

The Nonskeletal Effects of Vitamin D: An Endocrine Society Scientific Statement

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Significant controversy has emerged over the last decade concerning the effects of vitamin D on skeletal and nonskeletal tissues. The demonstration that the vitamin D receptor is expressed in virtually all cells of the body and the growing body of observational data supporting a relationship of serum 25-hydroxyvitamin D to chronic metabolic, cardiovascular, and neoplastic diseases have led to widespread utilization of vitamin D supplementation for the prevention and treatment of numerous disorders. In this paper, we review both the basic and clinical aspects of vitamin D in relation to nonskeletal organ systems. We begin by focusing on the molecular aspects of vitamin D, primarily by examining the structure and function of the vitamin D receptor. This is followed by a systematic review according to tissue type of the inherent biological plausibility, the strength of the observational data, and the levels of evidence that support or refute an association between vitamin D levels or supplementation and maternal/child health as well as various disease states. Although observational studies support a strong case for an association between vitamin D and musculoskeletal, cardiovascular, neoplastic, and metabolic disorders, there remains a paucity of large-scale and long-term randomized clinical trials. Thus, at this time, more studies are needed to definitively conclude that vitamin D can offer preventive and therapeutic benefits across a wide range of physiological states and chronic nonskeletal disorders. (*Endocrine Reviews* 33: 456–492, 2012)

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Abbreviations: BMI, Body mass index; CI, confidence interval; CV, cardiovascular; CVD, CV disease; 1,25D-MARRSBP, 1,25-(OH)₂D membrane-associated rapid response steroid-binding protein; DRIP, VDR-interacting protein; HAT, histone acetyl transferase; HR, hazard ratio; IFN- γ , interferon- γ ; LBD, ligand-binding domain; LEF1, lymphoid enhancer-binding factor-1; LPS, lipopolysaccharide; mTB, *Mycobacterium tuberculosis*; NR, nuclear receptor; NOD, nonobese diabetic; 1,25-(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; PRR, pattern recognition receptor; RCT, randomized clinical trial; RR, relative risk; RXR, retinoid X receptor; SRC, steroid receptor coactivator; TLR, Toll-like receptor; UCP, uncoupling protein; VDR, vitamin D receptor; VDRE, vitamin D response element.

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I. Introduction

The 100th anniversary of the identification of a factor whose deficiency was linked to the development of rickets, and was later found to be cholecalciferol, is approaching. Painstaking work by a number of laboratories over the last nine decades convincingly demonstrated that cholecalciferol not only was essential for skeletal health but also was a hormone mediating nonclassical tissue effects across a wide range of homeostatic functions. The physiology of vitamin D from its synthesis in the skin to its active form, 1,25-dihydroxyvitamin D [1,25-(OH)₂D], was fully defined by the mid-1970s (Fig. 1, A and B). However, the cloning of the vitamin D receptor (VDR) did not occur until 1987, and its subsequent identification in virtually all tissues spurred further basic and clinical studies and led to a much greater appreciation of the physiological role of vitamin D (Fig. 2). At the same time, interest in vitamin D as a therapeutic modality for the prevention of chronic diseases grew exponentially. Indeed, in a 2-month span during the summer of 2011, there were more than 500 publications centered on vitamin D, most of which were related to its relationship to nonskeletal tissues. However, the results from those studies, as well as others, are confounded and difficult to interpret. In this Scientific Statement we seek to outline the evidence that defines the effects of vitamin D on epidermal, neuromuscular, cardiovascular (CV), metabolic, immunological, maternal/fetal, and neoplastic tissues. Before reviewing the evidence in these areas, we first present an overview of the VDR

because this molecule represents the final common pathway through which vitamin D works on nonskeletal tissues. We next critically evaluate the literature for each organ system, beginning with the biological plausibility of an association, followed by utilization of the available evidence from observational studies and randomized trials, to delineate the strength of associations between serum 25-hydroxyvitamin D [25(OH)D] and/or dose of vitamin D supplementation and tissue-specific outcomes.

Several reviews of the skeletal effects of vitamin D have recently been published, including The Endocrine Society's Clinical Practice Guideline on vitamin D deficiency and the complete Institute of Medicine (IOM) Report on Calcium and Vitamin D (1–3). It is important to note that after the publication of these two summaries, our work took on additional significance, particularly in relation to nonskeletal effects of vitamin D, as the controversy surrounding the definition of a target serum level of 25(OH)D reached new heights. Importantly, this Scientific Statement represents the first comprehensive evaluation of both the basic and clinical evidence related to the effects of vitamin D on nonskeletal tissues.

II. Distribution, Structure, and Function of the Vitamin D Receptor

A. Background

Vitamin D is a steroid hormone, and the active metabolite, 1,25-(OH)₂D, is the ligand for a transcription factor and intracellular receptor called the “vitamin D receptor.” The VDR is widely distributed across many tissues. Indeed, cells lacking the VDR are the exception rather than the rule, and this widespread distribution underlies the potential myriad of physiological actions for vitamin D. Not surprisingly, most if not all effects of 1,25-(OH)₂D are mediated by the VDR acting primarily by regulating the expression of genes whose promoters contain specific DNA sequences known as vitamin D response elements (VDRE). The VDR works in partnership with other transcription factors, the best-studied of which is the retinoid X receptor (RXR), and a number of coactivators and corepressors that provide context, tissue, and target gene specificity. However, some actions of 1,25-(OH)₂D are more immediate and may be mediated by a membrane-bound VDR that has been less well characterized than the nuclear VDR. Our understanding of the mechanism by which VDR regulates gene expression has increased enormously over the past few years.

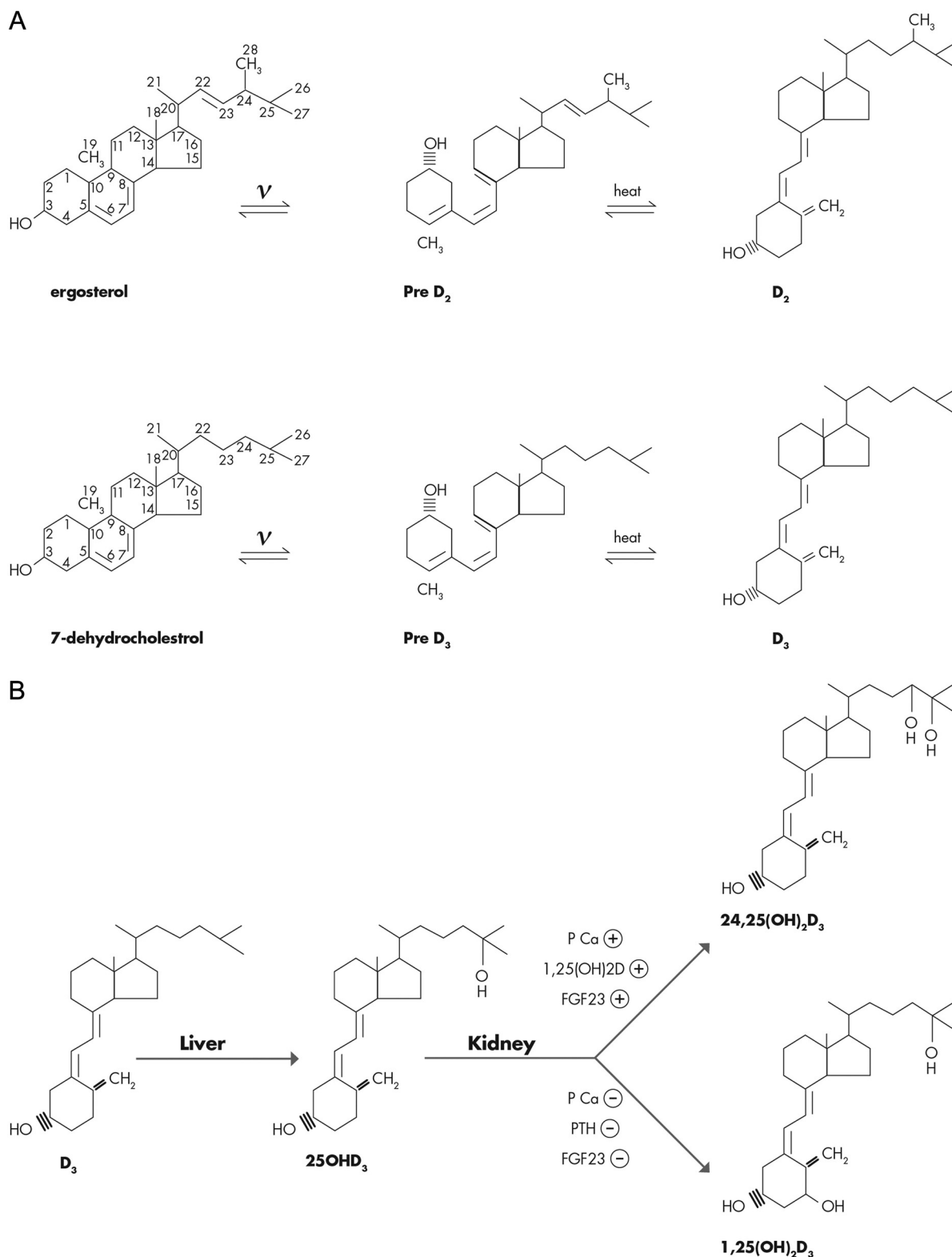
Figure 1.

Figure 1. A, Production of vitamin D from the skin via ultraviolet radiation (290–330 nm) in a nonenzymatic manner. B, The synthesis of vitamin D metabolites including the inactive form, 24,25-dihydroxyvitamin D, and the active form, 1,25-(OH)₂D. This process is controlled at several levels, including the liver, kidney, and peripheral tissues, and is regulated by systemic hormones including PTH, 1,25-(OH)₂D, and FGF23. Calcium and phosphorus are also major modulators of 1 α -hydroxylase and 24,25-hydroxylase activity through their effects on PTH and FGF23. FGF 23, Fibroblast growth factor 23.

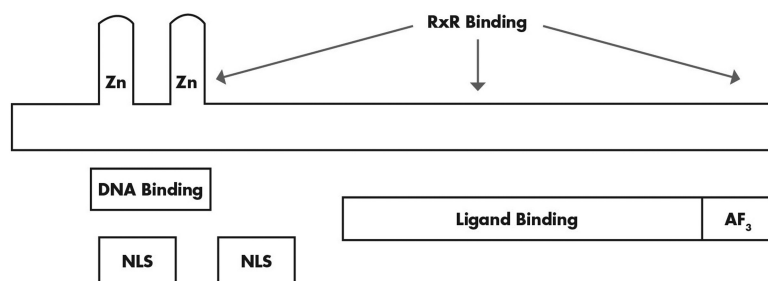
Figure 2.

Figure 2. Model of the VDR. The N-terminal region is short relative to other steroid hormone receptors. This region is followed by two zinc fingers, which constitute the principal DNA-binding domain. NLS are found within and just C-terminal to the DNA-binding domain. The LBD makes up the bulk of the C-terminal half of the molecule, with the AF-2 domain occupying the most C-terminal region. The AF-2 domain is largely responsible for binding to coactivators such as the SRC family and DRIP in the presence of ligand. Regions on the second zinc finger and within the LBD facilitate heterodimerization with RXR. Corepressor binding is less well characterized but appears to overlap that of coactivators in helices 3 and 5, a region blocked by helix 12 in the presence of ligand. NLS, Nuclear localization signals.

B. VDR distribution

The VDR was discovered in 1969 [although only as a binding protein for an as-yet unknown vitamin D metabolite subsequently identified as 1,25-(OH)₂D]; this binding protein was eventually cloned and sequenced in 1987 (4–6). It is a member of a large family of proteins (more than 150 members) that includes receptors for the steroid hormones, T₄, the vitamin A family of metabolites (retinoids), and a variety of cholesterol metabolites, bile acids, isoprenoids, fatty acids, and eicosanoids. A large number of family members have no known ligands and are called orphan receptors.

VDR is widely, although not universally, distributed throughout different tissues of the body (7). Many of these tissues were not originally considered targets for 1,25-(OH)₂D. The discovery of VDR in many cell types along with the demonstration that 1,25-(OH)₂D altered the function of these tissues has markedly increased our appreciation of the protean effects of 1,25-(OH)₂D. Inactivating mutations in the hereditary vitamin D resistant rickets result in hereditary vitamin D resistant rickets (8). An animal model in which the VDR has been deleted has the full phenotype of severe vitamin D deficiency, indicating that VDR is the major mediator of vitamin D action (9). The one major difference is the alopecia seen in hereditary vitamin D-resistant rickets and VDR knockout animals, a feature not associated with vitamin D deficiency, suggesting that the VDR may have functions independent of 1,25-(OH)₂D, at least in hair follicle cycling.

C. VDR structure

The VDR is a molecule of approximately 50–60 kDa, depending on species. The basic structure is shown in Fig. 2.

The VDR is unusual in that it has a very short N-terminal domain before the DNA-binding domain when compared with other nuclear hormone receptors. The human VDR has two potential start sites. A common polymorphism (Fok 1) alters the first ATG start site to ACG. Individuals with this polymorphism begin translation three codons downstream such that in these individuals the VDR is three amino acids shorter (424 aas *vs.* 427 aas). This polymorphism has been correlated with reduced bone mineral density in some studies, whereas other genome-wide association studies have not found a strong signal for polymorphisms in the VDR gene and bone mass or fractures (10). The most conserved domain in VDR from different species and among the nuclear hormone receptors

in general is the DNA-binding domain. This domain comprises two zinc fingers. The name derives from the cysteines within this stretch of amino acids that form tetrahedral complexes with zinc in a manner that creates a loop or finger of amino acids with the zinc complex at its base. The proximal (N-terminal) zinc finger confers specificity for DNA binding to the VDRE, whereas the second zinc finger and the region following provide at least one of the sites for heterodimerization of the VDR to the RXR. The second half of the molecule is the ligand-binding domain (LBD), the region responsible for binding 1,25-(OH)₂D, but that also contains regions necessary for heterodimerization to RXR. At the C-terminal end is the major activation domain, AF-2, which is critical for the binding to coactivators such as those in the steroid receptor coactivator (SRC) and VDR-interacting protein (DRIP, also known as Mediator) families (11). In mutation studies of the homologous thyroid receptor, corepressors were found to bind in overlapping regions with coactivators in helices 3 and 5, a region blocked by helix 12 (the terminal portion of the AF-2 domain) in the presence of ligand (12). Deletion of helix 12 promoted corepressor binding while preventing that of coactivators (12).

The LBD for VDR has been crystallized, and its structure solved (13). It shows a high degree of structural homology to other nuclear hormone receptors. It comprises 12 helices joined primarily by β -sheets. The ligand 1,25-(OH)₂D is buried deep in the ligand-binding pocket and covered with helix 12 (the terminal portion of the AF-2 domain). Assuming analogy with the unliganded LBD of RXR α and the ligand-bound LBD of RAR γ , the binding of 1,25-(OH)₂D to the VDR triggers a substantial movement

of helix 12 from an open position to a closed position, covering the ligand-binding pocket and putting helix 12 in position with critical residues from helices 3, 4, and 5 to bind coactivators (14). Some coactivator complexes such as DRIP bridge the gap from the VDRE to the transcription machinery at the transcription start site. Other coactivator complexes with histone acetyl transferase (HAT) activity such as the SRC family facilitate the opening of the chromatin structure, allowing transcription to occur. Although these two coactivator complexes are essential for VDR function, their interaction with each other remains unclear (11). Both will be discussed further in *Sections II.D. and II.E.*

D. Role of coactivators and corepressors

Nuclear hormone receptors including the VDR are further regulated by protein complexes that can be activators or repressors (15, 16). The role of corepressors in VDR function has been demonstrated but is less well studied than the role of coactivators (17). One such corepressor, hairless, is found in the skin and may regulate 1,25-(OH)₂D-mediated epidermal proliferation and differentiation as well as 1,25-(OH)₂D-independent VDR regulation of hair follicle cycling (18–20). The SRC family of coactivators has three members, SRC 1–3, all of which can bind to the VDR in the presence of ligand [1,25-(OH)₂D] (21). These coactivators recruit additional coactivators such as CBP/p300 and p/CAF that have HAT, an enzyme that appears to help unravel the chromatin allowing the transcriptional machinery to do its job. The domain in these molecules critical for binding to the VDR and other nuclear hormone receptors is called the nuclear receptor (NR) box. The NR box harbors a central LxxLL motif where L stands for leucine and x for any amino acid. Each SRC family member contains three well-conserved NR boxes in the region critical for nuclear hormone receptor binding. The DRIP complex of coactivators comprises 15 or so proteins, several of which contain LxxLL motifs (22). However, DRIP205 is the protein critical for binding the complex to VDR. It contains two NR boxes. Different NR boxes in these coactivators show specificity for different nuclear hormone receptors (23). Unlike the SRC complex, the DRIP complex does not have HAT activity (11). Rather the DRIP complex spans the gene from the VDRE to the transcription start site linking directly with RNA polymerase II and its associated transcription factors. DRIP and SRC appear to compete for binding to the VDR. In keratinocytes, DRIP binds preferentially to the VDR in undifferentiated cells, whereas SRC2 and SRC3 bind in the more differentiated cells in which DRIP levels have declined (24). Thus, in these cells DRIP may regulate the early stages of 1,25-(OH)₂D-induced differ-

entiation, whereas SRC may be more important in the later stages, although overlap in gene specificity is also observed (25–27). SMAD3, a transcription factor in the TGF- β pathway, has been found to complex with the SRC family members and the VDR, enhancing the coactivation process (28). Phosphorylation of the VDR may also control VDR function (29). Furthermore, VDR has been shown to suppress β -catenin transcriptional activity (30), whereas β -catenin enhances that of VDR (31). Thus, control of VDR activity may involve crosstalk between signaling pathways originating in receptors at the plasma membrane and in the nucleus.

