Vitamin D: Extraskeletal Health

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KEYWORDS

• Vitamin D • Extraskeletal effects • Psoriasis • Cancer
• Diabetes • Autoimmune diseases • Cardiovascular

Vitamin D is one of the oldest hormones.[1] Early in evolution as unicellular organisms evolved and took advantage of the sun's energy for photosynthesis of sugars, they also began to photosynthesize vitamin D.[1] A phytoplankton species that has existed in the Sargasso sea (Atlantic Ocean) for more than 500 million years unchanged was found to have more than 1% of its total dry weight as provitamin D_2 (ergosterol). When this organism was cultured and exposed to simulated sunlight it produced vitamin D_2.[2] As life forms evolved in the ocean, which has a high calcium content, and ventured onto land where calcium was stored in the soil, they needed to develop a method to efficiently absorb calcium from the plants and roots that they ate. It is likely that these organisms when exposed to sunlight produced vitamin D in their skin, which was critical for them to be able to absorb their dietary calcium efficiently. Vitamin D has evolved over millions of years to play and essential role in vertebrate evolution not only for bone health but for their overall health and well being.

SOURCES OF VITAMIN D

Humans have always depended on the sun for their vitamin D requirement.[1,3] Thus the major source of vitamin D for children and adults is exposure of the skin to sunlight.[3] Adults in a bathing suit exposed to an amount of sunlight that causes a slight pinkness to the skin 24 hours later (1MED) is equivalent to ingesting about 20,000 IU of vitamin D.[3] There are few foods that naturally contain vitamin D. Because vitamin D is fat-soluble it is found in oily fish, including salmon, mackerel, and herring. Fish that have little fat in their flesh concentrate their fat in their liver, which is why cod liver oil and oil from other nonoily fish are good sources of vitamin D. Yeast and mushrooms make huge quantities of ergosterol and when exposed to sunlight or ultraviolet irradiation are excellent sources of vitamin D. In the United States and Canada, milk and

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several other dairy products are fortified with vitamin D. Some orange juices are also fortified with calcium and vitamin D.[4]

HISTORICAL PERSPECTIVE ON EXTRASKELETAL EFFECTS OF VITAMIN D

At the turn of the twentieth century it was estimated that more than 90% of children in the industrialized cities of northern Europe and 80% of children living in the northeastern United States had skeletal evidence of rickets.[5,6] Besides the obvious deformities associated with rickets, it was noted that these children had severe muscle weakness, poor tooth eruption with dental caries, and were plagued by upper respiratory tract infections.[5,7] In the early 1900s Finsen observed that exposure to sunlight was effective in treating several skin disorders, including lupus vulgaris, which is caused by a tuberculosis infection of the skin. His remarkable observations resulted in him receiving the Nobel prize in 1903. In 1915 Hoffman compared cancer mortality in cities according to latitude, and demonstrated that cancer mortality increased with increasing distance from the equator (**Table 1**).[8] In 1941 Apperly[9] reported that people who lived in the Northeast were more likely to die of cancer than people who lived in the South. In the 1980s it was reported that there was a latitudinal association with colorectal cancer risk.[10]

In the 1970s it was appreciated that vitamin D (D represents D_2 or D_3) that came from the diet or was synthesized in the skin required a hydroxylation in the liver to form the major circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D).[11] 25(OH)D is metabolized in the kidneys to its active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).[3] Because $1,25(OH)_2D$ is fat-soluble it was assumed that it functioned by interacting with a nuclear vitamin D receptor (VDR) to up- and down-regulate genes responsible for calcium and bone metabolism.[3,11–13] It was quickly demonstrated that kidneys, small intestine, and osteoblasts had a VDR and that several genes, including calbindin9k, epithelial calcium channel, and receptor activator of nuclear factor-κB (RANKL) were up-regulated to control calcium and phosphorus absorption in the small intestine as well as calcium and phosphorus metabolism in the kidneys, and to enhance bone calcium mobilization from the skeleton.[3,12,13]

When radiolabeled $1,25(OH)_2D_3$ was given to vitamin D–deficient rats it had been assumed that it would concentrate only in the organs that were responsible for calcium and bone metabolism that had a VDR. However, when other tissues in the body were recovered to serve as a negative control it was found that nuclei in essentially every tissue and organ in the body were able to concentrate and localize

Table 1
Mortality from cancer in cities according to latitude measured between 1908 and 1912

Number of Cities	Latitude	Deaths from Cancer	Rate (per 100,000)
35	60N–50N	119374	105.7
48	50N–40N	121216	92.4
24	40N–30N	37451	78.1
7	30N–10N	5696	42.3
4	10N–10S	1056	40.9
7	10S–30S	3040	37.7
5	30S–40S	11048	89.8

Modified from Hoffman FL. The mortality of cancer throughout the world. Appendix E. Prudential Press; 1915.

^{3}H-1,25(OH)$_2$D$_3$, including the skin, colon, brain, and pancreas, among many other organs.[14] Within a decade a multitude of laboratories demonstrated the presence of a VDR in essentially every tissue and cell in the body including skin, colon, brain, pancreas, and breast as well as activated T and B lymphocytes, monocytes, and macrophages.[2,13]

The first insight into the noncalcium, nonskeletal effects of vitamin D was reported in the early 1980s, when it was observed that mouse and human leukemia cells had a VDR and when they were exposed to 1,25(OH)$_2$D$_3$ their proliferative activity was reduced, and the leukemic cells differentiated into normal-appearing macrophages.[15] This observation was quickly followed by reports that a variety of cancer cell lines developed from melanoma, colon cancer and prostate cancer had a VDR, and when these cell lines were incubated with 1,25(OH)$_2$D$_3$ their cellular proliferation was reduced and they showed signs of differentiation.[16–19]

