High Prevalence of Vitamin D Inadequacy and Implications for Health

MICHAEL F. HOLICK, PHD, MD

During the past decade, major advances have been made in vitamin D research that transcend the simple concept that vitamin D is important for the prevention of rickets in children and has little physiologic relevance for adults. Inadequate vitamin D, in addition to causing rickets, prevents children from attaining their genetically programmed peak bone mass, contributes to and exacerbates osteoporosis in adults, and causes the often painful bone disease osteomalacia. Adequate vitamin D is also important for proper muscle functioning, and controversial evidence suggests it may help prevent type 1 diabetes mellitus, hypertension, and many common cancers. Vitamin D inadequacy has been reported in approximately 36% of otherwise healthy young adults and up to 57% of general medicine inpatients in the United States and in even higher percentages in Europe. Recent epidemiological data document the high prevalence of vitamin D inadequacy among elderly patients and especially among patients with osteoporosis. Factors such as low sunlight exposure, age-related decreases in cutaneous synthesis, and diets low in vitamin D contribute to the high prevalence of vitamin D inadequacy. Vitamin D production from cutaneous synthesis or intake from the few vitamin D–rich or enriched foods typically occurs only intermittently. Supplemental doses of vitamin D and sensible sun exposure could prevent deficiency in most of the general population. The purposes of this article are to examine the prevalence of vitamin D inadequacy and to review the potential implications for skeletal and extraskeletal health.

***Mayo Clin Proc.* 2006;81(3):353-373**

1α(OH)D_3 = 1α-hydroxyvitamin D_3; 1,25$(OH)_2$D = 1,25-dihydroxyvitamin D; 25(OH)D = 25-hydroxyvitamin D; BMD = bone mineral density; PTH = parathyroid hormone; RCT = randomized controlled trial; RECORD = Record Evaluation of Calcium or Vitamin D; VDR = vitamin D receptor

During the past decade, important advances in the study of vitamin D have been made. In addition to its important role in skeletal development and maintenance, evidence is mounting that vitamin D produces beneficial effects on extraskeletal tissues and that the amounts needed for optimal health are probably higher than previously thought.[1] At the same time, numerous reports have shown that relatively high proportions of people have inadequate levels of vitamin D. The extraskeletal health benefits of vitamin D and high prevalence of inadequate levels of vitamin D have been largely unrecognized by both physicians and patients.[2] The purposes of this review article are to examine the prevalence of vitamin D inadequacy as defined by low serum 25-hydroxyvitamin D (25[OH]D), the major circulating form of vitamin D and standard indicator of vitamin D status, and to review the potential implications on both skeletal and extraskeletal health.

From the Vitamin D, Skin and Bone Research Laboratory, Section of Endocrinology, Diabetes, and Nutrition, Department of Medicine, Boston University School of Medicine, Boston, Mass.

Dr Holick is an Academic Associate for Nichols Institute Diagnostics.

This work was supported in part by National Institutes of Health grants MOIRR00533 and AR369637 and the UV Foundation.

Individual reprints of this article are not available. Address correspondence to Michael F. Holick, PhD, MD, Vitamin D, Skin and Bone Research Laboratory, Section of Endocrinology, Diabetes, and Nutrition, Department of Medicine, Boston University School of Medicine, 715 Albany St, Room M-1013, Boston, MA 02118 (e-mail: mfholick@bu.edu).

SOURCES OF VITAMIN D

Solar UV-B (wavelengths of 290-315 nm) irradiation is the primary source of vitamin D (other than diet supplements) for most people.[1,3,4] Dietary sources of vitamin D are limited. They include oily fish such as salmon (approximately 400 IU per 3.5 oz), mackerel, and sardines; some fish oils such as cod liver oil (400 IU/tsp); and egg yolks (approximately 20 IU). Some foods are fortified in the United States, including milk (100 IU per 8 oz) and some cereals (100 IU per serving), orange juice (100 IU per 8 oz), some yogurts (100 IU per serving), and margarine.[4-6] Milk is not vitamin D enriched in most European countries; however, margarine and some cereals are. There are 2 forms of vitamin D. Vitamin D_2 (ergocalciferol) comes from irradiation of the yeast and plant sterol ergosterol, and vitamin D_3 (cholecalciferol) is found in oily fish and cod liver oil and is made in the skin. Vitamin D represents vitamin D_2 and vitamin D_3.

For editorial comment, see page 297

Vitamin D from cutaneous synthesis or dietary sources typically occurs only intermittently. Irregular intake of vitamin D, irrespective of the source, can lead to chronic vitamin D inadequacy. This condition has been reported across all age groups, geographic regions, and seasons.[7-16] Enhancing vitamin D levels by taking supplements is usually necessary to achieve the minimum recommended daily intakes; however, compliance is often problematic. In particular, some groups who may be at high risk of vitamin D inadequacy often do not follow regular daily dosing guidelines. Adherence to vitamin D supplementation recommendations is low among elderly patients with osteoporosis. One study showed that, despite receiving counseling on the importance of vitamin D and calcium supplementation, 76% of elderly patients with hip fractures did not comply with recommendations.[17] This is not surprising given that

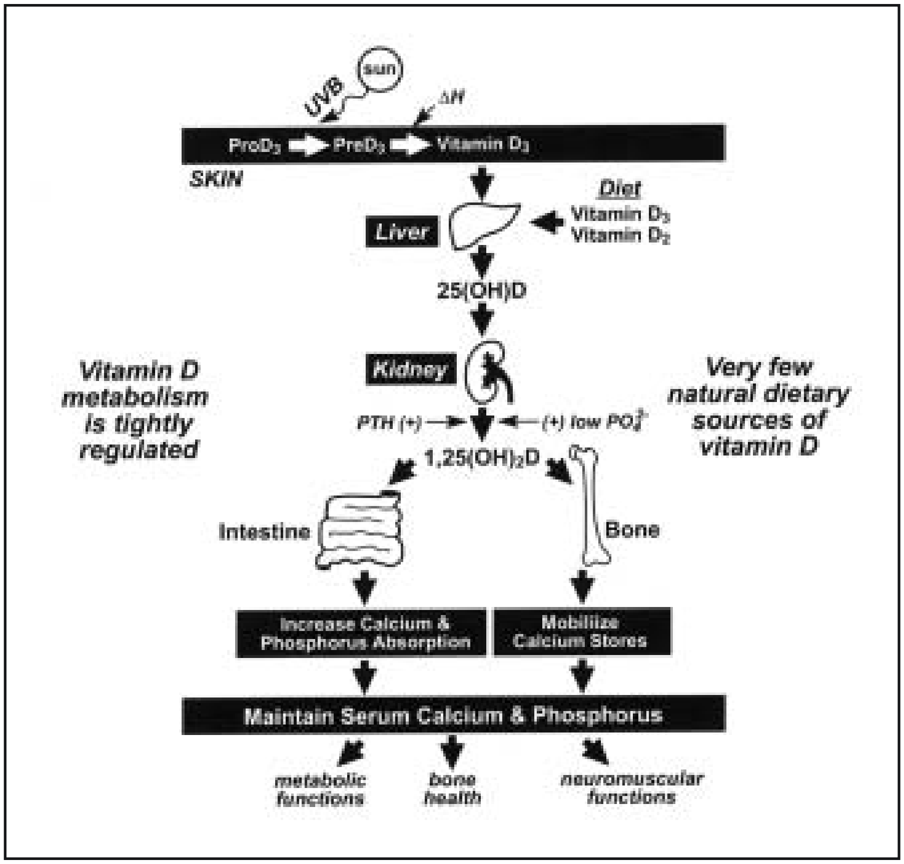

FIGURE 1. Cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth. 7-Dehydrocholesterol or provitamin D_3 (proD_3) in the skin absorbs solar UV-B radiation and is converted to previtamin D_3 (preD_3). D_3 undergoes thermally induced (ΔH) transformation to vitamin D_3. Vitamin D from the diet or from the skin is metabolized in the liver by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D_3 (25[OH]D_3). 25(OH)D_3 is converted in the kidney by the 25(OH)D_3-1α-hydroxylase to 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2D_3$]. A variety of factors, including serum phosphorus (PO_4^{2-}) and parathyroid hormone (PTH), regulate the renal production of 1,25(OH)$_2D_3$. 1,25(OH)$_2$D regulates calcium metabolism through its interaction with its major target tissues, the bone and the intestine. From *Osteoporos Int*,[21] with permission from Springer Science and Business Media.

compliance declines as the number of medications increases, and elderly patients often take many medications. Similarly, achieving adequate vitamin D intake through milk consumption is unreliable among elderly patients because of the high prevalence of lactose intolerance among this population and the often low levels of vitamin D in the milk supply.[6]

VITAMIN D PHOTOBIOCHEMISTRY, METABOLISM, AND FUNCTIONS

UV-B irradiation of skin triggers photolysis of 7-dehydrocholesterol (provitamin D_3) to previtamin D_3 in the plasma membrane of human skin keratinocytes.[18-20] Once formed in the skin, cell plasma membrane previtamin D_3 is rapidly converted to vitamin D_3 by the skin's temperature. Vitamin D_3 from the skin and vitamin D from the diet undergo 2 sequential hydroxylations, first in the liver to 25(OH)D and then in the kidney to its biologically active form, 1,25-dihydroxyvitamin D (1,25[OH]$_2$D) (Figure 1). Excessive solar UV-B irradiation will not cause vitamin D intoxication because excess vitamin D_3 and previtamin D_3 are photolyzed to biologically inactive photoproducts.[19,20,22] Melanin skin pigmentation is an effective natural sunscreen, and increased skin pigment can greatly reduce UV-B–mediated cutaneous synthesis of vitamin D_3 by as much as 99%, similar to applying a sunscreen with a sun protection factor of 15.[23,24] Keratinocytes are also capable of hydroxylating 25(OH)D to produce 1,25(OH)$_2$D.[25] The 1,25(OH)$_2$D (from keratinocyte or renal sources) may regulate keratinocyte differentiation, melanocyte apoptosis, and melanin production,[25-27] and this may be another mechanism for regulating the cutaneous synthesis of vitamin D_3 by negative feedback.

The $1,25(OH)_2D$ ligand binds with high affinity to the vitamin D receptor (VDR) and triggers an increase in intestinal absorption of both calcium and phosphorus. In addition, vitamin D is involved in bone formation, resorption, and mineralization and in maintaining neuromuscular function[1,3] (Figure 1). Circulating $1,25(OH)_2D$ reduces serum parathyroid hormone (PTH) levels directly by decreasing parathyroid gland activity and indirectly by increasing serum calcium. The $1,25(OH)_2D$ regulates bone metabolism in part by interacting with the VDR in osteoblasts to release biochemical signals, leading to formation of mature osteoclasts. The osteoclasts release collagenases and hydrochloric acid to dissolve the matrix and mineral, releasing calcium into the blood.[1,3,4]

When vitamin D levels are inadequate, calcium and phosphorus homeostasis becomes impaired. Vitamin D is primarily responsible for regulating the efficiency of intestinal calcium absorption. In a low vitamin D state, the small intestine can absorb approximately 10% to 15% of dietary calcium. When vitamin D levels are adequate, intestinal absorption of dietary calcium more than doubles, rising to approximately 30% to 40%.[1,3,4,28] Thus, when vitamin D levels (25[OH]D) are low, calcium absorption is insufficient to satisfy the calcium requirements not only for bone health but also for most metabolic functions and neuromuscular activity. The body responds by increasing the production and release of PTH into the circulation (Figure 1). The increase in PTH restores calcium homeostasis by increasing tubular reabsorption of calcium in the kidney, increasing bone calcium mobilization from the bone, and enhancing the production of $1,25(OH)_2D$.[1,3]

ASSESSMENT OF VITAMIN D STATUS

Serum 25(OH)D is the major circulating metabolite of vitamin D and reflects vitamin D inputs from cutaneous synthesis and dietary intake. The serum 25(OH)D level is the standard clinical measure of vitamin D status.[1,14] Although $1,25(OH)_2D$ is the active form of vitamin D, it should not be measured to determine vitamin D status. It usually is normal or even elevated in patients with vitamin D deficiency.[1,3,4] Testing of serum 25(OH)D is most useful in patients who are at risk of vitamin D deficiency, including elderly patients, infirm patients, children and adults with increased skin pigmentation, patients with fat malabsorption syndromes, and patients with osteoporosis. This measurement is also useful for purposes of planning or monitoring vitamin D therapy. Clinical assays of 25(OH)D include the Nichols Advantage Assay (chemiluminescence protein-binding assay, the DiaSorin radioimmunoassay, and the benchmark high-performance liquid chromatography assays[29] and liquid chromatography mass spectroscopy assays.[30] The chemiluminescence protein-binding assay and the radioimmunoassay are most commonly used to determine patient vitamin D status. Recent reports have raised concerns about the degree of variability between assays and between laboratories, even when using the same assay.[29-33] Although reliable and consistent evaluation of serum 25(OH)D levels remains an issue, reliable laboratories currently exist, and efforts are in progress to improve and standardize assays to enhance accuracy and reproducibility at other laboratories.[30,32,33]

As noted previously, vitamin D plays a central role in calcium and phosphorus homeostasis and skeletal health. Since impaired calcium metabolism due to low serum 25(OH)D levels triggers secondary hyperparathyroidism, increased bone turnover, and progressive bone loss,[1,34-38] the optimal range of circulating 25(OH)D for skeletal health has been proposed as the range that reduces PTH levels to a minimum[9,11,35] and calcium absorption is maximal.[28] Several studies have shown that PTH levels plateau to a minimum steady-state level as serum 25(OH)D levels approach and rise above approximately 30 ng/mL (75 nmol/L)[9,30,35-38] (Figure 2, left).