E. Plasticity of the VDRE

VDR acts in concert with other nuclear hormone receptors, in particular RXR (32). Unlike VDR, RXR has three forms— α , β , and γ —and all three are capable of binding to VDR with no obvious differences in terms of functional effect. RXR and VDR form heterodimers that optimize their affinity for the VDRE in the promoters of the genes being regulated. RXR appears to be responsible for keeping VDR in the nucleus in the absence of ligand (33). VDR may also partner with other receptors including the thyroid receptor and the retinoic acid receptor (34, 35), but these are the exceptions, whereas RXR is the rule. The VDR/RXR heterodimers bind to VDRE, which typically comprise two half sites, each with six nucleotides separated by three nucleotides of nonspecific type; this type of VDRE is known as a DR3 (direct repeats with three nucleotide spacing). RXR binds to the upstream half site, whereas VDR binds to the downstream site (36). However, a wide range of VDRE configurations have been found (31). 1,25-(OH)₂D is required for high-affinity binding and activation, but the RXR ligand, 9-cis retinoic acid, may either inhibit (37) or activate (38) 1,25-(OH)₂D stimulation of gene transcription. A DR6 has been identified in the phospholipase C- γ 1 gene that recognizes VDR/retinoic acid receptor heterodimers, and a DR4 has been found in the mouse calbindin 28k gene (34, 39). Inverted palindromes with seven to 12 bases between half sites have also been found (31). Furthermore, the half sites of the various known VDRE show remarkable degeneracy. The G in the second position of each site appears to be the only nearly invariant nucleotide. 1,25-(OH)₂D can also inhibit gene transcription through its VDR. This may occur by direct binding of the VDR to negative VDRE that in the PTH and PTHrP genes are remarkably similar in sequence to positive VDRE of other genes (40, 41). However, inhibition may also be indirect. For example, 1,25-(OH)₂D inhibits IL-2 production by blocking the NFATp/AP-1 complex of transcription factors from activating this gene (42) through a mechanism not yet clear. Similarly,

1,25-(OH)₂D inhibits CYP27B1 in some cells by an indirect mechanism (43). In the latter case, this has been shown to involve the role of two DNA methyl-transferases in the inhibitory complex that in the presence of 1,25-(OH)₂D serve to methylate CpG sites in the CYP27B1 promoter (44). Thus, a variety of factors including the flanking sequences of the genes around the VDRE and tissue-specific factors play a large role in dictating the ability of 1,25-(OH)₂D to regulate gene expression.

F. Nongenomic actions of vitamin D

A variety of hormones that serve as ligands for nuclear hormone receptors also exert biological effects that do not appear to require gene regulation and may work through membrane receptors or the VDR situated outside of the nucleus, rather than their cognate nuclear hormone receptors. Examples include estrogen, progesterone, testosterone, corticosteroids, and thyroid hormone (45–49). 1,25-(OH)₂D has also been shown to have rapid effects on selected cells that are not likely to involve gene regulation and that appear to be mediated by a distinct receptor which is likely on the membrane receptor. Similar to other steroid hormones, 1,25-(OH)₂D has been shown to regulate calcium and chloride channel activity, protein kinase C activation and distribution, and phospholipase C activity in a number of cells including osteoblasts, liver, muscle, and intestine (50–54). A putative membrane receptor for 1,25-(OH)₂D—*i.e.*, 1,25-(OH)₂D membrane-associated rapid response steroid-binding protein (1,25D-MARRSBP), also known as endoplasmic reticulum stress protein 57—has been purified from the intestine, cloned, and sequenced, and blocking antibodies have been prepared that block the rapid actions of 1,25-(OH)₂D (55–58). More recently, a mouse null for 1,25D-MARRSBP in the intestine has been developed and shown to lack the rapid response of intestinal cells to 1,25-(OH)₂D (59). However, these rapid actions of 1,25-(OH)₂D appear to require the VDR (ineffective in VDR null mice), which suggests that 1,25D-MARRSBP and VDR cooperate in mediating these acute actions of 1,25-(OH)₂D but without the need for new protein synthesis. In the latter case, analogs of 1,25-(OH)₂D that do not support genomic actions of 1,25-(OH)₂D do support these nongenomic actions, which suggests that the membrane VDR may have a different three-dimensional structure with a different binding pocket for its activating ligands.

III. Vitamin D and the Skin

A. Introduction

The skin is unique in that it is the only organ system identified thus far that is able to synthesize all the critical

components of the vitamin D-signaling pathway. The skin is capable of synthesizing the vitamin D prohormone in response to UV radiation, it expresses the hydroxylases required to generate 25(OH)D and 1,25-(OH)₂D, as well as the nuclear VDR that mediates the effects of the active hormone on target gene expression. CYP24A1, which inactivates 1,25-(OH)₂D by 24-hydroxylation, is also expressed in the skin. The evolutionary importance of the autocrine and paracrine actions of vitamin D in skin is exemplified by the observation that, in *Xenopus*, the highest levels of VDR are expressed in the skin (60).

The liganded VDR exerts prodifferentiation and anti-proliferative effects on epidermal keratinocytes (61). These actions are critical for expression of proteins that are involved in formation of the cornified envelope, which is an important contributor to the epidermal barrier. In addition, the liganded VDR is important for production of lipids that play a role in barrier function. In contrast, the effects of the VDR on cyclic regeneration of the hair follicle are 1,25-(OH)₂D-independent and may involve interactions with a distinct group of coregulators (62), such as hairless.

B. Proliferation, differentiation, barrier function of skin

Like calcium, 1,25-(OH)₂D exerts antiproliferative and prodifferentiative effects on skin keratinocytes (63). Although *in vitro* investigations demonstrate that the effects of 1,25-(OH)₂D and calcium partially overlap, it is not known whether they exert these effects by regulating the same target genes and pathways. However, studies in keratinocytes isolated from VDR knockout mice demonstrate normal acquisition of markers of keratinocyte differentiation in response to calcium, but not 1,25-(OH)₂D (64). Investigations in mice lacking the VDR demonstrate impaired keratinocyte differentiation after the second week of life, which correlates with the development of impaired calcium absorption and hypocalcemia (65). However, this impaired differentiation is not observed in VDR knockout mice in which normal calcium levels are maintained by a special diet; thus, calcium and 1,25-(OH)₂D may have redundant roles in keratinocyte differentiation *in vivo*. Similarly, the impaired keratinocyte differentiation in mice lacking the vitamin D 1 α -hydroxylase CYP27B1 is lessened by maintenance of normal mineral ion levels (61).

Epidermal keratinocytes are in contact with a basal lamina that separates the epidermis from the underlying dermis. Proliferation of these basal keratinocytes results in differentiation of cells that give rise to the population of keratinocytes that are present in the external or upper layers. These more differentiated keratinocytes are characterized by a specific profile of gene expression that cor-

relates with their function: to provide a barrier that prevents water loss and contributes to host defense against environmental pathogens and toxins. As cells differentiate from basal to spinous layer keratinocytes, expression of keratins 5 and 14 decreases, and they start to express keratins 1 and 10 as well as involucrin. In addition to these proteins, lipids produced by these differentiating keratinocytes form the cornified layer. The production of glucosylceramides, which also contribute to the physical epidermal barrier, is decreased in mice lacking the VDR. The impaired lipid barrier observed in the VDR null mice and mice lacking CYP27B1 is not rescued by normalization of mineral ion homeostasis (27); thus, calcium and the VDR do not exert overlapping effects on lipid barrier formation.

In addition to contributing to the formation of a physical barrier, the VDR regulates genes involved in host defense. Disruption of the epidermal barrier results in exposure of the dermis and underlying structures to infectious agents. Activation of Toll-like receptors (TLR) activates vitamin D signaling in keratinocytes and monocytes by activation of CYP27B1 and induction of VDR expression (66). In humans, this leads to induction of cathelicidin, a peptide involved in host defense, as well as enhancing TLR expression in a positive feedback loop (67). This feature of the epidermal barrier also requires the ligand-dependent effects of $1,25\text{-(OH)}_2\text{D}$ (66) (see *Section VIII*).

C. Coactivators and corepressors of vitamin D in skin

Investigations directed at identifying the molecular basis for the differing gene expression profiles associated with keratinocyte differentiation revealed that the VDR associates with a different set of nuclear receptor coactivators, depending on the state of keratinocyte differentiation (68). In proliferating keratinocytes, the VDR interacts with the DRIP/Mediator complex. Impairing expression of DRIP 205/Med1 or Med21, key components of this coactivator complex, leads to an increase in proliferation accompanied by impaired acquisition of markers of keratinocyte differentiation. The DRIP/Mediator complex is critical for responsiveness of keratinocytes to both calcium and $1,25\text{-(OH)}_2\text{D}$, which suggests that these two prodifferentiation agents converge on a common molecular pathway to exert their effects.

Keratinocyte differentiation is characterized by a decrease in the expression of proteins that make up the DRIP/Mediator complex and an increase in expression of SRC3. The VDR-SRC3 interaction is critical for induction of proteins and lipids that contribute to formation of the epidermal barrier (27). *In vitro* knockdown of SRC3 or of VDR in keratinocytes leads to a similar reduction in the expression of lipids that contribute to epidermal barrier function.

D. Hair follicle phenotype, $1,25\text{-(OH)}_2\text{D}$ independence, molecular interactors, and targets

The observation that humans and mice with mutations in the VDR develop alopecia, whereas those with mutations in CYP27B1 do not, was the first indication that the actions of the VDR on the hair follicle do not require $1,25\text{-(OH)}_2\text{D}$. The availability of mice with ablation of the VDR or CYP27B1 provided invaluable tools for dissecting the effects of the VDR in the hair follicle (69–73). The VDR is expressed by the outer root sheath and hair bulb keratinocytes of the hair follicle, as well as by the sebaceous gland. During embryogenesis, the hair follicle develops in response to reciprocal signaling between dermal cells, which give rise to the dermal papilla, and the epidermal placode, which then invaginates to form the hair follicle. Postnatally, the hair follicle goes through cycles of growth, characterized by proliferation of cells from the bulge, which lies below the sebaceous gland and is thought to contain keratinocyte stem cells. The end of this proliferative anagen phase is characterized by the formation of a mature hair shaft. This is followed by catagen, characterized by apoptosis of the keratinocytes that lie below the bulge (74). This is thought to bring the dermal papilla in close proximity to the bulge, during the telogen phase, to permit reciprocal communication that results in the initiation of a new anagen phase. In humans, the hair cycle can last from months to years, depending on the location of the hair follicle, and is thought to contribute to the differing lengths of hair on various parts of the body. In mice, hair cycles occur approximately every 4 wk. Studies in mice lacking the VDR demonstrate that development of hair follicles proceeds normally, but hair cycles are absent after the morphogenic period (64). In contrast to epidermal keratinocytes, where calcium and $1,25\text{-(OH)}_2\text{D}$ play redundant roles in the regulation of proliferation and differentiation, normocalcemia was unable to prevent the defect in postmorphogenic hair cycles. This suggested that the actions of the VDR that maintain hair cycling differed from those required for keratinocyte differentiation.

Hair reconstitution assays, in which the hair follicle is reconstituted by implantation of morphogenic dermal papilla cells and keratinocytes into a nude mouse host, demonstrated that keratinocytes lacking the VDR were unable to support postmorphogenic hair cycles, whereas the absence of the VDR in the dermal papilla had no untoward effects (75). Transgenic expression of the VDR in the keratinocytes of VDR null mice prevented alopecia, demonstrating that the effects of the VDR in keratinocytes are critical for the maintenance of cutaneous homeostasis (76). Furthermore, expression of a VDR transgene with a mutation that prevents $1,25\text{-(OH)}_2\text{D}$ binding and transactivation also prevents alopecia, demonstrating that the

effects of the VDR on the hair follicle are 1,25-(OH)₂D-independent (62). Mice with deletion of the first zinc finger and AF-1 domain of the VDR phenocopy mice that express no VDR protein, demonstrating that this region of the receptor is critical for cutaneous integrity (69).

In addition to the absence of postmorphogenic hair cycles, the VDR null mice develop lipid-laden dermal cysts with epidermal markers and expansion of sebaceous glands, which suggests that an abnormality in the stem cells gives rise to these cells. The effect of VDR ablation on keratinocyte stem cell number and function was examined. A progressive decline in keratinocyte stem cell number was observed with age in the VDR null mice; however, at 28 d, when the number of these cells is normal, the keratinocyte stem cells are unable to form colonies *in vitro* or regenerate a hair follicle *in vivo*, demonstrating a functional abnormality in the keratinocyte stem cells as well (77).

Studies directed at identifying molecular partners of the unliganded VDR that play a role in the regulation of keratinocyte stem cell function demonstrated that, in contrast to investigations demonstrating that the liganded VDR impairs canonical Wnt signaling (30, 78), the unliganded VDR is essential for canonical Wnt signaling in keratinocytes. Absence of the VDR impairs expression of a Wnt reporter in primary keratinocytes as well as that of Wnt target genes in keratinocytes *in vitro* (77, 79). The effect of VDR ablation on Wnt target gene expression *in vivo* is dependent upon the age of the mice examined and the stage of the hair cycle (79, 80).

In keratinocytes, the unliganded VDR interacts with lymphoid enhancer-binding factor-1 (LEF1) but not with other effectors of the canonical Wnt signaling pathway, including β -catenin or Tcf3. Interactions of the VDR with LEF1 were mapped to the first zinc finger of the DNA-binding domain, an interesting finding based on the alopecia observed in mice lacking this region of the VDR (79). The importance of LEF1 in maintenance of the hair follicle is evidenced by the alopecia observed in mice lacking LEF1 and the hair loss, accompanied by the development of lipid-laden dermal cysts, in mice with keratinocyte-specific expression of a dominant negative LEF1 transgene (81). Whether impairment of VDR/LEF1 interactions underlies the alopecia observed in VDR null mice remains to be determined. However, the interaction of liganded VDR with β -catenin appears to have different effects on the hair follicle than the unliganded VDR (31, 82), which suggests that, in the absence of 1,25-(OH)₂D, the VDR may recruit LEF1 and/or other comodulators to regulate hair cycling.

Other transcriptional regulators that interact with the VDR have been shown to be critical for the maintenance of the postmorphogenic hair follicle. Mice with keratino-

cyte-specific ablation of RXR- α , the dominant RXR isoform in skin, develop a phenotype analogous to that seen in the VDR null mice, including progressive alopecia and the formation of lipid-laden dermal cysts (83). The phenotype, which also includes an inflammatory response, is more extensive than that of the VDR null mice, suggesting that ablation of RXR- α impairs the action of additional nuclear receptor heterodimerization partners. Ablation of the nuclear receptor corepressor hairless also leads to the development of alopecia with severe skin wrinkling and lipid-laden dermal cysts (84, 85). Interestingly, the interactions of the VDR with LEF1, RXR- α , and hairless do not involve the AF-2 region, which is required for interactions with classical nuclear receptor comodulators (19).