In the 1980s the first reports for extrarenal synthesis of 1,25(OH)$_2$D came from observations that patients with sarcoidosis or tuberculosis who had hypercalcemia had inappropriately normal or elevated levels of 1,25(OH)$_2$D$_3$. Initially it was believed that this was due to a unregulated synthesis of 1,25(OH)$_2$D by the kidneys.[3,20] When it was reported that a sarcoid patient who developed nephritis and lost all kidney function remained hypercalcemic with an elevated blood level of 1,25(OH)$_2$D, it was suggested that there was a nonrenal source for this metabolite.[20] This result was quickly followed by the observation that macrophages converted 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$.[21] Within a decade several investigators began reporting that cultured cells from the skin, colon, prostate, breast, lung, and brain all had the enzymatic machinery to produce 1,25(OH)$_2$D$_3$.[3,13,16–18,22–25]

CANCER PREVENTION

Epidemiologic studies over the past decade have confirmed the observations of Garland and colleagues[25] Hanchette and Schwartz,[26] who reported that adults who lived at higher latitudes were more likely to develop and die of colorectal and prostate cancer. Other observations revealed that living at higher latitudes increased the risk of dying of ovarian,[27] breast,[28] lung,[29] and esophageal cancer[30] among many others. Compelling retrospective and prospective epidemiologic studies have demonstrated that when 25(OH)D levels are less than 20 ng/mL there is a 30% to 50% increased risk of developing and dying of colorectal, prostate, breast, pancreatic, and esophageal cancer, among others (**Fig. 1**).[10,29,31–33] Men who had the most exposure to sunlight had a 3- to 5-year reprieve from developing prostate cancer compared with men who worked indoors.[34] When 972 women in Canada who had a history of breast cancer were asked about their sun exposure history as teenagers and young adults and compared their sun exposure to 1135 women matched for age and location who did not have breast cancer, it was revealed that the women with breast cancer had much less sun exposure as teenagers and young adults compared with women with no history of breast cancer. It was estimated that women who had had the most sun exposure during their teens and 20s reduced their risk of developing breast cancer by 69%, and young and middle-aged women who had the most sun exposure reduced their risk by 51%.[35] Women older than 45 years received no benefit in reducing their risk for breast cancer by being exposed to more sunlight.

The Women's Health Initiative reported that 1000 mg calcium and 400 IU vitamin D/d did not decrease the risk of developing colorectal cancer, raising questions about the benefits of vitamin D in reducing the risk of this deadly cancer.[36] The study results, however, came into question because most of the women admitted that they were not

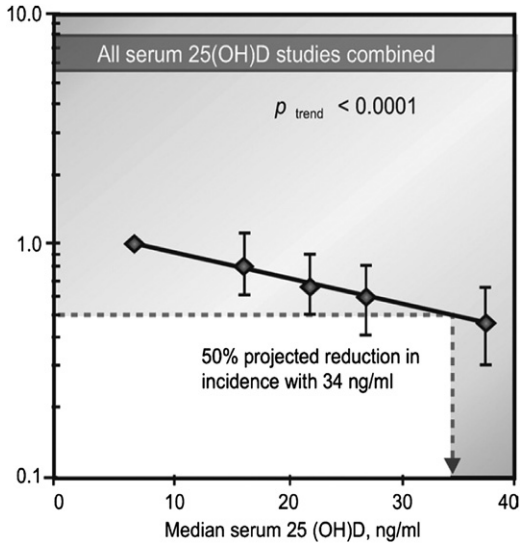


Fig. 1. Dose-response gradient for colorectal cancer according to serum 25(OH)D concentration, of 5 studies combined. The 5 points are the odds ratios for each quintile of 25(OH)D based on the combined data from the 5 studies. (*From* Gorham ED, Garland CF, Garland FC, et al. Optimal Vitamin D Status for Colorectal Cancer Prevention: A Quantitative Meta Analysis. Am J Prev Med 2007;32(3):210–6; with permission.)

taking their calcium and vitamin D more than 40% of the time during the study. More importantly, a review of the data revealed that women who had a blood level of 25(OH)D less than 12 ng/mL at the start of the study and followed for 8 years on suboptimal doses of vitamin D compared with women who had an initial blood level of 25(OH)D of 24 ng/mL had a 253% increased risk of developing colorectal cancer.[37] Pooled data of 1761 women found the highest vitamin D consumption correlated with a 50% lower risk of breast cancer (they had on average a blood level of 48 ng/mL).[31]

Lappe and colleagues[38] reported that 1179 postmenopausal women who received 1500 mg of calcium a day with 1100 IU of vitamin D_3 a day and followed for 4 years reduced their risk of developing all cancers by more than 60%. When women during the first year were removed from the analysis because of the likelihood that these women had a small undetectable cancer at the initiation of the trial, there was a dramatic 77% reduced risk of developing cancer when taking 1100 IU of vitamin D_3 a day along with calcium supplementation compared with the group that received either calcium or placebo (**Fig. 2**). In the Physician Health Study, men who had the highest levels of 25(OH)D had a lower risk of developing several cancers, including colorectal, esophageal, pancreatic, and leukemia.[33] It has also been suggested that one possible cause for the health disparity in blacks who are at a higher risk for developing and dying of cancer is due to their high incidence of vitamin D deficiency, which not only could increase their risk of developing deadly cancers but also might make the cancers more aggressive and more difficult to treat.[39,40]

Nagpal and colleagues[41] reported that $1,25(OH)_2D_3$ through its transcriptional activity was capable of regulating directly or indirectly at least 200 genes. Among these genes are those that control proliferation, differentiation, apoptosis, and angiogenesis (**Fig. 3**).[3,41] $1,25(OH)_2D_3$ increased the expression of cell cycle inhibitors and decreased activators of cyclin-cyclin dependent kinase complexes, in addition to

