



Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease¹⁻⁴

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ABSTRACT

Most humans depend on sun exposure to satisfy their requirements for vitamin D. Solar ultraviolet B photons are absorbed by 7-dehydrocholesterol in the skin, leading to its transformation to previtamin D₃, which is rapidly converted to vitamin D₃. Season, latitude, time of day, skin pigmentation, aging, sunscreen use, and glass all influence the cutaneous production of vitamin D₃. Once formed, vitamin D₃ is metabolized in the liver to 25-hydroxyvitamin D₃ and then in the kidney to its biologically active form, 1,25-dihydroxyvitamin D₃. Vitamin D deficiency is an unrecognized epidemic among both children and adults in the United States. Vitamin D deficiency not only causes rickets among children but also precipitates and exacerbates osteoporosis among adults and causes the painful bone disease osteomalacia. Vitamin D deficiency has been associated with increased risks of deadly cancers, cardiovascular disease, multiple sclerosis, rheumatoid arthritis, and type 1 diabetes mellitus. Maintaining blood concentrations of 25-hydroxyvitamin D above 80 nmol/L (~30 ng/mL) not only is important for maximizing intestinal calcium absorption but also may be important for providing the extrarenal 1 α -hydroxylase that is present in most tissues to produce 1,25-dihydroxyvitamin D₃. Although chronic excessive exposure to sunlight increases the risk of nonmelanoma skin cancer, the avoidance of all direct sun exposure increases the risk of vitamin D deficiency, which can have serious consequences. Monitoring serum 25-hydroxyvitamin D concentrations yearly should help reveal vitamin D deficiencies. Sensible sun exposure (usually 5–10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3 times per week) and increased dietary and supplemental vitamin D intakes are reasonable approaches to guarantee vitamin D sufficiency. *Am J Clin Nutr* 2004;80(suppl):1678S–88S.

KEY WORDS Vitamin D, sunlight, cancer, diabetes, bone

INTRODUCTION

Vitamin D is taken for granted and is assumed to be plentiful in a healthy diet. Unfortunately, very few foods naturally contain vitamin D, and only a few foods are fortified with vitamin D. This is the reason why vitamin D deficiency has become epidemic for all age groups in the United States and Europe. Vitamin D deficiency not only causes metabolic bone disease among children and adults but also may increase the risk of many common chronic diseases. The goal of this review is to provide a broad prospective on the evaluation, evolution, discovery, and many biological functions of vitamin D.

HISTORY

Vitamin D is recognized as the sunshine vitamin. From an evolutionary perspective, phytoplankton and zooplankton that have existed in our oceans for > 500 million years produced vitamin D when exposed to sunlight (1, 2). Although the function of vitamin D in these early life forms is not well understood, it is possible that the vitamin D photosynthetic process was used by early life forms to provide the organism with information about exposure to solar ultraviolet (UV)B (290–315 nm) radiation (1, 2).

The precursor provitamin D (either ergosterol or 7-dehydrocholesterol), which is a relatively rigid, 4-ringed structure, is incorporated into the lipid bilayer of the plasma membrane (**Figure 1**). During the production of previtamin D during exposure to solar UVB radiation, the B ring opens and becomes a less-rigid open structure, which may provide the membrane with increased permeability to various ions, including calcium. This may be why vitamin D has remained so important, throughout evolution, for the maintenance of calcium metabolism and ultimately for the evolution of life forms to develop endoskeletons and to venture onto land (1).

The earliest cave paintings indicate that humans appreciated not only the warmth but also the life-giving properties of the sun. The first evidence of the importance of sunlight for human health began with the industrial revolution in northern Europe. People began congregating in cities and living in dwellings that were built in close proximity to each other. The burning of coal and wood polluted the atmosphere and, as a result, children living in these industrialized cities had little direct exposure to sunlight. Glissen, DeBoot, and Whistler recognized that children living in the inner cities throughout Europe demonstrated growth retardation and developed skeletal deformities, including bony projections along the rib cage (rachitic rosary) and either bowed legs or knocked knees (**Figure 2**); they called this disease rickets (1–3). The disease migrated to the northeastern United States, where children in New York City and Boston were raised in a similar polluted sunless environment. By 1900, the disease was

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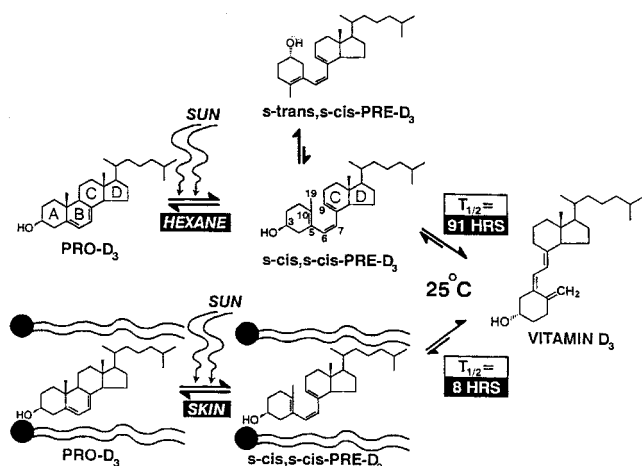


FIGURE 1. Photolysis of provitamin D₃ (pro-D₃) to previtamin D₃ (pre-D₃) and thermal isomerization of vitamin D₃ in hexane and in lizard skin. In hexane, provitamin D₃ is photolyzed to *S-cis,S-cis*-previtamin D₃. Once formed, this energetically unstable conformation undergoes a conformational change to *S-trans,S-cis*-previtamin D₃. Only *S-cis,S-cis*-previtamin D₃ can undergo thermal isomerization to vitamin D₃. The *S-cis,S-cis*-conformer of provitamin D₃ is stabilized in the phospholipid bilayer through hydrophilic interactions between the 3 β -hydroxyl group and the polar head group of the lipids, as well as through van der Waals interactions between the steroid ring and side-chain structure and the hydrophobic tail of the lipids. These interactions significantly decrease conversion of the *S-cis,S-cis*-conformer to the *S-trans,S-cis*-conformer, thus facilitating the thermal isomerization of *S-cis,S-cis*-previtamin D₃ to vitamin D₃. Reproduced with permission from *Proc Natl Acad Sci USA* 1995; 92:3124–6. Copyright 1995 National Academy of Sciences, U.S.A.

so common and devastating that it was estimated that > 90% of children in Leiden, the Netherlands, and 80% of children in Boston suffered from rickets (2, 3).

In 1822, Sniadecki (4) published his clinical observations that children who lived in the inner city in Warsaw had a high prevalence of this disease, whereas children who lived in the rural areas outside Warsaw did not. He hypothesized that it was lack of adequate sun exposure that was most responsible for the development of rickets among children. In 1890, Palm (5) followed up on this observation with his own account of recognizing that children who lived in the industrialized cities of Great Britain were at high risk of developing rickets, whereas his colleagues and friends who wrote to him from India and China noted that children who had poor nutrition and lived in squalor were free of this disease. Palm concluded that it was critically important to recognize that sunbathing could prevent rickets and that some type of sunshine recorder should be developed to measure the bone-healing properties of the sun.

In 1919, Huldschinsky (6) exposed children with rickets to a mercury arc lamp and reported the dramatic healing of rickets. In 1921, Hess and Unger (7) observed children in New York City who were exposed to sunlight on the roof of the hospital for a period of several months, and they noted effective treatment of rickets. These findings led Steenbock and Black (8) and Hess and Weinstock (9) to irradiate a wide variety of substances, including grasses and various vegetable oils; they reported that the irradiation process imparted antirachitic activity to the foods. Hess et al (10) also demonstrated that sun exposure prevented rickets in rats. This led Steenbock (11) to introduce the concept of irradiating foods with UV radiation for the treatment and prevention of rickets. Milk was initially fortified with ergosterol and irradiated



FIGURE 2. Typical presentation of 2 children with rickets. The child in the middle is normal; the children on both sides have severe muscle weakness and bone deformities, including bowed legs (right) and knock knees (left).

for antirachitic activity. This led to the fortification of milk with synthetically produced vitamin D₂. This simple fortification process essentially eradicated rickets in countries that adopted this practice. In the 1930s, vitamin D was the new miracle vitamin and many products were fortified with vitamin D₂, including peanut butter, hot dogs, soda pop, and bread. Schlitz Brewery (Milwaukee, WI) introduced beer containing vitamin D₂ (100 IU or 2.5 μ g per 8-ounce can) and marketed it as the beer with sunny energy in both summer and winter. Europe also fortified dairy products with vitamin D₂. After World War II, however, the vitamin D fortification process was not carefully monitored and large excess amounts of vitamin D were added to some milk products, causing an outbreak of vitamin D intoxication among infants and young children (12, 13). This led to the banning of vitamin D fortification of dairy products in most European countries that remains to this day. In Europe, margarine and some cereals are fortified with vitamin D.

