* 92: 3124–3126
46. Tian X, Holick MF (1995) Catalyzed thermal isomerization between previtamin D₃ and vitamin D₃ via β -cyclodextrin complexation. *J Biol Chem* 270:8706–8711
47. Albers E, Muller BW (1992) Complexation of steroid hormones with cyclodextrin derivatives: substituent effects of the guest molecule on solubility and stability in aqueous solution. *Pharm Sci* 81: 756–761
48. Chen ET, Pardue HL (1993) Analytical applications of catalytic properties of modified cyclodextrins. *Anal Chem* 65:2563–2567
49. Bogoslovsky NA, Kurganov BI, Samochvalova NG, Isaeva TA, Sugrobova NP, Gurevich VM, Valashek IE, Samochvalov GI (1988) Vitamin D: molecular, cellular and clinical endocrinology. *Walter de Gruyter & Co, Berlin*, pp 1021–1023
50. Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J (1993) Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* 91:2552–2555
51. Webb AR, de Costa B, Holick MF (1989) Sunlight regulates the cutaneous production of vitamin D₃ by causing its photodegradation. *J Clin Endocrinol Metab* 68:882–887
52. Holick MF, MacLaughlin JA, Dopplet SH (1981) Regulation of cutaneous previtamin D₃ photosynthesis in man: skin pigment is not an essential regulator. *Science* 211:590–593
53. Loomis F (1967) Skin-pigment regulation of vitamin D biosynthesis in man. *Science* 157:501–506
54. Clemens TL, Henderson SL, Adams JS, Holick MF (1982) Increased skin pigment reduces the capacity of skin to synthesize vitamin D₃. *Lancet* 1:74–76
55. Lo C, Paris PW, Holick MF (1986) Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to ultraviolet irradiation. *Am J Clin Nutr* 44:683–685
56. Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, Kohn N, Martinello S, Berkowitz R, Holick MF (2007) Factors that influence the cutaneous synthesis and dietary sources of vitamin D. *Arch Biochem Biophys* 460:213–217

57. Kassowitz M (1897) Tetanie and autointoxication in kindersalter. *Wien Med Presse* XXXViii 97:139
58. Schmorl G (1909) Die pathologische anatomie der rachitischen knochenerkrankung mit besonderer berucksichtigung ihrer histologie und pathogenese. *Ergeb d Inn Med Kinderheilkd* IV 403
59. Hansemann D (1906) Einfluss der domestikation auf die entstehung der krankheiten. *Berl Klin Wochenschr* XLiii 629:670
60. Matsuoka LY, Wortsman J, Dannenberg MJ, Hollis B, Lu Z, Holick MF (1992) Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D₃. *J Clin Endocrinol Metab* 75: 1099–1103
61. Webb AR, Kline L, Holick MF (1988) 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. *J Clin Endocrinol Metab* 67:373–378
62. Lu Z, Chen TC, Holick MF (1992) Influence of season and time of day on the synthesis of vitamin D₃. In: Holick MF and Kligman AM (eds) *Biological effects of light*. Walter de Gruyter, Berlin/New York, pp 57–61.