EPIDEMIOLOGY OF VITAMIN D INADEQUACY

Vitamin D inadequacy constitutes a largely unrecognized epidemic in many populations worldwide.[39-47] It has been reported in healthy children,[7,8,13,15,48] young adults,[38,39,49] especially African Americans,[7,41,42,49,50] and middle-aged and elderly adults.[9-12,14,36,37,40,43-49,51-56] Typically, the prevalence of low 25(OH)D levels (<20 ng/mL [50 nmol/L]) is approximately 36% in otherwise healthy young adults aged 18 to 29 years,[49] 42% in black women aged 15 to 49 years,[50] 41% in outpatients aged 49 to 83 years,[11] up to 57% in general medicine inpatients in the United States,[57] and even higher in Europe (28%-100% of healthy and 70%-100% of hospitalized adults).[40,55,58]

Vitamin D inadequacy is particularly common among patients with osteoporosis (Table 1). A recent systematic review by Gaugris et al[39] concluded that the prevalence of inadequate 25(OH)D levels appears to be high in postmenopausal women and especially those with osteoporosis and a history of fracture. This review, which included 30 studies published between January 1994 and April 2004, examined the prevalence of vitamin D inadequacy reported as serum 25(OH)D levels below various values. The results of a recent cross-sectional, observational study conducted at 61 sites across North America showed that 52% of postmenopausal women receiving therapy for osteoporosis had 25(OH)D levels of less than 30 ng/mL (75 nmol/L).[30]

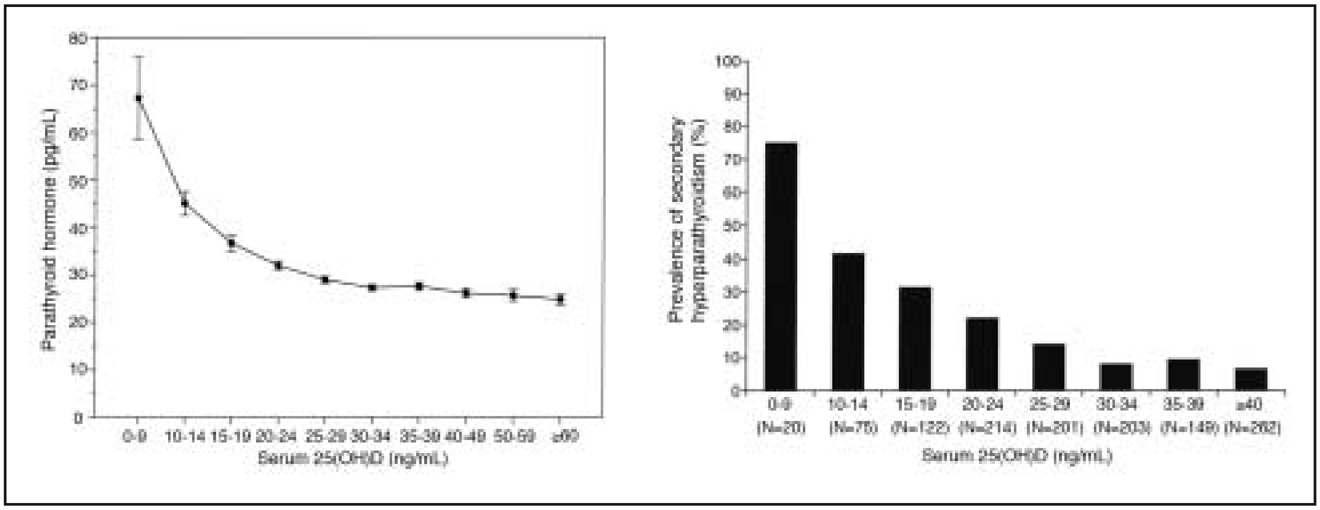

FIGURE 2. Left, Relationship between serum 25-hydroxyvitamin D (25[OH]D) concentrations and mean ± SE (error bars) serum concentrations of parathyroid hormone in patients with osteoporosis receiving treatment. Right, Percentage of subjects with secondary hyperparathyroidism by 25(OH)D level. The percentage of subjects with secondary hyperparathyroidism (parathyroid hormone level >40 pg/mL) sorted by subgroups with serum 25(OH)D concentrations delineated by predefined cutoffs for analyses of 25(OH)D inadequacy. Left and right, From *J Endocrinol Metab*,[30] with permission from The Endocrine Society, Copyright 2005.

The high prevalence of vitamin D inadequacy in that study was consistent across all age groups and North American geographic regions studied.[30] The prevalence of very low serum 25(OH)D levels (<12 ng/mL [30 nmol/L]) was 76% among patients with osteoporosis in another study.[55] A global study of vitamin D status in postmenopausal women with osteoporosis showed that 24% had 25(OH)D levels less than 10 ng/mL (25 nmol/L), with the highest prevalence reported in central and southern Europe.[14] Vitamin D inadequacy is common even among patients with osteoporosis living at lower latitudes in highly sunny climates. For instance, 53% of community-dwelling women with osteoporosis living in Southern California had 25(OH)D levels less than 30 ng/mL (75 nmol/L).[46] In a study of patients 50 years and older hospitalized for nontraumatic fractures, 97% had 25(OH)D levels less than 30 ng/mL (75 nmol/L).[59] Studies in the United Kingdom and South Africa reported that 13% to 33% of patients with hip fractures had histological evidence of osteomalacia that may have been caused by chronic vitamin D deficiency.[60-63]

Vitamin D inadequacy is also common among nonwhite populations and populations with low dietary or supplementary vitamin D intake or minimal exposure to sunlight. A study of Asian adults in the United Kingdom showed that 82% had 25(OH)D levels less than 12 ng/mL (30 nmol/L) during the summer season, with the proportion increasing to 94% during the winter months.[64] A study of 1546 African American women in the United States, ranging in age from 15 to 49 years, showed that more than 40% had serum 25(OH)D levels less than 15 ng/mL (37 nmol/L).[50] A much higher proportion (84%) of elderly black adults in Boston, Mass, had serum 25(OH)D levels less than 20 ng/mL (50 nmol/L).[3] Even children are at risk. A cross-sectional clinic-based study of 307 children (11-18 years old) in Boston reported that 52% of African American and Hispanic children had 25(OH)D levels of 20 ng/mL (50 nmol/L) or less.[7] Sullivan et al[15] observed that at the end of winter and summer 48% and 17%, respectively, of white girls (9-11 years of age) in Maine also had 25(OH)D levels less than 20 ng/mL (50 nmol/L). Even in sunny countries such as Lebanon, vitamin D inadequacy is common in schoolchildren.[48]

FACTORS THAT CONTRIBUTE TO VITAMIN D INADEQUACY

Physical factors that attenuate UV-B exposure, including clothing, sunscreens, and glass shielding, markedly reduce or completely eliminate the production of vitamin D_3 in the skin.[18] At latitudes above 37°N and below 37°S, sunlight is insufficient to induce cutaneous vitamin D_3 synthesis during the winter months.[16,65,66] Nevertheless, latitude is not the only determinant of 25(OH)D levels.[1,3,8,67-69] The high prevalence of osteomalacia in Saudi Arabian women, rickets in Saudi children, and vitamin D deficiencies in both may be attributable to their cultural practice of wearing clothing that covers the entire body and avoiding direct sunlight.[70,71]

Biological factors that inhibit cutaneous vitamin D synthesis and bioavailability include skin pigmentation,[23,72,73]

TABLE 1. **Vitamin D Inadequacy in Osteoporosis: Summary of Reports Published in 2003 and 2004***

Reference	Population characteristics	Location	Season	Sample size	Mean age (y)	Prevalence of low serum 25(OH)D (%)	Definition of low serum vitamin D (ng/mL)
Isaia et al,[40] 2003	Elderly women referred to an osteoporosis center	Italy	B	700	68	27 76	<5 <12
Plotnikoff et al,[41] 2003	Various ethnic groups referred for chronic musculoskeletal pain	Minnesota	B	150	10-65 (range)	33 93	<8 <20
Carnevale et al,[42] 2004	Patients with primary hyperparathyroidism	Italy	B	62	50	27	<12
Harwood et al,[43] 2004	Female patients with hip fractures	United Kingdom	NA	150	81	70	<12
Glowacki et al,[44] 2003	Postmenopausal osteoarthritic white women	Boston, Mass	B	68	66	22	<15
Segal et al,[17] 2004	Patients with hip fracture at time of hospitalization	Israel	B	96	72	60	<15
Gomez-Alonso et al,[45] 2003	Healthy population in osteoporosis study	Spain	B	268	69	67	<18
Holick et al,[30] 2005	Postmenopausal women receiving antiresorptive or anabolic therapy for osteoporosis	North America	L	1536	71	52	<30
Blau et al,[46] 2004	Community-dwelling women referred to osteoporosis clinic	Southern California	L	252	NA	53	<30
Simonelli et al,[47] 2005	Patients hospitalized for nontraumatic fracture	Minnesota	L	82	≥50	97	<30

*25(OH)D = 25-hydroxyvitamin D; B = both low sun/winter-spring and high sun/summer-fall; L = low sun/winter-spring; NA = not available.

medication use,[74] body fat content,[75] fat malabsorption,[76] and age.[77,78] Increased skin pigmentation can reduce cutaneous vitamin D_3 production as much as 99.9%.[23,72,73] Certain drugs (eg, anticonvulsants, corticosteroids, rifampin, and cholestyramine) may adversely affect metabolism or bioavailability of vitamin D.[74,79,80] Recent studies have shown that body mass index and body fat content are inversely related to serum 25(OH)D levels and directly related to PTH levels,[75,81-83] which is likely due to vitamin D sequestration in body fat compartments.[75] Dietary sources of vitamin D are limited, and obtaining a sufficient amount from regular diet is often problematic for many people whose diet does not normally include the few foods that are naturally rich in vitamin D. Patients with fat malabsorption syndromes, including sprue, cystic fibrosis, and Crohn disease, are at especially high risk of vitamin D deficiency.[76,84] Among elderly patients, multiple factors contribute to vitamin D inadequacy, including dietary deficiencies and decreased cutaneous synthesis due to reduced ability of the skin to synthesize vitamin D_3. A 70-year-old produces approximately 4 times less vitamin D via cutaneous synthesis compared with a 20-year-old.[77,78] Increasing age has been associated with lower 25(OH)D levels regardless of season.[85] Age does not alter dietary vitamin D absorption, but if an individual is taking cholestyramine, vitamin D will not be absorbed efficiently.[84]

SKELETAL CONSEQUENCES OF VITAMIN D INADEQUACY

Chronic severe vitamin D deficiency in infants and children causes bone deformation due to poor mineralization, commonly known as rickets.[1,4] In adults, proximal muscle weakness, bone pain, and osteomalacia may develop.[55,86-89] Less severe vitamin D inadequacy prevents children and adolescents from attaining their optimal genetically programmed peak bone mass and in adults leads to secondary hyperparathyroidism, increased bone turnover, and progressive loss of bone, increasing the risk of osteoporosis.

Vitamin D deficiency during skeletal maturation disrupts chondrocyte maturation and inhibits the normal mineralization of the growth plates. This causes a widening of the epiphyseal plates at the end of the long bones in rachitic children and bulging of costochondral junctions (rachitic rosary).[1,4] Secondary hyperparathyroidism causes phosphaturia and hypophosphatemia. The resulting inadequate calcium-phosphorus product results in poor mineralization, making the skeleton less rigid. When the rachitic child begins to stand, gravity causes bowing of the long bones in the lower extremities, resulting in bowed legs or knocked knees.[4,18]

In adults, the epiphyseal plates are fused, and secondary hyperparathyroidism and resulting phosphaturia have more

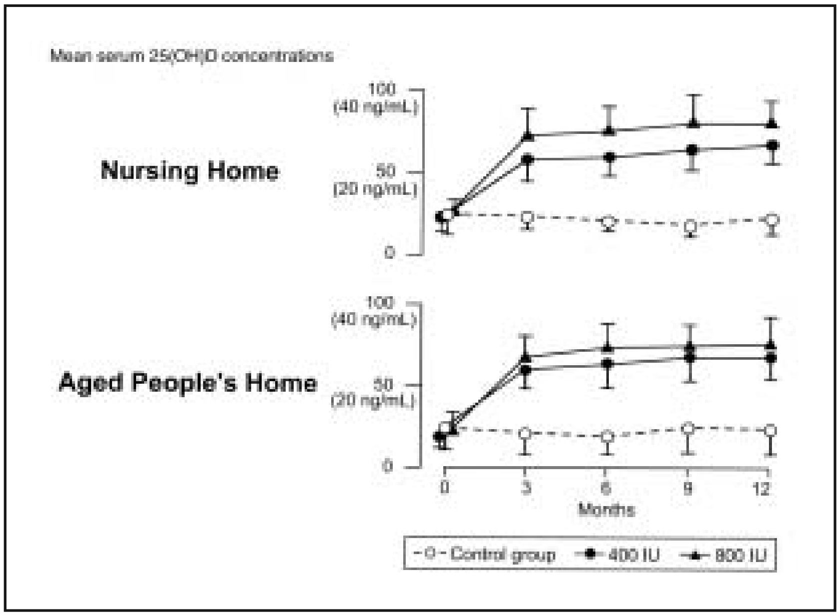

Figure 3. Mean ± SD (error bars) serum 25-hydroxyvitamin D (25[OH]D) concentrations (shown as nmol / L and ng / mL) in women older than 70 years, stratified by supplement use and residential status. Adapted from *J Clin Endocrinol Metab*,[97] with permission from The Endocrine Society, Copyright 1988.

subtle, but equally devastating, skeletal consequences. Chronic vitamin D inadequacy in adults can result in secondary hyperparathyroidism, increased bone turnover, enhanced bone loss, increased risk of fragility fracture, and (rarely) hypocalcemic tetany.[34-37,87-89] The increase in PTH-mediated osteoclastogenesis results in increased numbers and activity of osteoclasts. The osteoclasts resorb bone via enzymatic degradation of the collagen matrix and secretion of hydrochloric acid, releasing calcium and phosphorus into the extracellular space. The result is increased skeletal porosity, defective bone mineralization, decreased bone mineral density (BMD), osteoporosis, and increased fragility-fracture risk.[4,30,50,87,88] When 25(OH)D levels are less than approximately 10 ng/mL (25 nmol/L), osteomalacia is usually present.[7,9,61,88-90] Some studies suggest that serum 25(OH)D levels greater than 30 ng/mL (75 nmol/L) may be required to maximize intestinal calcium absorption[28] and prevent secondary hyperparathyroidism-induced skeletal conditions[14,30,53,81,89,90] (Figure 2).