PHOTOSYNTHESIS OF VITAMIN D

During exposure to sunlight, UVB radiation (290–315 nm) is absorbed by 7-dehydrocholesterol that is present in the plasma membranes of both epidermal keratinocytes and dermal fibroblasts (14). The energy is absorbed by the double bonds in the B ring, which results in rearrangement of the double bonds and opening of the B ring to form previtamin D₃ (Figure 1).

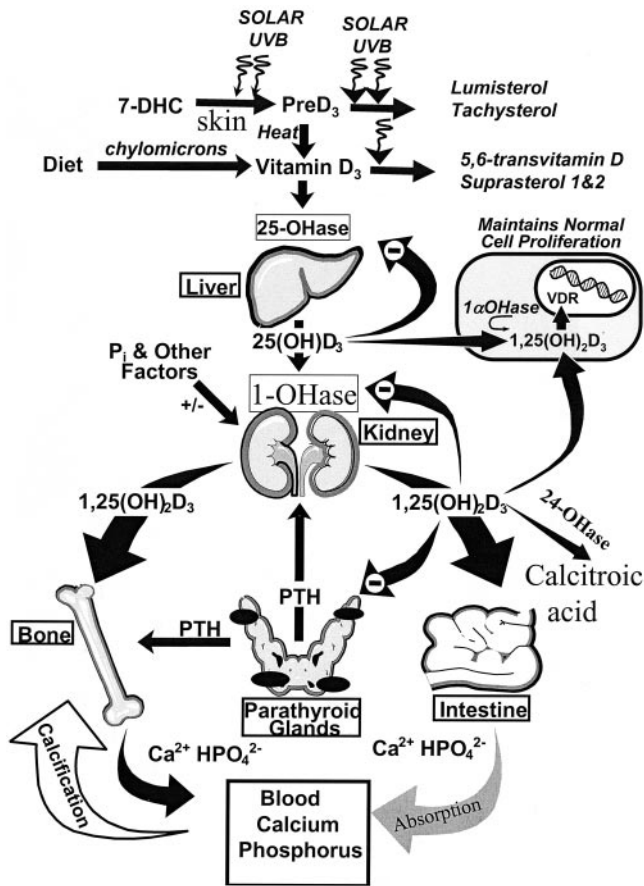
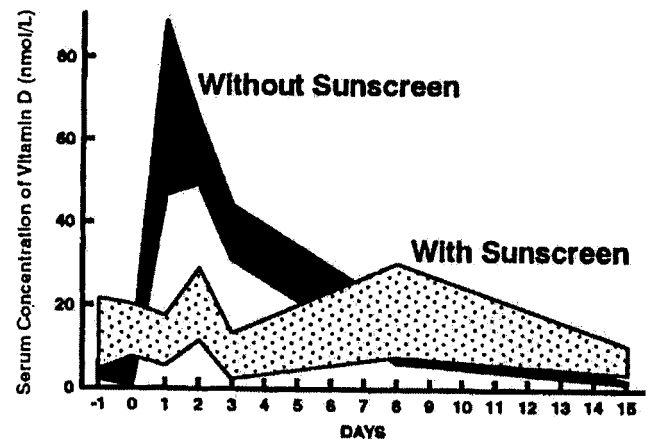


FIGURE 3. Schematic diagram of cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth. During exposure to sunlight, 7-dehydrocholesterol (7-DHC) in the skin absorbs solar UVB radiation and is converted to previtamin D₃ (preD₃). Once formed, previtamin D₃ undergoes thermally induced transformation to vitamin D₃. Additional exposure to sunlight converts previtamin D₃ and vitamin D₃ to biologically inert photoproducts. Vitamin D originating from the diet or from the skin enters the circulation and is metabolized to 25(OH)D₃ in the liver by vitamin D 25-hydroxylase (25-OHase). 25(OH)D₃ reenters the circulation and is converted to 1,25(OH)₂D₃ in the kidney by 25(OH)D₃ 1α-hydroxylase (1-OHase). A variety of factors, including serum phosphorus (P_i) and PTH, regulate the renal production of 1,25(OH)₂D₃. 1,25(OH)₂D₃ regulates calcium metabolism through interactions with its major target tissues, ie, bone and intestine. 1,25(OH)₂D₃ also induces its own destruction by enhancing the expression of 25(OH)D 24-hydroxylase (24-OHase). 25(OH)D is metabolized in other tissues for regulation of cellular growth.

Once formed, previtamin D₃, which is entrapped within the plasma membrane lipid bilayer, rapidly undergoes rearrangement of its double bonds to form the more thermodynamically stable vitamin D₃. During this transformation process, vitamin D₃ is ejected from the plasma membrane into the extracellular space (14). The vitamin D-binding protein in the dermal capillary bed has an affinity for vitamin D₃ (14–16) and draws it into the circulation.

Prolonged sun exposure does not result in the production of excess quantities of vitamin D₃ to cause intoxication. The reason for this is that, during sun exposure, the previtamin D₃ that is formed and the thermal isomerization product vitamin D₃ that does not escape into the circulation absorb solar UV radiation and isomerize to several photoproducts that are thought to have little activity on calcium metabolism (2, 3, 14, 15) (Figure 3).

A



B

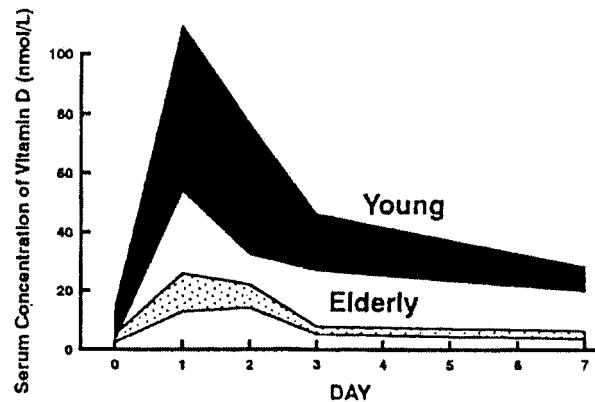


FIGURE 4. A: Circulating concentrations of vitamin D₃ after a single exposure to 1 MED of simulated sunlight, with a sunscreen (SPF 8) or a topical placebo cream. B: Circulating concentrations of vitamin D in response to whole-body exposure to 1 MED among healthy young and elderly subjects. Reproduced with permission from reference 3.

FACTORS THAT ALTER THE CUTANEOUS PRODUCTION OF VITAMIN D₃

Anything that either influences the number of solar UVB photons that penetrate the skin or alters the amount of 7-dehydrocholesterol in the skin influences the cutaneous production of vitamin D₃. The amount of 7-dehydrocholesterol in the epidermis is relatively constant until later in life, when it begins to decline (17, 18). A person 70 years of age exposed to the same amount of sunlight as a 20-year-old person can make ~25% of the vitamin D₃ that the 20-year-old person can make (Figure 4).

Melanin evolved as an effective natural sunscreen. Because it efficiently absorbs UVB photons, people with increased skin



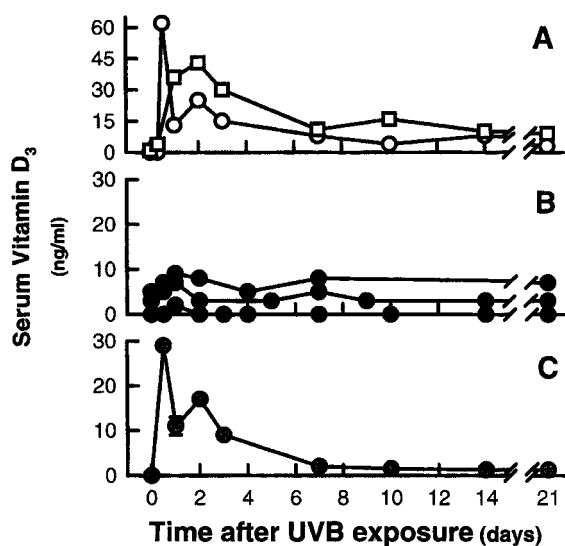


FIGURE 5. A and B: Changes in serum concentrations of vitamin D among 2 lightly pigmented white subjects (skin type II) (A) and 3 heavily pigmented black subjects (skin type V) (B) after total-body exposure to UVB radiation (54 mJ/cm^2). C: Serial changes in circulating vitamin D concentrations after reexposure of one black subject in B to a 320 mJ/cm^2 dose of UVB radiation. Reproduced with permission from Elsevier (*The Lancet* 1982;1:74–6).

melanin pigmentation require longer exposures to sunlight to make the same amount of vitamin D₃, compared with light-skinned people. For example, a young adult with skin type III (always burns, always tans) who was exposed to 1 minimal erythemal dose (MED) of 54 mJ/cm^2 exhibited a 50-fold increase in blood concentrations of vitamin D₃ within 8 h, whereas an adult of the same age with skin type V (an African American who never burns and always tans) who was exposed to 54 mJ/cm^2 did not exhibit any significant increase in circulating concentrations of vitamin D₃. The adult with skin type V required 5–10 times the exposure and exhibited only a 30-fold increase in the blood concentration of vitamin D₃, to $\sim 30 \text{ ng/mL}$ (Figure 5) (19).

