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The Role of Vitamin D in Human Health: A Paradigm Shift

Joan M. Lappe, PhD, RN, FAAN[1]

Abstract
Vitamin D deficiency is pandemic, spanning many continents and including all ages, genders and racial/ethnic groups. Currently, world-wide attention is focused on the importance of vitamin D in optimizing health and preventing disease. This focus is largely the result of the scientific discovery that vitamin D receptors are present in nearly every tissue and cell in the body and that adequate vitamin D status is essential for optimal functioning of these tissues and cells. An impressive body of research has accumulated over the past two decades providing new information about the role of vitamin D in prevention of a broad range of diseases. The purpose of this paper is to provide a review of this new information.

Vitamin D Metabolism

Vitamin D (which includes both D_2 and D_3) carries out essential biologic functions through both an endocrine mechanism and an autocrine mechanism. It is found in all animals, and in humans, it is made in skin exposed to ultraviolet (UV)-B radiation. It is derived from a cholesterol precursor in the skin, 7-dehydrocholesterol. When the skin absorbs UV-B radiation, the precursor is converted to previtamin D_3, which undergoes thermally induced transformation to vitamin D_3 (cholecalciferol). Vitamin D_2 (ergocalciferol) is a synthetic product produced by irradiation of plant sterols. Vitamin D, whether from the diet or the skin, is metabolized in the liver to 25(OH)D by 25-hydroxylase. Since 25(OH)D is the most plentiful and stable metabolite of vitamin D in the human bloodstream, it has been accepted as the functional indicator of vitamin D status.[1]

25(OH)D is a prohormone that serves as an immediate precursor to the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D; calcitriol). A single enzyme, 25(OH)D-1-α-hydroxylase (encoded by CYP27B1), is responsible for production of 1,25(OH)$_2$D, which serves as a high-affinity ligand for the vitamin D receptor.[2] In its endocrine action, 25(OH)D is converted by hydroxylation in the kidney to 1,25(OH)$_2$D, which circulates in the blood as a hormone to regulate mineral and skeletal homeostasis. The primary target of 1,25(OH)$_2$D is the intestinal mucosa in which it directs the calcium transport system to adapt to varying calcium intakes.

Renewed interest in vitamin D has been stimulated by the discovery that vitamin D also acts through an autocrine pathway. In this system, 25(OH)D is converted to 1,25(OH)$_2$D intracellularly by 25(OH)D 1-α-hydroxylases in various cells of the immune system as well as in many epithelial cell types, such as breast, colon, lung, skin, and prostate.[3-7] When these cells receive an extracellular signal to produce certain proteins, enzymes, or signaling molecules, 1,25(OH)$_2$D binds to the vitamin D receptor and, in combination with tissue-specific and stimulus-specific proteins, binds to vitamin D response elements on the chromosomes, inducing transcription of needed substances. In this manner, 1,25(OH)$_2$D serves as an intermediary between external stimuli and genomic response. Since the tissue level 1-α-hydroxylase operates well below its k_M, this local conversion of 25(OH)D to 1,25(OH)$_2$D is dependent on adequate levels of circulating 25(OH)D. Also produced in these cells that have 1-α-hydroxylases is vitamin D 24-hydroxylase, which degrades excess 1,25(OH)$_2$D intracellularly and prevents excess accumulation of 1,25(OH)$_2$D.[8] Thus, vitamin D serves as a quick on-off switch, necessary for expression of certain cellular actions but also limiting their duration and extent. This well-worked out model illustrates the key role of vitamin D in mediating certain cellular responses to external signals (see Figure 1).

[1] Creighton University, Omaha, NE, USA

Corresponding Author:
Joan M. Lappe, PhD, RN, FAAN, Creighton University, Suite 4820, 601 N 30th St, Omaha NE 68131, USA
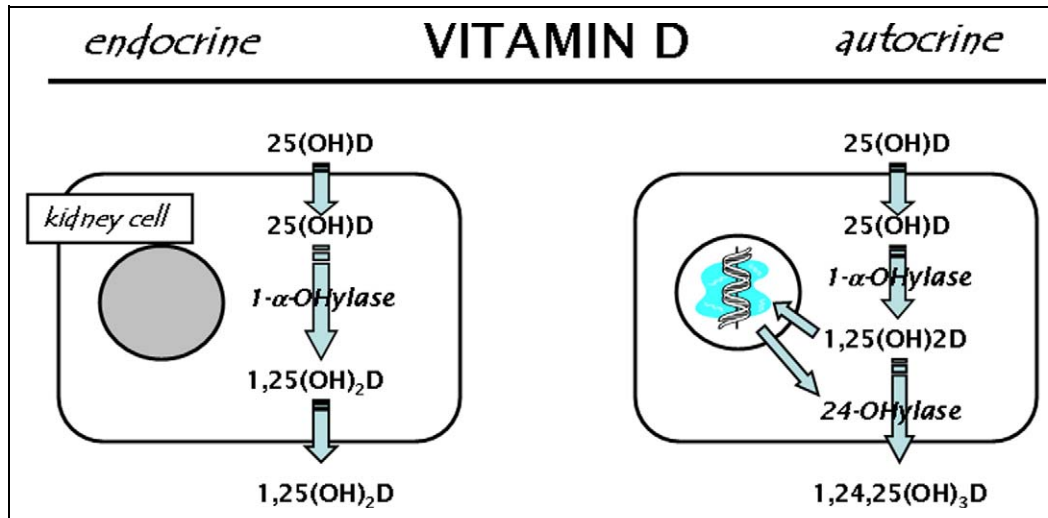Email: jmlappe@creighton.edu

Figure 1. Schematic depiction of the 2 faces of vitamin D function. The principal outputs of each are designated by the symbol ★. In the case of the endocrine side, the stimulus to expression and action of the 1-α-hydroxylase is typically parathyroid hormone (PTH) and FGF23, and the principal output is calcitriol (1,25(OH)$_2$D). On the autocrine side the stimulus to expression of the 1-α-hydroxylase will vary from tissue to tissue, and the principal output of the process (but not released from the cell) will be the proteins signaling, for example, cell differentiation and apoptosis. For the autocrine function the principal input variable will be serum 25(OH)D, as circulating calcitriol levels are not usually high enough to elicit the full autocrine response. Instead, each tissue controls its own autocrine activation independently of other tissues but is dependent on an adequate circulating level of 25(OH)D. (Copyright 2006, Robert P. Heaney, MD)