The hedgehog pathway also plays a role in the hair follicle. Absence of hedgehog signaling impairs hair follicle development, whereas activation of this pathway postnatally induces anagen both in wild-type and, to a lesser extent, in VDR null mice (80, 86). The VDR interacts directly with effectors of hedgehog signaling by binding to the regulatory regions of the GLI1 and Sonic hedgehog genes (79) and regulating their expression (87). Like the expression of Wnt target genes, the effect of VDR ablation on expression of genes in the hedgehog pathway depends upon the stage of the hair cycle, suggesting that the VDR may exert differential effects in epidermal *vs.* hair follicle keratinocytes (79, 80). Consistent with this hypothesis is that, in addition to being dysregulated in the skin of VDR null mice at the time of anagen, these genes are induced by the canonical Wnt signaling pathway. It remains to be determined whether VDR-LEF1 interactions are critical for induction of this pathway in the postnatal hair follicle.

E. Translational studies and clinical trials of vitamin D and skin

The antiproliferative and prodifferentiation effects of 1,25-(OH)₂D on keratinocytes led to an interest in its therapeutic potential for the treatment of skin disorders. Many investigations have examined the effects of vitamin D analogs on psoriasis, a disorder associated with keratinocyte hyperproliferation. Although these studies do suggest that topical treatment with combined glucocorticoids and vitamin D metabolites are superior to either alone, large, double-blind, placebo-controlled clinical trials demonstrating the effects of active vitamin D metabolites are required.

Exposure to UV light increases vitamin D synthesis as well as the risk of skin cancers. Investigations in animal models demonstrate that the VDR attenuates cutaneous malignancies. Mice lacking the VDR are more susceptible to skin cancers induced by either chemical carcinogens or UV radiation (87–89). Interestingly, mice lacking

CYP27B1, the enzyme required for 1α -hydroxylation of vitamin D metabolites, are not more susceptible to chemically or UV-induced tumors. Thus, the effects of the VDR on prevention of skin cancer in this model do not require $1,25\text{-(OH)}_2\text{D}$. Whether this is due to direct target gene regulation or is a reflection of the role of the VDR in regulating keratinocyte stem cell function remains to be determined.

F. Conclusions

All the elements of the vitamin D regulatory system are present in skin, and studies in humans and animals with mutations in key elements of this system support the biological role of vitamin D in regulation of the skin barrier and hair follicles. $1,25\text{-(OH)}_2\text{D}$ is strongly prodifferentiative and antiproliferative for keratinocytes, thereby supporting the use of topical and oral vitamin D in skin disorders such as psoriasis. Moreover, mice lacking the VDR gene are more susceptible to skin cancers induced by UV radiation. However, there are no large-scale, randomized, placebo-controlled clinical trials demonstrating that vitamin D metabolites are superior to other types of treatment for various proliferative skin disorders or for the prevention of skin cancer.

IV. Vitamin D and Its Relationship to Obesity and Diabetes Mellitus

A. Introduction

Low serum levels of 25(OH)D have been linked through observational studies to the pathophysiology of obesity, diabetes mellitus, and the metabolic syndrome. A number of mechanisms are plausible (90, 91). First, the VDR is highly expressed in adipocytes and is responsive to activation by $1,25\text{-(OH)}_2\text{D}$ (92–94). Second, vitamin D is fat soluble and can be stored in adipose tissues, although questions remain about the dynamics of its reentry into the circulation and subsequent fate (91, 95). Third, large cohort studies have shown that an increased percentage body fat and high body mass index (BMI) are strongly and inversely correlated with serum 25(OH)D concentrations, particularly in Caucasians (96, 97). Fourth, in rodent models, vitamin D modulates insulin synthesis and secretion (98, 99). Importantly, $1,25\text{-(OH)}_2\text{D}$ regulates calcium trafficking in β -cells *in vitro* and in mouse models (100, 101). There is also strong evidence that $1,25\text{-(OH)}_2\text{D}$ modulates intracellular ionized calcium signaling in the adipocyte, which in turn promotes increased lipogenesis and decreased lipolysis, possibly through the inhibition of uncoupling protein-2 (UCP2) (92, 100).

Thus, it is plausible that vitamin D could play a role in the pathogenesis of the metabolic syndrome and other obesity syndromes. However, *in vivo* data from mouse models add to the complexity of that relationship. For example, VDR null mice exhibit atrophy of adipose tissue in mammary and prostate glands (94, 102). And decreased overall fat mass, reduced serum leptin, and increased energy expenditure have been demonstrated in VDR $-/-$ mice (102–104). These changes, which are age dependent, are accompanied by an increase in UCP1 gene expression and a lean phenotype (104). In fact, recently, de Paula *et al.* (92) showed that VDR $+/-$ heterozygous mice also demonstrate a modest but significant lean phenotype. However, the mechanisms responsible for the remarkable changes in energy expenditure in VDR $-/-$ mice have not been fully clarified (100). Notwithstanding the mouse data, there remains an evidence gap in regard to the precise physiology of vitamin D in adipose tissue. It seems certain that there is an active role for vitamin D in adipocyte physiology, but the clinical data that obesity consistently is associated with low 25(OH)D levels lie in sharp contrast to the animal models in which absence of vitamin D is related to increased resting energy expenditure. Despite this paradox, there have been several observational and controlled trials of vitamin D in preventing or treating obesity and type 2 diabetes mellitus.

B. Observational studies of the relationship of vitamin D to obesity and the metabolic syndrome

Numerous observational studies (mostly cross-sectional, but some longitudinal) demonstrate a consistent association of low serum 25(OH)D levels with diabetes, prediabetes, metabolic syndrome, obesity, and fat content (adiposity) (105–107). This relationship is noted in adults and in children, in both sexes, and in various ethnic backgrounds (97, 108–114). Pittas *et al.* (115) performed a prospective cohort analysis of the Nurses Health Study in women followed for 20 yr relative to serum 25(OH)D levels and glucose intolerance. They found that total vitamin D and calcium intake was inversely associated with the risk of type 2 diabetes. Moreover, women who consumed three or more dairy servings per day were at a lower risk of developing diabetes compared with those consuming only one dairy serving per day. More recently, Devaraj *et al.* (97) noted that the first quartile of serum 25(OH)D level, compared with the fourth quartile, was associated with an adjusted odds ratio (OR) of prediabetes (defined as a 2-h glucose concentration of 140–199 mg/dl, a fasting glucose concentration of 110–125 mg/dl, or a glycosylated hemoglobin value of 5.7–6.4%) of 1.47 [95% confidence interval (CI), 1.16–1.85]. In that study, 25(OH)D levels were significantly and inversely correlated with fast-

ing glucose ($r = -0.29$; $P = 0.04$) and homeostasis model of assessment ($r = -0.34$; $P = 0.04$) in North American adults with the metabolic syndrome (97). A population-based study from Norway showed a similarly strong inverse association between elevated BMI and serum 25(OH)D (116).

In children and adolescents, the association seems more consistent and prominent. More than 50% of Norwegian children and adolescents with excess body weight had a low 25(OH)D status, and 19% had vitamin D deficiency (117). In obese African-American adolescents, low 25(OH)D levels correlated with low adiponectin levels, obesity, and insulin resistance (118). The association with increased adiposity was demonstrated in another study of both black and Caucasian youth (119). Analogous results (association of low vitamin D status with BMI and adiposity) were demonstrated in children in tropical environments such as Malaysia and Columbia and in adults in the Mediterranean region, such as Spain and France (105, 120–122). Meta-analysis of observational studies confirms the association of low 25(OH)D with incident diabetes (OR, 0.82; 95% CI, 0.72–0.93) (106).

Nevertheless, these studies remain observational and only document an association without causality, despite attempts to control for known confounders. For example, in one study the investigators adjusted for age, sex, race/ethnicity, season, geographic region, smoking, alcohol intake, BMI, outdoor physical activity, milk consumption, dietary vitamin D, blood pressure, serum cholesterol, C-reactive protein, and glomerular filtration rate to identify an association (114). The inability of these studies to evaluate temporality (*i.e.*, which occurred first, the vitamin D deficiency or obesity) and confounding (*i.e.*, an unknown factor may have caused both conditions) precludes conclusions about causality and whether vitamin D replacement would actually resolve or mitigate the observed outcome (obesity or glucose intolerance).

C. Randomized trials of vitamin D in obesity, type 2 diabetes mellitus

Until recently, there were no randomized trials testing the efficacy of vitamin D supplementation on the risk of developing type 2 diabetes mellitus. In 2008, de Boer *et al.* (107) evaluated the effect of calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative (WHI) trial. Postmenopausal women received 1000 mg/d elemental calcium plus 400 IU/d of vitamin D₃ or placebo in a double-blind fashion. The 2291 women with newly diagnosed type 2 diabetes were followed a median of 7 yr (107). The hazard ratio (HR) for incident diabetes mellitus associated with calcium/vitamin D treatment was 1.01 (95% CI, 0.94–1.10) based on intention-to-treat principles.

This null result was robust in subgroup analyses, efficacy analyses accounting for nonadherence, and analyses examining change in laboratory measurements (107). However, the supplement contained only 400 IU/d of vitamin D, and many women in the WHI were already taking upwards of 400 IU/d in their diet and with supplements.

A systematic review and meta-analysis was commissioned by The Endocrine Society to support the development of the Society's guidelines on vitamin D (123). Other than the trial by DeBoer *et al.* (107), this systematic review did not identify any other randomized controlled trials that reported the incidence of diabetes. Furthermore, that systematic review demonstrated that vitamin D supplementation did not affect glycemia (eight trials; weighted mean difference, -0.10 mg/dl; 95% CI, $-0.31, 0.12$; $P = 0.38$; $I^2 = 82\%$) (123).

However, other surrogate end points have been examined, and subgroup analyses have been performed in randomized controlled trials of vitamin D. Von Hurst *et al.* (124) supplemented the diets of nondiabetic overweight South Asian women with 4000 IU/d vitamin D₃ for 6 months and found a significant improvement in insulin sensitivity compared with a placebo group. Notably, it was the women with the lowest 25(OH)D levels at study initiation who achieved levels greater than 80 nmol/liter who had the greatest response with respect to glucose tolerance. In a subgroup analysis of the RECORD trial in which calcium (1000 mg/d), vitamin D (800 IU/d), both, or neither was randomly assigned to elderly people in Scotland, there was no difference in the incidence of self-reported development of type 2 diabetes among groups (125). Finally, Jorde *et al.* (116) performed a 1-yr, randomized, placebo-controlled trial in Norway of 438 obese women [ages 21–70 yr with a baseline 25(OH)D of 58 nmol/liter] using 40,000, 20,000, or 0 IU/wk of vitamin D₃ and found no differences in glucose tolerance among any of the groups despite an increase in serum 25(OH)D to 140 nmol/liter in the highest-dose vitamin D group.

D. Conclusions

At both the cellular and physiological level, the precise relationship between vitamin D and adiposity is not certain, although it remains an area of intense investigation. The ever-expanding obesity epidemic has been associated with a rising prevalence of vitamin D deficiency, but a cause-and-effect relationship has not been established; neither has a direct relationship been proven between low 25(OH)D levels and the pathogenesis of type 2 diabetes mellitus. Most of the evidence to date is correlational (*i.e.*, noninterventional) and derived from observational and longitudinal cohort studies of various populations. There remains a paucity of randomized controlled trials of vitamin D for the prevention of diabetes; hence, few conclu-

sions can be firmly established. At present, strong evidence does not exist to support the tenet that vitamin D supplementation reduces the risk of type 2 diabetes or the metabolic syndrome.

V. Vitamin D for the Prevention of Falls and Improvement in Quality of Life

A. Introduction

Rickets in children and osteomalacia in adults are characterized by undermineralized osteoid, resulting in “soft” bones (95, 126). Clinically, osteomalacia is associated with very low bone mass, bone pain, fractures, and muscle weakness. The myopathy associated with hypovitaminosis D includes type II muscle fiber atrophy and in some cases fatty infiltration of the muscles. However, these changes are nonspecific and can be found in other types of myopathy. Serum 25(OH)D levels are usually very low, which makes that measurement an extremely sensitive but not specific predictor of disease status (127). However, very low calcium intake in the face of normal vitamin D stores can also lead to osteomalacia. Conversely, in some forms of osteomalacia, calcium levels may be low normal, but with very low serum phosphorus, there is undermineralized osteoid and severe proximal muscle weakness. Supplementation with high doses of vitamin D rescues the phenotypic manifestations of osteomalacia due to dietary deficiency, including correction of low serum calcium and phosphorus, albeit only when serum 25(OH)D levels are restored to normal ranges. However, improvement in symptoms of muscle weakness and pain with osteomalacia can take up to 18 months after initiation of therapy, and these changes do not significantly correlate with the rise in serum 25(OH)D (126).

Several lines of evidence support the concept that there is a strong and direct effect of vitamin D on muscle function. First, the syndrome of vitamin D deficiency [*i.e.*, serum 25(OH)D levels <10 ng/ml or 25 nmol/liter] is frequently accompanied by profound muscle weakness that responds to vitamin D treatment, although as noted the myopathy is nonspecific (102, 126, 127). In children, the proximal muscle weakness is readily reversible with cholecalciferol supplementation. With the adult syndrome, there is relatively strong evidence that elders living in an institutional setting are more prone to vitamin D deficiency due to reduced solar and dietary exposure to vitamin D. These men and women often exhibit signs of muscle weakness, bone pain, frailty, and fractures that also respond to vitamin D replacement, although it is unclear whether this is direct or indirect, due to the effect of vitamin D on calcium entry into skeletal muscle cells and the

marked reduction in phosphate stores (128). Second, this phenotype is recapitulated in the heritable conditions of vitamin D resistance and impaired receptor function where muscle weakness, bone pain, and poor skeletal mineralization are quite common (73, 127, 129). Third, the VDR is widely expressed in many tissues, and genetic deletion of this receptor can lead to poor muscle function in mice (130). Some, but not all, studies have demonstrated by immunohistochemistry with several different antibodies that the VDR is expressed in adult muscle tissue, although this recently has come into question (131). Finally, there is biochemical evidence that activation of the VDR by 1,25-(OH)₂D in skeletal muscle induces fast, nontranscriptional responses involving stimulation of the transmembrane second messenger systems, including adenylyl cyclase/cAMP/PKA, PLC/DAG+ IP(3)/PKC/Ca(2+), and MAPK cascades. Short treatment with 1 α ,25(OH)₂D₃ also induces reverse translocation of the VDR from the nucleus to plasma membranes (132). Hence, there is some support for a direct relationship between vitamin D and muscle function.

There is also experimental evidence to suggest that the effects of vitamin D on muscle function may be indirect. First and foremost, Wang and DeLuca (131), using a highly specific antibody to VDR, recently reported that they could not identify strong VDR positivity by immunohistochemistry in adult muscle from either mouse or man. Second, genetic deletion of the VDR in intestinal tissue results in a phenocopy of the VDR null mouse with dramatic musculoskeletal and skin changes. Furthermore, high-dose supplementation with calcium and phosphorus rescues the skeletal phenotype in VDR^{-/-} mice. And a knock-in of the VDR in the intestine of VDR^{-/-} mice rescues the musculoskeletal phenotype (133). Third, some individuals with very low serum 25(OH)D levels (*i.e.*, <10 ng/ml) do not exhibit signs of osteomalacia either clinically or histologically, most likely because they have adequate calcium intake (134). These observations, plus data from both mice and humans that high doses of calcium alone can reverse the clinical syndrome of osteomalacia, suggest that the effects of vitamin D on muscle function may be mediated in part through changes in calcium absorption rather than directly via a putative muscle VDR. In summary, there is significant controversy about the role of vitamin D in adult muscle function. It remains to be determined whether the effects are mediated directly by VDR activation of second messengers in stem cells or skeletal muscle cells or through changes in calcium absorption that affect PTH secretion and ultimately determine intracellular calcium levels.

Conflicting data about the effects of vitamin D on muscle function translate into heterogeneous results from clin-

ical trials and produce significantly different conclusions from meta-analyses of vitamin D for fall prevention (see *Section V.C.*). Attempts to define an absolute threshold level of 25(OH)D above which muscle function is optimal or falls can be prevented are fraught with significant issues. First, as noted, although serum 25(OH)D is a sensitive indicator of osteomalacia, it is not specific likely because of variability in calcium intake. In a recent autopsy series from Priemel *et al.* (134) of more than 600 individuals, nobody had histological evidence of osteomalacia with levels greater than 30 ng/ml, but many subjects with values less than 15 ng/ml had no evidence of osteomalacia on bone biopsy. Notably, daily calcium intakes were not available in this study, making it difficult to discern the reason why so many individuals with low vitamin D levels had no histological evidence of osteomalacia. Second, according to the recent IOM report, serum 25(OH)D is only a measure of exposure to vitamin D, not a biomarker of a disease state (135). This is also clearly illustrated in the Priemel *et al.* study (134), as well as in clinical observations in which calcium insufficiency alone with normal levels of 25(OH)D can rarely cause osteomalacia. Third, there is some evidence to suggest that the protective effects of calcium plus vitamin D in respect to hip fracture risk reduction are more apparent in individuals with baseline low vitamin D levels (136, 137). Finally, the etiology of falls is multifactorial and involves numerous confounding determinants, including neurological factors, gait speed, mental status, and medications. As such, the complex interaction between calcium and vitamin D makes it difficult to define an absolute threshold for serum 25(OH)D below which muscle function is impaired or, conversely, above which, falls are prevented. Notwithstanding, it is noteworthy that in virtually every observational study, individuals with the lowest levels of serum 25(OH)D are at the greatest risk of falls and fractures (3).