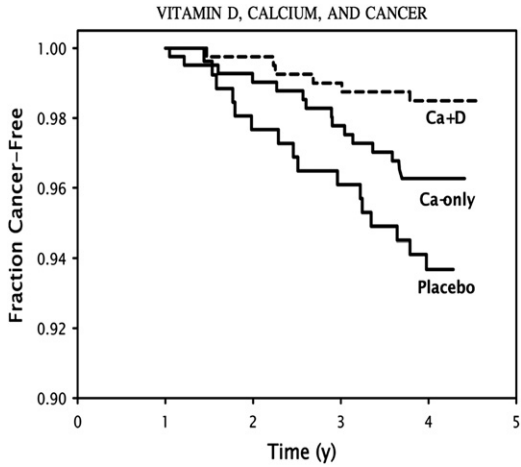


Fig. 2. Kaplan-Meier survival curves (ie, free of cancer) for the 3 treatment groups randomly assigned in the cohort of women who were free of cancer at 1 year after intervention (n = 1085). Sample sizes are 266 for the placebo group, 416 for the calcium-only (Ca-only) group, and 403 for the calcium plus vitamin D (Ca+D) group. The survival at the end of study for the Ca + D group is significantly higher than that for the placebo group, by logistic regression. (Copyright Robert P. Heaney, 2006. Used with permission.)

increasing levels of cyclin-dependent kinase inhibitors Cip/Kip proteins P21 and P27, which are known to keep the cell cycle in the G1/S phase, thus preventing DNA synthesis and cellular growth (**Fig. 4**). In addition, $1,25(OH)_2D_3$ increased the expression of the cell adhesion molecule E-cadherin and inhibited the expression of β-catenin.[42,43]

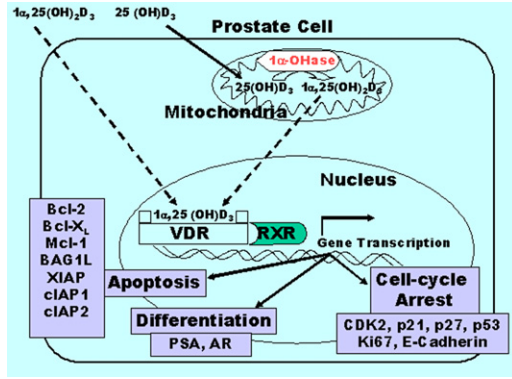


Fig. 3. Vitamin D maintains cellular growth by controlling several genes that control cellular proliferation and differentiation. 25-hydroxyvitamin D (25(OH)D) is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in a wide variety of nonrenal cells, including cells in the colon and prostate. $1,25(OH)_2D$ interacts with the vitamin D receptor (VDR) and regulates a variety of genes that control apoptosis, proliferation, and differentiation. (*Courtesy of* Michael F. Holick, PhD, MD; Copyright © 2009.)

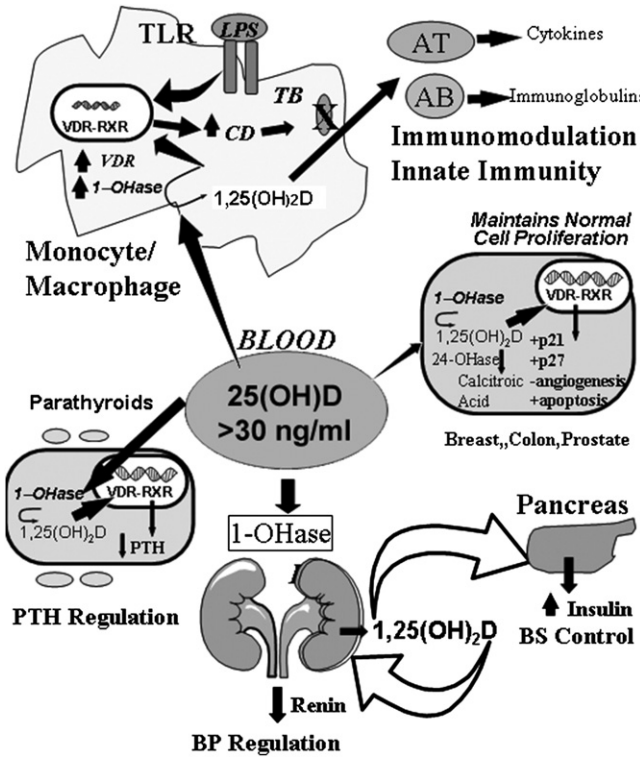


Fig. 4. Metabolism of 25-hydroxyvitamin D (25(OH)D) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) for nonskeletal functions. When a monocyte/macrophage is stimulated through its toll-like receptor 2/1 (TLR2/1) by an infective agent such as *Mycobacterium tuberculosis* (TB), or its lipopolysaccharide (LPS), the signal up-regulates the expression of vitamin D receptor (VDR) and the 25-hydroxyvitamin D1-hydroxylase (1-OHase). A 25(OH)D level greater than 30 ng/mL provides adequate substrate for the 1-OHase to convert it to $1,25(OH)_2D$. $1,25(OH)_2D$ returns to the nucleus where it increases the expression of cathelicidin (CD), which is a peptide capable of promoting innate immunity and inducing the destruction of infective agents such as TB. It is also likely that the $1,25(OH)_2D$ produced in the monocytes/macrophage is released to act locally on activated T (AT) and activated B (AB) lymphocytes, which regulate cytokine and immunoglobulin synthesis, respectively. When 25(OH)D levels are approximately 30 ng/mL, it reduces the risk of many common cancers. It is believed that the local production of $1,25(OH)_2D$ in the breast, colon, prostate, and other cells regulates a variety of genes that control proliferation, including p21 and p27 as well as genes that inhibit angiogenesis and induced apoptosis. Once $1,25(OH)_2D$ completes the task of maintaining normal cellular proliferation and differentiation, it induces the 25-hydroxyvitamin D24-hydroxylase (24-OHase). The 24-OHase enhances the metabolism of $1,25(OH)_2D$ to calcitroic acid, which is biologically inert. Thus, the local production of $1,25(OH)_2D$ does not enter the circulation and has no influence on calcium metabolism. The parathyroid glands have 1-OHase activity and the local production of $1,25(OH)_2D$ inhibits the expression and synthesis of parathyroid hormone (PTH). The production of 1,25(OH)2D in the kidney enters the circulation, and is able to down-regulate renin production in the kidney and to stimulate insulin secretion in the β-islet cells of the pancreas. (*Courtesy of* Michael F. Holick, PhD, MD; Copyright © 2007.)