Optimal Levels of Serum 25(OH)D

As mentioned earlier, the indicator of vitamin D status is serum 25(OH)D. There is lack of agreement on the definition of optimal 25(OH)D and the cutoff for low vitamin D status. Until recently, the index disease for vitamin D deficiency in adults has been osteomalacia, which is associated with serum 25(OH)D concentrations <8 ng/mL (<20 nmol/L).[9] However, it is now recognized that 25(OH)D ≥8 ng/mL (≥20 nmol/L) can also be associated with skeletal disease. Although "normal ranges" in US laboratories vary between 20 and 100 ng/mL (50-250 nmol/L), there is a growing consensus that the optimal range for 25(OH)D values lies above 30 to 32 ng/mL (75-80 nmol/L) for most populations. This is based on studies of the inverse relationship between serum parathyroid hormone and 25(OH)D, showing that parathyroid hormone concentrations plateau at serum 25(OH)D levels of 28 to 40 ng/mL (70-100 nmol/L).[10-14] Further evidence is provided by studies that show calcium absorption efficiency increases with rising 25(OH)D and plateaus at 25(OH)D levels about 32 ng/mL (80 nmol/L).[15-17]

An optimal level of at least 30 to 32 ng/mL (75-80 nmol/L) is also suggested by the relationship between 25(OH)D and both bone mineral density and lower extremity neuromuscular function in National Health and Nutrition Examination Survey III (NHANES III).[18,19] Numerous other disorders, in addition to skeletal diseases, have been associated with low 25(OH)D, although these conditions have not been linked definitively to specific 25(OH)D levels. For example, observational studies show that 25(OH)D levels above 30 to 32 ng/mL (75-80 nmol/L) are associated with reduced incidence of colorectal adenomas and cancer.[20,21] A study in nondiabetics found that insulin sensitivity is inversely associated with vitamin D status and that at 25(OH)D levels of about 46 ng/mL (114 nmol/L) no further lowering of serum glucose is observed.[22]

That higher levels of 25(OH)D are optimal is also supported by values found in persons who spend time outdoors. Dark-skinned workers in tropical climates have 25(OH)D values of about 60 ng/mL (150 nmol/L).[23] Others have shown that 25(OH)D as high as 80 ng/mL (200 nmol/L) can be achieved by cutaneous production.[16,24] Thus, the preponderance of evidence points to optimal serum 25(OH)D levels of at least 32 ng/mL (80 nmol/L). Furthermore, emerging data suggest that higher levels are needed to prevent some of the nonskeletal disorders associated with inadequate vitamin D.

Vitamin D Deficiency

Low vitamin D status is prevalent across all age-groups, geographic regions, and seasons.[7,25,26] The NHANES data shows that the number of persons with 25(OH)D levels below 30 ng/mL (75 nmol/L) nearly doubled from the 1994 survey to the 2004 survey.[27] The most recent survey (2004) found that 75% to 80% of the NHANES population has 25(OH)D levels <30 ng/mL (<75 nmol/L), whereas 65% to 75% of the population has levels <20 ng/mL (<501 nmol/L).[26] More than 90% of the black and Latino population have levels <30 ng/mL (<75 nmol/L). Of great concern are findings that less than 3% of African American mothers are vitamin D sufficient, and the mean cord blood levels of 25(OH)D in their infants is very low (10 ± 6 ng/mL or 25 ± 15 nmol/L).[27,28]

Factors associated with vitamin D deficiency include low sunlight exposure, age-related decreases in cutaneous

synthesis, low vitamin D oral intakes, obesity, and high degree of skin pigmentation.[7,25,29] The reasons for the dramatic increase in vitamin D insufficiency are not clear, but decline in 25(OH)D levels are associated with decreased consumption of vitamin D–fortified milk, increased use of sun screen, and the upward trend in body mass index.[30] It has been hypothesized that the worldwide increase in obesity is a major contributory factor to growing epidemic of vitamin D insufficiency.[31]

Achieving Optimal Levels of Vitamin D

It is very difficult to achieve and maintain optimal levels of serum 25(OH)D by diet alone since few foods are natural sources of vitamin D and fortified foods contain limited amounts. Oily fish, such as salmon, mackerel, and herring, and sun-dried mushrooms are a rich source of vitamin D. Ocean-raised fish, which feed on vitamin D–rich plankton, have much higher levels of vitamin D than farm-raised varieties. Cod liver oil is a rich source of vitamin D, but many available preparations of cod liver oil also contain large amounts of vitamin A, which antagonizes the action of vitamin D and can cause toxicity.[32] In the United States, milk is fortified with a little more than 100 International Units (IU) of vitamin D, whereas other foods, such as yogurt, juices, cereals, and soy, are fortified with varying amounts (see Table 1 of sources of vitamin D).

The major source of vitamin D is sunlight exposure. For a Caucasian adult, sunlight exposure in a bathing suit long enough to cause the skin to turn pink raises the serum 25(OH)D to a level comparable to a person taking 10 000 to 20 000 IU of vitamin D_2.[33] Exposure to arms and legs for 5 to 30 minutes between 10:00 AM and 3:00 PM is often adequate to meet vitamin D requirements. However, variables such as time of day, season, latitude, clothing, sunscreen use, skin pigmentation, and age affect the amount of vitamin D converted in the skin. For example, at latitudes above 37°N, from about mid-October to mid-March the solar angle is such that no vitamin D is converted in the skin. Although humans store some vitamin D obtained from summer sunlight, many individuals do not store enough to supply adequate amounts through the winter months.[34] Also, aging decreases the amount of 7-dehydrocholesterol in the skin by about 50% between the ages of 20 and 80 years,[35] which decreases the amount of vitamin D_3 older persons can make. It is well established that persons with greater skin pigmentation produce lower amounts of vitamin D_3 compared with their lighter skinned counterparts, and this largely accounts for the higher prevalence of vitamin D deficiency in darker-skinned persons.[36-38] Sunscreen with a sun protective factor as low as 8 decreases conversion of vitamin D_3 in the skin by 95%.[39] Probably the greatest barrier to obtaining optimal vitamin D from sunlight exposure is the widespread concern about skin cancer and photoaging resulting in sun avoidance and widespread use of sunscreen.