B. Observational studies of vitamin D and falls

Several observational studies have pointed to an association between serum 25(OH)D levels and falls and/or frailty. However, analysis in the most recent Agency for Healthcare Research and Quality (AHRQ) systematic review identified a significant inconsistency across studies (138). In part this relates to defining a threshold value for serum 25(OH)D that would prevent falls and improve muscle function. For example, in the Longitudinal Aging Study in Amsterdam, a prospective cohort study, the investigators found that serum levels less than 25 nmol/liter were associated with the greatest risk of falling in subjects who had experienced multiple falls (139). On the other hand, in National Health and Nutrition Examination Survey (NHANES) III, higher serum 25(OH)D concentra-

tions among 4100 older adults were associated with better lower extremity function, with the greatest effect occurring in those individuals with serum levels between 20 and 40 nmol/liter (137). In a Dutch study of men and women more than age 65 yr, serum levels below 20 nmol/liter were associated with a significant decline in physical function over a 3-yr period (140). More recently, Ensrud *et al.* (141) examined indices of frailty both cross-sectionally and after 6 yr in the large Study of Osteoporotic Fractures (SOF) cohort of elderly women and found increased frailty indices for women with levels below 20 ng/ml and a plateau for risk of frailty between 20 and 30 ng/ml. Additionally, in the Osteoporotic Fractures in Men Study (MrOS), a prospective cohort study in older men, serum levels of 25(OH)D below 20 ng/ml were independently associated with greater evidence of frailty at baseline, but unlike the Dutch study, did not predict greater frailty status at 4.6 yr (142).

In sum, observational data from cross-sectional and cohort studies suggest that serum 25(OH)D levels that are often considered deficient (*i.e.*, <20 ng/ml) are associated with greater frailty indices and likely increased the risk of falls among elderly individuals. However, as noted, there is considerable heterogeneity among the subjects, their calcium intake, the assay used for measuring serum 25(OH)D, and the primary outcome that was measured. In respect to the serum measurement of 25(OH)D, several distinct assays (*e.g.*, RIA, ELISA, liquid chromatography-mass spectrometry) have been used in large observational studies, and each has its own strengths and limitations. Notwithstanding, it is apparent that comparisons across studies to define a single 25(OH)D threshold level for falls are likely to be confounded by significant variations in the measurement tool.

C. Randomized trials of vitamin D on falls

There have been several randomized controlled trials of vitamin D or vitamin D plus calcium to prevent falls and improve frailty indices, although the quality of the evidence has been rated as “fair” by two AHRQ reviews (138). Surprisingly, more meta-analyses of vitamin D and falls have been published recently, such that the ratio of randomized clinical trial (RCT) publications to meta-analyses is now a mere 1.9:1. Thus, with fewer clinical trials and more meta-analyses, the results become less clear-cut. For example, outcome measurements (*i.e.*, falls *vs.* fallers), population heterogeneity, and serum measurements of 25(OH)D all complicate interpretation. More importantly, selection of the most appropriate studies for analysis based on *a priori* criteria is essential because the number of well-executed randomized trials is limited.

The most recent systematic review and meta-analysis by Murad *et al.* (143), also commissioned by The Endocrine Society to support the development of clinical practice guidelines, found a statistically significant reduction in the risk of falls in 26 randomized trials of vitamin D supplementation (OR = 0.85; 95% CI, 0.77–0.95; $I^2 = 60\%$). This effect was more prominent in patients who were vitamin D deficient at baseline, a finding consistent with previous observational studies ($P < 0.05$) (143). Interestingly, the effect of vitamin D on fall reduction was only noted in studies that used both calcium and vitamin D supplementation. Not surprisingly, the evidence supporting a reduction in falls with supplementation among individuals with very low levels of 25(OH)D has become more robust and relatively consistent in systematic reviews, including the recent report from the U.S. Public Health Services Task Force (137, 143–145).

The optimal dose and timing of supplementation with vitamin D has not been settled, in part due to issues related to compliance. Two recent well-designed, randomized, placebo-controlled trials in older individuals using high-dose intermittent cholecalciferol have been reported. Saunders *et al.* (146) administered 500,000 U vitamin D once yearly for 3 yr or placebo to older postmenopausal women (mean age, 76 yr) at high risk for falling (one third had also suffered previous fractures). The authors found that vitamin D raised serum levels of 25(OH)D from 53 nmol/liter (approximately 21 ng/ml) at baseline to 120 nmol/liter at 1 month, 90 nmol/liter at 3 months, and 75 nmol/liter (28 ng/ml) at 1 yr, but was not associated with fewer fractures or falls compared with placebo (146). Glendenning *et al.* (147) performed a 9-month, randomized, placebo-controlled trial in older Australian postmenopausal women (mean age, 76 yr) using 150,000 U cholecalciferol every 3 months and also found no reduction in falls among the vitamin D group despite an increase in serum 25(OH)D from 65 to 74 nmol/liter at 3 months. In both of these studies, the risk of falling was greater in the vitamin D-treated group than placebo, although the difference was only significant in the former trial (HR, 1.16; $P = 0.003$). However, caution must be exercised in extrapolating serum 25(OH)D levels from these studies to adverse events because peak levels were never ascertained in either of the two “high-dose” trials.

D. Effects of vitamin D supplementation on pain and quality of life

The effect of vitamin D supplementation on other functional outcomes such as pain and quality of life is less clear. Six studies assessed the effect of vitamin D on patients' quality of life using standardized instruments (SF-36, SF-12, and the Medical Outcome Survey Short Form-8)

(148–153). Meta-analysis demonstrated no significant change in the physical component score (standardized mean difference, 0.07; 95% CI, -0.03 to 0.16 ; $I^2 = 54\%$) or the mental component score (standardized mean difference, 0.02; 95% CI, -0.05 to 0.09 ; $I^2 = 29\%$). The individual domains of quality-of-life surveys were reported in three studies and did not significantly differ at the end of follow-up (150, 153, 154). This includes follow-up of the WHI, which is fairly large and better powered to demonstrate a difference (154). Lastly, vitamin D administration in elderly patients with congestive heart failure resulted in no significant benefit in terms of physical performance using a timed up-and-go test, subjective measures of function, or daily activity. Quality of life measured by a disease-specific tool (the Minnesota Living with Heart Failure questionnaire), worsened by a small, but significant amount in the treatment group (148).

The effect of vitamin D on pain was reported in five studies, but the results were too heterogeneous to be pooled in a meta-analysis (150, 153, 155–157). Three of these studies showed a possible beneficial effect. Arvold *et al.* (158) randomized patients with mild-to-moderate vitamin D deficiency (level, 10–25 ng/dl) to vitamin D₃ 50,000 U/wk for 8 wk or placebo. The study measured scores on the Fibromyalgia Impact Questionnaire and reported that those with mild-to-moderate deficiency had more fatigue and joint and muscle aches at baseline than placebo but no impairments in terms of the activities of daily living. Supplementation led to statistically significant improvements in fatigue symptoms compared with placebo. A third arm of severe deficiency (not randomized) had more severe baseline symptoms and marked improvements with supplementation. Brohult and Jonson (157) reported decreased pain and analgesic use by rheumatoid arthritis patients after using a large dose of vitamin D for 1 yr (67% of patients in the vitamin D group improved *vs.* 36% of control patients; $P < 0.05$). Grove and Halver (159) showed similar results in postmenopausal women with osteoporotic fractures who took vitamin D, calcium, and fluoride compared with placebo (83% of patients in the vitamin D group improved *vs.* 31% of control patients; $P = 0.05$).

The other studies did not demonstrate a benefit of vitamin D on pain scores in the elderly at risk of falls (as a component of SF-36), in postmenopausal osteoporotic women with vertebral fractures, or in patients with diffuse musculoskeletal pain and osteoarthritis who have 25(OH)D levels no greater than 20 ng/ml (150, 153, 155, 156). In summary, conclusions from studies that evaluated the effects of vitamin D on pain and quality of life are quite limited due to their heterogeneous nature in terms of population, cohort size, outcome definition, and imprecision.

E. Conclusions

In a somewhat distinct vein from the IOM report, we believe vitamin D supplementation is likely to reduce the risk of falls, particularly in those individuals who have low baseline levels (<20 ng/ml) and are supplemented with calcium as well (3). However, the absolute threshold level of 25(OH)D needed to prevent falls in an elderly population is not known in part because of the lack of true dose-ranging studies. Importantly, recommendations for vitamin D intake in a given individual must also be considered within the context of optimal calcium intake. Notwithstanding, the effect of vitamin D supplementation on falls could have important public health implications considering the morbidity associated with falls, particularly in the frail elderly. Selecting patients at risk for falls and defining the appropriate dose remain as areas in need of further research.

VI. Vitamin D and Cancer

A. Introduction

Vitamin D has received widespread attention in the medical literature and popular press for its potential role in cancer prevention. Thus, it surprised many in the biomedical community that this research did not have a prominent role in establishing new Dietary Reference Intakes for vitamin D by the IOM (3, 135). After a comprehensive and rigorous review of the scientific research, the IOM Committee concluded that the evidence that vitamin D prevented cancer was inconsistent and did not meet criteria for establishing a cause-effect relationship. A systematic review conducted by the AHRQ in 2009 (160) and in 2011 (161), as well as other recent reviews summarized in the report (3, 162), have reached similar conclusions. Importantly, no previous large-scale RCT of vitamin D had been completed with cancer as the primary prespecified outcome (3, 163). Most of the available evidence on vitamin D and cancer was derived from laboratory studies, ecological correlations, and observational investigations of serum 25(OH)D levels in association with cancer outcomes. Although measures of serum 25(OH)D concentrations were considered to be a useful marker of current vitamin D exposure, the committee was concerned about the limitations of association studies. Specifically, low serum 25(OH)D levels may be linked with numerous confounding factors that are known to relate to higher cancer risk, including obesity (due to vitamin D sequestration in adipose tissue), lack of physical activity (correlated with less time outdoors and less incidental solar exposure), race/dark skin pigmentation (less skin synthesis of vitamin D in response to sun), and diet or supplement-taking prac-

tices (3, 135, 163). Reverse causation bias is also a concern if poor health reduces outdoor activities and sun exposure or adversely affects diet, thereby resulting in lower serum 25(OH)D levels. Because of these potential biases and limitations, association cannot prove causation (163, 164).

There is strong biological plausibility for a role of vitamin D in cancer prevention. The VDR is expressed in most tissues. Studies of *in vitro* cell culture and *in vivo* experimental models suggest that 1,25-(OH)₂D promotes cell differentiation, inhibits cancer cell proliferation, and exhibits antiinflammatory, proapoptotic, and antiangiogenic properties (163, 165, 166). Through binding to the VDR, 1,25-(OH)₂D has been shown in laboratory studies to inhibit the growth of cancer cells by regulating several genes responsible for cell proliferation—*e.g.*, activating cyclin-dependent kinase inhibitors such as p21 and p27; repressing growth factors such as IGF-I and epidermal growth factor receptor; and activating growth regulatory genes such as TGF- β . 1,25-(OH)₂D-VDR transcriptional signaling may also exert antiinflammatory effects on cancer cells by down-regulating the prostaglandin pathway and cyclooxygenase-2, leading to growth inhibition. In addition, 1,25-(OH)₂D exhibits proapoptotic effects in cancer cells by repressing several prosurvival proteins such as BCL2 and telomerase reverse transcriptase and by activating proapoptotic proteins such as BAK. Recent *in vivo* and *in vitro* studies have further suggested that vitamin D signaling is particularly relevant for advanced-stage or high-grade tumors because of its inhibitory effects on angiogenesis, invasion, and metastatic potential. Treatment of cancer cells with 1,25-(OH)₂D may inhibit cell tube formation and tumor growth by repressing vascular endothelial growth factor and IL-8. Although the mechanistic studies are promising, they cannot provide conclusive evidence that vitamin D prevents the development of cancer in humans or slows its progression to invasive and metastatic forms.

B. Total cancer and cancer mortality: research findings

Although several observational studies have linked low serum levels of 25(OH)D with increased cancer incidence and mortality, no previous randomized trial has assessed cancer as a primary prespecified outcome (3, 135, 163). Three previous trials of vitamin D have assessed incident cancer or cancer mortality as secondary outcomes, but the results were null (167–169) (Table 1). For example, in a British trial of 2686 men and women aged 65–85, in which 100,000 IU of vitamin D₃ every 4 months (average intake, ~833 mg/d) was compared with placebo, the relative risk (RR) for cancer incidence over 5 yr was 1.09 (95% CI, 0.86–1.36) (167). In a 4-yr trial among 1179 postmenopausal women (mean age, 67 yr) in Nebraska, women

TABLE 1. RCT of vitamin D supplementation and total cancer incidence

Site of study	Cohort	Intervention dose	No. of subjects (treated/control)	RR (95% CI)
Oxford, United Kingdom	2,686 men and women, ages 65–85 yr	Vitamin D ₃ 100,000 IU every 4 months (~833 IU/d) vs. placebo	188/173	1.09 (0.86–1.36)
Nebraska, United States	1,179 postmenopausal women, mean age 67 yr	Vitamin D ₃ 1,100 IU/d + calcium vs. calcium alone	13/17	0.76 (0.38–1.55)
WHI, United States	36,282 postmenopausal women, ages 50–79 yr	Vitamin D ₃ 400 IU/d + calcium vs. placebo	1634/1655	0.98 (0.91–1.05)

Data are from Refs. 160 and 163.

receiving calcium plus vitamin D (1000 IU/d) had a lower rate of malignancies than those receiving placebo but did not have a significantly lower risk of cancer than those receiving calcium alone (13 vs. 17 cases; RR = 0.76; 95% CI, 0.38–1.55) (168). Interestingly, calcium alone tended to reduce cancer incidence vs. placebo, although this was not statistically significant. In that trial, assignment to vitamin D raised mean serum 25(OH)D by 24 nmol/liter, from 72 nmol/liter at baseline to 96 nmol/liter after 1 yr of treatment. Among 36,000 postmenopausal women aged 50–79 in the WHI trial of calcium (1000 mg/d) plus low-dose vitamin D₃ (400 IU/d), the 7-yr intervention did not reduce the incidence of total cancer (RR = 0.98; 95% CI, 0.91–1.05) or cancer mortality (RR = 0.89; 95% CI, 0.77–1.03) (169, 170).

C. Vitamin D and the risk of site-specific cancers

1. Breast cancer

a. Overview. The influence of 1,25-(OH)₂D on breast cancer cells *in vitro* includes anticancer effects such as cell cycle inhibition, reduced proliferation, enhanced sensitivity to apoptosis, and induction of differentiation markers (171). These responses appear to be mediated by the VDR, which is expressed on nearly all established breast cancer cell lines (172). Although target genes regulated by vitamin D show variability in different model systems, some common features include inducing cellular differentiation, remodeling of the extracellular matrix, and enhancing innate immunity (172). Many of the genes identified show a consensus VDRE in their promoter elements, suggesting that they are specific targets of the VDR complex (172, 173).

b. Observational studies. The 2009 AHRQ report did not find any qualified systematic reviews that evaluated associations between vitamin D intake or serum 25(OH)D and risk for breast cancer (160). Three observational studies of sufficient methodological quality were identified that assessed the association between 25(OH)D levels and breast cancer risk. A prospective cohort study within a subgroup of NHANES III reported that women with higher

25(OH)D levels were at significantly lower risk for breast cancer. In this study, however, only eight women were in the higher 25(OH)D category, and a linear trend analysis was nonsignificant (174). In a nested case-control study using data from the Nurses' Health Study, no significant relationship between higher plasma 25(OH)D concentrations and lower risk for breast cancer was observed overall, but a significant trend was seen for women above age 60 (175). Another nested case-control cohort study of postmenopausal women participating in the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial found no evidence that higher plasma 25(OH)D concentrations were associated with reduced risk of breast cancer (176). More recently, a large case-control study in Italy of women aged 20–74 yr found an inverse association between vitamin D intake and risk for breast cancer, with an apparent threshold at intakes of 188 IU/d or greater (177). In contrast, a study of Canadian women found no association between dietary intake of vitamin D or calcium with breast cancer risk, except for a reduced risk associated with vitamin D supplements greater than 400 IU/d (178). In the WHI, an inverse association between baseline 25(OH)D levels and incident breast cancer disappeared after adjustment for BMI and physical activity levels (170).

c. Randomized controlled trials. Only one randomized trial (the WHI calcium/vitamin D trial of 1000 mg calcium combined with 400 IU/d of vitamin D₃) was large enough to assess breast cancer as a separate, although secondary, outcome (170). Overall, the WHI showed no significant effect of the intervention on breast cancer incidence (HR, 0.96; 95% CI, 0.86–1.07) or breast cancer mortality (HR, 0.99) over 7 yr. When the study population was stratified by baseline vitamin D intake (diet plus supplements), evidence for effect modification was seen. Women who had the lowest baseline intakes of vitamin D had a reduced risk of breast cancer with the intervention (HR, 0.79; 95% CI, 0.65–0.97), whereas women with the highest baseline intakes (≥600 IU/d) had significantly increased breast cancer risk (HR, 1.34; 95% CI, 1.01–1.78) (*P* for interac-

tion = 0.003). Thus, randomized trial data on vitamin D and breast cancer suggest possible benefits from supplementation among women with low baseline intake but raise the possibility of harm at higher (*e.g.*, above recommended dietary allowance) levels of intake.

d. Conclusions. Vitamin D and breast cancer. Although experimental laboratory studies are suggestive of a role for vitamin D in breast biology, available observational research is inconsistent, and randomized trial evidence is limited and not supportive of benefit.