The recognition that many human cancer cell lines had a VDR prompted an investigation to determine whether $1,25(OH)_2D_3$ could be used as a treatment for preleukemia. In a double-blind placebo-controlled trial, patients with preleukemia who received $1,25(OH)_2D_3$ initially responded well.[44] However, the trial proved to be unsuccessful due to the observation that patients on $1,25(OH)_2D_3$ not only developed hypercalcemia but ultimately went into blastic crisis.

There have been several thousand analogues of $1,25(OH)_2D_3$ that have been made and evaluated for their antiproliferative and calcemic activities.[45,46] Many of these analogues appeared to have great clinical promise in that they demonstrated 100 to 1000 times higher antiproliferative activity while having minimum calcemic activity. In animal models, some of these analogues including those with 2 side arms known as Gemini compounds, were shown to be effective in inhibiting MC-26 tumor cell growth progression in mice, with minimum calcemic activity.[47]

It was observed that men with metastatic prostate cancer who received 2000 IU of vitamin D_3 a day for up to 21 months showed a more than 50% reduction in rise in their prostate-specific antigen (PSA) levels compared with before receiving the vitamin D_3.[48] Men with prostate cancer who received daily $1,25(OH)_2D_3$ had a significant decrease in the rise of their PSA levels compared with men who were on placebo.[49] This prompted a phase 2 clinical trial in which a single oral dose of 45 μg of $1,25(OH)_2D_3$ was given once a week. The study was halted as a result of hypercalcemia and increased death rate in men who were taking $1,25(OH)_2D_3$.[50]

Cancer cells have developed several strategies to decrease the effectiveness of $1,25(OH)_2D_3$ from keeping cell growth in check. A human prostate cancer cell line, DU-145, is able to resist the antiproliferative activity of $1,25(OH)_2D_3$ by increasing the expression of the 25-hydroxyvitamin D24-hydroxylase (24-OHase).[51,52] This enzyme hydroxylates the side arm on carbons 24 and 23, causing a cleavage of the carbon bond at carbon 23 that results in the formation of a water-soluble carboxylic acid metabolite, calcitroic acid.[53]

Another clever strategy that malignant cells have developed to mitigate the antiproliferative activity of $1,25(OH)_2D_3$ is to increase the expression of the transcriptional factor Snail.[42] Snail is a zinc finger transcription factor that is involved in cell movement, and exists in both invertebrates and vertebrates. Snail-1 induces epithelial-to-mesenchymal transition and was found to not only inhibit the expression of VDR but also E-cadherin. Palmer and colleagues[42] observed that a human colon cancer cell line, SW-480–ADH, transfected with the Snail gene prevented the antiproliferative and prodifferentiating activity of $1,25(OH)_2D_3$ (**Fig. 5**).

PSORIASIS

In the 1980s it was appreciated that keratinocytes in the skin was not only the major source for 7-dehydrocholesterol, which could be converted to vitamin D_3 when exposed to sunlight, but also that this cell had a VDR and was able to convert 25(OH)D to $1,25(OH)_2D_3$.[2,43,53] Studies revealed that incubating keratinocytes with $1,25(OH)_2D_3$ resulted in marked decrease in DNA synthesis and proliferation, and a marked increase in markers of differentiation, including transglutaminase activity.[43,54]

It was reasoned that because $1,25(OH)_2D_3$ was such a potent inhibitor of keratinocyte proliferation in vitro, it could be used for the treatment of the nonmalignant hyperproliferative disease psoriasis (**Fig. 6**). Topically applied $1,25(OH)_2D_3$ was found to be both safe and effective for treating psoriasis.[55] Topically applied $1,25(OH)_2D_3$ resulted in marked reduction in the thickness of plaques, scaling, and erythema. Several