Sunscreens work by absorbing UVB radiation and some UVA (321–400 nm) radiation before it enters the skin. Therefore, it is not surprising that a sunscreen with a sun protection factor (SPF) of 8 reduces the capacity of the skin to produce vitamin D₃ by $> 95\%$ (Figure 4) (20); properly used sunscreen with a SPF of 15 reduces the capacity by $> 98\%$.

Time of day, season, and latitude also dramatically influence the cutaneous production of vitamin D₃ (2, 3, 21). The reason is that, although the sun is closest to the earth in the winter, the sun's rays are entering at a more oblique angle (zenith angle) and more UVB photons are efficiently absorbed by the ozone layer, because the more oblique angle causes the UVB photons to pass through the ozone for a greater distance. In addition, with the more oblique angle there are fewer photons per unit area striking the earth. Time of day, season, and latitude all influence the zenith angle of the sun. Above 37° latitude during the months of November through February, there are marked decreases (~ 80 – 100% , depending on latitude) in the number of UVB photons reaching the earth's surface. Therefore, very little if any vitamin D₃ is produced in the skin during the winter.

However, below 37° and closer to the equator, more vitamin D₃ synthesis occurs in the skin throughout the year. Similarly, in the early morning or late afternoon, the zenith angle is so oblique

that very little if any vitamin D₃ is produced in the skin even in the summer (2, 3, 21, 22). This is why it is important to have safe sun exposure between the hours of 1000 and 1500 in the spring, summer, and autumn, because this is the only time when enough UVB photons reach the earth's surface to produce vitamin D₃ in the skin (2, 3, 21, 22).

Vitamin D₃ is fat soluble and is stored in the body fat. Any excess vitamin D₃ that is produced during exposure to sunlight can be stored in the body fat and used during the winter, when little vitamin D₃ is produced in the skin. We recently determined that there was 4–400 ng/g vitamin D₂ and vitamin D₃ in abdominal fat obtained from obese patients undergoing gastric bypass surgery. Therefore, for obese individuals, the fat can be an irreversible sink for vitamin D, increasing the risk of vitamin D deficiency (23, 24). We observed that, when we gave nonobese and obese subjects a 50 000 IU dose of vitamin D₂ orally or exposed them to simulated sunlight in a tanning bed for the same periods of time, the obese subjects exhibited increases in blood vitamin D concentrations of no more than 50%, compared with nonobese individuals (24).

SOURCES OF VITAMIN D

Very few foods naturally contain vitamin D. Oily fish such as salmon (360 IU per 3.5-ounce serving), mackerel, and sardines are good sources of vitamin D₃, as are irradiated mushrooms. Although egg yolks are reported to contain vitamin D, amounts are highly variable (usually no more than 50 IU per yolk), and the cholesterol content of egg yolks makes this a poor source of vitamin D. Cod liver oil, which has been considered for > 3 centuries to be critically important for bone health, is an excellent source of vitamin D₃. Very few foods are fortified with vitamin D. Fortified foods include milk (100 IU per 8-ounce serving), orange juice (100 IU per 8-ounce serving) and other juice products, and some breads and cereals (22, 25).

More than 90% of the vitamin D requirement for most people comes from casual exposure to sunlight (2, 3, 22). The skin has a large capacity to produce vitamin D. Young adults exposed to 1 MED of UVB radiation in a tanning bed underwent measurement of their blood vitamin D₃ concentrations, compared with their blood vitamin D₂ concentrations after an oral dose of vitamin D₂. Men and women in bathing suits who were exposed to a 1-MED dose of UVB radiation exhibited increases in blood concentrations of vitamin D that were equivalent to those observed with doses of 10 000–20 000 IU of vitamin D (2, 22, 26). Therefore, 1 MED is equivalent to ~ 10 – 50 times the recommended adequate intakes, which are 200, 400, and 600 IU for children and adults < 50 y, 51–70 y, and ≥ 71 y of age, respectively (2, 22, 26, 27). Studies reported that exposure of $\sim 20\%$ of the body's surface to either direct sunlight or tanning bed radiation was effective in increasing blood concentrations of vitamin D₃ and 25-hydroxyvitamin D₃ [25(OH)D₃] among both young adults and older adults (28–33). Indeed, Chuck et al (33) suggested that the use of UVB lamps in nursing homes in Great Britain was the most effective means of maintaining blood concentrations of 25(OH)D. There appears to be a benefit of higher blood concentrations of 25(OH)D for bone health, because the bone density of teenagers and adults was directly related to their 25(OH)D concentrations (30, 32–35). We found that tanners in Boston had 25(OH)D concentrations ($\sim 100 \text{ nmol/L}$) that were $> 150\%$ higher than those of nontanners ($\sim 40 \text{ nmol/L}$) at the end of the

winter. Furthermore, the average bone density of the tanners was greater than that of the nontanners (32).

SKIN CANCER, SUNLIGHT, AND VITAMIN D

There is great concern about any exposure to sunlight causing skin damage, including skin cancer and wrinkling (35–40). Chronic excessive exposure to sunlight and sunburn incidents during childhood and young adult life significantly increase the risk of nonmelanoma basal and squamous cell carcinomas (36–39).

The most serious form of skin cancer is melanoma. It should be recognized that most melanomas occur on non—sun-exposed areas (41) and that having more sunburn experiences, having more moles, and having red hair increase the risk of the deadly disease (36).

Chronic excessive sun exposure also damages the elastic structure of the skin, increasing the risk of wrinkling (35). However, on the basis of our understanding of the efficiency of sun exposure for producing vitamin D₃ in the skin, it is reasonable to allow some sun exposure without sun protection, for production of adequate amounts of vitamin D₃. When adults topically applied a sunscreen properly (2 mg/cm², ie, ~1 ounce over an adult body wearing a bathing suit), the amount of vitamin D₃ produced in the skin was reduced by > 95% (2, 3, 22, 40). Exposure to sunlight for 5–15 min between the hours of 1000 and 1500 during the spring, summer, and autumn is usually enough exposure for individuals with skin type II or III (2, 3, 22, 42–44). This is ~25% of what would cause a minimal erythematous response, ie, a slight pinkness to the skin. After this exposure, application of a sunscreen with a SPF of ≥ 15 is recommended, to prevent the damaging effects of chronic excessive exposure to sunlight.

PREVALENCE AND DETECTION OF VITAMIN D DEFICIENCY

The only way to determine whether a person is vitamin D (vitamin D represents either vitamin D₂ or vitamin D₃) sufficient, deficient, or intoxicated is to measure the circulating concentrations of 25(OH)D (2, 3, 22). 25(OH)D, which is produced in the liver, is the major circulating form of vitamin D (Figure 3). Its half-life in the circulation is ~2 wk, and it is a measure of vitamin D status. Although 25(OH)D requires additional hydroxylation in the kidney to become active as 1,25-dihydroxyvitamin D [1,25(OH)₂D] (Figure 3), serum concentrations of 1,25(OH)₂D should never be used to determine vitamin D status (45–47). The reasons for this are that the half-life of 1,25(OH)₂D in the circulation is <4 h, its concentrations are ~1000-fold less than those of 25(OH)D, and, most importantly, as a person becomes vitamin D deficient, there is a compensatory increase in parathyroid hormone (PTH) secretion, which stimulates the kidney to produce more 1,25(OH)₂D. As a person becomes vitamin D deficient and 25(OH)D concentrations decrease, 1,25(OH)₂D concentrations are maintained in the normal range and sometimes are even elevated. Therefore, 1,25(OH)₂D concentrations are not useful and can mislead physicians into thinking that patients are vitamin D sufficient when they can be severely vitamin D deficient (45–51).

Typically, normal ranges for assay results are considered to be the mean ± 2 SDs for a healthy population. Unfortunately, it was not appreciated that the general population is at risk of vitamin D deficiency, and so-called normal values included in the normal range often indicate vitamin D deficiency (49–51).