2. Colorectal cancer

a. Overview. The VDR and the enzyme 1α -hydroxylase, which converts 25(OH)D to $1,25\text{-(OH)}_2\text{D}$, are expressed in colorectal tissue (179, 180). $1,25\text{-(OH)}_2\text{D}$ and its analogs have been shown to regulate cell proliferation and differentiation in human colon cancer cell lines (181, 182). Injection of colon cancer cells into vitamin D-sufficient and vitamin D-deficient mice led to significantly greater tumor growth in the vitamin D-deficient animals (183). $1,25\text{-(OH)}_2\text{D}$ may also affect the development and progression of colon cancer by acting directly on calcium homeostasis, increasing intracellular calcium flux (182, 184).

b. Observational studies. Observational studies of serum 25(OH)D levels in relation to colorectal cancer incidence generally support an inverse association (3, 135, 162). In a meta-analysis of prospective data from five studies with a total of 535 cases (including the large-scale Nurses' Health Study and WHI), those with serum 25(OH)D of at least 33 ng/ml (≥ 83 nmol/liter) had about half the risk for colorectal cancer of those with levels of 12 ng/ml or less (≤ 30 nmol/liter) (185). The large-scale European Prospective Investigation into Cancer and Nutrition study reported a similarly strong inverse association (162, 186). The Japan Public Health Centre-based Prospective Study did not find an inverse relation between plasma 25(OH)D and colon cancer in either men or women, although an inverse association with rectal cancer was apparent (187). A 2008 meta-analysis by the International Agency for Research on Cancer (IARC) found a significant inverse association between serum 25(OH)D and colorectal cancer risk, although there was significant between-study heterogeneity (162). Similarly, a recent systematic review by AHRQ (161) found a 6% (95% CI, 3 to 9%) reduction in colorectal cancer risk for each 10-nmol/liter increase in 25(OH)D concentrations in observational studies but concluded that the evidence was not sufficiently robust to draw conclusions regarding a cause-effect relationship.

c. Randomized controlled trials. Randomized trial evidence for vitamin D in the prevention of colorectal cancer is limited. In a 5-yr British trial, in which 2686 older men and women were randomized to 100,000 IU of vitamin D₃ or placebo every 4 months (~ 833 IU/d), the intervention was not associated with a reduction in colorectal cancer incidence, a secondary outcome (28 colorectal cancers in the vitamin D group *vs.* 27 in the placebo group; RR = 1.02; 95% CI, 0.60–1.74) (167). Similarly, among 36,000 women in the WHI calcium-vitamin D trial, a combination of calcium (1000 mg/d) plus low-dose vitamin D₃ (400 IU/d) for a mean of 7 yr did not reduce colorectal cancer incidence (168 *vs.* 154 cases; RR = 1.08; 95% CI, 0.86–1.34) or deaths from colorectal cancer (34 *vs.* 41 cases; RR = 0.82; 95% CI, 0.52–1.29; *P* = 0.39) (169).

d. Conclusions: Vitamin D and colorectal cancers. The experimental laboratory and observational research on vitamin D is more compelling for colorectal cancer than for other cancers. However, randomized trial evidence remains limited and has not demonstrated benefits to date. Whether randomized trials testing higher doses of vitamin D and providing longer duration of treatment will demonstrate efficacy in colorectal cancer prevention remains unknown.

3. Prostate cancer

a. Overview. Studies *in vitro* demonstrate that prostate cancer and epithelial cells in culture respond to $1,25\text{-(OH)}_2\text{D}$ with antiproliferative effects and increased cell differentiation (3, 188). Like epithelial cells of other tissue origins, these effects appear to be mediated by the VDR expressed in prostate cells (3). Gene expression array studies suggest that $1,25\text{-(OH)}_2\text{D}$ inhibits growth factor signaling and cell cycle progression, promotes differentiation, and has antiinflammatory and antiangiogenic effects (3, 189, 190).

b. Observational studies. Although ecological studies suggest that prostate cancer mortality is inversely related to sun exposure, observational analytic studies of serum 25(OH)D and prostate cancer have been inconsistent. Eight of 12 nested case-control studies found no association between baseline serum 25(OH)D and risk for prostate cancer, whereas one reported a significant inverse association [for baseline serum 25(OH)D levels < 30 compared with levels > 55 nmol/liter] (163, 191). A more recent case-control analysis of data from the α -Tocopherol, β -Carotene Prevention Study found no association between serum 25(OH)D levels and incidence of prostate cancer (192). Moreover, a meta-analysis of 45 observational studies of dairy and milk intake in relation to risk of

prostate cancer showed no significant association with dietary intake of vitamin D (193).

c. Randomized controlled trials. No relevant RCT of vitamin D supplementation and risk of prostate cancer were identified.

d. Conclusions: Vitamin D and prostate cancer. Although laboratory data suggest a role for vitamin D in inhibiting prostate carcinogenesis, observational studies of serum 25(OH)D and risk of prostate cancer have provided mixed results, and data from randomized controlled clinical trials are lacking.

D. Other site-specific cancers

The large-scale Cohort Consortium Vitamin D Pooling Project of Rarer Cancers found no evidence linking higher serum concentrations of 25(OH)D to reduced risk of less common cancers, including endometrial, esophageal, gastric, kidney, pancreatic, and ovarian cancers, and non-Hodgkin lymphoma (194). In aggregate, these cancers represent approximately half of all cancers worldwide. Moreover, the report provided suggestive evidence for a significantly increased risk of pancreatic cancer at high levels [≥ 40 ng/ml (≥ 100 nmol/liter)] of 25(OH)D (194). An increased risk of esophageal cancer at higher levels of 25(OH)D also has been reported (3, 195).

E. Conclusions

Despite biological plausibility for a role of vitamin D in cancer prevention, most recent systematic reviews and meta-analyses, as well as a comprehensive review by the IOM Committee, have found that the evidence that vitamin D reduces cancer incidence and/or mortality is inconsistent and inconclusive as to causality. Importantly, no large-scale randomized trials have been completed with cancer as the primary prespecified outcome, and trials with cancer as a secondary outcome have been sparse and generally unresponsive. Observational evidence is strongest for colorectal cancer but is weak or inconsistent for breast, prostate, other cancer sites, and total cancer. Moreover, concerns about potential increased risk for selected cancers with high levels of 25(OH)D have been raised. New trials assessing the role of moderate- to high-dose vitamin D supplementation in cancer prevention, including the large-scale VITamin D and OmegaA-3 Trial (196), are in progress and should provide additional information within 5–6 yr. It is worth noting that many micronutrients that seemed promising in observational studies (*e.g.*, β -carotene, vitamins C and E, folic acid, and selenium) were not found to reduce the risk of cancer in RCT and some were found to cause harm at high levels of

supplementation (135, 163). Although future research may demonstrate clear benefits for vitamin D in relation to cancer and possibly support higher intake requirements for this purpose, the existing evidence has not reached that threshold.

VII. Vitamin D and Cardiovascular Disease

A. Introduction

For CV diseases (CVD), as with other outcomes, the assessment of 25(OH)D levels, intakes and supplementation is somewhat more complex due to a number of issues, including the relationship of sun exposure to serum 25(OH)D, the seasonal fluctuation in serum levels, the lack of information about the relationship between vitamin D dietary and supplement intake to serum levels, and, perhaps most importantly, the possibility that the benefits or risks of vitamin D supplementation may depend on initial levels of 25(OH)D. The assessment of vitamin D is further complicated by the fact that CVD is a heterogeneous category, and it is possible that vitamin D has a different relationship to individual types of clinical endpoints [*e.g.*, stroke *vs.* myocardial infarction (MI) *vs.* hypertension]. Notwithstanding, there is biological plausibility to the concept that vitamin D could impact CV events such as MI or hypertension, either directly through actions of the VDR in smooth muscle cells of the vasculature or cardiac muscle in the heart, or indirectly by promoting calcium absorption at the expense of lipid absorption or lipid excretion in the gut (197). Several basic mechanisms have been proposed, including endothelial dysfunction from lack of adequate vitamin D, vascular compliance impairment due to smooth muscle changes, enhanced inflammation or effects related to high levels of PTH, or the renin-angiotensin system. Although the VDR null mouse has been studied with respect to CVD outcomes and evidence has suggested that these mice might be at higher risk of vascular disease, several animal studies have not shown a relationship of vitamin D supplementation to development of CVD, and one animal study found increased thrombogenicity associated with vitamin D supplementation (198). On the other hand, vitamin D supplementation could lower vascular risk by improving glucose tolerance and/or inhibiting inflammatory components in the metabolic syndrome. However, vitamin D supplementation in animals with impaired renal function may actually worsen vascular responsiveness. And a recent meta-analysis of calcium use alone has suggested the possibility that enhanced calcium absorption (either from calcium supplements or possibly through increased vitamin D) may increase the risk of CV events (199).

B. Studies of hypertension and lipids

Ecological studies have suggested that rates of CVD and hypertension may increase with increasing distance from the equator (200), which suggests the possibility that lower vitamin D levels are associated with higher risk of CVD. Pittas *et al.* (115) reviewed prospective observational studies of vitamin D levels in relationship to incident hypertension and identified three cohort studies for this question. In a meta-analysis of these three, they found a significant association between lowest levels of 25(OH)D (<37 to 51 nmol/liter) and incidence of hypertension over 7 to 8 yr. A recently performed systematic review of randomized trials that studied vitamin D and its impact on mean blood pressure and lipids (total cholesterol, triglycerides, low-density lipoproteins, and high-density lipoproteins) (123) included between 11 and 14 relevant studies (depending on the endpoint). The authors found no significant effect of vitamin D on any of these endpoints (the significance levels varied from 0.27 to 0.91), although they saw significant heterogeneity of meta-analytic estimates of low-density lipoprotein and high-density lipoprotein analyses. Thus, whereas current data do not support an effect of vitamin D on blood pressure and lipids, further studies of the effect of vitamin D on lipids are warranted.

C. Studies of other CVD endpoints

1. Observational studies

There are a number of prospective observational studies that examined vitamin D status and risk of CVD using other endpoints for CVD. The Pittas *et al.* review (115) identified a total of seven studies, which included nine different analyses in six different cohorts. The outcomes used in these studies have varied and included MI, combined CVD, stroke, and CV mortality. The primary predictor in all of these studies was serum 25(OH)D concentration. These cohorts together have included more than 43,500 people with a mean follow-up ranging from 5 to 27 yr. Pittas *et al.* (115) judged five of the seven to be of good and two of poor quality.

Of the nine studies, five found that low vitamin D levels were associated with a high risk of CVD. The Framingham Offspring Study included 1739 participants without prior CVD (201). Over an average follow-up of 5 yr, the adjusted HR for overall incident CV events was 1.62 (95% CI, 1.11 to 2.36; $P = 0.01$) in the 28% of the cohort with low 25(OH)D levels (<15 ng/ml) *vs.* the remainder. A secondary analysis suggested that this association may have been significant only in those with initial hypertension. Similarly, the Health Professional Follow-up Study using a nested case-control study in 18,225 men found an

increased risk of MI in those with 25(OH)D levels below 15 ng/ml compared with those with levels above 30 ng/ml (RR = 2.42; 95% CI, 1.53–3.84) (202). In a cohort study of 3258 patients undergoing coronary angiography, after 8 yr, those at the lowest 25(OH)D levels [<8 ng/ml (20 nmol/liter)] had significantly higher CV mortality compared with those with higher levels [>28 ng/ml (69 nmol/liter)] (203). An analysis of 13,331 women and men over 8.7 yr from NHANES III found only a trend toward an increased risk (RR = 1.2; nonsignificant) in the lowest (<17.8 ng/ml) compared with the highest 25(OH)D levels but lower risk (nonsignificant) in the intermediate quartiles (204). However, they found that overall mortality was significantly higher (RR = 1.26; $P < 0.001$) in the lowest *vs.* highest quartiles (201). Marniemi *et al.* (205) found no significant relationship of serum 25(OH)D levels (lowest tertile) to MI but did find a relationship to stroke incidence. In a prospective cohort study of 1490 men over age 65 yr, followed for an average of 7.5 yr in the MrOs study (206) published since the Pittas review, there was no relationship of 25(OH)D level to CV mortality (HR = 1.01; 95% CI, 0.89–1.14) across its entire range. However, there was a trend (nonsignificant) toward a higher risk in those at the lowest level (<20 ng/ml) *vs.* those above 30 ng/ml (HR = 1.51; 95% CI, 0.82–2.76). Pittas *et al.* (115) did not perform a meta-analysis of observational cohort studies due to heterogeneity of outcomes.

Although most cohort studies have focused on risk of CVD among those with the lowest levels of serum 25(OH)D, several analyses allowed an examination of the higher levels and have suggested that risk does not continue to decrease at levels above 30 ng/ml. This includes the Framingham Osteoporosis Study for all CV events (201). The NHANES study found a higher risk for CVD mortality in those with 25(OH)D levels above 30 ng/ml overall, although risk began to increase with levels above 30 ng/ml and then declined with levels above 40 ng/ml (207). There are also a number of observational studies suggesting that overall mortality does not decrease further and may increase in those with higher levels of 25(OH)D. The IOM report suggested the possibility of increased risk of CV risk and mortality at the highest levels of 25(OH)D and that this possibility should be studied further (135).

Grandi *et al.* (208) pooled data from prospective observational studies and demonstrated an overall association of 25(OH)D baseline levels in the lowest compared with the highest 25(OH)D categories defined in each study. There was a significant relationship for incident composite CV events (pooled HR = 1.54; 95% CI, 1.22–1.95) and for CV mortality (HR = 1.83; 95% CI, 1.19–2.80). There was, however, significant heterogeneity and an indication for a possible publication bias in some of

these analyses (particularly for mortality), making the reported HR less reliable. The IOM report summarized the observational data as showing overall positive evidence for a relationship of low 25(OH)D levels to CVD but does not support the view that higher levels of 25(OH)D are associated with further lowering of risk (3). In sum, there is some evidence from observational data to suggest that low levels of 25(OH)D are associated with a greater risk of CVD. On the other hand, modestly higher levels of 25(OH)D may be associated with better health indices such as nutrition, sunlight exposure, and physical activity, which in turn could lead to a lower risk of CVD. Thus, confounding factors, even when controlled for, make it difficult to draw conclusions from these observational studies.

2. Randomized trials

There is a limited amount of evidence from randomized trials of vitamin D alone (*i.e.*, not in combination with calcium) *vs.* placebo to address the relationship to CVD. The Pittas *et al.* (115) review lists two such trials. Trivedi *et al.* (167) performed a randomized trial of vitamin D₃ (100,000 U every 4 months for 5 yr) *vs.* placebo. The primary endpoints were fractures and cause-specific mortality. They found a nonsignificant trend (RR = 0.84; 95% CI, 0.055–1.10) toward a reduction in CV deaths. Another RCT studied added vitamin D to ongoing calcium supplementation in 302 women; the primary endpoint was risk of falls (209). They reported as adverse events ischemic heart disease event rates of 1.3% in those on vitamin D *vs.* 2.0% for placebo (two *vs.* three events). Combining these two trials into a meta-analysis, Wang *et al.* report a pooled RR of 0.90 (0.77–1.05, not statistically significant) (210).