Fortunately, vitamin D dietary supplements, which are safe and inexpensive, are becoming widely available. Many

Table 1. Dietary Sources of Vitamin D

Food	IUs per Serving[a]
Cod liver oil, 1 tablespoon	1360
Salmon (sockeye), cooked, 3 ounces	794
Mackerel, cooked, 3 ounces	388
Tuna fish, canned in water, drained, 3 ounces	154
Milk, nonfat, reduced fat, and whole, vitamin D–fortified, 1 cup	115-124
Sardines, canned in oil, drained, 2 sardines	46
Liver, beef, cooked, 3.5 ounces	46
Egg, 1 whole (vitamin D is found in yolk)	25

Abbreviation: IU, International Unit.
[a]Adapted from *Selected Food Sources of Vitamin D*. US Department of Agriculture, Agricultural Research Service. USDA Nutrient Database for Standard Reference, Release 22; 2009.

multiple-vitamin preparations contain 200 to 400 IU/capsule. Also, calcium supplement pills commonly contain 200 to 400 IU of vitamin D. Stand-alone vitamin D supplements range in dose from 400 to 50 000 IU and can be found in various formulations such as tablets, gelcaps, liquid drops, and chewables. The American Academy of Pediatrics' currently recommended intake level of vitamin D for infants and children is 400 IU/day.[40] In November, 2010, the United States Institute of Medicine released revised recommendations for daily intake of vitamin D based on the body's need for skeletal health. (www.iom.edu/vitamind) Their recommended adequate intake levels are 600 IU/day for everyone from age one through 70 years. The IOM report stated that persons age 71 and older may require as much as 800 IUs per day because of potential changes in their bodies as they age However, the new IOM recommendations are controversial and have stimulated considerable debate.

Vitamin D_2 Versus Vitamin D_3

Vitamin D_3 (cholecalciferol) is synthesized in the skin on exposure to sunlight, whereas vitamin D_2 (ergocalciferol) is a synthetic product obtained by irradiation of plant sterols. Because of differences in the chemistry of their side chains, the 2 forms differ in their metabolism and in their binding to vitamin D binding protein, which transports both isoforms through the circulation[42] (see Figure 2). Probably due to these differences, recent studies suggest that vitamin D_3 is more potent than vitamin D_2.[43-45] Holick et al demonstrated that daily doses of 1000 IU of vitamin D_2 are equally as effective as daily doses of 1000 IU of vitamin D_3 in maintaining 25-hydroxyvitamin.[46] However, 2 studies found that with intermittent dosing vitamin D_3 stayed in the circulation longer than vitamin D_2.[43,45] Thus, vitamin D_3 would be a better choice for persons who prefer intermittent dosing. Both vitamin D_2 and D_3 are available commercially, and on average, vitamin D_3 is less expensive.
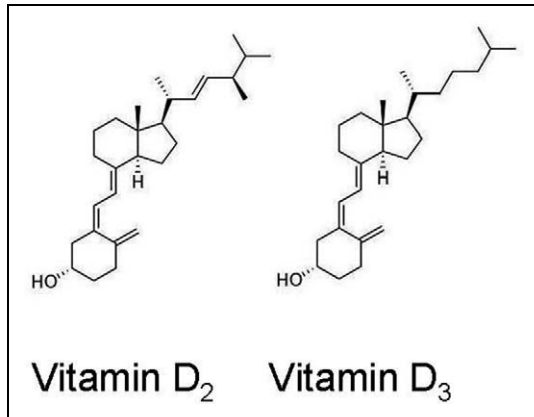
Figure 2. Isomers of vitamin D_2 and Vitamin D_3. Ergosterol in plants and 7-dehydrocholesterol in skin are the precursors for vitamin D_2 and vitamin D_3, respectively. Ultraviolet light B breaks the B chain of each molecule to form the pre-D isomer, which then undergoes isomerization to D. D_2 and D_3 differ only in the side chain in which D_2 has a double bond between C22 and C23 and a methyl group at C24. These differences result in somewhat different binding to D binding protein and metabolism.

How Much Vitamin D Supplementation Is Needed?

Heaney established the 25(OH)D response to various doses of vitamin D_3 (up to 10 000 IU).[34,47] In brief, the serum 25(OH)D rises by about 1 ng/mL (2.5 nmol/L) for each 100 IU of additional D_3. If, for example, one wants to increase serum 25(OH)D by 15 ng/mL (37.5 nmol/L)—for example, from 15 to 30 ng/mL (37.5-75 nmol/L)—this would require additional vitamin D_3 intake of about 1500 IU (37.5 μg) per day. Applying this information to the NHANES III national distribution data for serum 25(OH)D, Heaney showed explicitly what the distribution would be if everyone in the US population received an additional 2000 IU vitamin D_3 per day.[48] The mean would rise by about 14 ng/mL (35 nmol/L), and about 80% to 85% of the population would have 25(OH)D value above 32 ng/mL (80 nmol/L).

There is considerable variation in how individuals respond to vitamin D supplementation. This is seen in clinical studies as well as clinical practice.[43,49] Starting at the same baseline 25(OH)D level, some persons will require higher doses than others to achieve an identical target value. Thus, the only definitive way to assess for deficiency and to achieve the target serum 25(OH)D is to measure 25(OH)D. However, serum 25(OH)D assays are expensive, and the need for universal screening has not been established as of yet. Currently, third party payer coverage of vitamin D measurement is limited. Table 2 shows some of the clinical conditions for which measurement of serum 25(OH)D is indicated and can be reimbursed by Medicare. Of course, paying out-of-pocket costs for the assay is always an option and is prudent for otherwise healthy persons who are at risk of vitamin D insufficiency (eg, dark skin, advanced age, obesity).