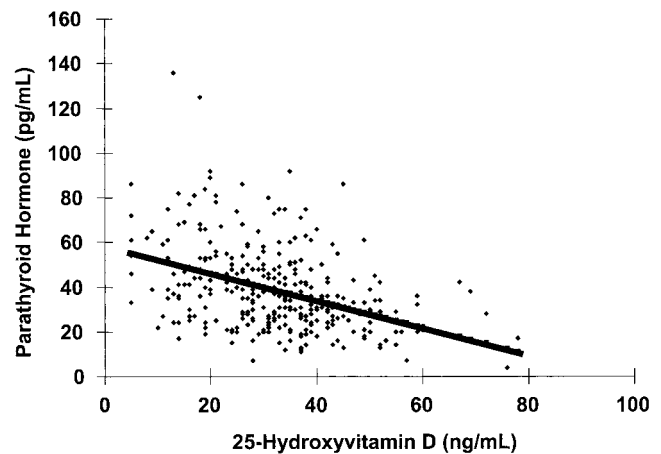


FIGURE 6. Relationship between 25(OH)D and PTH concentrations. Serum concentrations of 25(OH)D were inversely correlated with PTH concentrations ($r = 0.40$, $P < 0.001$). Reproduced from *Am J Med* 2002;112:659–62 with permission from Excerpta Medica Inc.

The upper limit of 25(OH)D concentrations for most commercial assays is ~125 nmol/L (50 ng/mL). It is known that lifeguards and sunbathers can have blood concentrations of 25(OH)D of > 250 nmol/L (100 ng/mL), and they are not vitamin D intoxicated. Indeed, 25(OH)D concentrations of > 325 nmol/L (150 ng/mL) with associated hypercalcemia are pathognomonic for hypervitaminosis D (vitamin D intoxication) (52–55).

The lower limit of the normal range for 25(OH)D assays is also suspect. Several reports suggested that, if PTH concentrations are plotted as a function of 25(OH)D concentrations, then they begin to plateau when the 25(OH)D concentrations are ~78 nmol/L (30 ng/mL) (56–59) (Figure 6). Malabanan et al (49) conducted provocative testing with vitamin D₂ among healthy adults with 25(OH)D concentrations of 25–62.5 nmol/L (10–25 ng/mL). All subjects were given 50 000 units of vitamin D₂ once per week for 8 wk, with calcium supplementation. The 25(OH)D concentrations increased by > 100% and the PTH concentrations decreased by 22%, on average. When the baseline 25(OH)D concentrations were related to the PTH concentrations at the end of the study, it was observed that subjects with 25(OH)D concentrations of 25–37.5 nmol/L (11–15 ng/mL) and 38–30 nmol/L (16–20 ng/mL) exhibited 55% and 35% decreases in PTH concentrations, respectively, whereas healthy adults with 25(OH)D concentrations of ≥ 50 nmol/L (≥ 20 ng/mL) demonstrated no significant change. It was concluded that concentrations of at least 50 nmol/L were required to minimally satisfy the body's vitamin D requirement and that concentrations of 30 ng/mL were preferred (49, 57, 58, 60).

CONSEQUENCES OF VITAMIN D DEFICIENCY

Vitamin D deficiency among children not only causes overt rickets but also can prevent children from reaching their genetically programmed height and peak bone mass. For adults, vitamin D deficiency has more subtle effects on the skeleton. The secondary hyperparathyroidism mobilizes calcium from the skeleton and thus can reduce bone mineral density and ultimately

precipitate or exacerbate osteoporosis. In addition, vitamin D deficiency causes a mineralization defect of the collagen matrix that is laid down by osteoblasts. The rubbery matrix does not provide structural support, which increases the risk of fracture. Collagen matrix that is not properly mineralized becomes hydrated, causing an outward expansion on the periosteal covering, which is highly innervated with sensory pain fibers. The consequence is that people with osteomalacia often complain of an aching in their bones (48, 59, 61–64). In addition, skeletal muscles have receptors for $1,25(\text{OH})_2\text{D}$, and vitamin D deficiency not only causes muscle weakness among children with rickets but also causes muscle weakness among adults with osteomalacia (62–66). Patients often complain of aching bones and muscle discomfort. Such patients are often misdiagnosed with fibromyalgia, chronic fatigue syndrome, myositis, or other nonspecific collagen vascular diseases. It is estimated that 40–60% of patients with fibromyalgia may have some component of vitamin D deficiency and osteomalacia (22, 47, 59, 61–66). Glerup et al (59) reported that 88% of Danish Arab women with muscle weakness and pain were vitamin D deficient. More than 90% of 150 children and adults 10–65 y of age who presented with nonspecific muscle aches and bone aches and pains at a Minnesota hospital were found to be vitamin D deficient (62).

It is estimated that > 50% of African Americans in the United States are either chronically or seasonally at risk of vitamin D deficiency. Nesby-O'Dell et al (67) reported that, in the third National Health and Nutrition Examination Survey, 42% of African American women 15–49 y of age were found to be vitamin D deficient at the end of the winter. Holick (22, 26) observed that 84% of African American men and women > 65 y of age were vitamin D deficient at the end of the summer in Boston. The reasons for this are that African Americans often have a lactase deficiency and do not drink milk, they have markedly decreased efficiency in making vitamin D_3 in their skin, and they avoid the sun because they do not want to increase their skin pigmentation. Women in Saudi Arabia and their children have high prevalences of osteomalacia and rickets, respectively, and vitamin D deficiency because of their practice of wearing clothing over the whole body and avoiding direct sunlight (68, 69).

Vitamin D deficiency is well recognized as a major health problem for adults > 50 y of age (49, 70–73). Gloth et al (70) reported that 54% of community dwellers and 38% of nursing home residents were vitamin D deficient. In a hospital setting, Thomas et al (73) reported that > 40% of hospitalized patients in Boston were vitamin D deficient. In an outpatient setting, 41% of 169 otherwise healthy adults 49–83 y of age were found to be vitamin D deficient throughout the year (49). Young adults, especially of African American origin, are at high risk of vitamin D deficiency (58). However, students and young adults who never see daylight and are always working or who always wear sun protection are also at risk. At the Boston Medical Center, it was observed that 32% of the students and doctors 18–29 y of age were vitamin D deficient at the end of the winter (58) (Figure 7). Even teenagers and young children are at risk. Gordon et al (74) reported that > 50% of African American teenagers in Boston were found to be vitamin D deficient. Sullivan et al (75) observed that 48% of white girls 9–13 y of age were vitamin D deficient at the end of the winter and 17% were still vitamin D deficient at the end of the summer, because of the sunscreen use and complete sun protection they practiced.

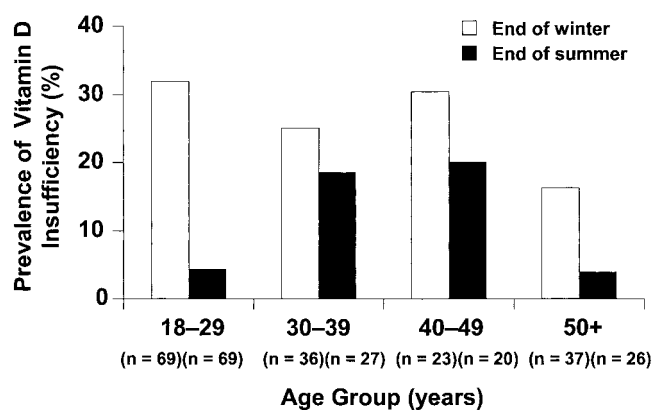


FIGURE 7. Percentages of subjects in the 4 age groups who were vitamin D deficient [$25(\text{OH})\text{D}$ concentrations of ≤ 20 ng/mL] at the end of winter and at the end of summer. There was a significant difference in the proportions of subjects with vitamin D insufficiency between the end-of-winter and end-of-summer groups ($P < 0.05$). Reproduced from *Am J Med* 2002;112:659–62 with permission from Excerpta Medica Inc.

EFFECTS OF VITAMIN D DEFICIENCY ON BONE HEALTH AND CALCIUM AND PHOSPHORUS METABOLISM

Vitamin D deficiency results in abnormalities in both calcium and phosphorus metabolism. The major function of vitamin D is to maintain serum calcium concentrations within the physiologically acceptable range. It accomplishes this by increasing intestinal calcium absorption (Figure 5). In a vitamin D-deficient state, the intestine typically absorbs 10–15% of dietary calcium (3, 22). In a vitamin D-sufficient state, 30% typically is absorbed from the diet; as much as 60–80% can be absorbed during periods of growth and pregnancy or lactation, with increased demand for calcium.