The Pittas *et al.* (115) review reports on two other randomized trials using vitamin D in combination with calcium *vs.* double placebo. In the largest of these, the WHI, more than 36,000 women were randomized to receive both 400 IU of vitamin D₃/d and 1000 mg of calcium/d or placebo (211, 212). After a 7-yr follow-up, no significant effect was reported on any of three CV outcomes (MI, coronary heart disease death, or stroke). The investigators also reported a nearly significant increased risk (RR = 1.09; 95% CI, 0.99 to 1.19) for a combined endpoint of nonfatal MI, coronary heart disease death, or revascularization. One other small trial (n = 192) of vitamin D (800 IU) in combination with calcium reported 11 CV events, which did not differ by treatment (213).

A recent meta-analysis by Elamin *et al.* (123) examined randomized trials of vitamin D (with or without calcium) for the endpoints of MI and stroke, as well as all-cause mortality. The authors performed a comprehensive literature search and found six studies (the four above plus two

others) that have reported on MI and/or stroke (including trials with nonfatal events as well as those with only fatal events). For MI, the pooled RR was 1.02 (95% CI, 0.93–1.13), and for stroke it was 1.05 (95% CI, 0.88–1.25). It is worth noting that the WHI accounted for approximately 90% of the participants in the pooled studies. Based on the result for MI and stroke, as well as the null results for overall mortality, the authors concluded that current trial evidence was not consistent with recommending vitamin D to patients to reduce CV risk.

3. Conclusions

The results from many, although not all, prospective observational studies of the relationship between CVD and 25(OH)D levels suggest that low levels of serum 25(OH)D are associated with future increased risk of CV outcomes. However, the interpretation of these findings is limited by the different outcomes assessed in the studies. More importantly, whereas the observational studies might suggest an association between low levels of 25(OH)D and future CVD, this association may not be causal, and therefore it cannot be assumed that increasing 25(OH)D levels through supplementation will reduce CV risk. As has been shown with several antioxidant vitamins and supplements (*e.g.*, vitamin E and selenium), well-conducted randomized trials can yield results that are inconsistent with previous positive observational evidence (214). Therefore, caution is required in generalizing observational evidence directly into clinical practice.

The randomized trial evidence is currently inadequate to define the relationship between vitamin D and reduction in CV events. The two trials of vitamin D alone discussed in *Section VII.C.* suggest a trend but not a significant reduction in CV events. The Trivedi *et al.* (167) trial used 100,000 IU four times per year, and therefore results may not be generalizable to more frequent/lower dose vitamin D supplementation. Although the WHI did not find a relationship between vitamin D supplementation and CVD, it used a low dose of vitamin D (400 IU) in combination with calcium. A recent meta-analysis raises the possibility that calcium supplementation might increase CV risk (199). Therefore, it is conceivable that vitamin D, by facilitating calcium delivery, might increase CV risk. Furthermore, the possibility that calcium increases CV risk complicates the interpretation of trials of vitamin D in combination with calcium with respect to CVD and suggests greater reliance on the limited evidence from trials that used vitamin D alone *vs.* placebo.

In conclusion, whereas there is a possibility that vitamin D supplementation may lower CVD risk, the limitations of applying observational data to clinical practice

and the insufficiency of the evidence from clinical trials do not support recommending vitamin D supplementation for lowering CVD risk at this time. This is consistent with the conclusions from the recent trial meta-analysis as well as the recent IOM report (3, 123). Additional research, particularly from randomized trials, is needed—particularly research examining whether there is a dose-response relationship of vitamin D supplementation to CV outcomes or whether high levels of supplementation might increase CVD.

VIII. Vitamin D and Immune Function

A. Introduction

The human immune response to invading microbes and cells is composed of two basic elements, the innate and adaptive immune response. “Innate” derives from the Latin word “innātus” meaning inborn or not conditioned by an acquired event. The human innate immune response is modulated principally by two cell types: the monocyte/macrophage and the dendritic cell. These two cell types are purposed to recognize, inactivate, or kill invaders as well as to draft cells of the adaptive immune response to protect the host from that invader. The so-called adaptive arm of the human immune response is composed of T and B lymphocytes. Through directed cell-to-cell contact or by the elaboration of cell-targeted, specific lymphokines T (Th₁, Th₁₇) and B Ig-producing lymphocytes act to promote destruction of the offending invader. On the other hand, Treg and Th₂ lymphocytes act to quell what might otherwise be an overzealous immune response to the invader that could illicit off-target damage to the host.

The first hint that cells of the innate and adaptive immune response in humans might be potential targets for 1,25-(OH)₂D-directed gene expression and the subject of functional consequences regulated by vitamin D balance in the host came from observations of human immune cell populations *in vitro*: 1) that human lymphocytes and monocyte-macrophage cells expressed the VDR when exposed to mitogens or specific antigens (215–219); 2) that the major effect on lymphocytes and monocytes was to limit their proliferation in response to mitogen and antigen stimulation (218, 219); 3) that disease-activated macrophages harvested from the lungs of patients with active granuloma-forming diseases, such as sarcoidosis and tuberculosis, were extremely efficient at synthesizing 1,25-(OH)₂D when incubated with substrate 25(OH)D (220); and 4) that 1,25-(OH)₂D production by the macrophage was driven by the T-cell cytokine interferon- γ (IFN- γ) (221). *In vivo* clinical correlates of these findings were: 1) the utility of 1-hydroxylated vitamin D metabolites or an-

alogs as therapeutic agents to control hyperproliferative disorders such as psoriasis (222); 2) the course of patients (lacking a renal source for the vitamin D hormone) with widespread, active granuloma-forming diseases can be complicated clinically by hypercalciuria or frank hypercalcemia if macrophage-produced 1,25-(OH)₂D escapes the local inflammatory microenvironment to the general circulation (223, 224); 3) going back to the preantibiotic era in the late 1800s and the early 1900s, observations that direct sunlight exposure at high altitudes was beneficial in the management of cutaneous and pulmonary tuberculosis (225); and 4) colocalization and concentration of the 25(OH)D-CYP27B1-hydroxylase stimulating Th1 cytokine IFN- γ and product 1,25-(OH)₂D at sites of inflammation in the human host with active granuloma-forming disease (226, 227).

What was not clearly recognized until recently was the molecular means by which macrophages could be activated to express the VDR and CYP27B1-hydroxylase in the presence of a disease-causing agent. For insight in this direction, lessons learned from the human macrophage exposed to the human pathogen *Mycobacterium tuberculosis* (mTB) will be the focus of much of the subsequent discussion. Liu *et al.* (67) investigated the consequence of the interaction of human macrophages with a pathogen-associated membrane pattern molecule from mTB recognized to activate a pair of pattern recognition receptors (PRR), the TLR 2/1 dimer pair (67, 228); PRR such as the TLR are unique in that they are activated not by specific antigens but by nonspecific products of microbes and other cells (229). The TLR, like other PRR in the human macrophage, are known to engage an intracellular adaptor proteins (*e.g.*, myD88), which signals through the cell's kinase systems to activate and translocate nuclear signaling molecules (*e.g.*, nuclear factor κ B) to transcribe monokine gene products that are then used to regulate (either up or down) the innate immune response in that cell or to direct the adaptive immune response (230). Working from previous studies that showed that the cathelicidin gene and its endogenous antibiotic-like gene product LL37 were under stimulatory control of a VDRE in the promoter of the cathelicidin gene, Liu *et al.* (67, 231) showed that inhibition of the CYP27B1-hydroxylating activity, blockade of the VDR with a competitive nonacting analog of 1,25-(OH)₂D, and small interfering RNA-directed knock-down of the LL37 mRNA translation all resulted in failure to synthesize LL37. These and more recent data suggest that: 1) the synthesis of 1,25-(OH)₂D and the VDR inside the human macrophage represents a self-contained, intracrine-acting system capable of killing mTB inside that cell by a combination of antimicrobial gene expression and co-opting the cell's autophagy pathway (232–234); 2) this

antimicrobial action is amplified under the influence of IFN- γ -driven, autocrine IL-15 and IL-1 β production (234, 235); and 3) this is dependent on extracellular availability of free 25(OH)D to the macrophage (236–238).

B. Clinical observations and trials

To date, clinical observations imputing a role for the vitamin D synthetic-metabolic system as a *bona fide* regulator of the human immune response *in vivo* has been largely confined to cross-sectional studies, many very large, in which a disease is associated with low 25(OH)D levels (239). For the most part, these diseases are ones in which TLR have been implicated in pathogenesis of the particular disorder and for which VDR and CYP27B1 expression lie downstream in the innate immune response elicited by that disease. For example, atherosclerosis, an inflammatory disease of the vasculature in which the TLR are implicated in its pathogenesis, has been shown to be significantly associated with 25(OH)D less than 30 ng/ml (240). In fact, an increase in all-cause mortality in the U.S. population has been linked to low population serum 25(OH)D levels (204). Most of this mortality is attributed to the consequences of atherosclerotic vascular disease, the number one killer in the United States. The occurrence of cancer, particularly colon cancer, in which the TLR2 and TLR4 signaling pathways are activated, has been associated with vitamin D deficiency (241). Infectious mycobacterial (TLR2/1 and TLR4) (233, 235), bacterial (TLR4 and TLR6) (242, 243), and viral diseases (TLR 7) (244), as well as autoimmune diseases (TLR7) (245) have all been significantly associated with low serum 25(OH)D levels in cross-sectional studies.

The IOM found inadequate data from clinical trials to support the utility of vitamin D supplementation in the treatment and prevention of infectious, inflammatory, hyperproliferative, and autoimmune disorders (135). One area of recent investigation that is at the intersection of immunology and metabolism has been the association of type 1 diabetes mellitus with vitamin D status. Although there are few high-quality randomized trials of vitamin D supplementation for the prevention of this type of diabetes, theoretically, changes in immune status (*i.e.*, self-recognition) could prevent or forestall the onset of β -cell dysfunction (246). Alternatively, vitamin D supplementation could alter the innate immune response to latent viruses, thereby impacting the disease course in a different manner (247). Intuitively, boosting the endogenous innate response with vitamin D and/or 25(OH)D supplementation in subjects susceptible to chronic diseases should be a safe and relatively inexpensive intervention. The 20,000-subject VITAL RCT (163) will likely provide important insights as to whether supplemental vitamin D will lower

the risk for infections, inflammatory disease, autoimmune disease, musculoskeletal deficiency, type 2 diabetes, and hypertension (secondary outcomes). Barring larger preliminary studies in type 1 diabetes mellitus, it is unlikely that a vitamin D intervention trial will be performed in younger individuals at high risk for developing type 1 diabetes mellitus.

C. Conclusions

There is a large body of evidence being generated *in vitro* and *ex vivo* to implicate the substrate-dependent, intracellular conversion of 25(OH)D to 1,25-(OH) $_2$ D with subsequent modulation in the bioactions of activated, VDR-expressing monocytes, macrophages, dendritic cells, and lymphocytes to control both the innate and adaptive immune response in man (Fig. 3). With the possible exception of psoriasis, in which the topical administration of 1-hydroxylated vitamin D metabolites and analogs has been shown to be both efficacious and safe, delivery of such vitamin D metabolites and analogs parenterally or orally to achieve an immunomodulatory effect in the host has been thwarted by off-target activation of the VDR in tissues that contribute an influx of calcium into the general circulation, resulting in hypercalciuria and hypercalcemia. These disappointing results and general failure to discover a nonhypercalcemic analog with immunomodulatory potential have led others to begin to employ the use of vitamin D and 25(OH)D supplements to boost the availability of 25(OH)D to the disease (TLR)-activated monocyte-macrophage. This approach would permit the host macrophage to generate 1,25-(OH) $_2$ D in a regulated fashion, which may in turn engage the VDR and turn on the host immune innate immune response. The expectation would be that this macrophage will be enhanced in its ability to: 1) neutralize invading microbes and foreign cells; 2) instruct the adaptive immune response in promoting this neutralization response at same time; and 3) prevent what might turn into an overzealous adaptive immune response that would prove detrimental, not beneficial, to the health of the host.

IX. Vitamin D, the Placenta, and Maternal/Fetal Health

A. Introduction

The placenta forms a physical and functional barrier between the maternal and fetal circulations. Within it, 1,25-(OH) $_2$ D could conceivably play autocrine, paracrine, or endocrine roles in regulating host defenses, trophoblast invasion, nutrient and gas exchange, hematopoiesis, hormone production, and fetal growth

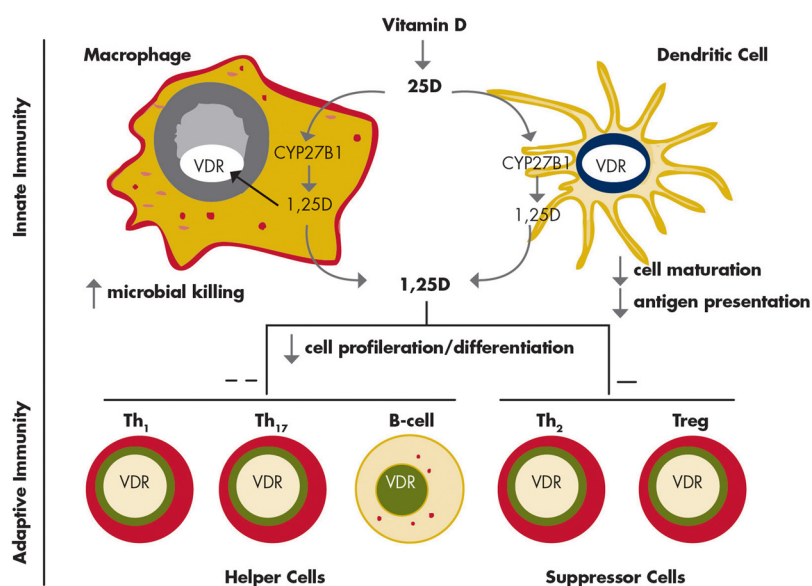
Figure 3.

Figure 3. Impact of vitamin D on the human innate (*upper panel*) and adaptive immune response (*lower panel*). When activated by mitogen or specific antigen, macrophages, dendritic cells, and lymphocytes express the VDR, thereby becoming targets for the active vitamin D metabolite, 1,25-(OH)₂D. Macrophages and dendritic cells can also express the CYP27B1-hydroxylase that synthesizes 1,25-(OH)₂D from substrate 25(OH)D, the major circulating metabolite of vitamin D and acknowledged best indicator of the amount of vitamin D entering the host via cutaneous synthesis or that ingested in the diet. Operating in an intracrine mode, 1,25-(OH)₂D promotes microbial killing in the macrophage, whereas it inhibits maturation and the antigen-presenting capacity of the dendritic cell. If 1,25-(OH)₂D escapes the confines of the macrophage or dendritic cell in sufficient amount, it can act on VDR-expressing lymphocytes recruited to the local inflammatory microenvironment. The major bioaction of 1,25-(OH)₂D acting through the VDR in lymphocytes is to inhibit their proliferation and differentiation to maturity; this antiproliferative effect is more profound on the classes of helper than suppressor cells, leading to generalized suppression of the adaptive immune response. 1,25D, 1,25-dihydroxyvitamin D; 25D, 25-hydroxyvitamin D.

and development. Since the 1970s, we have known that trophoblasts and maternal decidua convert 25(OH)D to 1,25-(OH)₂D, whereas more recent studies have confirmed that this is due to expression of CYP27B1 (248–252). In 1983, placental expression of VDR was inferred by binding of radiolabeled calcitriol to rat trophoblasts (253), and subsequent studies confirmed that trophoblasts, yolk sac, and decidua of humans, sheep, mouse, and rat express VDR (254–256). CYP24A1 is also expressed by trophoblasts, yolk sac, and decidua, where it converts 25(OH)D and 1,25-(OH)₂D into inactive forms (251, 257, 258).