Table 2. Clinical Conditions for Which Measurement of Serum 25(OH)D Is Indicated and May Be Reimbursed by Medicare

Hypo/hyperparathyroidism
Rickets
Osteoporosis and osteomalacia
Paget's bone disease
Disorders of phosphorus metabolism
Disorders of calcium metabolism
Chronic kidney disease
History or risk of falls
Malabsorption syndromes
Fibromyalgia
Gastric bypass and bariatric surgery
Psoriasis
Unspecified vitamin D deficiency
Liver disease
Use of anticonvulsants

Safety of Vitamin D

The Institute of Medicine (IOM) National Academy of Sciences recently raised the tolerable upper intake level of vitamin D from 2000 IU/day to 4000 IU/day.[1] The recent IOM report indicated that serum levels of 25(OH)D above 50 ng/mL may be associated with adverse events, but did not set that as a threshold for vitamin D toxicity.[50] Barger-Lux and Heaney[16] found that healthy men who completed a summer season of outdoor work had a mean serum 25(OH)D of 45 ng/mL (122 nmol/L), with some men exceeding 80 ng/mL (200 nmol/L). Others have found similarly high levels of serum 25(OH)D in other populations exposed to the sun.[24,51] In the Barger-Lux and Heaney study, the mean sun exposure response was equivalent in dosing to 2800 IU/d (70 μg/d). This means that natural sunlight exposure provides healthy young people with considerably higher doses of vitamin D than currently considered safe for oral dosing. In fact, there is no evidence of adverse effects of vitamin D_3 intake at or below 10 000 IU/d.[52] Based on the sunlight studies, Binkley has concluded that designating the upper limits of serum 25(OH)D as between 80 and 100 ng/mL (200 and 250 nmol/L) is appropriate.[53]

Vitamin D toxicity, which is very rare, is a clinical syndrome of both hypervitaminosis D and hypercalcemia.[54] Hyperphosphatemia and hypercalciuria are often, but not always, present. Clinical symptoms include nausea and vomiting, dehydration, muscle weakness, lethargy, and confusion. No cases of vitamin D toxicity have been reported in vitamin D supplementation doses less than 10 000 IU/d.[52] Usually, the large doses have been for prolonged periods of time and often concomitant with excessive amounts of calcium. In a recent study, vitamin D supplementation of 1600 IU/d or 50 000 IU monthly was not associated with any signs of toxicity.[55] In another study, Hollis supplemented women during pregnancy with 4000 IU/d vitamin D_3 without any adverse clinical or laboratory events.[56] This provides confidence that we can increase vitamin D intake from prevailing levels without incurring significant risk.

Persons with vitamin D toxicity usually have serum 25(OH)D levels above 150 ng/mL (375 nmol/L).[57] In fact, Heaney pointed out that "no credible reports" show vitamin D toxicity at serum 25(OH)D levels below 200 ng/mL (500 nmol/L).[58] However, individuals with metastatic cancer or granulomatous diseases, such as tuberculosis or sarcoidosis, can develop hypercalcemia at 25(OH)D levels that are somewhat lower.[49]

Vitamin D and Disease Prevention

Two major scientific findings in the past decade have revolutionized the field of vitamin D: (*a*) vitamin D receptors are present in nearly every tissue and cell in the body and (*b*) 25(OH)D-1α-hydroxylase, the enzyme responsible for conversion of 25(OH)D to the biologically active form of vitamin D (1,25(OH)$_2$D), has been identified in a multitude of cells outside the kidney. Subsequently, clinical studies have suggested a preventive effect of vitamin D on a broad range of disorders. Furthermore, preclinical research has advanced the field by elucidating mechanisms underlying the preventive effects of vitamin D. This section will review the disorders associated with vitamin D that are supported with a substantial amount of scientific evidence.

Skeletal Disorders

It is well established that vitamin D deficiency in adults leads to secondary hyperparathyroidism, causing a loss of bone matrix and minerals and subsequent increased risk of osteoporosis and low-trauma fractures. In severe vitamin D deficiency, accumulation of poorly mineralized bone leads to osteomalacia, a painful bone disease also associated with fractures.

Although osteomalacia can be prevented by maintaining relatively low levels of serum 25(OH)D (>10 ng/mL or >25 nmol/L),[11,59] studies suggest that higher 25(OH)D levels are needed to decrease the risk of osteoporosis. Among 13 432 individuals in NHANES III, higher serum 25(OH)D was associated with higher bone density throughout the reference range 9 to 37.6 ng/mL (22.5-94.0 nmol/L).[19] A meta-analysis of randomized trials of the vitamin D effect on fractures found that serum 25(OH)D levels more than 30 ng/mL (75 nmol/L) prevented fractures in the treatment group.[60] The antifracture effect increased with higher achieved levels of 25(OH)D. Only trials that gave at least 700 to 800 IU of vitamin D achieved serum 25(OH)D levels more than 30 ng/mL (75 nmol/L). These trials showed that vitamin D reduced the incidence of nonverteberal fractures by at least 20%.

Recent studies have also established that vitamin D supplementation decreases the risk of falls, a frequent event underlying osteoporotic fractures.[61] This is not surprising since proximal muscle weakness is a major sign of clinical vitamin D deficiency. Also, muscle tissue expresses vitamin D receptor, and vitamin D receptor activation can promote synthesis of new muscle protein.[62,63] Observational studies have found a positive association between serum 25(OH)D and muscle strength and lower extremity function.[64,65] Randomized trials have shown the efficacy of vitamin D supplementation for increasing muscle strength and balance[65,66] and reducing the risk of falls.[67-70] A minimum 25(OH)D level of 24 ng/mL (60 nmol/L) is needed for fall prevention, and higher levels of 25(OH)D are associated with greater fall reduction.

Cancer

An impressive body of evidence suggests that vitamin D decreases the risk of cancer. Actually, it has long been recognized that there is an inverse association between sunlight exposure and malignancy. In the 1930s, it was reported that US Navy personnel with abundant sunlight exposure had higher rates of skin cancer but lower rates of other malignancies.[71] In 1941, Apperly noted an inverse association between latitude and cancer mortality rates.[72] However, no further reports were available until 1980 when Garland and Garland noted that rates of cancer were higher in the northeast US states than in the southwest states and attributed this to greater sunlight exposure in the southwest.[73] They further proposed that that the apparent benefit of sunlight exposure was mediated by vitamin D. Since then a remarkable number of research studies have been designed to elucidate the role of vitamin D in cancer development and prevention. Subsequently, an inverse association between cancer mortality rates and regional solar UV-B radiation exposure has been found for cancers of the breast, colon, rectum, ovary, prostate, stomach, bladder, esophagus, kidney, lung, gallbladder, thyroid, rectum, pancreas, and uterus, as well as non-Hodgkin's lymphoma and multiple myeloma.[74-77]