Unlike patients with osteoporosis, patients with osteomalacia often complain of skeletal pain.[41,86,89] This pain can be elicited on physical examination by applying minimal pressure with the thumb or forefinger on the sternum or anterior tibia. Although the exact cause of the aching sensation that patients often complain of is unknown, it is possible that the collagen-rich osteoid that is laid down on the periosteal surface of the skeleton may become swollen similar to the hydration of gelatin-based food products (eg, Jell-O). This swelling could put outward pressure on the periosteal covering that is innervated with nocioceptors.[91] Patients with osteomalacia are often misdiagnosed as having fibromyalgia, chronic fatigue syndrome, or myocytis and treated inappropriately with nonsteroidal anti-inflammatory agents.[41,91]

Some, but not all, observational studies have linked vitamin D inadequacy (or lower vitamin D intake) to an increased risk of hip and other nonvertebral fractures.[60,90] Moreover some, but not all, clinical trials and observational studies have reported that dietary vitamin D supplementation (often given together with calcium) lowers fracture risk[34,92-96] (Figure 3). Bischoff-Ferrari et al[98] recently conducted a systematic review and meta-analysis of double-blind randomized controlled trials (RCTs), the highest level of evidence, to assess the efficacy of vitamin D (vitamin D_3 [cholecalciferol] or vitamin D_2 [ergocalciferol]) supplementation with or without calcium supplementation vs calcium supplementation alone or placebo for preventing hip and nonvertebral fractures in elderly patients (≥60 years of age). Statistical justification was provided for pooling trials with higher vitamin D doses separately from those with lower doses. On the basis of the analysis of 3 RCTs for hip fracture risk involving 5572 subjects and 5 RCTs for nonvertebreal fracture risk involving 6098 subjects, the authors concluded that daily vitamin D supplementation between 700 and 800 IU with or without calcium appears to reduce hip fracture risk by 26% and nonvertebral fracture risk by 23% vs calcium alone or placebo in ambulatory or institutionalized elderly persons. No effect on fracture risk was observed in 2 trials that used a lower dose of 400 IU/d. A population-based, 3-year cluster randomized intervention study involving 9605 community-dwelling elderly adults (≥66 years of age) found that 400 IU/d of vitamin D with 1000 mg of calcium produced a 16% fracture risk reduction,[95] although this lower-quality trial did not meet the inclusion criteria for the meta-analysis described herein. A separate systematic review and meta-analysis conducted several years earlier that included RCTs involving either vitamin D or its analogues reported a 37% reduction in the relative risk of vertebral fracture.[99,100]

Two trials that failed to detect an effect on fracture risk were published soon after the meta-analysis was conducted. Porthouse et al[101] conducted an open-label RCT to assess whether 1000 mg of calcium daily with 800 IU of vitamin D_3 supplementation reduced fracture risk among 3314 women 70 years and older with one or more risk factors for hip fracture. The incidence of hip and other clinical fractures did not differ significantly between groups after a median follow-up of 25 months. Another randomized, double-blinded, controlled trial with a factorial design examined the effect on fracture risk.[102] A total of 5292 patients were randomized to receive vitamin D with or without calcium, calcium alone, or placebo. After a 24-month follow-up, the authors found no significant differences in fracture rates among the 4 groups. However, compliance with the medication had declined to 63% after 24 months and may have been as low as 45% if nonresponders to the evaluation questionnaire were included. Data from a randomized, double-blind, placebo-controlled trial of 9440 community-dwelling adults (75-100 years old) randomized to receive either an annual injection of 300,000 IU of cholecalciferol (comparable to a 822-IU daily dose) or matching placebo disclosed no effect on fracture occurrence between groups.[103] However, since 25(OH)D levels were not evaluated, it is unknown whether the intramuscular vitamin D_3 was completely bioavailable. Most intramuscular preparations are not very bioavailable, which is why they are no longer available in the United States.

Decreased BMD is a major risk factor for fractures,[104] and some studies have linked vitamin D inadequacy or low intake of vitamin D to low BMD.[90,105,106] Some randomized trials have also shown a benefit.[99,100,105,107,108] For example, a double-blinded RCT randomized 249 healthy ambulatory postmenopausal women with usual daily intakes of 100 IU of vitamin D to receive 400 IU of vitamin D supplements or placebo daily. All participants also received 377 mg/d of calcium. At the end of 1 year, the vitamin D group had significantly reduced wintertime bone loss and improved net BMD of the spine.[109] By contrast, however, another RCT reported no effect of vitamin D supplementation on bone loss or bone turnover markers in calcium-replete postmenopausal African American women.[110] The earlier meta-analysis that pooled data from RCTs that included vitamin D analogues found a small nonsignificant BMD increase of 0.4% relative to the control groups.[99,100]

Many of the vitamin D supplementation studies reported herein included concurrent calcium supplementation; therefore, the observed benefits of vitamin D supplementation may be confounded or obscured by the effects of concurrent calcium supplements and cannot be ascribed to vitamin D alone. Although the meta-analysis by Bischoff-Ferrari et al[98] reported that vitamin D supplementation with or without calcium supplementation reduced fracture risks, the factorial design of the Record Evaluation of Calcium or Vitamin D (RECORD) trial concluded that vitamin D supplementation with or without calcium supplementation had no significant effect on fracture risk reduction.[102] It is also possible that the benefits of vitamin D on fracture risk reduction (and BMD) may be greater in those with vitamin D deficiency or low calcium intake at baseline. In the RECORD trial, only 60 participants (1.1%) had their serum baseline 25(OH)D levels measured. Thus, we cannot know if the lack of effect on fracture risk in the RECORD trial might be related to pretreatment levels of vitamin D and/or calcium. The hierarchy of evidence for the role of vitamin D in BMD[102-117] changes and fracture reduction is given in Table 2.

NEUROMUSCULAR FUNCTION

The VDR has been identified in skeletal muscle tissue,[167-169] and low serum 25(OH)D levels have been associated with reversible myopathy in patients with osteomalacia.[89,170] Patients with nonspecific muscle weakness, muscle aches and pains, and bone pain are sometimes discounted or diagnosed as having fibromyalgia or chronic fatigue syndrome despite strong scientific evidence that they have vitamin D inadequacy.[41,86,89,91] Several studies support the hypothesis that vitamin D inadequacy contributes to age-related muscle weakness[143,171] and falls.[120,126,172-174] A compelling meta-analysis of RCTs of vitamin D supplementation showed a greater than 20% reduced risk of falls among ambulatory or institutionalized older individuals treated with supplements.[173] Additional supporting evidence comes from a study that investigated factors leading to fracture in postmenopausal women with osteoporosis; vitamin D inadequacy was associated with increased body sway, increased risk of falls, and fall-related fractures.[172] A prospective study

TABLE 2. **Hierarchy of Evidence for Studies Relating Vitamin D to Pathologic Conditions***

Level	Study type	Data source	Treatment or predictor	Outcome	Result	Notes
1a	Meta-analyses	RCTs	Vitamin D and calcium	BMD[100]	Small ↑	Effect seen with low baseline vitamin D
				Fracture risk[99,100]	Small ↓ or no effect	Effect seen with low baseline 25(OH)D
				Falls[173]	Approximately 20% ↓	Low baseline 25(OH)D
1b	RCTs		Vitamin D (or analogue) alone	BMD changes[100]	Small ↑	Effect seen with vitamin D or 1-H-vitamin D_3
				Fracture risk[94]	Small ↓	
				Neuromuscular function[118,119]	Small ↓ in sway and reaction time	Single small study in fallers
				Falls[119-121]	↓ number of fallers	Benefit only with good calcium intake[116]
				CRP[122]	25% ↓ with approximately 500 IU; ICU patients	Unblinded study
			Vitamin D (or analogue) and calcium	BMD changes[111,112]	Small ↑	Benefits mostly seen with vitamin D deficiency at baseline
				Fracture risk[101,102]	No effect	
				Falls[43]	Approximately 50% ↓ in falls in post–hip fracture post–hip fracture women	Nonblinded
				BP[201]	9% ↓	Low baseline 25(OH)D; small trial
				Cytokines in patients with MS[200]	↑ TGF-β1	No effect on TNF-α, IFN-γ, or IL-13
			Exposure to UV-B	BP[125]	BP ↓ to normal	Very small double-blinded single study
			Exposure to UV-A	BP[201]	No effect on BP	
2	Cohort studies	Prospective epidemiological studies	Serum 25(OH)D	Fracture risk[113]	↑ risk of hip fracture when low	Hemiplegic stroke patients
			Serum 25(OH)D	Fracture risk, gait speed, balance[114]	No effect on fracture; poorer balance and gait speed when low	
			Serum $1,25(OH)_2D$	Fracture risk[115]	↑ risk of hip fracture when low	
			Vitamin D supplements	Fracture risk[116]	No effect	
			Serum 25(OH)D	Falls[126]	↑ risk when low	
			Serum PTH	Falls[126]	↑ risk when high	
			Serum 25(OH)D	Sarcopenia[127]	↑ risk when low	
			Dietary vitamin D and calcium	Colon cancer[164]	↑ risk when low	
			Serum 25(OH)D	Colon cancer[202]	↑ risk when low	
			Dietary vitamin D and calcium	Colorectal cancer[164]	↑ risk when low	Small effect
			Dietary vitamin D and sunlight exposure	Breast cancer risk[132,165,166]	↑ risk when low	NHANES I
			Latitude	MS[205]	↑ risk at higher latitudes	NHS I and II—effect seen in earlier cohort
			Dietary vitamin D	IHD mortality[219]	No effect on MI or IHD	
			Dietary vitamin D in first year of life	Type 1 DM incidence[207]	↓ risk with higher intake	Birth cohort, Finland, 11-year follow-up
			Dietary vitamin D	Rheumatoid arthritis[208]	33% ↓ risk in highest tertile of intake	
3	Case-control studies	Fracture cases and nonfracture controls	Fracture vs no fracture	Serum 25(OH)D[60]	↓ levels in women with hip fracture	
		D-deficient cases, "normal" controls	Case vs control	Muscle function[135]	Reduced vitamin D deficiency	
		Colorectal cancer cases (nested in cohort)	Cancer vs control	Serum 25(OH)D and serum $1,25(OH)_2D$[123]	↑ risk with low 25(OH)D level	Blood drawn at baseline, before diagnosis
		Breast cancer cases at diagnosis, clinic controls	Cancer vs control	Serum 25(OH)D and serum $1,25(OH)_2D$[124]	↑ race-$1,25(OH)_2D$ interaction in black cases, ↓ in white, compared with controls	No difference in 25(OH)D
		Prostate cancer cases (nested in cohort)	Cancer vs control	Serum 25(OH)D[125]	↑ risk with low levels	149 cases
		Prostate cancer cases (nested in cohort)	Cancer vs control	Serum 25(OH)D and serum $1,25(OH)_2D$[128]	No difference	61 cases
		MS cases, community controls	MS case vs control	Level of sunlight exposure during childhood and adolescence[129,205,225]	↑ risk with less exposure	

(*level 3 continued on next page*)

TABLE 2. **Continued***

Level	Study type	Data source	Treatment or predictor	Outcome	Result	Notes
3		Childhood-onset type 1 DM, community controls, nationwide	Diabetic vs control	Cod liver oil during pregnancy or first year of life[130]	26% ↓ risk with use in first year of life; no effect with prenatal use	Norway
		Childhood-onset type 1 DM, community controls, 1 county	Diabetic vs control	Cod liver oil during pregnancy or first year of life[131]	70% ↓ risk with prenatal use; no effect during first year	Norway
4a	Cross-sectional surveys, ecological studies	Older adults	Serum 25(OH)D	Lower-extremity function[143]	Better walking speed and sit-to-stand time with higher levels	
		Breast cancer cases	Sunlight	Cancer mortality [132,165,166,204]	Higher in areas with less sunlight	
		Ovarian cancer cases	Sunlight	Cancer mortality [133,165]	Higher in areas with less sunlight	
		Population-based, geographic area	Serum $1,25(OH)_2D$	BP[134]	Higher systolic and diastolic BP with higher levels	
		Population-based, Tromsø	Vitamin D intake	BP[136]	No effect	
		Population-based, NHANES III	Serum 25(OH)D	Periodontal disease[137]	↑ risk with low level, independently of BMD	
4b	Case series (and poor-quality cohort and case-control studies)	Patients with nonvertebral fracture	NA	Serum 25(OH)D inadequacy[117]	98% <28 ng/mL	
		Patients with minimal trauma fracture	NA	Serum 25(OH)D inadequacy[47]	97% <30 ng/mL	
5	Expert opinion without explicit critical appraisal or based on physiology, bench research, or "first principles"	Laboratory or animal studies	$1,25(OH)_2D_3$	Leukemia (mouse)[138]	Longer survival with treatment	
			$1,25(OH)_2D_3$	Lung cancer cell growth[150]	Regulates cell growth	
			$1,25(OH)_2D_3$ and analogues	Cancer cells proliferation (breast, osteosarcoma, melanoma)[3]	Antiproliferative effect	
			Vitamin D analogues	Prostate cancer[139]	Varying effects on serum calcium, depending on analogue used	
			Vitamin D and nicotine	Aortic calcification[140]	↑ with treatment	
			$1\alpha,25(OH)_2D$	Keratinocytes[27]	↓ proliferation	
			$1\alpha,25(OH)_2D$	Keratinocytes and fibroblasts of patients with psoriasis[141]	↓ proliferation, ↑ differentiation	
			$1\alpha,25(OH)_2D$	DM (NOD mice)[142,144,146]	Protection against developing DM	Up-regulates IL-4
			$1\alpha,25(OH)_2D$	EAE, mice[177]	Prevents EAE	
			$1\alpha,25(OH)_2D$	Lyme arthritis, mice[147]	Inhibits progression	IL-10 KO mice
			$1\alpha,25(OH)_2D$	IBD, mice[149]	↓ risk and severity	IL-2 KO mice
			$1\alpha,25(OH)_2D$ and calcium	IBD, mice[148]	↑ effect with both	IL-10 KO mice
			Vitamin D analogues	SLE[153]	Inhibits lupus nephritis	MRL/1 mice
			Vitamin D analogue	Aortic allograft intimal and advential damage[154]	↓ damage	
			$1\alpha,25(OH)_2D$	Heart allograft survival, mice; heterotopic graft, rats[155]	Prolongs survival without bone loss or ↑ risk of infection	
			Vitamin D analogue	Xenogenic pancreatic islets, mice[234]	Prolongs graft survival when used with cyclosporine	NOD mice
			Vitamin D analogue	Liver cancer[156]	Patients with inoperable liver cancer	Human

*References are provided for examples of each type of study. The most convincing evidence comes from randomized controlled trials. There is some evidence from clinical trials that vitamin D (often given with calcium) may reduce the risk of falls and fractures. Associations with most other diseases and conditions come from lower levels of evidence. $1\alpha,25(OH)_2D$ = 1α-25-hydroxyvitamin D; $1,25(OH)_2D$ = 1,25 hydroxyvitamin D; 25(OH)D = 25-hydroxyvitamin D; 1-H-vitamin D_3 = 1 hydroxylated vitamin D_3; BMD = bone mineral density; BP = blood pressure; CRP = C-reactive protein; DM = diabetes mellitus; EAE = experimental autoimmune encephalitis; IBD = inflammatory bowel disease; ICU = intensive care unit; IFN-γ = interferon-γ; IHD = ischemic heart disease; IL = interleukin; KO = knockout; MI = myocardial infarction; MS = multiple sclerosis; NA = not applicable; NHANES = National Health and Nutrition Examination Survey; NHS = Nurses' Health Study; NOD = nonobese diabetic; PTH = parathyroid hormone; RCT = randomized controlled trial; SLE = systemic lupus erythematosus; TNF-α = tumor necrosis factor α.