To achieve the maximal efficiency of vitamin D-induced intestinal calcium transport, the serum $25(\text{OH})\text{D}$ concentrations must be at least 78 nmol/L (30 ng/mL) (60). When there is inadequate calcium in the diet, $1,25(\text{OH})_2\text{D}$ interacts with its receptor in osteoblasts and induces the expression of receptor activator of nuclear factor- κB ligand, which interacts with its receptor on preosteoclasts, inducing them to become mature osteoclasts (76, 77) (Figure 8). The net effect is to enhance mobilization of calcium from the skeleton to maintain serum calcium concentrations in the normal range. Vitamin D deficiency results in decreased concentrations of ionized calcium, which are immediately recognized by the calcium sensor in the parathyroid glands (78). This results in increased expression, production, and secretion of PTH. PTH helps maintain calcium metabolism by increasing tubular reabsorption of calcium in the kidney, enhancing the production of $1,25(\text{OH})_2\text{D}$, and interacting with osteoblasts to increase the receptor activator of nuclear factor- κB ligand system, similar to $1,25(\text{OH})_2\text{D}$ (Figure 8).

Vitamin D-deficient children and adults with rickets and osteomalacia typically have normal serum calcium concentrations. This is one of the reasons why physicians often miss the diagnosis, since they are monitoring serum calcium concentrations for vitamin D deficiency and not $25(\text{OH})\text{D}$ concentrations. However, it is not low serum calcium concentrations that cause rickets among children and osteomalacia among adults. Instead, vitamin

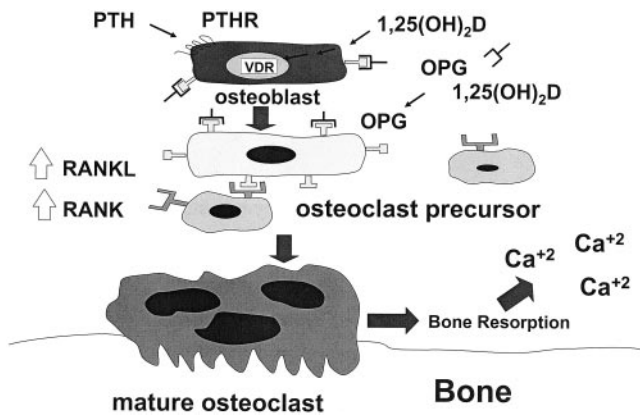


FIGURE 8. 1,25(OH)₂D and PTH stimulation of the mobilization of calcium from the skeleton through interactions with their respective receptors on osteoblasts, which induce expression of the receptor activator of nuclear factor- κ B (RANK) ligand (RANKL). The receptor activator of nuclear factor- κ B on immature osteoclasts binds to the receptor activator of nuclear factor- κ B ligand, which causes the cells to mature and coalesce with other osteoclast precursors to become mature multinuclear osteoclasts.

D deficiency, which causes secondary hyperparathyroidism, results in PTH-induced loss of phosphorus into the urine and decreased intestinal phosphorus absorption. This results in low or low-normal fasting serum phosphorus concentrations. The low-normal serum phosphorus concentrations with low-normal serum calcium concentrations often result in an inadequate calcium-phosphate product, which is important for the mineralization process. This is what causes the mineralization defects that result in rickets among children and osteomalacia among adults (2, 22, 26, 45, 46).

NONCALCEMIC ACTIONS OF VITAMIN D

The revelation in 1979 that most tissues and cells in the body localized [³H]-1,25(OH)₂D₃ opened an exciting new chapter on the multitude of biological functions of vitamin D (79). It is known that 1,25(OH)₂D interacts with a specific nuclear receptor similar to other steroid hormones (2, 22, 76, 79, 80). After 1,25(OH)₂D enters the cell, it is transported through the microtubular network to the nucleus (81). After it enters the nucleus bound to its vitamin D receptor (VDR), it complexes with the retinoic acid X receptor to form a heterodimeric complex that seeks out specific DNA sequences known as vitamin D-responsive elements. Once the 1,25(OH)₂D₃-VDR-retinoic acid X receptor complex binds to the vitamin D-responsive element, a variety of transcriptional factors, including DRIP, bind to it, resulting in expression of the vitamin D-responsive gene (Figure 9) (76, 80).

The VDR is present in the small intestine, colon, osteoblasts, activated T and B lymphocytes, β -islet cells, and most organs in the body, including brain, heart, skin, gonads, prostate, breast, and mononuclear cells (2, 22, 76, 80). One of the first insights into why so many tissues in the body have VDRs occurred when Tanaka et al (82) reported that mouse (M-1) and human (HL-60) leukemic cells with VDRs responded to 1,25(OH)₂D₃. Incubation of these cells with 1,25(OH)₂D₃ not only inhibited their proliferation but also stimulated them to differentiate into mature macrophages. Suda et al (83) then showed that mice given M-1

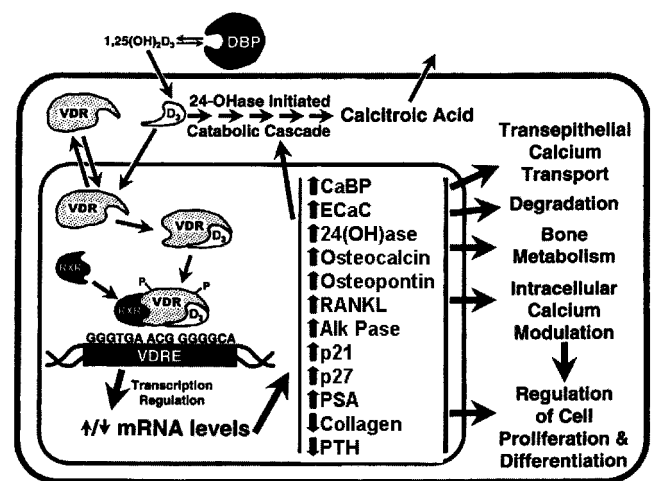


FIGURE 9. Schematic representation of the mechanism of action of 1,25(OH)₂D₃ in various target cells, resulting in a variety of biological responses. The free form of 1,25(OH)₂D₃ enters the target cell and interacts with its nuclear VDR, which is phosphorylated (Pi). The 1,25(OH)₂D₃-VDR complex combines with the retinoic acid X receptor (RXR) to form a heterodimer, which in turn interacts with the vitamin D-responsive element (VDRE), causing enhancement or inhibition of transcription of vitamin D-responsive genes, including calcium-binding protein (CaBP), epithelial calcium channel (ECaC), 25(OH)D 24-hydroxylase (24-OHase), receptor activator nuclear factor- κ B ligand (RANKL), alkaline phosphatase (alk PASE), prostate-specific antigen (PSA), and PTH (76). Reprinted from reference 76 with permission of the American Society for Bone and Mineral Research.

leukemia survived for longer times if they were treated with an analog of 1,25(OH)₂D₃, ie, 1 α -hydroxyvitamin D₃.

Studies were immediately implemented to determine whether 1,25(OH)₂D₃ could be used to treat leukemia. The studies were disappointing, because the drug caused severe hypercalcemia and, although some of the patients experienced remission, all eventually died in a blastic phase (84).

Although 1,25(OH)₂D₃ was a disappointment as an antitumor agent, the one proven clinical application for the potent antiproliferative activity of 1,25(OH)₂D₃ was in the treatment of psoriasis. Keratinocytes have a VDR and, when they are exposed to 1,25(OH)₂D₃, their growth is markedly inhibited and they are induced to differentiate (85). Initial clinical trials with topical 1,25(OH)₂D₃ treatment demonstrated remarkable improvement in scaling, erythema, and plaque thickness, which was sustained (86–88). There were no untoward side effects. As a result, 3 analogs, including calcipotriene, 1,24-dihydroxyvitamin D₃, and 22-oxo-1,25-dihydroxyvitamin D₃, were developed and were demonstrated to be clinically effective for the treatment of psoriasis (87). Topical activated vitamin D treatment is the first-line therapy for psoriasis throughout the world.

1,25(OH)₂D₃ has been shown to have a multitude of other physiologic functions (2, 22, 76, 89, 90), including stimulation of insulin production (91), modulation of activated T and B lymphocyte function (92, 93), effects on myocardial contractility (94, 95), prevention of inflammatory bowel disease (96), and promotion of thyroid-stimulating hormone secretion (2, 3, 22). These are just a few of the many physiologic functions that have been reported for 1,25(OH)₂D₃ that are not related to calcium metabolism (2, 3, 22, 79, 82, 96).