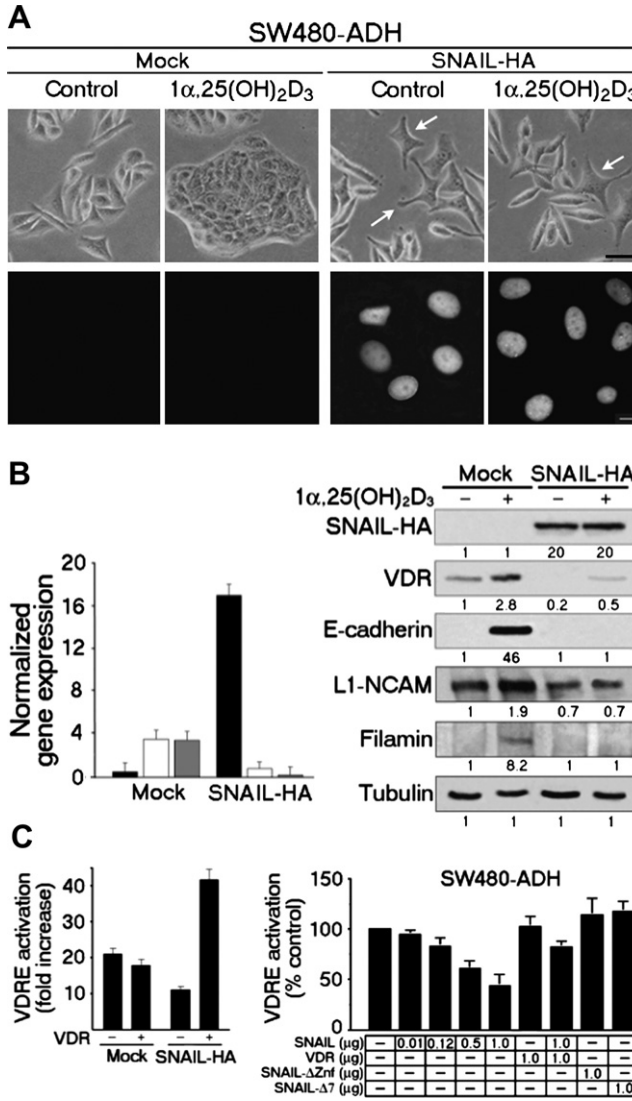


Fig. 5. (*A, top*) Micrographs of SNAIL-HA and mock-infected cells. Arrows indicate the phenotypic change induced by SNAIL. Bar, 50 μm. (*A, bottom*) Immunostaining of ectopic SNAIL expression using an antibody to HA. Bar, 10 μm. (*B, left*) normalized SNAIL. VDR and E-cadherin mRNA levels were measured by real-time reverse transcription-polymerase chain reaction. (*B, right*) Protein expression was estimated by Western blot. Numbers refer to fold increase over untreated mock-infected cells. (*C*) SNAIL inhibits the induction of L1-NCAM and filamin by 1,25$(OH)_2D_3$. Wild-type (*left*) but not mutant (*right*) SNAIL proteins inhibit VDR transcriptional activity (4XVDRE-tk-luciferase). (*From* Palmer HG, Larriba MJ, Garcia JM, et al. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. Nat Med 2004;10:917–9; with permission.)

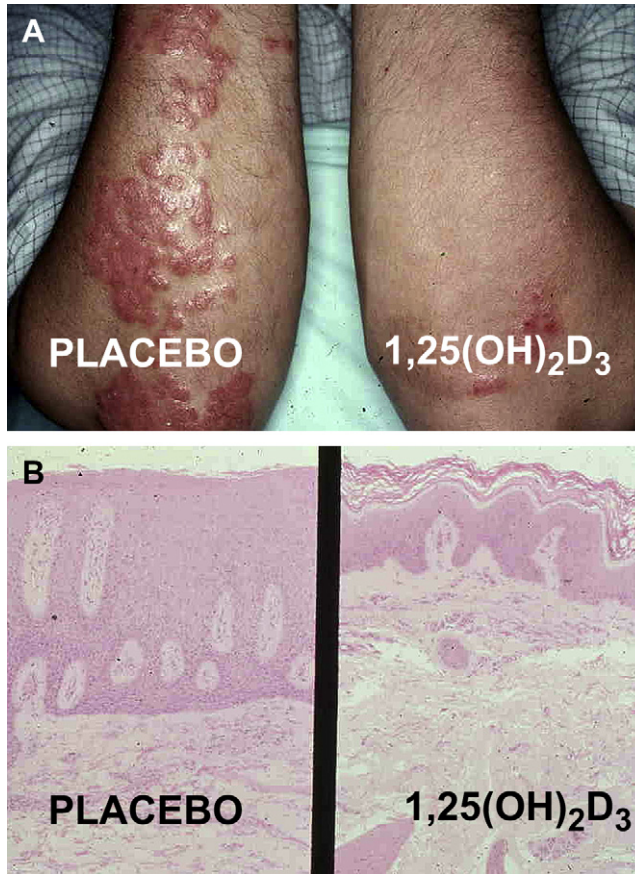


Fig. 6. (Top panel) A 28-year-old man with a more than 20-year history of psoriasis. The psoriatic lesions on the patient's right forearm were treated with placebo Vaseline and the psoriatic lesions on the left forearm were treated with Vaseline containing 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). (*Bottom panel*) Photomicrographs of biopsies from the right forearm and left forearm. (*Courtesy of* Michael F. Holick, PhD, MD; Copyright © 2009.)

analogues of $1,25(OH)_2D_3$, including calcipotriene, 1,24-dihydroxyvitamin D_3, and 22-oxo-$1,25(OH)_2D_3$, were also evaluated for their antiproliferative activity in cultured keratinocytes.[56,57] These substances were all found to inhibit keratinocyte proliferation and induced maturation; along with $1,25(OH)_2D_3$, they were consequently developed as a first-line therapy for the treatment of psoriasis.

VITAMIN D AND AUTOIMMUNE DISEASES

Living at a latitude above 35° for the first 10 years increases the risk of developing multiple sclerosis (MS) by 100% no matter where one lives thereafter.[58,59] A similar observation has been made for type I diabetes. There was a 10- to 15-fold increased risk of developing type 1 diabetes if living in far northern or southern regions of the globe compared with living near the equator.[60]

Epidemiologic evidence suggests that both men and women who have the highest blood levels of 25(OH)D had the lowest risk for developing MS.[61] In the Nurses' Health

Study it was observed that women who had the highest intake of vitamin D had a 42% reduced risk of developing MS.[62] A similar observation was made in that the women who had the highest intake of vitamin D and had a reduced risk of developing rheumatoid arthritis by 41%.[63]

In the 1960s children in Finland during their first year of life were recommended to take 2000 IU of vitamin D a day. A follow-up study 31 years later revealed that those children who took 2000 IU of vitamin D a day during their first year of life reduced their risk of developing type 1 diabetes by 88%.[64] Those children who had evidence of vitamin D deficiency had a 2.4-fold increased risk of developing type 1 diabetes. Wheezing disorders and asthma have been linked to vitamin D deficiency in utero. Children born from mothers who were vitamin D deficient had a 60% increased risk of having wheezing disorders during their first few years of life.[65,66]