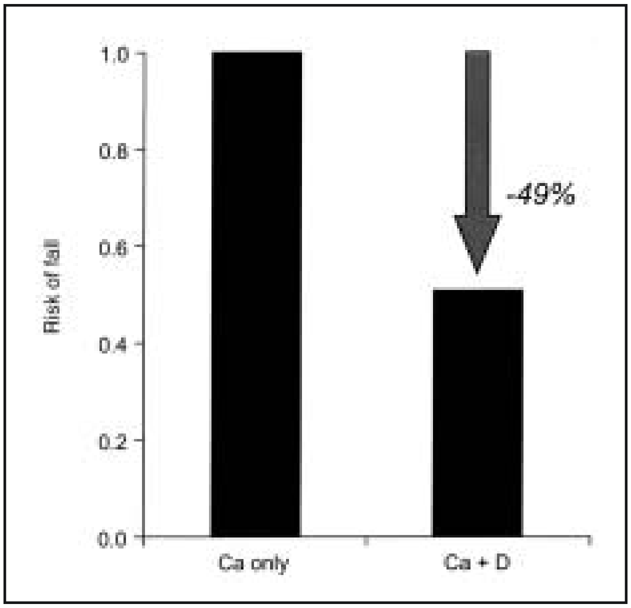

FIGURE 4. Relative to therapy with 1200 mg/d of calcium for 12 weeks, daily therapy with 800 IU of vitamin D and 1200 mg of calcium accounted for a 49% reduction in the relative risk of falls among older women (mean age, 85.3 years) in long-term geriatric care. Ca = calcium; Ca + D = Ca plus vitamin D. Adapted from *J Bone Miner Res*,[174] with permission from The American Society for Bone and Mineral Research.

that examined factors associated with falls in 637 institutionalized ambulatory subjects reported that a low baseline serum 25(OH)D level was significantly associated with increased risk of falling.[126] In a randomized, double-blind trial, treatment with vitamin D plus calcium daily for 3 months reduced the risk of falling by 49% compared with calcium alone among elderly women in long-stay geriatric care[174] (Figure 4). Similarly, a study of community-dwelling elderly adults in Switzerland showed that treatment with 1,25$(OH)_2D_3$ and also 1α-hydroxyvitamin D_3 (1α$[OH]D_3$) significantly reduced the number of falls for individuals with calcium intake of more than 512 mg/d.[120] These studies suggest that adequate serum 25(OH)D may prevent fractures not only by improving calcium homeostasis but also by improving musculoskeletal function.

Other recent evidence suggests that vitamin D inadequacy may be a factor in loss of muscle mass and muscle strength (sarcopenia).[127,175] One population-based study reported that 25(OH)D levels between 16 and 38 ng/mL (40 and 94 nmol/L) were associated with better musculoskeletal function in the lower extremities of both active and sedentary ambulatory adults 60 years or older compared with levels less than 16 ng/mL (40 nmol/L).[143] The hierarchy of evidence for the role of vitamin D in muscle function and fall prevention is summarized in Table 2.[118,119,121,135]

VITAMIN D AND EXTRASKELETAL HEALTH

The small intestine, kidneys, and bones are the primary organs and tissues responsive to vitamin D that are involved in mineral metabolism that affects skeletal health. However, the effects of vitamin D are not limited to mineral homeostasis and the maintenance of skeletal health. The presence of the VDR in other tissues and organs suggests that vitamin D may also be important in nonskeletal biological processes.[176-178] Additionally, the enzyme responsible for conversion of 25(OH)D to the biologically active form of vitamin D (1,25$[OH]_2D$) has been identified in tissues other than kidney[150,179-181] (Figure 5), and evidence is growing that extrarenal synthesis of 1,25$(OH)_2D$ may be important for regulating cell growth and cellular differentiation[1,4,176,177] via paracrine or autocrine regulatory mechanisms.

The VDR is a steroid hormone nuclear receptor that binds 1,25$(OH)_2D$ with high affinity and mediates transcriptional gene regulation.[1,4,176,177,182,183] Mounting biochemical and epidemiological evidence suggests that the VDR is also involved in mediating the noncalcemic effects of vitamin D and its analogues and may play a vital role in disease prevention and maintenance of extraskeletal health.[1-3] The VDR has been isolated from many cell types, tissues, and organs, including those not typically associated with calcium homeostasis and bone metabolism. Some of these include the heart, stomach, pancreas, brain, skin, gonads, and various cells of the immune system.[1,4,176,177] Genetic variants of the gene encoding the VDR have also been associated with differential risk of developing various cancers[184,185] and immune disorders, including type 1 diabetes mellitus.[186,187]

In addition, 1,25$(OH)_2D$ is involved in non–genomic-mediated intracellular signaling pathways.[188-191] Both 1,25$(OH)_2D$ and its synthetic analogues (collectively, VDR ligands) have demonstrated antiproliferative, prodifferentiative, and immunomodulatory activities (which may be mediated by both the genomic and the nongenomic mechanisms) in several clinical and experimental settings,[188] and are being investigated for the potential treatment of many pathologic conditions, including psoriasis, type 1 diabetes mellitus, rheumatoid arthritis, multiple sclerosis, Crohn disease, hypertension, cardiovascular heart disease, and many common cancers.[1,2,151,192,193]

Since the primary function of vitamin D is to modulate calcium homeostasis, the use of analogues for the treatment of conditions other than osteoporosis or osteomalacia could trigger hypercalcemia or other unwanted adverse effects.

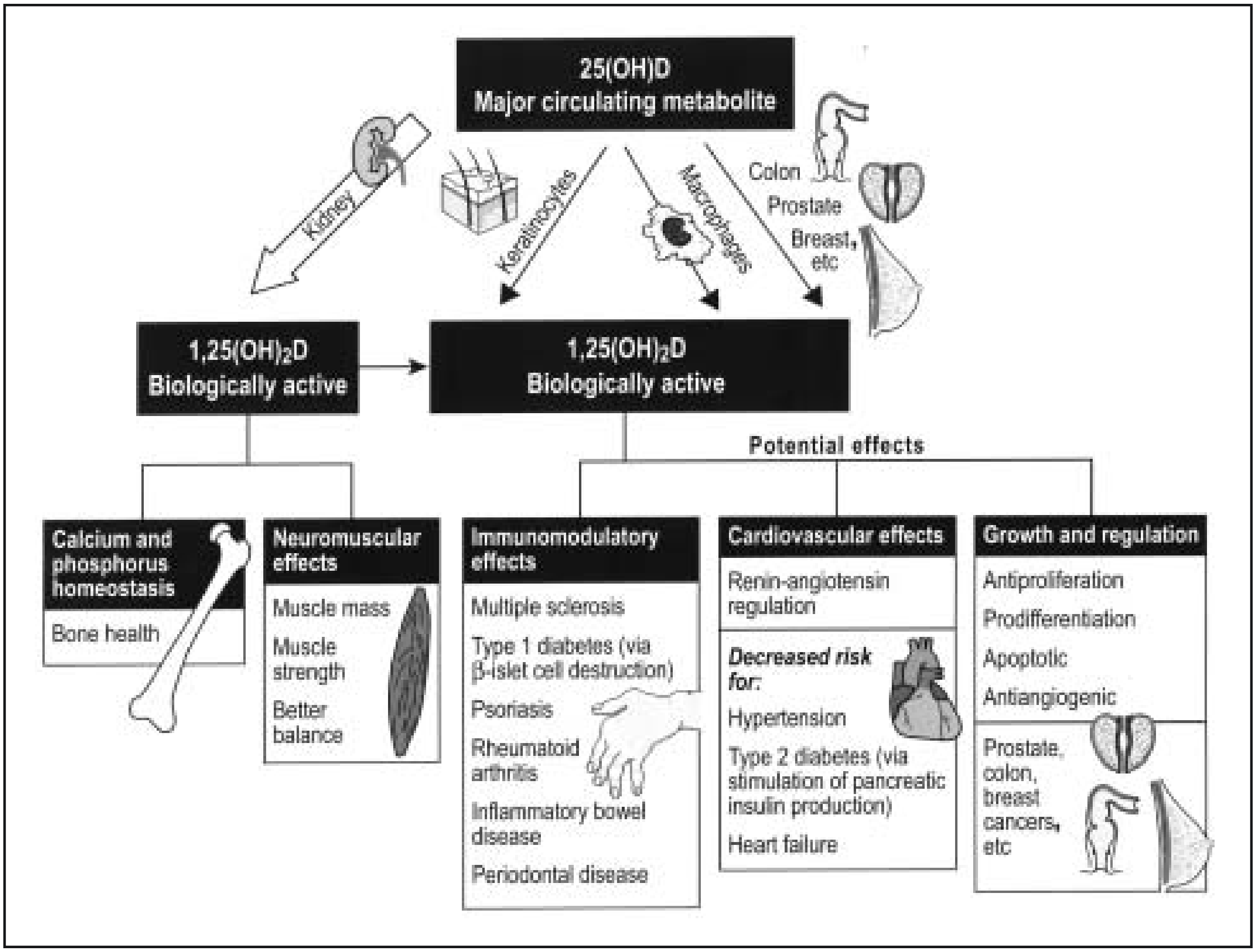

FIGURE 5. Endocrine and autocrine or paracrine functions of 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). The kidneys serve as the endocrine organ to convert 25-hydroxyvitamin D (25[OH]D) to 1,25(OH)$_2$D. 1,25(OH)$_2$D carries out its calcium-regulating functions for bone health by stimulating intestinal calcium and phosphorus absorption. The circulating levels of 1,25(OH)$_2$D can also potentially influence the activity of other tissues and cells that have a vitamin D receptor (VDR) and have no function in regulating calcium homeostasis and bone health. These include, among others, the heart skeletal muscle, active T and B lymphocytes, breast, colon, and prostate. In addition, a multitude of in vitro studies with human and animal cells have shown that most tissues and cells not only express the VDR but also express the same 1α-hydroxylase as the kidney. Thus, it has been suggested that most cells, including lung, colon, prostate, and breast, locally produce 1,25(OH)$_2$D$_3$ to help regulate a variety of cellular functions including growth and differentiation. This may help explain the epidemiological evidence that sun exposure at lower altitudes and higher serum levels of 25(OH)D are related to a decreased risk of a wide variety of chronic illnesses. It has been speculated that when 25(OH)D levels are above 30 ng/mL this serves as the substrate for the external 25(OH)D$_3$-1α-hydroxylase to produce 1,25(OH)$_2$D in the colon, prostate, breast, and lung to modulate cell growth and reduce risk of the cells becoming malignant.

Researching the development of VDR ligands with attenuated calcemic potential is a difficult challenge. More than 1000 VDR ligands with various bioactivities have been synthesized.[191,194] Analogues that are able to effect antiproliferative, prodifferentiative, blood pressure, and immunomodulation changes while maintaining calcium homeostasis are of particular interest as potential treatments for nonskeletal diseases. The hierarchy of evidence for the role of vitamin D and its analogues on these nonskeletal effects is summarized in Table 2.

VITAMIN D AND CANCER

Vitamin D is one of the most potent hormones for regulating cell growth; 1,25(OH)$_2$D inhibits proliferation and induces differentiation into normally functioning cells.[1,4,176,177,197] Some evidence suggests that 1,25(OH)$_2$D helps to regulate cell growth and prevent cancer progression[1,176,177,198] by reducing angiogenesis,[153] increasing cell differentiation and apoptosis of cancer cells, and reducing cell proliferation[156-158] and metastases.[1,4,126,151,156,176,177,179,181] The antiproliferative and prodifferentiative activity of VDR ligands was noted almost 3 decades ago.[158,176,177] Tanaka et al[159] showed that mouse and human leukemic cells that expressed the VDR had growth inhibition and were stimulated to differentiate into mature macrophages when treated with $1\alpha,25(OH)_2D_3$. Suda et al[138] were then able to show that leukemic mice survived longer if they were treated with $1\alpha(OH)D_3$, a $1,25(OH)_2D_3$ analogue. However, results of human trials for the treatment of preleukemia with VDR ligands were disappointing. Although some patients experienced remission, the treatment caused severe hypercalcemia, and all patients eventually died.[160]

Several in vitro studies have shown that breast, colon, and prostate cancer cells, osteosarcomas, and melanomas are responsive to the antiproliferative effects of $1,25(OH)_2D$,[1,4,161,162,176,177,182] and several epidemiological studies have reported that higher 25(OH)D levels are associated with reduced cancer incidence and decreased cancer-related mortality.[1,2,161,182,204] As early as 1941, people who live at higher latitudes in the United States were noted to have increased risks of breast, colon, and prostate cancers.[163] This insightful observation went unnoticed until the 1980s, when Garland et al[132,164,202] reported increased breast and colon cancer risks for those living at higher latitudes in the United States. Evidence now exists to support the link between increased sunlight exposure and a lower incidence of many cancers.[165,166,209-211] Such ecologic studies can be a weak form of evidence, particularly since latitude is not always a strong predictor of vitamin D status. Adding to these data are several retrospective and prospective observational studies that have reported decreases of 50% or greater in risk of large bowel cancer and prostate cancer when serum 25(OH)D levels are greater than 20 ng/mL (50 nmol/L) or vitamin D intake is increased.[2,123,125,162,164,182,202-204]

Similar results have been observed for breast cancer.[212] In one study, women in the highest quartile of serum $1,25(OH)_2D$ had one fifth the risk of breast cancer vs those in the lowest quartile.[124] Women in the National Health and Nutrition Examination Survey with self-reported high intake of vitamin D from supplements or high lifetime sun exposure had a significantly reduced risk of breast cancer.[204] Grant[166,209] postulated that roughly 25% of deaths due to breast cancer among women in northern Europe could be attributed to inadequate vitamin D levels, possibly due to living at higher latitudes, and that both men and women with greater sun exposure were less likely to die prematurely of cancer.