VITAMIN D DEFICIENCY AND LATITUDINAL ASSOCIATIONS WITH RISKS OF CANCER AND AUTOIMMUNE DISEASES

In 1941, Apperley (97) reported his remarkable observation that people in the United States who lived at higher latitudes, such as in New Hampshire, Vermont, and Massachusetts, had overall greater risks of dying as a result of cancer, compared with men and women of similar ages who lived in southern states, such as Texas, Georgia, and Alabama. He suggested that there needed to be a reexamination of the relationship between skin cancer and other cancers, noting that “the presence of skin cancer is really an occasional accompaniment of a relative cancer immunity in some way related to exposure to solar radiation” (p. 195). Little attention was paid to this remarkable observation until the 1980s, when Garland et al (98–100) reported that both colon and breast cancer risks were higher for those living at higher latitudes in the United States. A prospective study revealed that, if 25(OH)D concentrations were < 50 nmol/L (20 ng/mL), then there was a 2-fold increased risk of developing colon cancer (98). Hanchette and Schwartz (101) also demonstrated a gradient for prostate cancer, with the highest mortality rates among white men living at the highest latitudes in the United States. Since these observations, several investigators not only confirmed the observations but added to the list of cancers that may be associated with living at higher latitudes and thus may be related to vitamin D deficiency (102–105). Grant (102) examined latitudinal variations in breast cancer mortality rates in Europe and, controlling for diet, concluded that lack of UVB radiation from sunlight accounts for perhaps 25% of the breast cancer mortality rates in northern Europe (104). He also reported that both men and women with more sun exposure were less likely to die prematurely as a result of cancer (102, 104).

Interestingly, there is also a latitudinal association with increased risk of developing multiple sclerosis (106–108) and cardiovascular disease (109). Rostand (109) observed in 1979 that people who lived at higher latitudes in the United States, Europe, and Asia were more likely to have hypertension. It is well established that there is a latitudinal association with the prevalence of multiple sclerosis. People who were born below 35° N latitude and lived at or below that latitude for the first 10 years of their lives had decreased lifetime risks of developing multiple sclerosis, compared with those who were born above 35° N latitude (106, 107). There is compelling evidence that this is attributable to a decrease in UVB light exposure. One study suggested that the seasonal variation in multiple sclerosis was 50% less in the summer, compared with that in the winter (108, 110). Mahon et al (111) and Ponsonby et al (107) observed that increases in vitamin D intake were related to decreases in multiple sclerosis incidents.

Bodiwala et al (112) reported that men who worked outdoors and had increased sun exposure throughout their lifetimes had a 3–5-y “honeymoon” period before they developed prostate cancer, compared with age-matched control subjects who had little sun exposure and began developing prostate cancer at the age of 53 y. Tuohimaa et al (113) reported that the risk of prostate cancer was reduced by 50% with serum 25(OH)D concentrations of ≥ 50 nmol/L.

POSSIBLE MECHANISMS FOR THE ROLE OF VITAMIN D IN PREVENTING COMMON CANCERS, HEART DISEASE, AND AUTOIMMUNE DISEASES

There is good epidemiologic documentation that living at lower latitudes decreases the risks of many chronic diseases. It

has been assumed that, because the production of vitamin D is more efficient at lower latitudes, this is the explanation for these interesting observations. In addition, there is mounting scientific evidence suggesting that increasing vitamin D intake decreases the risks of developing chronic diseases. For example, it was shown that treatment of children with 2000 IU/d vitamin D from 1 y of age decreased their risk of developing type 1 diabetes mellitus by 80% throughout the next 20 y (114). Furthermore, children from the same cohort who were vitamin D deficient at 1 y of age had a 4-fold increased risk of developing type 1 diabetes. An increase in vitamin D intake has been associated with decreased risk of developing rheumatoid arthritis (115). Exposure to tanning bed UVB radiation, which resulted in a $> 100\%$ increase in blood concentrations of 25(OH)D, was effective in treating hypertension among adults. However, adults exposed to a tanning bed that transmitted only UVA radiation and did not increase blood concentrations of 25(OH)D demonstrated no effect on their hypertension (116). There is also evidence that increased intake of calcium and vitamin D decreases the risk of developing colon cancer (117).

How is it possible that vitamin D can have such a wide range of therapeutic and health-related benefits? The answer lies in the fact that the VDR is present in most cells and tissues in the body. 1,25(OH)₂D is one of the most potent regulators of cellular growth in both normal and cancer cells (2, 3, 22, 26, 76, 82, 83). It has been suggested that increased vitamin D intake or increased exposure to sunlight, raising blood concentrations of 25(OH)D above 78 nmol/L (30 ng/mL), is necessary for maximal extrarenal production of 1,25(OH)₂D in a wide variety of tissues and cells in the body, including colon, breast, prostate, lung, activated macrophages, and parathyroid cells. The local production of 1,25(OH)₂D is thought to be important for keeping cell growth in check and possible preventing the cell from becoming autonomous and developing into a unregulated cancer cell (2, 3, 118–120).

Activated T and B lymphocytes have VDRs. 1,25(OH)₂D is a very effective modulator of the immune system. In a variety of animal models, it has been demonstrated that pretreatment with 1,25(OH)₂D is effective in mitigating or preventing the onset of type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis,

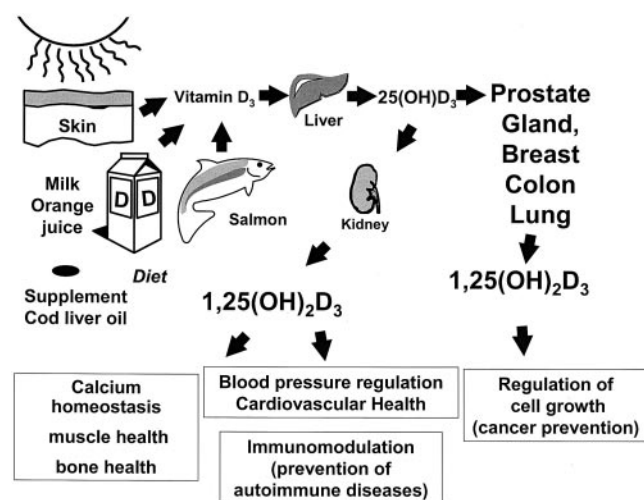



FIGURE 10. Metabolism of 25(OH)D₃ to 1,25(OH)₂D₃ in the kidney and other organs and the biological consequences.

and Crohn's disease (2, 22, 90, 121–123). In addition, Li et al (124) reported that, in a mouse model, $1,25(\text{OH})_2\text{D}$ was an effective inhibitor of the blood pressure hormone renin.

CONCLUSIONS

Vitamin D can no longer be thought of as a nutrient necessary for the prevention of rickets among children. Vitamin D should be considered essential for overall health and well-being (Figure 10). Vitamin D deficiency and decreased exposure to solar UVB radiation have been demonstrated to increase the risks of many common cancers, type 1 diabetes, rheumatoid arthritis, and multiple sclerosis, and there are indications that they may be associated with type 2 diabetes (125, 126) and schizophrenia (127–129). The photosynthesis of vitamin D has been occurring in living organisms for > 500 million years, and it is not surprising that vitamin D has evolved into such an important and necessary hormone, which acts as an indicator of overall health and well-being. Vigilance in maintaining a normal vitamin D status, ie, $25(\text{OH})\text{D}$ concentrations of 75–125 nmol/L, should be a high priority. Surveillance for vitamin D deficiency, with measurement of $25(\text{OH})\text{D}$ concentrations, should be part of normal yearly physical examinations. 