Although the mechanism by which enhancing vitamin D status reduces risk of developing autoimmune diseases is not fully understood, it is known that when resting T and B lymphocytes are stimulated, one of the first genes that is turned on is the gene for the VDR. Activated T and B lymphocytes have a VDR and $1,25(OH)_2D_3$ is a potent regulator of both T- and B-cell activity. $1,25(OH)_2D_3$ suppresses proliferation and immunoglobulin synthesis,[43,67] and has a multitude of effects on T-lymphocyte function and activity. $1,25(OH)_2D_3$ inhibits T-cell proliferation, in particular T-helper (Th1) cells capable of producing interferon (IFN)-γ and interleukin (IL)-2. These actions in turn prevent further antigen presentation to and recruitment of T lymphocytes. In addition, $1,25(OH)_2D_3$ enhances the production of IL-4, IL-5, and IL-10, shifting the balance from Th1 to Th2 cell phenotype.[43,68] In addition to its effects on activated T lymphocytes, $1,25(OH)_2D_3$ regulates dendritic cell activity, which plays a key role in antigen presentation. These cells have a VDR, and respond to the antiproliferative and immunomodulatory activities of $1,25(OH)_2D_3$. It is also recognized that $1,25(OH)_2D_3$ inhibits the formation of Th17 cells, which are now considered to play an important role in autoimmunity.[43,69]

It is curious that whereas most tissues and cells in the body are capable of producing $1,25(OH)_2D_3$, lymphocytes do not express the 1-OHase. Instead, activated macrophages produce $1,25(OH)_2D_3$ not only for the regulation of cathelicidin production[70,71] but also to act in a paracrine fashion to interact with the VDR in activated T and B lymphocytes, in order to modulate their immune functions (see **Fig. 4**).[3]

It has been suggested that the potent immunomodulatory activity of $1,25(OH)_2D_3$ will lead to an increased risk of autoimmune diseases.[72] However, what these investigators do not appreciate is that vitamin D is a modulator, not an inhibitor, of the immune system and that it plays a central role in maintaining a healthy immune system. Several animal models have been used to demonstrate that $1,25(OH)_2D_3$ is very effective in either preventing or significantly reducing the progression of autoimmune encephalitis in models of MS, type 1 diabetes, and Crohn disease,[73,74] all of which support the epidemiologic evidence that vitamin D is important for immune health.

INNATE IMMUNITY

In the mid-1800s it was recognized that cod liver oil was effective in treating tuberculosis (TB). In the early 1900s solariums were developed, in part to treat patients with TB, and Finsen demonstrated that exposure of the skin to sunlight was an effective therapy for treating *Mycobacterium* infections of the skin. More recent studies have associated vitamin D deficiency with increased risk of not only developing TB but also other infectious diseases, including otitis media,[75] upper respiratory tract

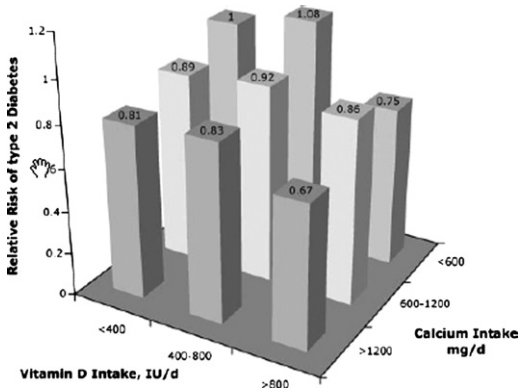


Fig. 7. Adjusted relative risk of incident type 2 DM in the Nurses' Health Study by calcium and vitamin D intake. (*From* Holick, MF. Diabetes and the Vitamin D Connection. Current Diabetes Reports 2008;8:393–8; with permission.)

infections,[76] and influenza infection.[77] It has been hypothesized that there is a seasonal stimulus for influenza infection; it usually appears in mid to end of winter, a time when the 25(OH)D levels are at the nadir.[77] Postmenopausal women who took 2000 IU of vitamin D a day for 1 year reduced their risk of upper respiratory tract infections by 90%.[78] Children and adults who had the highest blood levels of 25(OH)D had the lowest risk of developing upper respiratory tract infections throughout the year.[76]