One study of human placentas found high levels of VDR and CYP27B1 mRNA, but low levels of CYP24A1 mRNA, as compared with adjacent decidua (259). When these findings are taken together with evidence that CYP24A1 is methylated in human placenta (260), some investigators have concluded that trophoblast synthesis of 1,25-(OH)₂D is

unopposed or “unfettered” by degradation to 24-hydroxylated forms (260, 261). However, this conclusion is not supported by the burden of functional evidence. Human and rodent placentas preferentially metabolize 25(OH)D to 24,25-dihydroxyvitamin D over calcitriol (262–264), resulting in fetal levels of 24-hydroxylated forms that are up to 40-fold higher than 1,25-(OH)₂D in humans, rats, and sheep (263–267). The placenta controls passage of vitamin D metabolites such that 25(OH)D freely crosses hemochorial placentas whereas 1,25-(OH)₂D is blocked (263, 268). This explains why cord blood 25(OH)D levels are typically about 75–100% of maternal values at term whereas 1,25-(OH)₂D is 25–40% of maternal levels (269). Maternal nephrectomy did not alter fetal 24,25-dihydroxyvitamin D or 1,25-(OH)₂D levels, confirming that these metabolites are independently synthesized in the fetal-placental unit (264). Placenta and fetal kidneys both contribute to the low level of 1,25-(OH)₂D in the fetal circulation (270). On the other hand, low vitamin D binding protein in the fetal circulation means that the free 1,25-(OH)₂D level may be normal or even increased (271).

Maternal levels of 1,25-(OH)₂D double or triple during pregnancy, whereas free levels do not increase until the third trimester (269, 271.) Placental production of 1,25-(OH)₂D has often been assumed to explain the higher maternal levels, but this is incorrect. The rat placenta contributes a small amount of 1,25-(OH)₂D to the maternal circulation, whereas only one sixth remnant of maternal kidney is sufficient to enable the normal pregnancy-induced increase in 1,25-(OH)₂D (248, 272, 273). 1 α -Hydroxylase null pigs have very low levels of 1,25-(OH)₂D, with no increase during pregnancy despite bearing heterozygous placentas (274). The most compelling human data come from a pregnant anephric woman who had very low 1,25-(OH)₂D levels that did not change significantly during pregnancy (275). Overall, in pregnant mammals it appears that most or all of the 1,25-(OH)₂D comes from a 2- to 5-fold up-regulation in CYP27B1 within maternal kidneys (276, 277). 1,25-(OH)₂D that is produced by the fetal-placental unit likely doesn't affect the mother.

B. Biological plausibility

The placenta has immunoregulatory functions that enable successful implantation, block most maternal anti-

bodies and blood cells, and defend against microbial organisms. The importance of this role is underscored by the realization that intrauterine infections explain much of the risk of preterm birth and intrauterine growth retardation (278, 279). The TLR are essential components of the innate response to pathogen-associated microbial products, and trophoblasts express at least 10 TLR to some degree (280). Trophoblasts also express cathelicidin and other antimicrobial proteins, including bactericidal/permeability-increasing protein, secretory leukocyte protease inhibitor, human β -defensin 2, and acyloxyacyl hydrolase (281). Placenta and adjacent decidua also contain abundant immune cells (macrophages, dendritic cells, lymphocytes) that, as mentioned earlier, synthesize and respond to 1,25-(OH)₂D.

There is biological plausibility for 1,25-(OH)₂D to play a role in regulating placental defenses against infection. Injection of normal pregnant mice with lipopolysaccharide (LPS), a TLR ligand, caused marked elevation in placental expression of 1 α -hydroxylase and VDR (282). *Vdr* null and *Cyp27b1* null trophoblasts cultured *in vitro* had dysregulated inflammatory markers at baseline (increased IFN- γ , decreased IL-10) and in response to treatment with LPS (increased TLR2, IFN- γ , and IL-6) (282). Treatment of a trophoblast cell line with 25(OH)D before challenge with *Escherichia coli* protected against trophoblast cell death and led to fewer bacterial colony-forming units being formed (283). TLR2 was also increased in placentas obtained from pregnancies with documented preterm infection (284). In contrast to what occurs in macrophages, cathelicidin and other antimicrobial proteins were not induced by LPS or other TLR ligands (281, 283), and this was thought to be caused by absence of TLR4 in trophoblasts (281).

Because preeclampsia is considered a disease caused by dysfunctional trophoblasts, there is biological plausibility that altered vitamin D metabolism could locally predispose to preeclampsia. Several TLR (TLR-2, TLR-3, TLR-4, and TLR-9) were up-regulated in placentas from preeclamptic *vs.* normal women (285). Other investigators sought a causative role for altered placental expression of 1 α -hydroxylase but found conflicting results of decreased (286) and increased expression of 1 α -hydroxylase in preeclamptic *vs.* normal placentas (287).

These and other data support the hypothesis that 1,25-(OH)₂D may act in a paracrine or autocrine manner to influence trophoblast growth and responses to infection and inflammation. In turn, loss of such actions might predispose to preeclampsia, placental infections and insufficiency, preterm birth, and certain immune-related disorders (*e.g.*, type 1 diabetes). None of these studies address what intake of vitamin D or circulating level of 25(OH)D

is required to achieve the postulated effects of 1,25-(OH)₂D on trophoblasts *in vivo*.

C. Animal data

Pregnancy and fetal development have been examined in severely vitamin D-deficient rats (288–290), 1 α -hydroxylase null pigs (291), and *Vdr* null mice (292, 293). 1 α -Hydroxylase null (*Cyp27b1* null) mice are infertile. Fetal-placental mineral and skeletal homeostasis appears unaffected in all of these models, and these data are reviewed elsewhere (269, 294). Local production of 1,25-(OH)₂D by maternal decidua, immune cells, and invading trophoblasts has been proposed to critically regulate implantation and growth at the maternal-fetal interface (261, 295). At first glance, this theory appears to be supported by the findings that severely vitamin D-deficient rats and *Vdr* null mice conceive less often and bear fewer pups than normal and that *Cyp27b1* null mice were reported to be infertile (73, 289, 296, 298). However, the conception rate and litter sizes of *Vdr* null mice are normalized simply with a higher calcium diet (292, 293, 299, 301), whereas *Cyp27b1* null mice may have more fundamental problems of hypoplastic uteri and failure to ovulate (73). Although the size and weight of pups born of severely vitamin D-deficient rats are normal (289, 296, 298), pups born of *Vdr* null mothers are globally smaller than normal (293), which is consistent with a role for VDR in fetal growth or nutrition. Gestational length is normal in all mouse, rat, and pig models, with no reported evidence of infections, preeclampsia, or preterm births. Severe vitamin D deficiency beginning during gestation does not increase the risk of type 1 diabetes in otherwise normal rodents, but it causes diabetes to emerge earlier in nonobese diabetic (NOD) mice (302). Supraphysiological doses of vitamin D given *in utero* did not prevent diabetes in NOD mice (303). In contrast, *Vdr* null mice are not predisposed to develop type 1 diabetes, and *Vdr*-NOD double-mutants have the same risk of diabetes as NOD mice (304). These studies suggest that severe vitamin D deficiency increases the risk of type 1 diabetes only in genetically predisposed NOD mice and that the effect is not mediated by VDR. Vitamin D deficiency during rat gestation has been shown to cause subtle changes in brain development that may lead to impaired dopaminergic and cognitive function as adults (305–307).

D. Observational and association studies

Available observational data come from vitamin D-deficient to -sufficient pregnant women who participated in observational studies and in the placebo arms of clinical trials of vitamin D supplementation (269). In none of these reports were increased adverse obstetrical/neonatal out-

comes reported, but low numbers of subjects meant that few adverse events occurred. Similarly, women with genetic absence of 1α -hydroxylase or VDR (vitamin D-dependent rickets types I and II, respectively) have been reported to have normal fertility and uneventful pregnancies (269). Numerous associations have been examined between single measurements of maternal 25(OH)D or estimates of vitamin D intake and various obstetrical/neonatal outcomes. At best, these studies indirectly implicate altered fetal-placental vitamin D physiology as causing the outcome of interest. Some studies suggest that low maternal 25(OH)D predicts increased risk of preterm birth (308), threatened preterm delivery (308), and cesarean section (309), whereas other studies found no significant associations with these outcomes (310–313). These studies had low power, differed in their methods to measure 25(OH)D, and differed in the cut-points used to define vitamin D deficiency. They were also confounded by factors that predict low 25(OH)D and the outcomes of interest, including race/ethnicity, maternal overweight/obesity, lower socioeconomic status, poor nutrition, *etc.* For example, overweight/obesity predisposes to preeclampsia, gestational diabetes, vaginal infections, macrosomia, and other obstetrical complications (314).

Preeclampsia is associated with normal maternal ionized and albumin-corrected serum calcium, but lower calcium intake, hypocalciuria, lower $1,25\text{-(OH)}_2\text{D}$ levels, and reduced creatinine clearance (294). $1,25\text{-(OH)}_2\text{D}$ may be reduced to the level of healthy nonpregnant women. Low maternal 25(OH)D and low estimated vitamin D intake have also been associated with preeclampsia (315–318), although an equal number of studies refuted this association (310, 319–321). Rather than causing preeclampsia, the low $1,25\text{-(OH)}_2\text{D}$ and hypocalciuria may result from the renal damage that occurs with the condition. Consistent with this, preeclamptic women had normal $1,25\text{-(OH)}_2\text{D}$ levels earlier in pregnancy and low calcitriol only after developing hypertension and proteinuria (322). Moreover, because serum levels of fat-soluble vitamins A, D, and E were all lower in preeclamptic women *vs.* normotensive pregnant and nonpregnant controls (323), a confounding factor such as overweight/obesity or nutrition may explain why the fat-soluble vitamins were reduced.

Other associational studies have explored “fetal programming,” the concept that low 25(OH)D stores during gestation program the emergence of disorders in the child or adult. (Studies that used season of birth to implicate vitamin D during late gestation are not specific enough to warrant consideration in this review.) For type 1 diabetes, a higher maternal dietary intake of vitamin D during pregnancy was associated with decreased prevalence of islet

cell antibodies and diabetes in the children; curiously, maternal use of vitamin D supplements had no effect (324). In other studies, recalled use of vitamin D supplements during pregnancy was associated with lower childhood incidence of type 1 diabetes (325, 326). But another study found no association between maternal 25(OH)D during pregnancy and type 1 diabetes in the offspring (327). For childhood asthma and allergy, some studies found that higher maternal intake of vitamin D during pregnancy decreased the risk (328, 329), whereas other studies found that higher maternal 25(OH)D levels during pregnancy increased the risk as much as 5-fold (330, 331). Investigators who found effects of fetal vitamin D deficiency on rat brain neurodevelopment looked at banked human serum and found no association between maternal 25(OH)D levels during late pregnancy and risk of schizophrenia in the offspring (307).

Associational and ecological studies are hypothesis-generating but do not prove causality. The studies described above provide inconsistent and conflicting evidence, and no proof that higher intakes of vitamin D during pregnancy will prevent any adverse nonskeletal outcomes.

E. Randomized interventional trials

The finding that preeclampsia is associated with low calcium intake and hypocalciuria prompted numerous randomized, placebo-controlled clinical trials of calcium supplementation in women at risk of preeclampsia. A recent meta-analysis found that 1 g of supplemental calcium significantly reduced the risk of preeclampsia in women who had low dietary calcium intake at baseline (RR = 0.36), or a high baseline risk of preeclampsia (RR = 0.22). There was no benefit when dietary calcium intake was adequate (332). In the same meta-analysis, preterm birth was also significantly decreased by the use of calcium supplements (RR = 0.76) (332). Multiple clinical trials have tested whether vitamin D supplementation improves maternal or fetal outcomes of pregnancy (269). The consistent finding is that supplemental vitamin D increases maternal and cord blood 25(OH)D levels. No study demonstrated any other obstetrical benefit, including those that specifically reported preeclampsia (333, 335–337) or preterm birth and low birth weight (333, 335, 338). The most extreme example raised the cord blood 25(OH)D level from 10 to 138 nmol/liter without obvious obstetrical benefits (334). However, none of these studies was sufficiently powered to detect a difference in many of the outcomes.

Two recent studies by Hollis *et al.* (333) warrant additional consideration. The first study randomized 494 women at 12–16 wk to receive 400, 2000, or 4000 IU of

vitamin D₃/d; 350 subjects (70.9%) completed the trial. Achieved mean maternal 25(OH)D was 78.9 ± 36.5 , 98.3 ± 34.2 , and 111.0 ± 43.0 nmol/liter, respectively (333). The second study enrolled 257 women at 12–16 wk to receive 2000 or 4000 IU of vitamin D₃/d; 160 subjects (62.3%) completed it. The first study has been published, whereas results of both studies have been presented at conferences and on YouTube (333, 335). In the intention-to-treat analysis for each study, no effect was seen on birth weight, gestational length, preterm birth, early preterm birth (<32 wk), preeclampsia, infections, cesarean sections, gestational diabetes, or other obstetrical outcomes (333, 335). Consequently, because no maternal or fetal/neonatal benefit was found, these two studies do not provide any evidence to justify a particular level of 25(OH)D or intake of vitamin D during pregnancy.

F. Conclusions

1,25-(OH)₂D and its receptor are well poised in the placenta to influence obstetrical and neonatal outcomes, but whether they truly play a significant role remains unproven. Consequently, there is insufficient evidence to recommend a particular maternal intake of vitamin D or 25(OH)D blood level during pregnancy to achieve any purported nonskeletal benefit of vitamin D. On the other hand, the biological plausibility may be sufficient to justify clinical trials to test whether vitamin D supplementation during pregnancy will prevent type 1 diabetes in the offspring.

X. Summary and Future Direction

Vitamin D is a pleiotropic hormone that affects classical and nonclassical tissues principally through the VDR. Its primary sites of action are still considered to be the intestine, bone, and kidneys. In regard to the former, changes in intestinal calcium absorption may play a major role in modulating cardiac and skeletal muscle function, although it is not absolutely clear whether in certain muscle cells, vitamin D may directly regulate function through the VDR. Nevertheless, it is likely that deficiencies in vitamin D may contribute to a modest risk for falls, particularly in older individuals. On the other hand, there is emerging evidence that vitamin D may directly regulate immune function, both innate and adaptive. However, it will require large well-designed clinical trials to prove that vitamin D supplementation could enhance innate immunity or reduce the severity of autoimmunity.

The role of vitamin D in the CV system is complex and will require further trials to define whether outcomes such as hypertension, MI, and stroke are directly related to

vitamin D supplementation. The *in vitro* antiproliferative effects of vitamin D on neoplastic tissue are well recognized, but the clinical evidence has been relatively modest due to the short duration of follow-up and the relatively small number of subjects in previous trials. The 20,000-person VITAL trial, which has just been initiated, may help determine what effect vitamin D supplementation has on both neoplastic and CV outcomes. Although all the elements of the vitamin D regulatory system are present in skin, randomized trials have not demonstrated that this hormone prevents skin cancer or is better than other agents for the treatment of proliferative skin disorders. In respect to the relationship between vitamin D and placenta and maternal/fetal health, the observational studies provide somewhat conflicting evidence, and the randomized trials for preeclampsia and neonatal outcome do not show clear benefits for mother or child.

In summary, not surprisingly there remains a persistent need for large randomized controlled trials and dose-response data to test the effects of vitamin D on chronic disease outcomes including autoimmunity, obesity, diabetes mellitus, hypertension, and heart disease. The VITAL trial, as noted above, could help determine whether higher doses of vitamin D (*i.e.*, 2000 IU/d) will reduce the risk of osteoporosis, cancer, and CVD. Similarly, a very large, placebo-controlled, randomized trial of vitamin D, 4000 IU/d, to prevent the onset of type 2 diabetes mellitus in prediabetics is currently in the planning stage. Any potential benefit of high-dose vitamin D supplementation on maternal or fetal outcomes will also await larger trials. Notwithstanding, large-scale clinical trials of a single nutrient may not fully answer the many questions inherent in vitamin D actions. Thus, the role of vitamin D supplementation in the prevention and treatment of chronic nonskeletal diseases remains to be determined.

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Review

Vitamin D—Effects on Skeletal and Extraskkeletal Health and the Need for Supplementation

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Abstract: Vitamin D, the sunshine vitamin, has received a lot of attention recently as a result of a meteoric rise in the number of publications showing that vitamin D plays a crucial role in a plethora of physiological functions and associating vitamin D deficiency with many acute and chronic illnesses including disorders of calcium metabolism, autoimmune diseases, some cancers, type 2 diabetes mellitus, cardiovascular disease and infectious diseases. Vitamin D deficiency is now recognized as a global pandemic. The major cause for vitamin D deficiency is the lack of appreciation that sun exposure has been and continues to be the major source of vitamin D for children and adults of all ages. Vitamin D plays a crucial role in the development and maintenance of a healthy skeleton throughout life. There remains some controversy regarding what blood level of 25-hydroxyvitamin D should be attained for both bone health and reducing risk for vitamin D deficiency associated acute and chronic diseases and how much vitamin D should be supplemented.