Even stronger evidence for the anticancer effect of vitamin D is provided by numerous cohort and case–control studies that show an inverse association between serum 25(OH)D and cancer incidence/mortality. For example, Pilz et al reported that low 25(OH)D predicts fatal cancer in general.[78] Other studies have found an inverse association between low serum 25(OH)D and cancer incidence/mortality specifically for colon, rectal, prostate, breast, and ovarian cancer.[79-83] The decrease in risk is dramatic. For example, Garland et al found that the risk of colon cancer in a group of 25 620 Maryland community volunteers was 80% lower if they were in the highest quintile of 25(OH)D compared with the lowest.[84] Abbas reported that the risk of breast cancer was 70% lower in women who were in the highest quartile of serum 25(OH)D (>30 ng/mL, or >75 nmol/L) compared with those in the lowest quartile (<18 ng/mL, or <45 nmol/L).[85] The preponderance of evidence supports an inverse association between vitamin D and cancer incidence/mortality. Although there were studies that found no effect,[86-88] none of the studies showed an increased risk between vitamin D and cancer.

The culmination of the decades of vitamin D research is a 4-year population-based randomized, placebo controlled trial by Lappe et al[89] showing that calcium and vitamin D supplementation significantly reduced the incidence of all types of
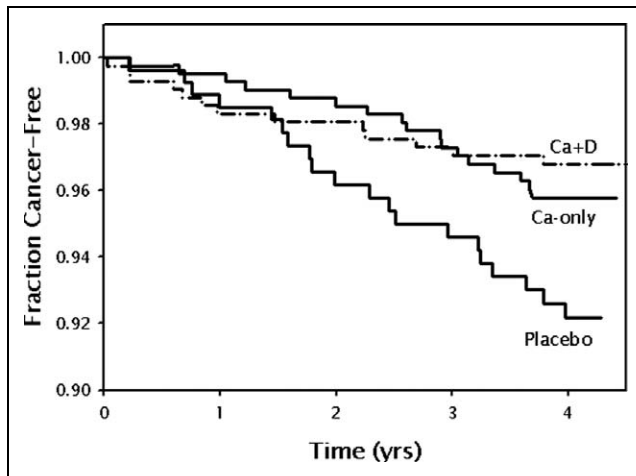
Figure 3. Kaplan–Meier survival curves (ie, free of cancer) for the 3 random treatment groups in the entire cohort of 1179 women. Sample sizes are 288 for placebo, 445 for calcium only, and 446 for calcium plus vitamin D. The survival at the end of study for the Ca + D group is significantly higher than that for placebo, by logistic regression. (Copyright Robert P. Heaney, 2006. Used with permission)

Figure 4. Kaplan–Meier survival curves (ie, free of cancer) for the 3 random treatment groups in the cohort of women free of cancer at 1 year of intervention (N = 1,085). Sample sizes are 266 for placebo, 416 for calcium only, and 403 for calcium plus vitamin D. The survival at the end of study for the Ca + D group is significantly higher than that for placebo, by logistic regression. (Copyright Robert P. Heaney, 2006. Used with permission)
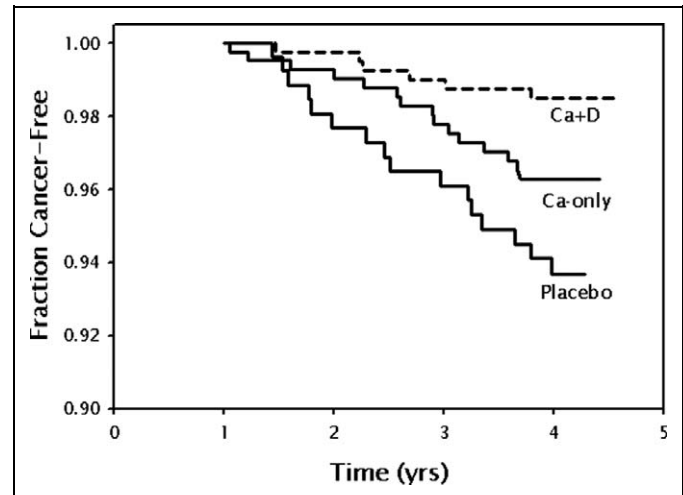
cancer by 60% to 77%. The study participants were 1179 community-dwelling women randomly selected from the population of healthy postmenopausal women aged 55 and older in a 9-county rural area of Nebraska. Participants were randomly assigned to receive 1400 to 1500 mg/d supplemental calcium alone (Ca-only), supplemental calcium plus 1100 IU/d vitamin D_3 (Ca + D), or double placebo. The mean serum 25(OH)D at baseline in the 3 treatment groups was 28.8 ng/mL (72 nmol/L). Vitamin D_3 produced an elevation in serum 25(OH)D in the Ca + D group of 9.56 ± 7.12 ng/mL (23.9 ± 17.8 nmol/L), whereas the placebo and Ca-only groups had no significant change. The intention-to-treat analysis showed that the Ca + D group had significantly fewer incident cancers of all types (relative risk [RR] = 0.40; 95% confidence interval [CI] = 0.20-0.82; P = .013) (see Figure 3). In a second analysis that excluded cancers diagnosed during the first year of the study, the RR for the Ca + D group was 0.23 (95% CI = 0.09-0.60; P < .005) (see Figure 4). Additionally, baseline and treatment-induced serum 25(OH)D concentrations themselves were strong predictors of cancer risk.

In a second randomized trial, the Women's Health Initiative (WHI), postmenopausal women were randomly assigned to 1000 mg calcium and 400 IU vitamin D per day or placebo pills.[90] The primary analysis by treatment group found no effect on colorectal cancer incidence. However, the 400 IU dose was inadequate to raise blood levels of 25(OH)D to an optimal level. Furthermore, the reported adherence to supplementation was only about 50%. Although the primary analysis found no association, a nested case–control study found a highly statistically significant inverse relationship between baseline 25(OH)D and incident cancer risk. The risk of colorectal cancer in the lowest quartile of serum 25(OH)D

(<12.4 ng/mL, or <31 nmol/L) was 253% higher than in the highest quartile (≥23.36 ng/mL, or ≥58.4 nmol/L) (RR = 2.53; 95% CI = 1.49-4.32).[90]

The principal weakness of the Lappe et al[89] study was that cancer was a secondary outcome. (Cancer was also a secondary outcome in the Women's Health Initiative).[90] Although the design and successful completion of the Lappe et al study render the findings quite strong, they need to be confirmed with a randomized controlled clinical trial designed with incidence of cancer as the primary outcome variable. Two such trials are currently under way.