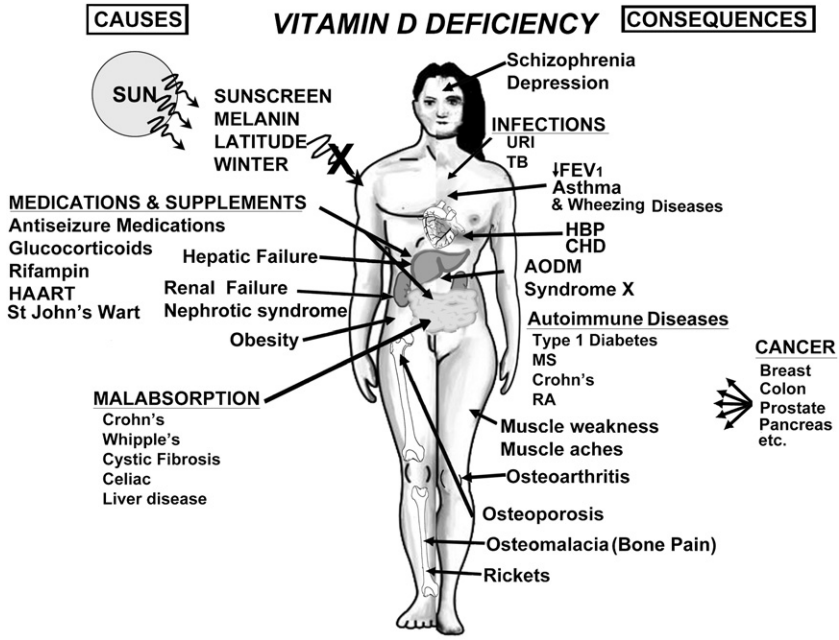
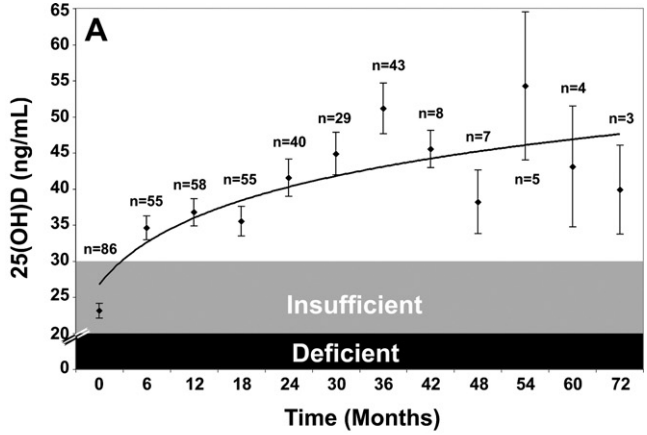
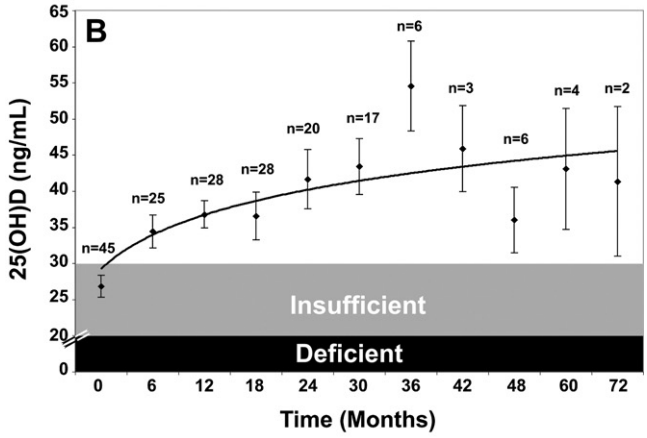
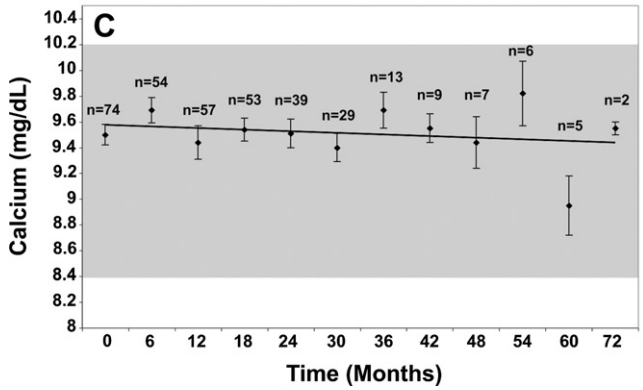


Fig. 8. Major Causes of vitamin D deficiency and potential health consequences. (*Courtesy of* Michael F. Holick, PhD, MD; Copyright © 2007.)

Although it was well known that activated T and B lymphocytes had a VDR and that $1,25(OH)_2D_3$ was a potent modulator of the immune response, it was unclear how this activity could reduce risk of infectious diseases. It was also known that circulating monocytes and macrophages have a VDR and also can produce $1,25(OH)_2D_3$.[3,43,79] Innate immunity is associated with the activation of toll-like receptors (TLRs), not







only on monocytes and macrophages but also in other barrier cells of the intestine, gingiva, bladder, lungs, and epidermis.[43] Activation of TLRs results in the production of antimicrobial peptides and reactive oxygen species, which in turn kill infective agents. When a macrophage ingests a mycobacterium the lipopolysaccharide on its cell wall interacts with the TLR2/1 receptor, resulting in the expression of VDR and 1-OHase.[70] The macrophage now has the capability of producing $1,25(OH)_2D_3$, which can in turn interact with its VDR to stimulate the production of the antimicrobial peptide cathelicidin. It has been demonstrated that monocytes infected with *Mycobacterium* and incubated in blood from an African American who had a 25(OH)D level of 8 ng/mL resulted in the death of the monocyte. When monocytes were exposed to the same mycobacterium but now incubated in blood that had added to it 25(OH)D to raise the level to 28 ng/mL, the monocyte was able to mount an effective response by enhancing cathelicidin production, resulting in the death of the mycobacterium. These results provide a mechanism by which vitamin D plays a crucial role in reducing the risk of infectious diseases.

CARDIOVASCULAR HEALTH

Adults who are vitamin D deficient have a 50% higher risk of developing a myocardial infarction.[80] Furthermore, patients who had a myocardial infarction and were vitamin D deficient were more likely to die from the event.[81] In 1979 Rostand[82] reported that living at higher latitudes increased the risk of hypertension. Studies have suggested that increasing vitamin D intake reduces the risk of hypertension. Exposure of patients to vitamin D producing simulated sunlight 3 times a week for 3 months on a tanning bed increased circulating levels of 25(OH)D by 180% and reduced systolic and diastolic blood pressure by 6 mm Hg, whereas hypertensive patients exposed to a tanning bed that only emitted ultraviolet A radiation and did not experience any increase in the blood level of 25(OH)D and had no change in their blood pressure.[83]