Although it was recognized that most cells, including the prostate, breast, and colon, had VDR, it was perplexing how increased exposure to sunlight, higher vitamin D intake, and higher serum 25(OH)D levels could reduce risk of cancer in these tissues. It was known that the kidney tightly controls the amount of $1,25(OH)_2D$ it produces. Although PTH and hypophosphatemia increase the renal production of $1,25(OH)_2D$, an increase in sun exposure or dietary vitamin D intake does not. In 1998, Schwartz et al[179] reported that normal prostate tissue and prostate cancer tissue from prostate biopsy specimens had the enzymatic machinery (25[OH]D-1α-hydroxylase; CYP27B) to convert 25(OH)D to $1,25(OH)_2D$. Similar observations have been made in breast, colon, lung, skin, and a multitude of other organs and tissues.[150,180,181] Thus, a new autocrine or paracrine function for vitamin D was revealed (Figure 5). This concept was supported by the observation that when a prostate cancer cell line LNCaP that did not express CYP27B was incubated with $25(OH)D_3$, there was no effect on the cancer cell's proliferation activity. When similar cells were transfected with a plasmid that contained the *CYP27B* gene, the cells were able to convert $25(OH)D_3$ to $1,25(OH)_2D_3$. When the CYP27B transfected cells were treated with $25(OH)D_3$, the cellular proliferative activity decreased similarly to cells treated with $1,25(OH)_2D_3$.[213] This observation supports the hypothesis that increasing circulating concentrations of 25(OH)D provide most cells with the substrate to make $1,25(OH)_2D$ locally to regulate cell growth and differentiation. Once $1,25(OH)_2D$ completes this task, it then induces the 25(OH)D-24-hydroxylase gene (*CYP24B*) to catabolize it to the biologically inactive calcitroic acid (Figure 1).[1,4,176,177,189]

VITAMIN D AND CARDIOVASCULAR DISEASE

Adding to the evidence of the effect of vitamin D on extraskeletal tissues are data that suggest that inadequate vitamin D and calcium and living at higher latitudes may be independent contributing factors in the pathogenesis and progression of hypertension and cardiovascular disease.[1,2,201,214] $1,25(OH)_2D$ is involved in controlling the production of renin, one of the most important hormones for regulating blood pressure.[215] Ecological evidence also exists that African Americans, who have been shown to be at greater risk of vitamin D deficiency, also have a greater risk of hypertension and cardiovascular disease.[199,214,216] These studies do not directly provide a cause-and-effect relationship between vitamin D and cardiovascular health but suggest a provocative hypothesis for further research.

In a small blinded study of 18 hypertensive patients randomized to receive repeated exposure to artificial UV-A radiation (which cannot produce vitamin D_3) or UV-B radiation (which leads to cutaneous vitamin D_3 synthesis) treatment, the UV-B treatment group showed an average 6-mm Hg decrease in both systolic and diastolic blood pressure and a 180% increase in serum 25(OH)D levels vs the group exposed to UV-A, who showed no change in serum

25(OH)D levels or blood pressure.[201] A randomized, placebo-controlled, double-blind clinical trial of 148 elderly women (mean age, 74 years) found that vitamin D and calcium were more effective in reducing systolic blood pressure than calcium alone.[216] Sowers et al[134] also reported a positive association between 1,25$(OH)_2$D and vitamin D inadequacy and hypertension. Although this evidence suggests that increased vitamin D is associated with reduced risk of hypertension, some studies have reported contradictory findings. Jorde and Bonaa[136] reported no link between vitamin D intake and blood pressure. The authors noted that most participants older than 50 years were receiving less than 400 IU of vitamin D from their diet, which meant that they were not receiving enough vitamin D. Furthermore, blood levels of 25(OH)D were not obtained, making it difficult to know the vitamin D status of the subjects who were studied.

Elevated C-reactive protein levels have been associated with increased cardiovascular events.[217] In one unblinded study, 22 patients with prolonged critical illnesses were compared with matched controls and then randomized to daily vitamin D supplement of either ±200 IU or ±500 IU. The results demonstrated that a 500-IU/d dose of vitamin D reduced C-reactive protein levels by more than 25% in the critically ill patients vs the matched controls who did not receive supplementation.[217] McCarty[218] hypothesized that the excess of coronary mortality observed in winter may be related to inadequate levels of vitamin D and suggested a possible role for vitamin D in maintaining vascular health.

However, as with hypertension, contradictory findings suggest that increased vitamin D may be a causative factor or play no role in cardiovascular disease. In an observational study of vitamin D and myocardial infarction, Lindén[219] reported that vitamin D intake was elevated in patients with myocardial infarctions vs randomly selected age- and sex-matched controls who had no prior myocardial infarction or angina pectoris. However, this study was confounded because the patients in general were at high risk of cardiovascular heart disease and there was no measure of vitamin D status to substantiate the “high intake.” Two other studies, one observational with age-matched controls[220] and a prospective cohort study of 34,486 postmenopausal women (55-69 years of age),[206,221] found that 25(OH)D levels were not elevated in patients with myocardial infarctions and ischemic heart disease.

VITAMIN D AND PSORIASIS

One of the great successes of vitamin D therapy for treating an extraskeletal disorder is in the treatment of psoriasis.[193,196] Smith et al[27] showed that 1,25$(OH)_2D_3$ inhibited the proliferation of human keratinocytes that express the VDR in vitro and accelerated their differentiation. This suggested that hyperproliferative skin disorders such as psoriasis might be responsive to treatment with 1,25$(OH)_2D_3$.[222] Initial treatments with topical 1,25$(OH)_2D_3$ showed great improvements in reducing the severity and area of psoriatic lesions, with little or no adverse effects.[25,141,193,223] Today, 3 vitamin D analogues including calcipotriene, 1,24$(OH)_2D_3$, and 22-oxo-1,25$(OH)_2D_3$, are among the first-line treatments used for psoriasis.[141,193,196,223]

VITAMIN D AND MULTIPLE SCLEROSIS

As with previous epidemiological data reporting a latitudinal risk gradient for cancer and cardiovascular disease,[2,161,163,166,204,209,215] a similar risk gradient exists for developing multiple sclerosis.[205,224-226] Subjects who were born and/or lived below 35°N latitude for the first decade of life had decreased overall lifetime risks of developing multiple sclerosis.[205,225] However, as with other ecological studies, the observed differences could be related to any number of other factors that were not measured.

One double-blinded RCT involving patients with multiple sclerosis who were randomized to receive either vitamin D supplementation or placebo showed that patients who received supplementation had increased serum transforming growth factor β1 levels vs those who did not receive supplementation.[200] Elevated transforming growth factor β1 levels have been associated with the stable phase of multiple sclerosis, whereas reduced levels have been associated with relapsing-remitting multiple sclerosis.[227,228] Two related observational studies[229] (the Nurses’ Health Study [N=92,253, from 1980 to 2000] and the Nurses’ Health Study II [N=95,310, from 1991 to 2001]) reported that higher intake of vitamin D was associated with a lower risk of developing multiple sclerosis.

VITAMIN D AND TYPE I DIABETES MELLITUS

1,25$(OH)_2$D acts as an immunomodulator, reducing cytokine production and lymphocyte proliferation, which have been implicated in the destruction of insulin-secreting β cells in the pancreas and the development of type 1 diabetes mellitus.[142] In addition, β-islet cells express the VDR and respond to 1,25$(OH)_2$D by increasing insulin production.[1,142,230,231]

In animals, the administration of 1,25$(OH)_2$D prevents the development of experimentally induced type 1 diabetes mellitus.[142,144] Zella and DeLuca[145] have also shown that very large doses of vitamin D were able to suppress the development of insulitis and diabetes in the nonobese diabetic mouse, a model of human type 1 diabetes mellitus. A birth cohort study involving 10,366 children conducted in Finland showed that higher dietary vitamin D supplementation was associated with reduced risk of type 1 diabetes mellitus. Children who regularly took the recommended supplemental dose of 2000 IU/d of vitamin D during their first year of life had a rate ratio of 0.22 (range, 0.05-0.89) for type 1 diabetes mellitus compared with those who regularly received less than 2000 IU/d.[207] Likewise, Stene et al[130] reported a lower risk of type 1 diabetes mellitus in the children of mothers who took cod liver oil during their pregnancy. These data do not support a direct cause-and-effect relationship but suggest that further studies are warranted.

VITAMIN D IN OTHER DISEASES

A possible role of vitamin D has also been implicated in several other diseases, including rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, osteoarthritis, and periodontal disease. Many of these studies are epidemiological studies and animal models, and the effect in humans is unknown. Recent findings suggest that vitamin D intake is inversely associated with rheumatoid arthritis[208] and that $1,25(OH)_2D_3$ supplementation can inhibit disease progression in mouse models of human Lyme arthritis.[147] Merlino et al[208] tracked approximately 30,000 women throughout 11 years and found that those whose daily vitamin D intake was less than 200 IU were 33% more likely to develop rheumatoid arthritis compared with women with higher intake levels. Cantorna et al[148,152] have shown that $1,25(OH)_2D_3$ can prevent and ameliorate symptoms of inflammatory bowel disease in a mouse model. Abe et al[153] were also able to alleviate symptoms in a mouse model of systemic lupus erythematosus without inducing hypercalcemia by using 22-oxa-$1,25(OH)_2D_3$, a synthetic analogue of $1,25(OH)_2D_3$. McAlindon et al[232] observed that a higher intake of vitamin D and higher blood levels of 25(OH)D decreased progression of osteoarthritis in men and women by more than 60%. Krall et al[233] found that calcium and vitamin D supplementation was associated with a reduced risk of tooth loss in elderly patients. More recently, Dietrich et al[137] reported that low serum levels of 25(OH)D may be associated with periodontal disease, independent of BMD.

Experimental evidence also exits for an immunomodulatory role of VDR ligands in attenuating rejection of rat aortic allografts[154,188] and both vascular and nonvascular transplants.[155] Treatment of nonobese diabetic mice with a vitamin D analogue KH1060 in combination with cyclosporine achieved 100% early graft success of xenogenic islets.[234] In addition, VDR ligands have been used experimentally to attenuate bone loss after organ transplantation.[235] Nagpal et al[188] postulate that the mechanism by which vitamin D prevents transplantation-induced osteoporosis may be comparable to the mechanism by which it suppresses secondary hyperparathyroidism brought on by immunosuppressive agents such as corticosteroids and/or cyclosporine; the mechanism appears to involve pregnane X receptor–enhanced CYP24 gene expression.[74]

VITAMIN D DOSING, SUPPLEMENTATION, AND UV IRRADIATION AND/OR SENSIBLE SUN EXPOSURE

Supplementation with vitamin D has been estimated to prevent vitamin D deficiency in approximately 98% of the general population.[1,16,236] Vitamin D supplementation and exposure to sunlight or simulated sunlight have been shown to increase serum 25(OH)D levels in elderly patients [11,84,87,89,90,92,94,97,102,107,237-240] (Figure 3).

The Institute of Medicine's adequate intake for the United States and Canada is 200 IU/d for all children and adults younger than 51 years, 400 IU/d for people aged 51 to 70 years, and 600 IU/d for those older than 70 years.[21,236] A report by the Scientific Committee for Food, established by the European Commission, indicated that adults 65 years and older should receive 400 IU/d of vitamin D_3 and suggested that the requirements of all adults, including those with inadequate sunlight exposure, would be met by this dietary intake.[241] This recommendation is consistent with that of the US Food and Drug Administration's daily recommended value of 400 IU/d (10 μg/d) of vitamin D_3 regardless of age.[242] Because it has been suggested that amounts up to 1000 IU/d of vitamin D_3 may be needed to maintain a healthy 25(OH)D level of more than 30 ng/mL (75 nmol/L),[5,28,243,244] an intake of 400 IU/d may represent a minimum. This is especially true in the winter or for children and adults not exposed to sunlight.

Vitamin D toxicity has not been reported from long-term exposure to sunlight[1,4] and has only been observed from dietary intake when daily doses exceed 10,000 IU.[245,246] Doses of 4000 IU/d for 3 months and 50,000 IU/wk for 2 months have been administered without toxicity.[11,247,248]

TREATMENT OF SEVERE VITAMIN D DEFICIENCY

Although severe vitamin D deficiency (25[OH] levels <10 ng/mL [25 nmol/L]) is much less common than inadequacy, it does occur, especially in elderly house-bound people. The best method for treating vitamin D deficiency is an oral dose of 50,000 IU/wk of vitamin D_2 for 8 weeks, then checking 25(OH)D levels.[1,11] In some cases, another once-weekly 8-week course of 50,000 IU of vitamin D_2 may be necessary to boost 25(OH)D levels into the desired range of more than 30 to 50 ng/mL (75-125 nmol/L). For patients prone to developing vitamin D deficiency, after correcting the deficiency, giving patients 50,000 IU every 2 weeks will sustain them in a vitamin D–sufficent state. Alternatively, 1000 IU of vitamin D_3 intake should be maintained. Cutaneous exposure to sunlight or artificial UV-B such as a tanning bed is also helpful, especially if the patient is prone to vitamin D deficiency.[76,165,238-240,248,249] Exposure to direct sunlight typically of no more than 5 to 10 minutes on the arms and legs between the hours of 10 AM and 3 PM during the spring, summer, and fall will prevent vitamin D inadequacy.[1,16,65]

CONCLUSION

Vitamin D is important for calcium and phosphorus homeostasis and musculoskeletal health. In children, severe vitamin D deficiency (25[OH]D, <10 ng/mL [24.9 nmol/L]) manifests as rickets, and vitamin D inadequacy (25[OH]D, 10-29 ng/mL [24.9-72.4 nmol/L]) can impair or retard attainment of peak bone mass. In adults, inadequate vitamin D can result in secondary hyperparathyroidism, decreased BMD, osteoporosis, osteomalacia, and increased risk of fragility fractures.[250-252]

Vitamin D inadequacy is a global problem, especially among elderly patients and patients with osteoporosis. Risk factors for low vitamin D include lack of exposure to sufficient sunlight, inadequate dietary intake and supplementation, and other factors, including obesity, age, medication use, sunscreen use, covering all skin with clothing, and skin pigmentation. Fortunately, vitamin D supplements are widely available and relatively inexpensive.

Despite some negative studies, the preponderance of evidence from RCTs supports a reduction in the risk of vertebral[100] and nonvertebral[92,107] fractures with vitamin D (given in combination with calcium in most trials), especially in populations with low vitamin D status and low calcium intake at baseline. Similarly, results of a meta-analysis of RCTs suggest that vitamin D can reduce the risk of falls.[143]

Many lines of research support the concept that inadequate vitamin D may be involved in the pathogenesis and/or progression of several disorders, including cancer, hypertension, cardiovascular disease, neuromuscular diseases, osteoarthritis, diabetes, and other autoimmune diseases (Table 2). Some of the mechanisms by which vitamin D exerts its noncalcemic effects include apoptosis, anti-angiogenesis, antiproliferation, prodifferentiation, and immunomodulation (Figure 5) (Table 2).[150,178-188,253]

However, the current level of evidence for associations of vitamin D with nonmusculoskeletal conditions is generally weaker than that for its calcemic and musculoskeletal effects (falls and fractures). The reported nonmusculoskeletal associations have come primarily from observational studies in humans or laboratory experiments (in vivo and in vitro). As indicated in Table 2, randomized, placebo-controlled, double-blind clinical trials (or meta-analyses of these trials) are considered to provide the highest level of evidence because randomization and blinding minimize the risk of bias in estimating treatment effects.[254,255] Even among observational studies, evidentiary value varies, with prospective cohort studies (such as the Framingham Study, the Nurses' Health Study, or the Study of Osteoporotic Fractures) yielding the most clinically useful information, whereas bench research and expert opinion provide the least. All types of non-RCT studies are subject to the risk of several biases, which are minimized by the design of RCTs: the qualitative and quantitative differences between observational and trial findings about hormone replacement therapy for postmenopausal women are probably the most noteworthy recent demonstration of the limitations of observational data.[256,257] However, results from nontrial studies are important because they identify associations and suggest hypotheses that can be tested more rigorously in RCTs. The presence of VDR in tissues other than bone and muscle suggests the possibility of important effects of vitamin D; however, the available supporting data are sometimes conflicting and generally represent a low level of evidence. The putative nonmusculoskeletal effects of vitamin D may become better understood if appropriate trials are conducted, but at present inferences about such effects must be made with caution.