The optimal levels of 25(OH)D for prevention of cancer have not been established. A review of several studies of serum 25(OH)D and colorectal cancer showed that the dose–response curve is linear up to a 25(OH)D value of 36 ng/mL (90 nmol/L).[20,21,89,91,92] Garland et al[93] combined data from observational studies to estimate the dose–response gradient between serum 25(OH)D and colon and breast cancer. They confirmed the estimate with an analysis of modeled and reported 25(OH)D levels and estimated age-standardized cancer incidence rates for 177 countries from the International Agency for Research on Cancer GLOBOCAN database. The first apparent increase in prevention of colorectal cancer is seen at serum 25(OH)D levels ≥22 ng/mL (≥55 nmol/L). The first apparent increase in prevention of breast cancer is at ≥32 ng/mL (≥80 nmol/L). The authors concluded that differences in serum 25(OH)D below those levels are unlikely to affect cancer risk.

Garland et al further estimated that if serum 25(OH)D levels in the US population were maintained ≥34 ng/mL (≥85 nmol/L), 50% of colon cancer incidence could be

prevented.[93] Maintaining serum 25(OH)D levels ≥42 ng/mL (≥105 nmol/L) would prevent about 30% of breast cancers. Using linear extrapolation of the known data points, an estimated 50% of breast cancer could be prevented with serum 25(OH)D levels ≥52 ng/mL (≥130 nmol/L). In the randomized trial of Lappe et al,[89] the mean achieved 25(OH)D level in the group with the lowest risk of cancer was 39 ± 8.6 ng/mL (96.0 ± 21.4 nmol/L). Thus, it is apparent that serum 25(OH)D levels higher than the currently accepted optimum of 32 ng/mL (80 nmol/L) are needed to provide the maximum effect on cancer reduction. According to Garland et al, a 50% reduction in colorectal cancer risk would require a population intake of 2000 IU of vitamin D_3 per day. However, a 50% reduction in breast cancer would require a higher dose, 3500 IU/d.[93] These doses are much higher than the currently recommended levels of 400 to 600 IU/d.

The mechanism for vitamin D's effect on cancer works through its autocrine mode of action. Via the autocrine pathway, in various cells of the immune system as well as in many epithelial cell types (breast, colon, lung, skin, and prostate), tissue-level 1-α-hydroxylases convert 25(OH)D to 1,25(OH)$_2$D intracellularly.[3-7,75,94,95] Then this freshly synthesized 1,25(OH)$_2$D binds to the vitamin D receptor and, in combination with tissue-specific and stimulus-specific proteins, binds to 1 of more than 1000 vitamin D response elements on the chromosomes, inducing transcription of the corresponding proteins. These include proteins responsible for cell proliferation, differentiation, and apoptosis, activities that are necessary for initiation and promotion of cancer.[94,96] Because the tissue-level 1-α-hydroxylase operates well below its k_M, this local conversion of 25(OH)D to 1,25(OH)$_2$D is dependent on circulating 25(OH)D levels.

In further support of the role of vitamin D in cancer, a convincing body of animal data, summarized by Welsh,[97] as well as by Holick,[75] shows that vitamin D–deficient animals are more prone both to spontaneous cancer and to chemical carcinogenesis by carcinogenic agents. The ecologic and serum 25(OH)D studies and the animal and in vitro reports are consistent with and strongly support the randomized trial findings of the chemopreventive effect of optimal vitamin D nutrition. Adequate serum 25(OH)D is essential for the anticancer effect of vitamin D.

Hypertension and Cardiovascular Disease

Evidence is accumulating for the association between inadequate vitamin D and hypertension and cardiovascular disease.[98-103] Since the 1980s, it has been recognized that the incidence of cardiovascular disease increases in winter with increasing latitude and decreases at higher altitudes.[104,105] It was hypothesized that sunlight exposure, by raising vitamin D levels, decreases the risk of cardiovascular disease. This has been a consistent finding. For example, the US National Register of Myocardial Infarction, including more than 250 000 cases,

showed 53% more myocardial infarctions in winter than in summer for all parts of the country.[106] Recent cohort studies have also shown that low vitamin D levels increase the risk of cardiovascular disease.[103,107-109] In the Framingham Offspring Study over 5 years of follow-up, those with baseline serum 25(OH)D levels <10 ng/mL (<25 nmol/L) had a statistically significant 80% greater risk for cardiovascular disease compared with those with >15 ng/mL (>37.5 nmol/L).[103] The effect was seen only in participants with hypertension, which suggests that hypertension somehow intensifies the negative effect of inadequate vitamin D. The only randomized clinical trial to date failed to find an effect of vitamin D and calcium on cardiovascular mortality or morbidity.[110] This was the Women's Health Initiative in which 36 282 postmenopausal women were randomly assigned to calcium carbonate 1000 mg/d and vitamin D 400 IU/d. However, the dose of vitamin D was likely too low to have an effect, and adherence to the vitamin D supplementation was poor.[110,111] Thus, rigorously conducted randomized clinical trials are needed to confirm the effect of vitamin D on cardiovascular events.

Epidemiological evidence from sunlight and cohort studies also suggests an inverse relationship between vitamin D and blood pressure.[112-114] For example, a prospective study that included subjects from both the Nurses' Health Study and the Health Professionals Follow-up Study found that the relative risk for incident hypertension was 3.18 for those with 25(OH)D levels <15 ng/mL (<37.5 nmol/L) compared with those with levels >30 ng/mL (>75 nmol/L).[114] NHANES III found that systolic blood pressure is 3 mm Hg higher in the lowest quintile of 25(OH)D compared with the highest quintile.[115] Researchers estimated that this modest decrease in blood pressure could account for a 10% to 15% decline in cardiovascular mortality in the population.[116] On the other hand, another large cohort study failed to find an inverse relationship between dietary vitamin D and hypertension.[117] One explanation for the lack of an effect in this study is that dietary sources of vitamin D provide much less vitamin D than sunlight exposure, and dietary intake is difficult to measure.[118]