Fig. 9. (*A*) Mean serum 25-hydroxyvitamin D (25(OH)D) levels in all patients: includes patients treated with 50,000 IU vitamin D_2 every 2 weeks (maintenance therapy, N = 81), including those patients with vitamin D insufficiency who were initially treated with 8 weeks of 50,000 IU vitamin D_2 weekly before maintenance therapy (N = 39). Error bars represent standard error of the mean; mean result over 5 years is shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. When mean 25(OH)D in each 6-month group was compared with mean initial 25(OH)D, $P<.001$ up until month 43; $P<.001$ when all remaining values after month 43 were compared with mean initial 25(OH)D. (*B*) Mean serum 25(OH)D levels in patients receiving maintenance therapy only: levels for 37 patients who were vitamin D insufficient (25(OH)D levels <30 ng/mL) and 5 patients who were vitamin D sufficient (25(OH)D levels $\geq$30 ng/mL) who were treated with maintenance therapy of 50,000 IU vitamin D_2 every 2 weeks. Error bars represent standard error of the mean; mean result over 5 years is shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. When mean 25(OH)D in each 6-month group were compared with mean initial 25(OH)D, $P<.001$ up until month 37; $P<.001$ when all remaining values after month 43 were compared with mean initial 25(OH)D. (*C*) Serum calcium levels: results for all 81 patients who were treated with 50,000 IU of vitamin D_2. Error bars represent standard error of the mean. Time 0 is initiation of treatment, results shown as mean values averaged for 6-month intervals. Normal serum calcium: 8.5 to 10.2 mg/dL. (*From* Pietras SM, Obayan BK, Cai MH, et al. Vitamin D2 treatment for vitamin D deficiency and insufficiency for up to 6 years. Arch Intern Med 2009;169:1806–8; with permission. Copyright © 2009 American Medical Association. All rights reserved.)

$1,25(OH)_2D_3$ is a potent down-regulator of renin production, a hormone that is responsible for regulating blood pressure.[84] Vascular smooth muscle and cardiomyocytes have a VDR, and it has been estimated that 200 genes that regulate cardiovascular health may be influenced by $1,25(OH)_2D_3$.[85,86] In addition to these cardioprotective effects $1,25(OH)_2D_3$ has anti-inflammatory activity, and reduces C-reactive protein (CRP) and IL-10 production.[85,86] In addition, $1,25(OH)_2D_3$ suppressed foam cell formation by reducing acetylated or oxidized low-density lipoprotein cholesterol uptake in macrophages obtained from diabetes patients.[87]

This finding may help explain the observation of an 80% reduction in development of peripheral vascular disease when the 25(OH)D was above 25 ng/mL.[88]

TYPE 2 DIABETES

β-Islet cells in the pancreas have a VDR, and $1,25(OH)_2D_3$ stimulates insulin production.[60,89] In addition, it has been reported that improvement in vitamin D status in type 2 diabetic patients improves insulin resistance.[60,89] Men and women who had an intake of calcium of greater than 1000 mg a day and more than 800 IU of vitamin D a day had a relative risk of reduction in developing type 2 diabetes of 33% (**Fig. 7**).[90] It has also been observed that there is an inverse relationship between blood levels of 25(OH)D and risk of type 2 diabetes, with a 75% reduction in whites and 83% reduction in Mexican Americans.[91]

SUMMARY

Vitamin D deficiency is the most common nutritional deficiency and likely the most common medical condition in the world.[3] There is a multitude of causes of vitamin D deficiency (**Fig. 8**), but the major cause has been the lack of appreciation that the body requires 5- to 10-fold higher intakes than is currently recommended by the Institute of Medicine and other health agencies.[92] It is likely that our hunter gatherer forefathers being exposed to sunlight on a daily basis were making several thousand IU of vitamin D a day. The fact that 100 IU of vitamin D prevented overt signs of rickets led to the false security that ingesting twice this amount was more than adequate to satisfy the body's vitamin D requirement.[93] Although this may be true for preventing overt skeletal deformities associated with rickets, there is now overwhelming and compelling scientific and epidemiologic data suggesting that the human body requires a blood level of 25(OH)D above 30 ng/mL for maximum health.[94] The likely reason is that essentially every tissue and cell in the body has a VDR and thus, to have enough vitamin D to satisfy all of these cellular requirements, the blood level of 25(OH)D needs to be above 30 ng/mL. It has been estimated that for every 100 IU of vitamin D ingested that the blood level of 25(OH)D increases by 1 ng/mL.[95,96] Thus to theoretically achieve a blood level above 30 ng/mL requires the ingestion of 3000 IU of vitamin D a day. There is evidence, however, that when the blood levels of 25(OH)D are less than 15 ng/mL, the body is able to more efficiently use vitamin D to raise the blood level to about 20 ng/mL.[97] To raise the blood level of 25(OH)D above 20 ng/mL requires the ingestion of 100 IU of vitamin D for every 1-ng increase; therefore to increase the blood level to the minimum 30 ng/mL requires the ingestion of at least 1000 IU of vitamin D a day for adults.

There is a great need to significantly increase the recommended adequate intakes of vitamin D. All neonates during the first year of life should take at least 400 IU/d of vitamin D, and increasing it to 1000 IU/d may provide additional health benefits. Children 1 year and older should take at least 400 IU/d of vitamin D as recently recommended by the American Academy of Pediatrics,[98] but they should consider increasing

intake up to 2000 IU/d derive maximum health benefits from vitamin D. Prepubertal and teenage girls who received 2000 IU of vitamin D per day for a year showed improvement in their musculoskeletal health with no untoward toxicity.[99] All adults should be taking 2000 IU of vitamin D per day. A recent study reported that adults who took 50,000 IU of vitamin D once every 2 weeks, which is equivalent to taking 3000 IU of vitamin D a day, for up to 6 years was effective in maintaining blood levels of 25(OH)D of between 40 and 60 ng/mL without any toxicity (**Fig. 9**).[100]

There is no downside to increasing either a child's or adult's vitamin D intake, with the exception of acquired disorders such as granulomatous diseases including sarcoidosis and tuberculosis, as well as some lymphomas with activated macrophages that produce $1,25(OH)_2D_3$ in an unregulated fashion.[3,79]

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