63. Ladizesky M, Lu Z, Oliver B, Roman NS, Diaz S, Holick MF, Mautalen C (1995) Solar ultraviolet B radiation and photoproduction of vitamin D₃ in Central and Southern areas of Argentina. *J Bone Miner Res* 10:545–549
64. Fitzpatrick TB (1988) The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 124:869–871
65. Ala-Houhala M, Parviainen MT, Pyykko K, Visakorpi JK (1984) Serum 25-hydroxyvitamin D levels in Finnish children aged 2–17 years. *Acta Paediatr Scand* 73:232–236
66. Oliveri MB, Ladizesky M, Mautalen CA, Alonso A, Martinez L (1993) Seasonal variations of 25-hydroxyvitamin D and parathyroid hormone in Ushuaia (Argentina), the southernmost city of the world. *Bone Miner* 20:99–108
67. Sherman SS, Hollis BW, Tobin JD (1990) Vitamin D status and related parameters in a healthy population: the effects of age, sex and season. *J Clin Endocrinol Metab* 71:405–413
68. Lester E, Skinner RK, Wills MR (1977) Seasonal variation in serum 25-hydroxyvitamin D in the elderly in Britain. *Lancet* i:979–980
69. Holick MF, Chen TC, Lu Z, Sauter E (2007) Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res Supply* 2:V28–V33
70. Chalmers J, Conacher DH, Gardner DL, Scott PJ (1967) Osteomalacia- a common disease in elderly women. *J Bone Joint Surg (Br)* 49B:403–423
71. Jenkins DH, Roberts JG, Webster D, Williams EO (1973) Osteomalacia in elderly patients with fracture of the femoral neck. *J Bone Joint Surg (Br)* 55B:575–580
72. Doppelt SH, Neer RM, Daly M, Bourret L, Schiller A, Holick MF (1983) Vitamin D deficiency and osteomalacia in patients with hip fractures. *Orthop Trans* 7:512–513
73. Sokoloff L (1978) Occult osteomalacia in American patients with fracture of the hip. *Am J Surg Pathol* 2:21–30
74. Whitelaw GP, Abramowitz AJ, Kavookjian H, Holick MF (1991) Fractures and vitamin D deficiency in the elderly. *Complications Ortho* 6:70–80
75. Omdahl JL, Garry PJ, Hunsaker LA, Junt WC, Goodwin JS (1982) Nutritional status in a healthy elderly population: vitamin. *Am J Clin Nutr* 36:1225–1233
76. Lamberg-Allard T (1984) Vitamin D intake, sunlight exposure, and 25-hydroxyvitamin D levels in elderly during one year. *Ann Nutr Metab* 28:144–150
77. Webb AR, Pilbeam C, Hanafin N, Holick MF (1990) An evaluation of the relative contributions of exposure to sunlight and diet on the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. *Am J Clin Nutr* 51:1075–1081
78. Tan CY, Strathum B, Marks R (1982) Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability. *Br J Dermatol* 106:657–667
79. MacLaughlin JA, Holick MF (1985) Aging decreases the capacity of human skin to produce vitamin D₃. *J Clin Invest* 76:1536–1538
80. Holick MF, Matsuoka LY, Wortsman J (1989) Age, vitamin D, and solar ultraviolet radiation. *Lancet* ii:1104–1105
81. Gilchrest BA (1993) Sunscreens – a public health opportunity. *N Engl J Med* 329(16):1193–1194

82. Matsuoko L, Ide L, Wortsman J, MacLaughlin JA, Holick MF (1987) Sunscreens suppress cutaneous vitamin D₃ synthesis. *J Clin Endocrinol Metab* 64:1165–1168
83. Matsuoko LY, Wortsman J, Hanifan N, Holick MF (1988) Chronic sunscreen use decreases circulating concentrations of 25-hydroxyvitamin D: a preliminary study. *Arch Dermatol* 124:1802–1804
84. Robson J, Diffey BL (1990) Textiles and sun protection. *Photodermatol Photoimmunol Photomed* 7:32–34
85. Tangpricha V, Pearce EN, Lu Z, Mathieu JS, Flanagan JN, Chen TC, Holick MF (2002) Vitamin D insufficiency among free-living young healthy adults. *Am J Med* 112:659–662
86. Koutkia P, Lu Z, Chen TC, Holick MF (2001) Treatment of vitamin D deficiency due to Crohn's disease with tanning bed ultraviolet B radiation. *Gastroenterology* 121:1485–1488
87. Chandra P, Wolfenden LL, Ziegler TR, Tian J, Luo M, Stecenko AA, Chen TC, Holick MF, Tangpricha V (2007) Treatment of vitamin D deficiency with UV light in patients with malabsorption syndromes: a case series. *Photodermato, Photoimmunol Photomed* 23:179–185
88. Tangpricha V, Turner A, Spina C, Decastro S, Chen TC, Holick MF (2004) Tanning is associated with optimal vitamin D status (serum 25-hydroxyvitamin D concentration) and higher bone mineral density. *Am J Clin Nutr* 80:1645–1649
89. Gamgee KML (1927) The artificial light treatment of children in rickets, anaemia & malnutrition. P.B. Hoeber Inc., New York, 172pp.