Despite evidence of its profound importance to human health, vitamin D inadequacy is not widely recognized as a problem by physicians and patients. These observations highlight the need for greater awareness among researchers, clinicians, and patients of the high prevalence of vitamin D inadequacy and more aggressive screening for vitamin D inadequacy with a serum 25(OH)D determination, particularly among high-risk populations such as elderly patients and patients with osteoporosis.

Finally, we should not forget the important role that sensible sun exposure has in providing both young and old people with their vitamin D requirement. It is well documented that excessive exposure to sunlight, especially the number of sunburning experiences, is related to increased risk of squamous and basal cell carcinoma.[258,259] These skin cancers, if detected early, are usually easily treated and often cured. Melanoma, however, is one of the most aggressive and deadly forms of skin cancer. This is because melanocytes are neurocrest cells and when they become malignant they express the Slug gene, which is responsible for its quick exit from skin metastasizing to various organs, making it difficult to detect and treat.[260] However, it should be appreciated that most melanomas occur on the least sun-exposed areas.[16,261,262] Furthermore, Kennedy et al[259] reported that lifetime sun exposure appeared to be associated with a lower risk of malignant melanoma, despite the fact that lifetime sun exposure did not diminish the number of melanocytic nevi or atypical nevi. Furthermore, Berwick et al[263] reported that sun exposure is associated with increased survival in patients with melanoma, and Chang et al[264] reported that a history of increased sun exposure was associated with reduced risk of non–Hodgkin lymphoma. These observations are consistent with the suggestion of Apperly[163] that sun exposure seems to provide an immunity for most deadly cancers, even though it is associated with increased risk of relatively benign skin cancer. No substantiated scientific evidence exists to suggest that sensible suberythemal sun exposure significantly increases risk of any type of skin cancer and certainly not melanoma. The negative publicity regarding sun exposure during the past 30 years has resulted in a vitamin D deficiency pandemic. The important role that sensible sun exposure has in providing vitamin D for the world's population needs to be reevaluated. Indeed, in Australia and New Zealand, where the incidence of skin cancer is the highest in the world, the New Zealand Bone and Mineral Society in collaboration with the Australian College of Dermatologists and the Cancer Council of Australia have recommended a balance between avoiding an increased risk of skin cancer and achieving enough UV radiation to maintain adequate vitamin D levels. It is hoped that this message will be heard loud and clear and that this recommendation will also be adopted worldwide.

I thank Dr Joseph West and Christine McCrary Sisk of Merck and Catherine St. Clair for assistance with preparation of the submitted manuscript.

REFERENCES

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr.* 2004;80(6, suppl):1678S-1688S.

2. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. *Altern Med Rev.* 2005;10:94-111.

3. 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.

4. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis [published correction appears in *Am J Clin Nutr*. 2004;79:890]. *Am J Clin Nutr.* 2004;79:362-371.

5. 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;77:1478-1483.

6. Holick MF, Shao Q, Liu WW, Chen TC. The vitamin D content of fortified milk and infant formula. *N Engl J Med.* 1992;326:1178-1181.

7. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med.* 2004;158:531-537.

8. Outila TA, Karkkainen MU, Lamberg-Allardt CJ. Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. *Am J Clin Nutr.* 2001;74:206-210.

9. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int.* 1997;7:439-443.

10. Kinyamu HK, Gallagher JC, Rafferty KA, Balhorn KE. Dietary calcium and vitamin D intake in elderly women: effect on serum parathyroid hormone and vitamin D metabolites. *Am J Clin Nutr.* 1998;67:342-348.

11. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency [letter]. *Lancet.* 1998;351:805-806.

12. Kauppinen-Mäkelin R, Tähtelä R, Löyttyniemi E, Kärkkäinen J, Välimäki MJ. High prevalence of hypovitaminosis D in Finnish medical in- and outpatients. *J Intern Med.* 2001;249:559-563.

13. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone.* 2002;30:771-777.

14. 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 [published correction appears in *J Clin Endocrinol Metab*. 2001;86:3008]. *J Clin Endocrinol Metab.* 2001;86:1212-1221.

15. Sullivan SS, Rosen CJ, Halteman WA, Chen TC, Holick MF. Adolescent girls in Maine are at risk for vitamin D insufficiency. *J Am Diet Assoc.* 2005;105:971-974.

16. Holick M, Jenkins M. *The UV Advantage*. NewYork, NY: iBooks; 2003.

17. Segal E, Zinnman H, Raz B, Tamir A, Ish-Shalom S. Adherence to vitamin D supplementation in elderly patients after hip fracture [letter]. *J Am Geriatr Soc*. 2004;52:474-475.

18. Holick MF. McCollum Award Lecture, 1994: vitamin D—new horizons for the 21st century. *Am J Clin Nutr.* 1994;60:619-630.

19. MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D_3 and its photoisomers in human skin. *Science.* 1982;216:1001-1003.

20. Holick MF, Tian XQ, Allen M. Evolutionary importance for the membrane enhancement of the production of vitamin D_3 in the skin of poikilothermic animals. *Proc Natl Acad Sci U S A.* 1995;92:3124-3126.

21. Holick MF. Vitamin D requirements for humans of all ages: new increased requirements for women and men 50 years and older. *Osteoporos Int.* 1998;8(suppl 2):S24-S29.

22. Webb AR, deCosta BR, Holick MF. Sunlight regulates the cutaneous production of vitamin D_3 by causing its photodegradation. *J Clin Endocrinol Metab.* 1989;68:882-887.

23. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D_3. *Lancet.* 1982;1:74-76.

24. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens supress cutaneous vitamin D_3 synthesis. *J Clin Endocrinol Metab.* 1987;64:1165-1168.

25. Bikle DD. Vitamin D: role in skin and hair. In: Feldman D, ed. *Vitamin D.* Vol 1. 2nd ed. San Diego, Calif: Elsevier Academic Press; 2005:609-630.

26. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T. Regulation of terminal differentiation of cultured mouse epidermal cells by 1 alpha,25-dihydroxyvitamin D_3. *Endocrinology*. 1983;113:1950-1957.

27. Smith EL, Walworth NC, Holick MF. Effect of 1α,25-dihydroxyvitamin D_3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. *J Invest Dermatol.* 1986;86:709-714.

28. 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;22:142-146.

29. Binkley N, Krueger D, Cowgill CS, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab.* 2004;89:3152-3157.

30. Holick MF, Siris ES, Binkley N, et al. Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab.* 2005;90:3215-3224.

31. Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF. An international comparision of serum 25-hydroxyvitamin D measurements. *Osteoporos Int.* 1999;9:394-397.

32. Holick MF. 25-OH-vitamin D assays [letter]. *J Clin Endocrinol Metab*. 2005;90:3128-3129.

33. Glendenning P, Fraser WD. 25-OH-vitamin D assays [letter]. *J Clin Endocrinol Metab.* 2005;90:3129.

34. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev.* 2001;22:477-501.

35. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. *Am J Clin Nutr.* 2004;80(6, suppl):1706S-1709S.

36. McKenna MJ, Freany R. Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. *Osteoporos Int.* 1998;8(suppl 2):S3-S6.

37. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med.* 1989;321:1777-1783.

38. Kinyamu HK, Gallagher JC, Balhorn KE, Petranick KM, Rafferty KA. Serum vitamin D metabolites and calcium absorption in normal young and elderly free-living women and in women living in nursing homes [published correction appears in *Am J Clin Nutr*. 1997;66:454]. *Am J Clin Nutr.* 1997;65: 790-797.

39. Gaugris S, Heaney RP, Boonen S, Kurth H, Bentkover JD, Sen SS. Vitamin D inadequacy among post-menopausal women: a systematic review. *QJM.* 2005;98:667-676.

40. Isaia G, Giorgino R, Rini GB, Bevilacqua M, Maugeri D, Adami S. Prevalence of hypovitaminosis D in elderly women in Italy: clinical consequences and risk factors. *Osteoporos Int.* 2003;14:577-582.

41. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc.* 2003;78:1463-1470.

42. Carnevale V, Manfredi G, Romagonoli E, et al. Vitamin D status in female patients with primary hyperparathyroidism: does it play a role in skeletal damage? *Clin Endocrinol (Oxf).* 2004;60:81-86.

43. Harwood RH, Sahota O, Gaynor K, Masud T, Hosking DJ. A randomised, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: the Nottingham Neck of Femur (NONOF) Study. *Age Ageing.* 2004;33:45-51.

44. Glowacki J, Hurwitz S, Thornhill TS, Kelly M, LeBoff MS. Osteoporosis and vitamin-D deficiency among postmenopausal women with osteoarthritis undergoing total hip arthroplasty. *J Bone Joint Surg Am.* 2003;85-A:2371-2377.

45. Gomez-Alonso C, Naves-Diaz ML, Fernandez-Martin JL, Diaz-Lopez JB, Fernandez-Coto MT, Cannata-Andia JB. Vitamin D status and secondary hyperparathyroidism: the importance of 25-hydroxyvitamin D cut-off levels. *Kidney Int Suppl.* 2003;85:S44-S48.

46. Blau EM, Brenneman SK, Bruning AL, Chen Y. Prevalence of vitamin D insufficiency in an osteoporosis population in Southern California [abstract]. *J Bone Miner Res.* 2004;19(suppl 1):S342. Abstract SU582.

47. Simonelli C, Weiss TW, Morancey J, Swanson L, Chen YT. Prevalence of vitamin D inadequacy in a minimal trauma fracture population. *Curr Med Res Opin.* 2005;21:1069-1074.

48. El-Hajj Fuleihan G, Nabulsi M, Choucair M, et al. Hypovitaminosis D in healthy schoolchildrren. *Pediatrics.* 2001;107:E53.

49. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. *Am J Med.* 2002;112:659-662.

50. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. 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;76:187-192.

51. Hanley DA, Davison KS. Vitamin D insufficiency in North America. *J Nutr*. 2005;135:332-337.

52. Romagnoli E, Caravella P, Scarnecchia L, Martinez P, Minisola S. Hypovitaminosis D in an Italian population of healthy subjects and hospitalized patients. *Br J Nutr.* 1999;81:133-137.

53. van der Wielen RP, Lowik MR, van den Berg H, et al. Serum vitamin D concentrations among elderly people in Europe. *Lancet.* 1995;346:207-210.

54. Gloth FM III, Gundberg CM, Hollis BW, Haddad JG Jr, Tobin JD. Vitamin D deficiency in homebound elderly persons. *JAMA.* 1995;274:1683-1686.

55. Passeri G, Pini G, Troiano L, et al. Low vitamin D status, high bone turnover, and bone fractures in centenarians. *J Clin Endocrinol Metab.* 2003; 88:5109-5115.

56. Harris SS, Soteriades E, Coolidge JA, Mudgal S, Dawson-Hughes B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. *J Clin Endocrinol Metab.* 2000;85:4125-4130.

57. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med.* 1998;338:777-783.

58. McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. *Am J Med.* 1992;93:69-77.

59. Simonelli C, Morancey JA, Swanson L, et al. A high prevalence of vitamin D insufficiency/deficiency in a minimal trauma fracture population [abstract]. *J Bone Miner Res.* 2004;19(suppl 1):S433. Abstract M373.

60. LeBoff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *JAMA*. 1999;281:1505-1511.

61. Aaron JE, Gallagher JC, Anderson J, et al. Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. *Lancet.* 1974;1:229-233.

62. Solomon L. Fracture of the femoral neck in the elderly: bone ageing or disease? *S Afr J Surg.* 1973;11:269-279.

63. Hordon LD, Peacock M. Osteomalacia and osteoporosis in femoral neck fracture. *Bone Miner.* 1990;11:247-259.

64. Pal BR, Marshall T, James C, Shaw NJ. Distribution analysis of vitamin D highlights differences in population subgroups: preliminary observations from a pilot study in UK adults. *J Endocrinol.* 2003;179:119-129.

65. 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;67:373-378.

66. Chen TC. Photobiology of vitamin D. In: Holick MF, ed. *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*. Totowa, NJ: Humana Press; 1999:17-37.

67. Park S, Johnson MA. Living in low-latitude regions in the United States does not prevent poor vitamin D status. *Nutr Rev*. 2005;63(6, pt 1):203-209.

68. Kimlin MG, Schallhorn KA. Estimations of the human 'vitamin D' UV exposure in the USA. *Photochem Photobiol Sci*. 2004;3:1067-1070.

69. Engelsen O, Brustad M, Aksnes L, Lund E. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochem Photobiol*. 2005 [Epub ahead of print].

70. 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;18:739-741.

71. Sedrani SH. Low 25-hydroxyvitamin D and normal serum calcium concentrations in Saudi Arabia: Riyadh region. *Ann Nutr Metab.* 1984;28:181-185.

72. Matsuoka LY, Wortsman J, Chen TC, Holick MF. Compensation for the interracial variance in the cutaneous synthesis of vitamin D. *J Lab Clin Med*. 1995;126:452-457.

73. Matsuoka LY, Wortsman J, Haddad JG, Kolm P, Hollis BW. Racial pigmentation and the cutaneous synthesis of vitamin D. *Arch Dermatol*. 1991; 127:536-538.

74. Pascussi JM, Robert A, Nguyen M, et al. Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. *J Clin Invest*. 2005;115:177-186.

75. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity [published correction appears in *Am J Clin Nutr.* 2003;77:1342]. *Am J Clin Nutr.* 2000;72:690-693.

76. Koutkia P, Lu Z, Chen TC, Holick MF. Treatment of vitamin D deficiency due to Crohn's disease with tanning bed ultraviolet B radiation. *Gastroenterology.* 2001;121:1485-1488.

77. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D_3. *J Clin Invest.* 1985;76:1536-1538.

78. Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. *Lancet.* 1989;2:1104-1105.

79. Walker-Bone K, Wood A, Hull R, et al. The prevention and treatment of glucocorticoid-induced osteoporosis in clinical practice. *Clin Med.* 2004;4: 431-436.