One small clinical trial found that administering vitamin D to older adults with existing high blood pressure caused both the systolic and diastolic blood pressure to decrease.[119] In this randomized, placebo-controlled study of 145 elderly women, 800 IU of vitamin D_3 plus 1200 mg of calcium per day significantly reduced blood pressure by 9.3% after 8 weeks, whereas supplementation with 1200 mg of calcium per day alone lowered blood pressure significantly by only 4.0%. These data suggest that vitamin D and calcium somehow work together to cause a reduction in blood pressure. On the other hand, at least 3 randomized trials found no effect of vitamin D alone or in combination with calcium on lowering blood pressure.[120-122] One study was short, with only 5 weeks of follow-up after a single vitamin D dose of 100 000 IU.[122] A second study was the Women's Health Initiative, in which the supplement dose of 400 IU/d was too low to increase serum 25(OH)D.[120] In the third study by Orwoll et al, blood pressure was a secondary outcome.[121] Normotensive men were randomly assigned to 1000

mg of calcium per day and cholecalciferol 1000 IU/d for 3 years. Although the dose of vitamin D was likely adequate, nearly 25% of the subjects dropped from study before the end of 3 years. Thus, there remains a lack of rigorous trials of the effect of vitamin D on blood pressure.

Several mechanisms have been proposed to play a role in prevention of hypertension and cardiovascular disease. The renin–angiotensin system plays an essential role in regulating blood pressure, and Li et al have shown that $1,25(OH)_2D$ is a potent suppressor of renin biosynthesis.[123] Vitamin D receptor knock out mice have increased production of renin and angiotensin II with resultant hypertension and cardiac hypertrophy. Hypertrophy of cardiac and smooth muscle could be caused by elevation of matrix metalloproteinases, associated with vitamin D deficiency.[124-126] Insufficient vitamin D levels increase the inflammatory process, which is associated with atherosclerosis, endothelial dysfunction, and insulin resistance.[127-129] Considerable research effort is underway to further elucidate the mechanisms underlying a vitamin D effect on the circulatory system.

Thus, the epidemiologic studies of vitamin D tend to support its effects on lowering/maintaining blood pressure, and plausible mechanisms for such an effect have been proposed. However, rigorous randomized clinical trials using adequate doses of vitamin D supplementation are needed to confirm the effect.

Optimal Functioning of the Immune System

A fascinating role of vitamin D is involvement in optimal functioning of the immune system. Evidence suggests that vitamin D insufficiency is linked to bacterial and viral infections as well as autoimmune diseases. This article will provide only a broad overview of this complex topic.

Regulation of Immune Function. Calcitriol ($1,25(OH)_2D$) is a hormone that regulates both adaptive and innate immunity.

Adaptive immunity. Adaptive immunity involves the production of cytokines by T-lymphocytes and immunoglobulins by B-lymphocytes to combat the antigens presented to them by cells such as macrophages and dendritic cells. $1,25(OH)_2D$ has been found to inhibit the adaptive immune system by suppressing immunoglobulin production and proliferation and retarding the differentiation of B-cell precursors into plasma cells.[130] Furthermore, $1,25(OH)_2D$ suppresses T-cell proliferation and function, particularly T helper-1 and T helper-17 cells.[131,132] This ability of $1,25(OH)_2D$ to suppress the adaptive immune response seems to account for the beneficial effect of vitamin D on autoimmune disorders. In fact, in experimental models for inflammatory arthritis, autoimmune diabetes, multiple sclerosis, and inflammatory bowel disease, $1,25(OH)_2D$ administration has prevented or diminished the disease process.[133,134] Evidence from clinical studies also show promise.

Clinical evidence of a vitamin D effect on autoimmune disorders. A considerable body of epidemiological evidence supports the effect of vitamin D on prevention of various autoimmune disorders such as diabetes, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, and systemic lupus erythematosus.[22,135-150] For example, data from Finland show that adults who had been given 2000 IU/d of vitamin D during the first year of life had greater than an 80% decrease in risk of type 1 diabetes compared with those who had not been supplemented.[136] In NHANES III, nondiabetic individuals were shown to have a higher prevalence of high blood sugar values if they had low vitamin D.[135] In animal studies, $1,25(OH)_2D$ administration prevents development of experimentally induced type 1 diabetes.[151,152]

The risk of multiple sclerosis seems to be decreased with adequate vitamin D. Studies show that persons who were born in an area below 35°N latitude and/or resided there during the first 10 years of life have a lower lifetime risk of multiple sclerosis.[137-140] Large observational studies have shown that higher intake of vitamin D is associated with lower risk of multiple sclerosis.[153] Burton et al[154] conducted an open-label randomized prospective controlled 52-week trial matching patients with multiple sclerosis for demographic and disease characteristics. The treatment group received increasing vitamin D doses up to 40 000 IU/d over 28 weeks to raise serum 25(OH)D rapidly, followed by 10 000 IU/d for 12 weeks, and further decreased to 0 IU/d. Calcium (1200 mg/d) was given throughout the study. The primary objective was to assess tolerability of high doses of vitamin D. Secondary endpoints included immunologic biomarkers and relapse events. Despite a mean peak 25(OH)D of 165.2 ng/mL (413 nmol/L), no significant adverse events occurred. Those in the treatment group appeared to have fewer relapse events and a persistent reduction in T-cell proliferation compared with controls. A second randomized trial found that supplemented multiple sclerosis patients had increased serum transforming growth factor β1 levels compared with controls.[155] Elevated β1 levels are associated with the stable phase of multiple sclerosis.[156,157]

Thus, evidence for the relationship between vitamin D and autoimmune diseases is accumulating rapidly. However, rigorous randomized trials have not been done. Because of the long latency of many of these disorders, designing and conducting such trials may be challenging.

Innate immunity. Innate immunity involves the activation of the Toll-like receptor (TLR) pathway in polymorphonuclear cells, monocytes, and macrophages, as well as in several types of epithelial cells.[158] TLRs are transmembrane pathogen recognition receptors that trigger the innate immune response of the host to infectious agents.[159] In this regard, TLRs serve as stimulators of inflammation and triggers for sepsis and immune exacerbation.[160] The mechanism of vitamin D's action in this context has been elucidated and shows that vitamin D is a necessary intermediate in the production of antimicrobial peptides, such as cathelicidin, by monocyte-macrophages.[161-168] It has

been shown that activation of the TLR pathway in human monocyte-macrophages by microbial agents, such as the *Mycobacterium tuberculosis*, stimulates expression of the CYP 27B (25(OH)D-1-α-hydroxylase) and vitamin D receptor genes in that cell. If there is inadequate 25(OH)D substrate available to the cell CYP 27B, there will be insufficient production of 1,25(OH)$_2$D locally.[162,169,170] This will result in decreased binding of 1,25(OH)$_2$D to the vitamin D receptor and limited activation of 1,25(OH)$_2$D-VDR-directed antimicrobial genes. The end result is decreased killing of ingested microbes.