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REFERENCES

- Holick MF. Phylogenetic and evolutionary aspects of vitamin D from phytoplankton to humans. In: Pang PKT, Schreiberman MP, eds. Vertebrate endocrinology: fundamentals and biomedical implications. Vol 3. Orlando, FL: Academic Press, 1989:7–43.
- Holick MF. Vitamin D: a millennium perspective. *J Cell Biochem* 2003;88:296–307.
- Holick MF. McCollum Award Lecture, 1994: vitamin D: new horizons for the 21st century. *Am J Clin Nutr* 1994;60:619–30.
- Sniadecki J, Jerdrzej Sniadecki (1768–1838) on the cure of rickets (1840); cited in Mozolowski W. *Nature* 1939;143:121–4.
- Palm TA. The geographical distribution and aetiology of rickets. *Practitioner* 1890;14:270–342.
- Huldschinsky K. Heilung von Rachitis durch Kunstliche Hohensonne. *Dtsch Med Wochenschr* 1919;45:712–3.
- Hess AF, Unger LJ. The cure of infantile rickets by sunlight. *JAMA* 1921;77:39–41.
- Steenbock H, Black A. The induction of growth-promoting and calcifying properties in a ration by exposure to ultraviolet light. *J Biol Chem* 1924;61:408–22.
- Hess AF, Weinstock M. Antirachitic properties imparted to inert fluids and to green vegetables by ultraviolet irradiation. *J Biol Chem* 1924; 62:301–13.
- Hess AF, Unger LJ, Pappenheimer AM. Experimental rickets in rats: the prevention of rickets in rats by exposure to sunlight. *J Biol Chem* 1922;50:77–83.
- Steenbock H. The induction of growth-promoting and calcifying properties in a ration by exposure to light. *Science* 1924;60:224–5.
- Bauer JM, Freyberg RH. Vitamin D intoxication and metastatic calcification. *JAMA* 1946;130:1208–15.
- British Pediatric Association. Hypercalcemia in infants and vitamin D. *Br Med J* 1956;2:149–51.
- Holick MF, Tian XQ, Allan M. Evolutionary importance for the membrane enhancement of the production of vitamin D_3 in the skin of poikilothermic animals. *Proc Natl Acad Sci USA* 1995;92:3124–6.
- Holick MF, MacLaughlin JA, Dobbelt SH. Regulation of cutaneous previtamin D_3 photosynthesis in man: skin pigment is not an essential regulator. *Science* 1981;211:590–3.
- Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J. Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* 1993;91:2552–5.
- MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D_3 . *J Clin Invest* 1985;76:1536–8.
- Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. *Lancet* 1989;2:1104–5.
- Clemens TL, Henderson SL, Adams JS, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D_3 . *Lancet* 1982;1:74–6.
- Matsuoka LY, Ide L, Wortsman J, MacLaughlin J, Holick MF. Sunscreens suppress cutaneous vitamin D_3 synthesis. *J Clin Endocrinol Metab* 1987;64:1165–8.
- Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D_3 : exposure to winter sunlight in Boston and Edmonton will not promote vitamin D_3 synthesis in human skin. *J Clin Endocrinol Metab* 1988;67:373–8.
- Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;79: 362–71.
- Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* 1985;76:370–3.
- Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72:690–3.
- Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick MF. Fortification of orange juice with vitamin D: a novel approach to enhance vitamin D nutritional health. *Am J Clin Nutr* 2003;77:1478–83.
- Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes* 2002;9:87–98.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press, 1999.
- Reid IR, Gallagher DJA, Bosworth J. Prophylaxis against vitamin D deficiency in the elderly by regular sunlight exposure. *Age Aging* 1985;15:35–40.
- Chel VGM, Ooms ME, Popp-Snijders C, et al. Ultraviolet irradiation corrects vitamin D deficiency and suppresses secondary hyperparathyroidism in the elderly. *J Bone Miner Res* 1998;13:1238–42.
- Jones G, Dwyer T. Bone mass in prepubertal children: gender differences and the role of physical activity and sunlight exposure. *J Clin Endocrinol Metab* 1998;83:4274–9.
- Shao Q, Chen TC, Holick MF. Sun-tanning bed radiation increases vitamin D synthesis in human skin in vivo. In: Holick MF, Klignman A, eds. *Biologic Effects of Light*. Berlin: Walter de Gruyter, 1992:62–6.
- Tangpricha V. Bone health and vitamin D status in men and women who frequent tanning salons. In: Proceedings of the 10th annual meeting of the International Society for Clinical Densitometry. Hartford, CT: International Society for Clinical Densitometry, 2004:B-44.
- Chuck A, Todd J, Diffey B. Subliminal ultraviolet-B irradiation for the prevention of vitamin D deficiency in the elderly: a feasibility study. *Photochem Photobiophys Photomed* 2001;17:168–71.
- Bischoff HA, Dietrich T, Orav JE, Dawson-Hughes B. Positive association between $25\text{-hydroxyvitamin D}$ levels and bone mineral density: a population-based study of younger and older US adults. In: Proceedings of the 5th International Symposium on Nutritional Aspects of Osteoporosis. Lausanne, Switzerland: 2003:12.
- Contet-Audonneau JL, Jeanmaire C, Pauly G. A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *Br J Dermatol* 1999;140:1038–47.
- Veierod MB, Weiderpass E, Thorn M, et al. A prospective study of pigmentation, sun exposure, and risk of cutaneous malignant melanoma in women. *J Natl Cancer Inst* 2003;95:1530–8.
- Grodstein F, Speizer FE, Hunter DJ. A prospective study of incident squamous cell carcinoma of the skin in the Nurses' Health Study. *J Natl Cancer Inst* 1995;87:1061–6.
- Kennedy C, Bajdik CD, Willemze R, de Gruij FR, Bavinck JN. The influence of painful sunburns and lifetime of sun exposure on the risk of actinic keratoses, seborrheic warts, melanocytic nevi, atypical nevi and skin cancer. *J Invest Dermatol* 2003;120:1087–93.
- Ziegler A, Jonason AS, Leffell DJ, et al. Sunburn and p53 in the onset of skin cancer. *Nature* 1994;372:773–6.
- Matsuoka LY, Ide L, Wortsman J, Hanifan N, Holick MF. Chronic

- sunscreen use decreases circulating concentrations of 25-hydroxyvitamin D: a preliminary study. *Arch Dermatol* 1988;124:1802-4.
41. Garland CF, Garland FC, Gorham ED. Rising trends in melanoma: an hypothesis concerning sunscreen effectiveness. *Ann Epidemiol* 1993; 3:103-10.
 42. Holick MF. *The UV advantage*. New York: ibooks, 2004.
 43. Chen TC. Photobiology of vitamin D. In: Holick MF, ed. *Vitamin D: physiology, molecular biology, and clinical applications*. Totowa, NJ: Humana Press, 1999:17-38.
 44. Lu Z, Chen TC, Kline L, et al. Photosynthesis of previtamin D₃ in cities around the world. In: Holick MF, Kligman A, eds. *Biological Effects of Light*. Berlin: Walter De Gruyter, 1992:48-52.
 45. Holick MF. Too little vitamin D in premenopausal women: why should we care? *Am J Clin Nutr* 2002;76:3-4.
 46. Holick MF. Sunlight and vitamin D: both good for cardiovascular health. *J Gen Intern Med* 2002;17:733-5.
 47. Holick MF. Sunlight "Dilemma": risk of skin cancer or bone disease and muscle weakness. *Lancet* 2001;357:4-6.
 48. Malabanan AO, Turner AK, Holick MF. Severe generalized bone pain and osteoporosis in a premenopausal black female: effect of vitamin D replacement. *J Clin Densitometr* 1998;1:201-4.
 49. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998;351:805-6.
 50. Souberbielle J, Lawson-body E, Hammadi B, Sarfati E, Kahan A, Cormier C. The use in clinical practice of parathyroid hormone normative values established in vitamin D-sufficient subjects. *J Clin Endocrinol Metab* 2003;88:3501-4.
 51. Holick MF. The parathyroid hormone D-lemma. *J Clin Endocrinol Metab* 2003;88:3499-500 (editorial).
 52. Pettifor JM, Bikle DD, Cavaleros M, Zachen D, Kamdar M, Ross FP. Serum levels of free 1,25-dihydroxyvitamin D in vitamin D toxicity. *Ann Intern Med* 1995;122:511-3.
 53. Koutkia P, Chen TC, Holick MF. Vitamin D intoxication associated with an over-the-counter supplement. *N Engl J Med* 2001;345:66-7.
 54. Adams JS, Lee G. Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Int Med* 1997;127:203-6.
 55. Jacobus CH, Holick MF, Shao Q, et al. Hypervitaminosis D associated with drinking milk. *N Engl J Med* 1992;326:1173-7.
 56. Chapuy MC, Schott AM, Garnero P, et al. Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. *J Clin Endocrinol Metab* 1996;81:1129-33.
 57. Chapuy MC, Preziosi P, Maaner M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997;7: 439-43.
 58. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. *Am J Med* 2002;112: 659-62.
 59. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *J Intern Med* 2000;247:260-8.
 60. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003;22:142-6.
 61. Gloth FM III, Lindsay JM, Zelesnick LB, Greenough WB III. Can vitamin D deficiency produce an unusual pain syndrome? *Arch Intern Med* 1991;151:1662-4.
 62. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc* 2003;78:1463-70.
 63. Holick MF. Vitamin D deficiency: what a pain it is. *Mayo Clin Proc* 2003;78:1457-9.
 64. Glerup H, Middelsen K, Poulsen L, et al. Hypovitaminosis D myopathy without biochemical signs of osteomalacia bone involvement. *Calcif Tissue Int* 2000;66:419-24.
 65. Bischoff HA, Stahelin HN, Dick W, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res* 2003;18:343-351.
 66. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C. Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. *Bone Miner Res* 2000;15:1113-5.
 67. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 2002;76:187-92.
 68. Taha SA, Dost SM, Sedrani SH. 25-Hydroxyvitamin D and total calcium: extraordinarily low plasma concentrations in Saudi mothers and their neonates. *Pediatr Res* 1984;18:739-41.
 69. Sedrani SH. Low 25-hydroxyvitamin D and normal serum calcium concentrations in Saudi Arabia: Riyadh region. *Ann Nutr Metab* 1984; 28:181-5.
 70. Gloth FM, Gundberg CM, Hollis BW, Haddad HG, Tobin JD. Vitamin D deficiency in homebound elderly persons. *JAMA* 1995;274:1683-6.
 71. Gloth FM, Tobin JD, Sherman SS, Hollis BW. Is the recommended daily allowance for vitamin D too low for the homebound elderly? *J Am Geriatr Soc* 1991;39:137-41.
 72. Lips P, Duong T, Oleksik A, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *J Clin Endocrinol Metab* 2001;86:1212-21.
 73. Thomas KK, Lloyd-Jones DH, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998;338:777-83.
 74. Gordon CM, DePeter KC, Estherann G, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. In: *Proceedings of Endo2003, the Endocrine Society Meeting*. Bethesda, MD: The Endocrine Society, 2003:87.
 75. Sullivan SS, Rosen CJ, Chen TC, Holick MF. Seasonal changes in serum 25(OH)D in adolescent girls in Maine. In: *Proceedings of the American Society for Bone and Mineral Research Annual Meeting*. Washington, DC: American Society for Bone and Mineral Research, 2003:S407.
 76. Holick MF. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus M, ed. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. 5th ed. Washington, DC: American Society for Bone and Mineral Research, 2003:129-137.
 77. Khosla S. The OPG/RANKL/RANK system. *Endocrinology* 2001; 142:5050-5.
 78. Brown EM, Pollak M, Seidman CE, et al. Calcium-ion-sensing cell-surface receptors. *N Engl J Med* 1995;333:234-40.
 79. Stumpf WE, Sar M, Reid FA, et al. Target cells for 1,25-dihydroxyvitamin D₃ in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. *Science* 1979;206:1188-90.
 80. MacDonald P. Molecular biology of the vitamin D receptor. In: Holick MF, ed. *Vitamin D: physiology, molecular biology, and clinical applications*. Totowa, NJ: Humana Press, 1999:109-28.
 81. Barsony J. Vitamin D receptor translocation. In: Holick MF, ed. *Vitamin D: physiology, molecular biology, and clinical applications*. Totowa, NJ: Humana Press, 1999:129-48.
 82. Tanaka H, Abe E, Miyaura C, et al. 1,25-Dihydroxycholecalciferol and human myeloid leukemia cell line (HL-60): the presence of cytosol receptor and induction of differentiation. *Biochem J* 1982;204:713-9.
 83. Suda T, Abe E, Miyaura C, et al. Induction of differentiation of human myeloid leukemia cells by 1,25-dihydroxyvitamin D₃. In: Norman A, Schaefer K, Herrath DV, Grigdeit H-G, eds. *Vitamin D: chemical, biochemical and clinical endocrinology of calcium metabolism*. New York: Walter de Gruyter, 1982:59-64.
 84. Koeffler HP, Hirjik J, Itri L, Southern California Leukemia Group. 1,25-Dihydroxyvitamin D₃: in vivo and in vitro effects on human pre-leukemic and leukemic cells. *Cancer Treat Rep* 1985;69:1399-407.
 85. Smith EL, Walworth ND, Holick MF. Effect of 1,25-dihydroxyvitamin D₃ on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. *J Invest Dermatol* 1986;86:709-14.
 86. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. *J Am Acad Dermatol* 1988;19: 516-28.
 87. Holick MF. Clinical efficacy of 1,25-dihydroxyvitamin D₃ and its analogues in the treatment of psoriasis. *Retinoids* 1998;14:12-7.
 88. Perez A, Chen TC, Turner A, Holick MF. Pilot study of topical calcitriol (1,25-dihydroxyvitamin D₃) for treating psoriasis in children. *Arch Dermatol* 1995;131:961-2.
 89. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D₃ analogs as immunomodulatory agents. *Trends Mol Med* 2002;8:174-9.
 90. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr* 1998;128:68-72.