80. Di Munno O, Mazzantini M, Delle Sedie A, Mosca M, Bombardieri S. Risk factors for osteoporosis in female patients with systemic lupus erythematosus. *Lupus.* 2004;13:724-730.

81. Snijder MB, van Dam RM, Visser M, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab.* 2005;90:4119-4123.

82. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab.* 2003;88: 157-161.

83. 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;76:370-373.

84. Lo CW, Paris PW, Clemens TL, Nolan J, Holick MF. Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. *Am J Clin Nutr.* 1985;42:644-649.

85. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians [published correction appears in *CMAJ*. 2002;167:850]. *CMAJ.* 2002;166:1517-1524.

86. 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;1:201-204.

87. Eriksen EF, Glerup H. Vitamin D deficiency and aging: implications for general health and osteoporosis. *Biogerontology.* 2002;3:73-77.

88. Exton-Smith AN, Hodkinson HM, Stanton BR. Nutrition and metabolic bone disease in old age. *Lancet.* 1966;2:999-1001.

89. 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;247:260-268.

90. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. *Am J Clin Nutr.* 2003;77:504-511.

91. Holick MF. Vitamin D deficiency: what a pain it is [editorial]. *Mayo Clin Proc.* 2003;78:1457-1459.

92. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med*. 1997;337:670-676.

93. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. *N Engl J Med.* 1992;327:1637-1642.

94. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. *BMJ.* 2003;326:469-475.

95. Larsen ER, Mosekilde L, Foldspang A. Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. *J Bone Miner Res.* 2004;19:370-378.

96. Vieth R. The pharmacology of vitamin D, including fortification strategies. In: Feldman D, ed. *Vitamin D*. Vol 2. 2nd ed. Amsterdam, Netherlands: Elsevier Academic Press; 2005:995-1015.

97. Lips P, Wiersinga A, van Ginkel FC, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab.* 1988;67:644-650.

98. Bischoff-Ferrari HA, Willet WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA.* 2005;293:2257-2264.

99. Cranney A, Guyatt G, Griffith L, Wells G, Tugwell P, Rosen C, Osteoporosis Methodology Group, Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis, IX: summary of meta-analyses of therapies for postmenopausal osteoporosis. *Endocr Rev.* 2002;23:570-578.

100. Papadimitropoulos E, Wells G, Shea B, et al, Osteoporosis Methodology Group, Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis, VIII: meta-analysis of the efficacy of vitamin D treatment in preventing osteoporosis in postmenopausal women. *Endocr Rev.* 2002;23:560-569.

101. Porthouse J, Cockayne S, King C, et al. Randomized controlled trial of calcium and supplementation with cholecalciferol (vitamin D_3) for prevention of fractures in primary care. *BMJ.* 2005;330:1003-1006.

102. Grant AM, Avenell A, Campbell MK, et al, RECORD Trial Group. Oral vitamin D_3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet.* 2005;365:1621-1628.

103. Anderson FH, Smith HE, Raphael HM, Crozier SR, Cooper C. Effect of annual intramuscular vitamin D supplementation on fracture risk in 9440 community-living older people: the Wessex Fracture Prevention Trial [abstract]. *J Bone Miner Res.* 2004;19(suppl 1):S57. Abstract 1220.

104. Cauley JA, Lui LY, Ensrud KE, et al. Bone mineral density and the risk of incident nonspinal fractures in black and white women. *JAMA.* 2005;293:2102-2108.

105. Khaw KT, Sneyd MJ, Compston J. Bone density parathyroid hormone and 25-hydroxyvitamin D concentrations in middle aged women. *BMJ.* 1992;305:273-277.

106. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ.* 1996;312:1254-1259.

107. Chapuy MC, Chapuy P, Thomas JL, Hazard MC, Meunier PJ. Biochemical effects of calcium and vitamin D supplementation in elderly, institutionalized, vitamin D-deficient patients. *Rev Rhum Engl Ed.* 1996;63:135-140.

108. Lips P, Graafmans WC, Ooms ME, Bezemer PD, Bouter LM. Vitamin D supplementation and fracture incidence in elderly persons: a randomized, placebo-controlled clinical trial. *Ann Intern Med.* 1996;124:400-406.

109. Dawson-Hughes B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Intern Med*. 1991;115:505-512.

110. Aloia JF, Talwar SA, Pollack S, Yeh J. A Randomized controlled trial of vitamin D_3 supplementation in African American women. *Arch Intern Med.* 2005;165:1618-1623.

111. Inanir A, Ozoran K, Tutkak H, Mermerci B. The effects of calcitriol therapy on serum interleukin-1, interleukin-6 and tumour necrosis factor-alpha concentrations in post-menopausal patients with osteoporosis. *J Int Med Res.* 2004;32:570-582.

112. Di Daniele N, Carbonelli MG, Candeloro N, Iacopino L, De Lorenzo A, Andreoli A. Effect of supplementation of calcium and vitamin D on bone mineral density and bone mineral content in peri- and post-menopause women; a double-blind, randomized, controlled trial. *Pharmacol Res.* 2004;50:637-641.

113. Sato Y, Asoh T, Kondo I, Satoh K. Vitamin D deficiency and risk of hip fractures among disabled elderly stroke patients. *Stroke.* 2001;32:1673-1677.

114. Gerdhem P, Ringsberg KA, Obrant KJ, Akesson K. Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. *Osteoporos Int.* 2005;16:1425-1431.

115. Cummings SR, Browner WS, Bauer D, et al, Study of Osteoporotic Fractures Research Group. Endogenous hormones and the risk of hip and vertebral fractures among older women. *N Engl J Med.* 1998;339:733-738.

116. Cumming RG, Cummings SR, Nevitt MC, et al. Calcium intake and fracture risk: results from the study of osteoporotic fractures. *Am J Epidemiol*. 1997;145:926-934.

117. Gallagher SJ, McQuillian C, Harkness M, Finlay F, Gallagher AP, Dixon T. Prevalence of vitamin D inadequacy in Scottish adults with non-vertebral fragility fractures. *Curr Med Res Opin*. 2005;21:1355-1361.

118. Dhesi JK, Jackson SH, Bearne LM, et al. Vitamin D supplementation improves neuromuscular function in older people who fall. *Age Ageing*. 2004; 33:589-595.

119. Dukas L, Schacht E, Mazor Z, Stahelin HB. Treatment with alfacalcidol in elderly people significantly decreases the high risk of falls associated with low creatinine clearance of <65 ml/min. *Osteoporos Int.* 2005;16:198-203.

120. Dukas L, Bischoff HA, Lindpainter LS, et al. Alfacalcidol reduces the number of fallers in a community-dwelling elderly population with a minimum calcium intake of more than 500 mg daily. *J Am Geriatr Soc.* 2004;52:230-236.

121. Gallagher JC. The effects of calcitriol on falls and fractures and physical performance tests. *J Steroid Biochem Mol Biol*. 2004;89-90:497-501.

122. van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab.* 2003;88:4623-4632.

123. Tangrea J, Helzlsouer K, Pietinen P, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. *Cancer Causes Control*. 1997;8:615-625.

124. Janowsky EC, Lester GE, Weinberg CR, et al. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutr*. 1999;2:283-291.

125. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control.* 2000;11:847-852.

126. Sambrook PN, Chen JS, March LM, et al. Serum parathyroid hormone predicts time to fall independent of vitamin D status in a frail elderly population. *J Clin Endocrinol Metab.* 2004;89:1572-1576.

127. Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocinol Metab.* 2003;88:5766-5772.

128. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). *Cancer Causes Control*. 1995;6:235-239.

129. van der Mei IA, Ponsonby AL, Dwyer T, et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. *BMJ*. 2003;327:316-317.

130. Stene LC, Joner G, Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr.* 2003;78:1128-1134.

131. Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of type I diabetes on the offspring [published correction appears in *Diabetologia*. 2000;43;1451]. *Diabetologia.* 2000;43:1093-1098.

132. 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;19:614-622.

133. Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. *Int J Epidemiol.* 1994;23:1133-1136.

134. Sowers MF, Wallace RB, Hollis BW, Lemke JH. Relationship between 1,25-dihydroxyvitamin D_3 and blood pressure in a geographically defined population. *Am J Clin Nutr.* 1988;48:1053-1056.

135. Glerup H, Mikkelsen K, Poulsen L, et al. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. *Calcif Tissue Int.* 2000;66:419-424.

136. Jorde R, Bonaa K. Calcium from dairy products, vitamin D intake, and blood pressure: the Tromso study. *Am J Clin Nutr.* 2000;71:1530-1535.

137. Dietrich T, Joshipura KJ, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D_3 and periodontal disease in the US population. *Am J Clin Nutr.* 2004;80:108-113.

138. Suda T, Abe E, Miyaura C, et al. Induction of differentiation of human myeloid leukemia cells by 1α,25-dihydroxyvitamin D_3. In: Norman AW, Schaefer K, Herrath DV, Grigdeit HG, eds. *Vitamin D, Chemical, Biochemical, and Clinical Endocrinology of Calcium Metabolism: Proceedings of the Fifth Workshop on Vitamin D, Williamsburg, VA, USA, February 1982.* Berlin, Germany: Walter de Gruyter; 1982:59–64.

139. Swamy N, Persons KS, Chen TC, Ray R. 1α,25-Dihydroxyvitamin D_3-3β-(2)-bromoacetate, an affinity labeling derivative of 1α,25-dihydroxyvitamin D_3 displays strong antiproliferative and cytotoxic behavior in prostate cancer cells. *J Cell Biochem.* 2003;89:909-916.

140. Niederhoffer N, Bobryshev YV, Lartaud-Idjouadiene I, Giummelly P, Atkinson J. Aortic calcification produced by vitamin D_3 plus nicotine. *J Vasc Res.* 1997;34:386-398.

141. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis: oral or topical use of 1,25-dihydroxyvitamin D_3 can be a safe and effective therapy for psoriasis. *J Am Acad Dermatol.* 1988;19:516-528.

142. Casteels K, Waer M, Bouillon R, et al. 1,25-Dihydroxyvitamin D_3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. *Clin Exp Immunol.* 1998; 112:181-187.

143. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged ≥60 y. *Am J Clin Nutr.* 2004;80:752-758.

144. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D_3. *Diabetologia.* 1994;37:552-558.

145. Zella JB, DeLuca HF. Vitamin D and autoimmune diabetes. *J Cell Biochem.* 2003;88:216-222.

146. Cantorna MT, Humpal-Winter J, DeLuca HF. In vivo upregulation of interleukin-4 is one mechanism underlying the immunoregulatory effects of 1,25-dihydroxyvitamin D_3. *Arch Biochem Biophys.* 2000;377:135-138.

147. 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.

148. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr.* 2000;130:2648-2652.

149. Bemiss CJ, Mahon BD, Henry A, Weaver V, Cantorna MT. Interleukin-2 is one of the targets of 1,25-dihydroxyvitamin D_3 in the immune system. *Arch Biochem Biophys*. 2002;402:249-254.

150. Mawer EB, Hayes ME, Heys SE, et al. Constitutive synthesis of 1,25-dihydroxyvitamin D_3 by a human small cell lung cancer cell line. *J Clin Endocrinol Metab.* 1994;79:554-560.

151. Chen TC, Holick MF, Lokeshwar BL, Burnstein KL, Schwartz GG. Evaluation of vitamin D analogs as therapeutic agents for prostate cancer. *Recent Results Cancer Res.* 2003;164:273-288.

152. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D_3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol.* 2005;35:217-224.

153. Abe J, Nakamura K, Takita Y, Nakano T, Irie H, Nishii Y. Prevention of immunological disorders in MRL/1 mice by a new synthetic analogue of vitamin D_3: 22-oxa-1α,25-dihydroxyvitamin D_3. *J Nutr Sci Vitaminol (Tokyo).* 1990;36:21-31.

154. Raisanen-Sokolowski AK, Pakkala IS, Samila SP, Binderup L, Hayry PJ, Pakkala ST. A vitamin D analog, MC1288, inhibits adventitial inflammation and suppresses intimal lesions in rat aortic allografts. *Transplantation.* 1997;63:936-941.

155. Hullett DA, Cantorna MT, Redaelli C, et al. Prolongation of allograft survival by 1,25-dihydroxyvitamin D_3. *Transplantation.* 1998;66:824-828.

156. Dalhoff K, Dancey J, Astrup L, et al. A phase II study of the vitamin D analogue seocalcitol in patients with inoperable hepatocellular carcinoma. *Br J Cancer.* 2003;89:252-257.

157. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1α,25-Dihydroxyvitamin D_3 inhibits angiogenesis in vitro and in vivo. *Circ Res.* 2000;87:214-220.

158. Ylikomi T, Laaksi I, Lou YR, et al. Antiproliferative action of vitamin D. *Vitam Horm.* 2002;64:357-406.

159. Tanaka H, Abe E, Miyaura C, et al. 1 alpha,25-Dihydroxycholecalciferol and a human myeloid leukaemia cell line (HL-60). *Biochem J*. 1982;204: 713-719.

160. Koeffler HP, Hirjik J, Itri L. 1,25-Dihydroxyvitamin D_3: in vivo and in vitro effects on human preleukemic and leukemic cells. *Cancer Treat Rep.* 1985;69:1399-1407.

161. Zhao XY, Feldman D. The role of vitamin D in prostate cancer. *Steroids*. 2001;66:293-300.

162. Polek TC, Weigel NL. Vitamin D and prostate cancer. *J Androl.* 2002; 23:9-17.

163. Apperly FL. The relation of solar radiation to cancer mortality in North America. *Cancer Res.* 1941;1:191-195.

164. Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Rossof AH, Paul O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet*. 1985;1:307-309.

165. Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. *Occup Environ Med.* 2002;59:257-262.

166. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer.* 2002;94:1867-1875.

167. Simpson RU, Thomas GA, Arnold AJ. Identification of 1,25-dihydroxyvitamin D_3 receptors and activities in muscle. *J Biol Chem.* 1985;260: 8882-8891.

168. Costa EM, Blau HM, Feldman D. 1,25-dihydroxyvitamin D_3 receptors and hormonal responses in cloned human skeletal muscle cells. *Endocrinology*. 1986;119:2214-2220.

169. Haddad JG, Walgate J, Min C, Hahn TJ. Vitamin D metabolite-binding proteins in human tissue. *Biochim Biophys Acta.* 1976;444:921-925.

170. Schott GD, Wills MR. Muscle weakness in osteomalacia. *Lancet.* 1976; 1:626-629.

171. Boland R. Role of vitamin D in skeletal muscle function. *Endocr Rev*. 1986;7:434-448.

172. Pfeifer M, Begerow B, Minne HW, et al. Vitamin D status, trunk muscle strength, body sway, falls, and fractures among 237 postmenopausal women with osteoporosis. *Exp Clin Endocrinol Diabetes*. 2001;109:87-92.

173. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, et al. Effect of vitamin D on falls: a meta-analysis. *JAMA.* 2004;291:1999-2006.

174. Bischoff HA, Stahelin HB, 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.

175. Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. Sarcopenia. *J Lab Clin Med.* 2001;137:231-243.

176. Holick MF. Vitamin D: a millenium perspective. *J Cell Biochem.* 2003; 88:296-307.

177. DeLuca H. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* 2004;80(6, suppl):1689S-1696S.

178. Stumpf WE, Sar M, Reid FA, Tanaka Y, DeLuca HF. Target cells for 1,25-dihydroxyvitamin D_3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. *Science*. 1979;206:1188-1190.

179. Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF. Human prostate cells synthesize 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3. *Cancer Epidemiol Biomarkers Prev.* 1998;7:391-395.

180. Cross HS, Bareis P, Hofer H, et al. 25-Hydroxyvitamin D_3-1α-hydroxlyase and vitamin D receptor gene expression in human colonic mucosa is elevated during early cancerogenesis. *Steroids.* 2001;66:287-292.

181. Tangpricha V, Flanagan JN, Whitlatch LW, et al. 25-Hydroxyvitamin D-1α-hydroxylase in normal and malignant colon tissue. *Lancet* . 2001;357:1673-1674.

182. Chen TC, Holick MF. Vitamin D and prostate cancer prevention and treatment. *Trends Endocrinol Metab.* 2003;14:423-430.

183. Rachez C, Freedman LP. Mechanisms of gene regulation by vitamin D_3 receptor: a network of coactivator interactions. *Gene.* 2000;246:9-21.

184. Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol.* 2004;89-90:187-193.
185. Slattery ML, Neuhausen SL, Hoffman M, et al. Dietary calcium, vitamin D, VDR genotypes and colorectal cancer [published correction appears in *Int J Cancer*. 2004;111:983]. *Int J Cancer*. 2004;111:750-756.
186. Pani MA, Knapp M, Donner H et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes*. 2000;49:504-507.
187. McDermott MF, Ramachandran A, Ogunkolade BW, et al. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians *Diabetologia.* 1997;40:971-975.
188. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev.* 2005;26:662-687.
189. Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annu Rev Nutr.* 2002;22:139-166.
190. Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones—a focus on rapid, nongenomic effects. *Pharmacol Rev.* 2000;52:513-556.
191. Norman AW, Bishop JE, Bula CM, et al. Molecular tools for study of genomic and rapid signal transduction responses initiated by $1\alpha,25(OH)_2$-vitamin D_3. *Steroids*. 2002;67:457-466.
192. Harris SS. Vitamin D and type 1 diabetes [letter]. *Am J Clin Nutr.* 2004; 79;889-890.
193. Holick MF. Clinical efficacy of 1,25-dihydroxyvitamin D_3 and its analogues in the treatment of psoriasis. *Retinoids* 1998;14:12-17.
194. Bouillon R, Okamura WH, Norman AW. Structure-function relationships in the vitamin D endocrine system. *Endocr Rev.* 1995;16:200-257.
195. Spina C, Tangpricha V, Yao M, et al. Colon cancer and solar ultraviolet B radiation and prevention and treatment of colon cancer in mice with vitamin D and its Gemini analogs. *J Steroid Biochem Mol Biol.* 2005;97:111-120.
196. Guenther L, Cambazard F, Van De Kerkhof PCM, et al. Efficacy and safety of a new combination of calcipotriol and betamethasone dipropionate (once or twice daily) compared to calcipotriol (twice daily) in the treatment of psoriasis vulgaris: a randomized, double-blind, vehicle-controlled clinical trial. *Br J Dermatol*. 2002;147:316-323.
197. Holick MF. Evolution and function of vitamin D. *Recent Results Cancer Res.* 2003;164:3-28.
198. van den Bemd GJ, Chang GT. Vitamin D and vitamin D analogs in cancer treatment. *Curr Drug Targets.* 2002;3:85-94.
199. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D_3 and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab.* 2001; 86:1633-1637.
200. Mahon BD, Gordon SA, Cruz J, Cosman F, Cantorna MT. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *J Neuroimmunol.* 2003;134:128-132.
201. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure [letter]. *Lancet.* 1998;352:709-710.
202. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet.* 1989;2:1176-1178.
203. Martinez ME, Giovannucci EL, Colditz GA, et al. Calcium, vitamin D, and the occurrence of colorectal cancer among women. *J Natl Cancer Inst*. 1996;88:1375-1382.
204. John EM, Schwartz GG, Dreon DM, Koo J. Vitamin D and breast cancer risk: the NHANES I epidemiologic follow-up study, 1971-1975 to 1992: National Health and Nutrition Examination Survey. *Cancer Epidemiol Biomarkers Prev.* 1999;8:399-406.
205. Hernan MA, Olek MJ, Ascherio A. Geographic variation of MS incidence in two prospective studies of US women. *Neurology.* 1999;53:1711-1718.
206. Bostick RM, Kushi LH, Wu Y, Meyer KA, Sellers TA, Folsom AR. Relation of calcium, vitamin D, and dietary food intake to ischemic heart disease mortality among postmenopausal women. *Am J Epidemiol.* 1999;149: 151-161.
207. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet.* 2001; 358:1500-1503.
208. Merlino LA, Curtis J, Mikuls TR, et al. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Womens's Health Study. *Arthritis Rheum.* 2004;50:72-77.
209. 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: Holick MF, ed. *Biologic Effects of Light 2001: Proceedings of a Symposium, Boston, Massachusetts.* Boston, Mass: Kluwer Academic Publishing; 2002:267-276.
210. Luscombe CJ, Fryer AA, French ME, et al. Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. *Lancet* . 2001;358:641-642.
211. Grant WB. A multicountry ecologic study of risk and risk reduction factors for prostate cancer mortality. *Eur Urol.* 2004;45:271-279.
212. Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2005;14:1991-1997.
213. Whitlatch LW, Young MV, Schwartz GG, et al. 25-Hydroxyvitamin D-1α-hydroxylase activity is diminished in human prostate cancer cells and is enhanced by gene transfer. *J Steroid Biochem Mol Biol.* 2002;81:135-140.
214. Rostand SG. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension.* 1997;30(2, pt 1):150-156.
215. Li YC. Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem.* 2003;88:327-331.
216. Fahrleitner A, Dobnig H, Obernosterer A, et al. Vitamin D deficiency and secondary hyperparathyroidism are common complications in patients with peripheral arterial disease. *J Gen Intern Med.* 2002;17:663-669.
217. Sepulveda JL, Mehta JL. C-reactive protein and cardiovascular disease: a critical appraisal. *Curr Opin Cardiol.* 2005;20:407-416.
218. McCarty MF. Secondary hyperparathyroidism promotes the acute phase response—a rationale for supplemental vitamin D in prevention of vascular events in the elderly. *Med Hypotheses.* 2005;64:1022-1026.
219. Lindén V. Vitamin D and myocardial infarction. *BMJ.* 1974;3:647-650.
220. Schmidt-Gayk H, Goossen J, Lendle F, Seidel D. Serum 25-hydroxy-cholecalciferol in myocardial infarction. *Atherosclerosis.* 1977;26:55-58.
221. Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Körfe R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? *J Am Coll Cardiol.* 2003;41:105-112.
222. MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF. Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D_3. *Proc Natl Acad Sci U S A.* 1985;82:5409-5412.
223. Perez A, Chen TC, Turner A, et al. Efficacy and safety of topical calcitriol (1,25-dihydroxyvitamin D_3) for the treatment of psoriasis. *Br J Dermatol.* 1996;134:238-246.
224. Leibowitz U, Sharon D, Alter M. Geographical considerations in multiple sclerosis. *Brain.* 1967;90:871-886.
225. Ponsonby AL, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology.* 2002;181-182:71-78.
226. Embry AF, Snowdon LR, Vieth R. Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis [letter]. *Ann Neurol.* 2000;48:271-272.
227. Carrieri PB, Provitera V, De Rosa T, Tartaglia G, Gorga F, Perrella O. Profile of cerebrospinal fluid and serum cytokines in patients with relapsing-remitting sclerosis: a correlation with clinical activity. *Immunopharmacol Immunotoxicol.* 1998;20:373-382.
228. Losy J, Michalowska-Wender G. In vivo effect of interferon-beta 1a on interleukin-12 and TGF-beta(1) cytokines in patients with relapsing-remitting multiple sclerosis. *Acta Neurol Scand.* 2002;106:44-46.
229. Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology.* 2004;62:60-65.
230. Lee S, Clark SA, Gill RK, Christakos S. 1,25-Dihydroxyvitamin D_3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology*. 1994;134:1602-1610.
231. Mathieu C, Badenhoop K. Vitamin D and type 1 diabetes mellitus: state of the art. *Trends Endocrinol Metab.* 2005;16:261-266.
232. McAlindon TE, Felson DT, Zhang Y, et al. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Ann Intern Med.* 1996;125:353-359.
233. Krall EA, Wehler C, Garcia RI, Harris SS, Dawson-Hughes B. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med.* 2001; 111:452-456.
234. Gysemans C, Waer M, Laureys J, Bouillon R, Mathieu C. A combination of KH1060, a vitamin D_3 analog, and cyclosporin prevents early graft failure and prolongs graft survival of xenogenic islets in nonobese diabetic mice. *Transplant Proc.* 2001;33:2365.

235. Sambrook P. Alfacalcidiol and calcitriol in the prevention of bone loss after organ transplantation. *Calcif Tissue Int.* 1999;65:341-343.

236. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board Institute of Medicine. Vitamin D. In: *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press; 1997:250-287.

237. 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;13:1238-1242.

238. Tangpricha V, Turner A, Spina C, Decastro S, Chen TC, Holick MF. Tanning is associated with optimal vitamin D status (serum 25-hydroxyvitamin D concentration) and higher bone mineral density. *Am J Clin Nutr.* 2004;80:1645-1649.

239. Chuck A, Todd J, Diffey B. Subliminal ultraviolet-B irradiation for the prevention of vitamin D deficiency in the elderly: a feasibility study. *Photodermatol Photoimmunol Photomed.* 2001;17:168-171.

240. Shao Q, Chen TC, Holick MF. Sun-tanning bed radiation increases vitamin D synthesis in human skin in vivo. In: Holick MF, Kligman A, eds. *Biological Effects of Light*. Berlin, Germany: Walter De Gruyter; 1992:62-66.

241. Scientific Committee on Food. Opinion on the tolerable upper intake level of vitamin D. December 4, 2002. Available at: www.imace.org/nutrition/pdf/poster.pdf. Accessibility verified January 31, 2006.

242. United States Food and Drug Administration Web site. Reference daily intakes, recommended dietary allowances. Available at: www.fda.gov/fdac. Accessibility verified February 13, 2006.

243. Vieth R. Why the optimal requirement for vitamin D_3 is probably much higher than what is officially recommended for adults. *J Steroid Biochem Mol Biol.* 2004;89-90:575-579.

244. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr.* 2005;135:317-322.

245. Jacobus CH, Holick MF, Shao Q, et al. Hypervitaminosis D associated with drinking milk. *N Engl J Med* . 1992;326:1173-1177.

246. Koutkia P, Chen TC, Holick MF. Vitamin D intoxication with an over-the-counter supplement [letter]. *N Engl J Med.* 2001;345:66-67.

247. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D_3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr.* 2001;73:288-294.

248. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol [published correction appears in *Am J Clin Nutr*. 2003;78:1047]. *Am J Clin Nutr.* 2003;77:204-210.

249. Matuulla-Nolte B, Krause B, Schmidt-Gayk H. Serial UVB irradiation can influence secondary hyperparathyroidism in vitamin D deficiency. In: Holick MF, Jung EG, eds. *Biologic Effects of Light: Proceedings of a Symposium, Basel, Switzerland, November 1-3, 1998*. Boston, Mass: Kluwer Academic Publishers; 1999;121-123.

250. Holick MF. The vitamin D epidemic and its health consequences. *J Nutr* 2005;135:2739S-2748S.

251. Tylavsky FA, Ryder KA, Lyytikäinen A, Cheng S. Vitamin D, parathyroid hormone, and bone mass in adolescents. *J Nutr.* 2005;135:2735S-2738S.

252. Cooper C, Javaid K, Westlake S, Harvey N, Dennison E. Developmental origins of osteoporotic fracture: the role of maternal vitamin D insufficiency. *J Nutr.* 2005;135:2728S-2734S.

253. Cantorna MT, Hullet DA, Redaelli C, et al. 1,25-Dihydroxyvitamin D_3 prolongs graft survival without compromising host resistance to infection or bone mineral density. *Transplantation.* 1998;66:828-831.

254. Devereaux PJ, Yusuf S. The evolution of the randomized controlled trial and its role in evidence-based decision making. *J Intern Med.* 2003;254:105-113.

255. McAlister FA, Laupacis A, Wells GA, Sackett DL, Evidence-Based Medicine Working Group. Users' guides to the medical literature, XIX: applying clinical trial results: B. guidelines for determining whether a drug is exerting (more than) a class effect. *JAMA*. 1999;282:1371-1377.

256. Rossouw JE, Anderson GL, Prentice RL, et al, Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288:321-333.

257. Anderson GL, Limacher M, Assaf AR, et al, Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *JAMA*. 2004;291:1701-1712.

258. Gilchrest BA. Sunscreens—a public health opportunity [editorial]. *N Engl J Med.* 1993;329:1193-1194.

259. Kennedy C, Bajdik CD, Willemze R, de Gruijl 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;120:1087-1093.

260. Gupta PB, Kuperwasser C, Brunet J, et al. The melanocytes differentiation program predisposes to metastasis after neoplastic transformation. *Nat Genet.* 2005;37:1047-1054.

261. Garland FC, White MR, Garland CF, Shaw E, Gorham ED. Occupational sunlight exposure and melanoma in the U.S. Navy. *Arch Environ Health*. 1990;45:261-267.

262. Garland CF, Garland FC, Gorham ED. Lack of efficacy of common sunscreens in melanoma prevention. In: Grob JJ, Stern RS, MacKie RM, Weinstock WA, eds. *Epidemiology, Causes, and Prevention of Skin Diseases*. Oxford, England: Blackwell Science; 1999:151-158.

263. Berwick M, Armstrong BK, Ben-Porat L, et al. Sun exposure and mortality from melanoma. *J Natl Cancer Inst.* 2005;97:195-199.

264. Chang ET, Smedby KE, Hjalgrim H, et al. Family history of hematopoietic malignancy and risk of lymphoma*. J Natl Cancer Inst.* 2005;97:1466-1474.