This antimicrobial mechanism is also used by epithelial cells in a variety of sites that serve as microbial barriers, such as the intestine, lung, placenta, and skin.[171-177] Both bacteria and viruses can activate TLR-induced pathways.[31] One of the human cathelicidins, LL-37, has bactericidal effects and is involved in inflammatory and tissue remodeling processes.[167,168] Furthermore, LL-37 stimulates angiogenesis, proliferation of lung epithelial cells, cytokine release, and cell migration.

That serum 25(OH)D is the key precursor is also indicated by the fact that the first gene expressed in the macrophage is the 25(OH)D-1-α−hydroxylase, a step that would make sense only if the cell were expecting to use circulating 25(OH)D. It has also been found that, in addition to the gene for cathelicidin, the macrophage also produces the vitamin D-24 hydroxylase (CYP 24), resulting in a rapid degradation of the 1,25(OH)$_2$D synthesized within the cell. It should be noted that dendritic cells and many epithelial cells, as well as monocyte-macrophages, express the vitamin D receptor and produce the 25(OH)D-1-α-hydroxylase and the 25(OH)D-24 hydroxylase.[162,178-180] Thus, a large body of preclinical research suggests that vitamin D, working through the autocrine pathway, is essential for resisting infectious disease.

Clinical evidence of a vitamin D effect on infections. The effect of vitamin D in prevention and treatment of the bacterial infection tuberculosis has been known for decades. In fact, the Nobel Prize was awarded to Niels Finsen in 1903 for discovering that ultraviolet radiation heals lupus vulgaris, a skin form of tuberculosis.[181] During the 1900s tuberculosis sanitariums with abundant sunshine exposure were common for treatment of tuberculosis patients.

A wealth of clinical evidence supports the effect of vitamin D on decreasing the risk of infectious diseases.[182-188] In the 1800s, it was recognized that patients with rickets, a vitamin D–deficiency disease, were prone to infections and often died of pneumonia.[189] More recently, high rates of tuberculosis are associated with vitamin D deficiency in various populations.[183,184] Viral infections such as the common cold, influenza, and respiratory syncytial virus peak during winter months when the serum 25(OH)D levels are the lowest.[182] This has also been shown for scarlet fever,[185] meningitis,[190] and viral infections of the gastrointestinal tract.[186-188] Pandemic influenza also appears to have a higher incidence during winter months.[191] Recently, Ginde et al[182] reported that the prevalence of upper respiratory infections in NHANES III was higher with lower 25(OH)D levels, regardless of season of the year. In a prospective cohort study, Sabetta et al[192] found that serum 25(OH)D levels of 38 ng/mL (95 nmol/L) or more were associated with a significant (*P* < .0001) 2-fold reduction in the risk of developing acute respiratory infections and with a marked reduction in the percentages of days ill.

A few randomized trials support the effect on vitamin D on infectious diseases. For example, 10 000 IU of native vitamin D given daily along with standard tuberculosis treatment resulted in a 24% greater clearance of mycobacterium from the sputum compared with the standard treatment alone.[193] In a study of African American women randomized to placebo or vitamin D$_3$ 800 IU for 2 years and 2000 IU for the third year, Aloia et al found a significantly lower incidence of common colds and influenza in the treated group.[194] However, colds and influenza were secondary outcomes in this study of bone health. Recently, young Finnish males in military training were randomly assigned to vitamin D$_3$ 400 IU/d or placebo for 6 months.[195] The primary outcome was number of days absent from duty due to respiratory infection. Although the mean number of days absent did not differ between groups, the proportion of men remaining healthy (without a respiratory infection) was significantly higher in the treatment group (51.3% vs 35.7%, *P* = .045).

In total, the evidence is very strong for vitamin D being essential for prevention of infectious diseases. However, rigorous randomized trials remain to be done to confirm the efficacy of vitamin D in this regard and to delineate the optimal levels of 25(OH)D for prevention of various diseases in a multitude of population groups.

Conclusion

In conclusion, scientists are generating a strong body of evidence to support a vitamin D paradigm shift. This evidence suggests that vitamin D is much more than a nutrient needed for bone health; it is an essential hormone required for regulation of a large number of physiologic functions. It is clear that sufficient levels of serum 25(OH)D are essential for optimizing human health. However, many questions remain unanswered. For example, what levels of serum 25(OH)D are optimal? Do these optimal levels vary for prevention of various disorders or in differing human populations? What amount of supplementation or sunlight exposure is needed to achieve and maintain these levels?

Although innumerable clinical studies support the effect of vitamin D in preventing a wide range of disorders, rigorous randomized clinical trials of vitamin D supplementation are sorely lacking. Successful completion of such trials is essential to establish the efficacy and safety of vitamin D supplementation on a population level.

However, since we are experiencing a global epidemic of vitamin D insufficiency, it is unacceptable to continue the status quo pending the outcome of long-term clinical trials. It is

imperative that all individuals be encouraged to obtain vitamin D from either sunlight or supplementation. Although relatively few clinical research reports are available in children, it is obvious that all age-groups require optimal levels of vitamin D to support physiologic functions that are dependent on circulating 25(OH)D. Public education should be provided about the safety of vitamin D supplementation and the value of sensible sunlight exposure. There is a growing consensus that the optimal range for 25(OH)D values lies above 30 to 32 ng/mL (75-80 nmol/L) for most populations, and it seems prudent that persons at high risk of vitamin D deficiency and/or vitamin D–deficiency disorders have their serum 25(OH)D assessed.

Vitamin D is truly remarkable in that it plays a key role in a wide range of physiologic functions. As scientists continue to solve the remaining mysteries related to vitamin D function and provide approaches for optimizing vitamin D status, we can expect dramatic improvement in a broad spectrum of human disorders.

Declaration of Conflicting Interests

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