91. Lee S, Clark SA, Gill RK, Christakos S. 1,25-Dihydroxyvitamin D₃ and pancreatic β -cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology* 1994;134:1602–10.
92. Tsoukas CD, Provedine DM, Manolagas SC. 1,25-Dihydroxyvitamin D₃, a novel immuno-regulatory hormone. *Science* 1984;221:1438–40.
93. Bhalla AK, Clemens T, Amento E, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D₃ in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab* 1983;57:1308–10.
94. Weishaar RE, Simpson RU. The involvement of the endocrine system in regulating cardiovascular function: emphasis on vitamin D₃. *Endocr Rev* 1989;10:1–15.
95. Weishaar RE, Simpson RU. Involvement of vitamin D₃ with cardiovascular function. II. Direct and indirect effects. *Am J Physiol* 1987;253:E675–83.
96. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr* 2000;130:2648–52.
97. Apperly FL. The relation of solar radiation to cancer mortality in North America. *Cancer Res* 1941;1:191–5.
98. Garland CF, Garland FC, Shaw EK, Comstock GW, Helsing KJ, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1989;18:1176–78.
99. Garland FC, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. *Prev Med* 1990;19:614–22.
100. Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Ross AH, Oglesby P. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985;1:307–9.
101. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality: evidence for a protective effect of ultraviolet radiation. *Cancer* 1992;70:2861–9.
102. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer* 2002;94:1867–75.
103. Bodiwala D, Luscombe CJ, Liu S, Saxby M, French M, Jones PW. Prostate cancer risk and exposure to ultraviolet radiation: further support for the protective effect of sunlight. *Cancer Lett* 2003;192:145–9.
104. Grant WB. An ecologic study of the role of solar UV-B radiation in reducing the risk of cancer using cancer mortality data, dietary supply data and latitude for European countries. In: MF Holick, ed. *Biologic effects of light 2001*. Boston: Kluwer Academic Publishing, 2002: 267–6.
105. Chen TC, Holick MF. Vitamin D and prostate cancer prevention and treatment. *Trends Endocrinol Metab* 2003;14:423–30.
106. Hernán MA, Olek MJ, Ascherio A. Geographic variation of MS incidence in two prospective studies of US women. *Neurology* 1999;51:1711–8.
107. Ponsonby A-L, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology* 2002;181:71–8.
108. Embry AF, Snowdon LR, Vieth R. Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Ann Neurol* 2000;48:271–2.
109. Rostand SG. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* 1979;30:150–6.
110. van der Mei I, Ponsonby A-L, Dwyer T, Blizzard L, Simmons R, Taylor BV. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. *Br Med J* 2003;327:316–7.
111. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *J Neuroimmunol* 2003;134:128–32.
112. Bodiwala D, Luscombe CJ, Liu S, Saxby M, French M, Jones PW. Prostate cancer risk and exposure to ultraviolet radiation: further support for the protective effect of sunlight. *Cancer Lett* 2003;192:145–9.
113. Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. *Int J Cancer* 2004;108:104–8.
114. Hyponen E, Laara E, Jarvelin M-R, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;358:1500–3.
115. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis. *Arthritis Rheum* 2004;50:72–7.
116. Krause R, Buhning M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998;352:709–10.
117. Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Ross AH, Oglesby P. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985;9:307–9.
118. Tangpricha V, Flanagan JN, Whitlatch LW, et al. 25-Hydroxyvitamin D-1 α -hydroxylase in normal and malignant colon tissue. *Lancet* 2001;357:1673–4.
119. Cross HS, Bareis P, Hofer H, Bischof MG, Bajna E, Kriwanek S. 25-Hydroxyvitamin D₃-1 α -hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early carcinogenesis. *Steroids* 2001;66:287–92.
120. Mawer EB, Hayes ME, Heys SE, et al. Constitutive synthesis of 1,25-dihydroxyvitamin D₃ by a human small cell lung cell line. *J Clin Endocrinol Metab* 1994;79:554–60.
121. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D₃. *Diabetologia* 1994;37:552–8.
122. Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D₃ analogs as immunomodulatory agents. *Trends Mol Med* 2002;8:174–9.
123. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D₃ reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci USA* 1996;93:7861–4.
124. Li Y, Kong J, Wei M, Chen ZF, Liu S, Cao LP. 1,25-Dihydroxyvitamin D₃ is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002;110:229–38.
125. Isai G, Giorgino R, Adami S. High prevalence of hypovitaminosis D in female type 2 diabetic population. *Diabetes Care* 2001;24:1496–8.
126. Ishida H, Seino Y, Matsukura S. Diabetic osteopenia and circulating levels of vitamin D metabolites in type 2 (noninsulin-dependent) diabetes. *Metabolism* 1985;34:797–801.
127. Davies G, Welham J, Chant D, Torrey EF, McGrath J. A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia. *Schizophr Bull* 2003;29:587–93.
128. Kendell RE, Adams W. Exposure to sunlight, vitamin D and schizophrenia. *Schizophr Res* 2002;54:193–8.
129. McGrath J, Selten JP, Chant D. Long-term trends in sunshine duration and its association with schizophrenia birth rates and age at first registration: data from Australia and the Netherlands. *Schizophr Res* 2002;54:199–212.