Keywords: vitamin D; 25-hydroxyvitamin D; vitamin D deficiency; osteoporosis; fractures; cancer; type 2 diabetes mellitus; cardiovascular diseases; autoimmune diseases; infectious diseases

1. Introduction

Vitamin D has been produced by phytoplankton for more than 500 million years [1] and is thought to be the oldest of all hormones whose function initially could have been the protection of ultraviolet-sensitive macromolecules including proteins, DNA and RNA, when these early forms of life were exposed to sunlight for photosynthesis. Later, after the evolution of ocean dwelling animals with vertebral skeletons ventured onto land, the maintenance of calcium homeostasis was a major physiological problem (as opposed to living in the calcium-rich ocean). It was vitamin D that ensured the efficient intestinal calcium absorption from dietary sources and ultimately was essential for the development and maintenance of a calcified mammalian skeleton [2]. Obtaining vitamin D from either sunlight or diet is still critical for most vertebrates for their skeletal health [1,3–5]. Over time, vitamin D has evolved into a hormone having numerous extraskeletal effects by regulating up to estimated 2000 genes [6,7].

Ethnic and gender differences in skin pigmentation indicate the evolutionary importance of a sufficient vitamin D supply. The varying degrees of depigmentation that evolved in order to permit UVB-induced synthesis of previtamin D₃ when hominids migrated outside the tropics can be considered as a compromise solution to the conflicting physiological requirements of vitamin D synthesis and photoprotection that differ depending on latitude and thus warrant different degrees of skin pigmentation. An evolutionary selection pressure towards a lighter skin coloration going along with a higher ability to produce vitamin D seems not only to be exerted by living in geographic regions with a lower UV intensity but also by being female. Gender differences in skin pigmentation with females being lighter skinned than males in all populations for which data about the skin reflectance was available could be explained by the higher needs of vitamin D during pregnancy and lactation [8].

2. Vitamin D—Sources

The main sources of vitamin D are sunlight, supplements and diet [7] (Table 1).

Table 1. Sources of vitamin D₂ and vitamin D₃ [7]. Note: This table is modified and reproduced with permission from [7], Copyright © 2007 Massachusetts Medical Society.

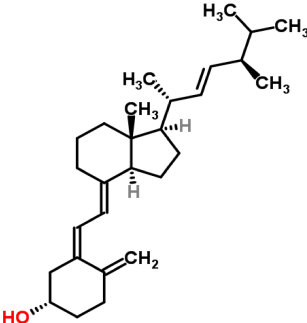
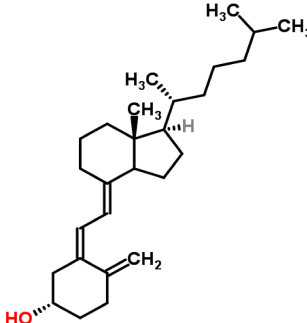
Source	Vitamin D Content IU = 25 ng
Chemical structures of vitamin D ₂ [9] and vitamin D ₃ [10]. Vitamin D ₂ (Ergocalciferol) Vitamin D ₃ (Cholecalciferol)	
	

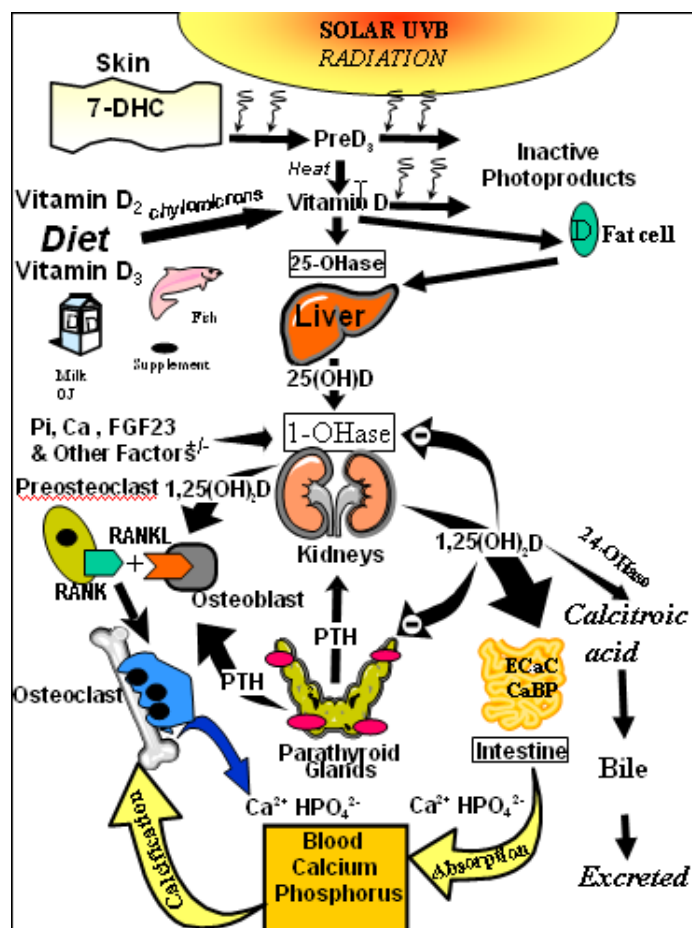
Table 1. Cont.

<i>Natural sources</i>	
Cod liver oil	~400–1000 IU/tsp vitamin D ₃
Egg yolk	~20 IU/yolk vitamin D ₃ or D ₂
Mackerel, canned	~250 IU/3.5 oz vitamin D ₃
Salmon, canned	~300–600 IU/3.5 oz vitamin D ₃
Salmon, fresh farmed	~100–250 IU/3.5 oz vitamin D ₃ , vitamin D ₂
Sardines, canned	~300 IU/3.5 oz vitamin D ₃
Shiitake mushrooms, fresh	~100 IU/3.5 oz vitamin D ₂
Shiitake mushrooms, sun dried	~1600 IU/3.5 oz vitamin D ₂
Sunlight/UVB radiation	~20,000 IU equivalent to exposure to 1 minimal erythral dose (MED) in a bathing suit. Thus, exposure of arms and legs to 0.5 MED is equivalent to ingesting ~3000 IU vitamin D ₃
Tuna, canned	236 IU/3.5 oz vitamin D ₃
<i>Fortified foods</i>	
Fortified breakfast cereals	~100 IU/serving usually vitamin D ₃
Fortified butter	56 IU/3.5 oz usually vitamin D ₃
Fortified cheeses	100 IU/3 oz usually vitamin D ₃
Fortified margarine	429/3.5 oz usually vitamin D ₃
Fortified milk	100 IU/8 oz usually vitamin D ₃
Fortified orange juice	100 IU/8 oz vitamin D ₃
Fortified yogurts	100 IU/8 oz usually vitamin D ₃
Infant formulas	100 IU/8 oz vitamin D ₃
<i>Pharmaceutical Sources in the United States</i>	
Drisdol (vitamin D ₂) liquid	8000 IU/mL
Vitamin D ₂ (Ergocalciferol)	50,000 IU/capsule
<i>Supplemental Sources</i>	
Multivitamin	400, 500, and 1000 IU vitamin D ₃ or vitamin D ₂
Vitamin D ₃	400, 800, 1000, 2000, 5000, 10,000, 14,000, and 50,000 IU

Exposure of human skin to solar UVB radiation (wavelengths: 290–315 nm) leads to the conversion of 7-dehydrocholesterol to previtamin D₃ in the skin. Previtamin D₃ is then rapidly converted to vitamin D₃ (cholecalciferol) by temperature- and membrane-dependent processes [7,11,12] (Figure 1).

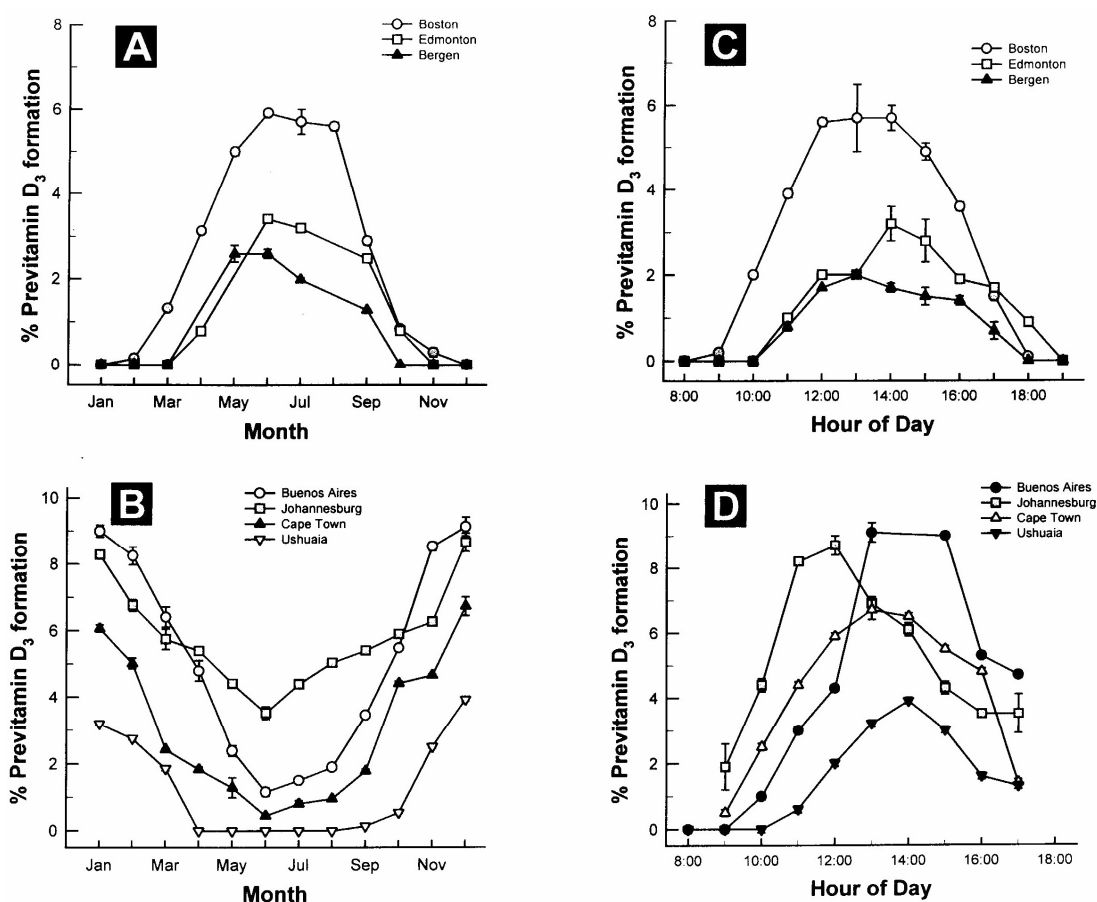
Figure 1. Schematic representation of the synthesis and metabolism of vitamin D for regulating calcium, phosphorus and bone metabolism [7]. During exposure to sunlight, 7-dehydrocholesterol in the skin is converted to previtamin D₃. Previtamin D₃ immediately converts by a heat dependent process to vitamin D₃ [7,11,12]. Excessive exposure to sunlight degrades previtamin D₃ and vitamin D₃ into inactive photoproducts [13]. Vitamin D₂ and vitamin D₃ from dietary sources is incorporated into chylomicrons, transported by the lymphatic system into the venous circulation [14]. Vitamin D (D represents D₂ or D₃) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein which transports it to the liver where vitamin D is converted by the vitamin D-25-hydroxylase to

25-hydroxyvitamin D [25(OH)D]. This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status [7,15] (although most reference laboratories report the normal range to be 20–100 ng/mL, the preferred healthful range is 30–60 ng/mL) [7]. It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1 α -hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [1,25(OH)₂D] [7,15–17]. Serum phosphorus, calcium, fibroblast growth factors (FGF-23) and other factors can either increase (+) or decrease (–) the renal production of 1,25(OH)₂D [7]. 1,25(OH)₂D feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands [6,7]. 1,25(OH)₂D increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize 1,25(OH)₂D to the water soluble biologically inactive calcitroic acid which is excreted in the bile [7,18]. 1,25(OH)₂D enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9K (calcium binding protein; CaBP) [7,19,20]. 1,25(OH)₂D is recognized by its receptor in osteoblasts causing an increase in the expression of receptor activator of NF κ B ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels [7,17]. Adequate calcium and phosphorus levels promote the mineralization of the skeleton [7]. Note: This figure is reproduced with permission from [21], Copyright © 2007 Michael F. Holick.



The amount of vitamin D production in the skin depends on the incident angle of the sun and thus on latitude, season and time of the day. It is highest when the sun is in the zenith and a flattening of the incident angle leads to a reduced vitamin D production [17]. Whole body exposure to sunlight with one minimal erythema dose (MED), *i.e.*, the minimal dose leading to pink coloration of the skin 24 h after exposure, leads to vitamin D levels comparable to oral intake of 10,000 to up to 25,000 IU vitamin D₂ [16,22]. However, sun exposure during most of the winter at latitudes above and below ~33 degrees North and South, respectively, doesn't lead to any production of vitamin D₃ in the skin [16,23] (Figure 2). Other factors influencing the cutaneous vitamin D production adversely are an increase in skin pigmentation, aging, especially age >65 years and the topical application of a sunscreen [17].

Figure 2. Influence of season, time of day, and latitude on the synthesis of previtamin D₃ in Northern (A and C) and Southern hemispheres (B and D). The hour indicated in C and D is the end of the 1-h exposure time. Note: This figure is reproduced with permission from [13], Copyright © 2010 Humana Press.



The number of foods naturally containing vitamin D in significant amounts is very limited. Among these are oily fish such as salmon, sardines and tuna, and oils of the liver of some fish such as cod as well as sun-exposed mushrooms [7] (Table 1). To increase the content of vitamin D₂ in mushrooms producers are irradiating them with UV radiation [24,25].

In the 1930s, the fortification of milk, sodas, bread and even beer became popular [26]; however, after several cases of presumed vitamin D intoxication in infants in the 1950s in Great Britain [27]

strict regulations limiting vitamin D fortification to only margarine were introduced in Europe [14,28]. Due to a relatively high prevalence of lactose intolerance leading to an avoidance of milk by many adults, the fortification of orange juice in the US was introduced as a novel approach of enhancing the vitamin D status of the public in the 2003 and proved to be as effective as oral supplementation [26,29]. Other fortified foods include margarine, yogurt, infant formula, butter, cheese and breakfast cereals [7] (Table 1).

Vitamin D₂ and vitamin D₃ are available as oral over-the-counter supplements. In the US, only vitamin D₂ is available as prescription drug [7,17]. Although there has been debate as to whether vitamin D₂ is as effective as vitamin D₃ in maintaining vitamin D status [30–36], other studies in children and adults have demonstrated that they are equally effective [29,37–40].

3. Vitamin D—Metabolism

Vitamin D from cutaneous synthesis or dietary/supplemental intake, is transported to the fat where it can be stored or to the liver for the first step of activation, the hydroxylation to 25-hydroxyvitamin D [25(OH)D], which is the major circulating form of vitamin D [7,15] and measured to assess a patient's vitamin D status [7,16,41,42] (Figure 1).

25(OH)D is metabolized in the kidneys by the mitochondrial enzyme 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1) to generate the systemically circulating active form, 1,25-dihydroxyvitamin D [1,25(OH)₂D] [7,15–17]. The renal synthesis of 1,25(OH)₂D is regulated by several factors including serum phosphorus, calcium, fibroblast growth factor 23 (FGF-23), parathormone (PTH) and itself [7]. CYP27B1 is also expressed extrarenally in a multitude of tissues [17,43], including bone, placenta, prostate, keratinocytes, macrophages, T-lymphocytes, dendritic cells, several cancer cells [44], and the parathyroid gland [45] and enables the production of 1,25(OH)₂D. This active form of vitamin D is locally active and exerts auto- or paracrine effects [15,17].

1,25(OH)₂D induces its own destruction by rapidly inducing the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which leads to the multistep catabolism of both 25(OH)D and 1,25(OH)₂D into biologically inactive, water-soluble metabolites including calcitric acid [7,18] (Figure 1).

4. Vitamin D Receptor (VDR)—Distribution and Function

1,25(OH)₂D, either produced in the kidneys [7] or extrarenally in the target tissues [15,17], is the ligand of the vitamin D receptor (VDR) whose widespread distribution across many tissues explains the myriad of physiological actions of vitamin D. By interacting with the VDR, a transcription factor [17,46], 1,25(OH)₂D regulates directly and indirectly the expression of up to 2000 genes [6,7], many of whose promoters contain specific vitamin D response elements (VDRE). The VDR partners with other transcription factors, most importantly the retinoid X receptor (RXR) [47], and coactivators and corepressors provide target gene specificity [48–50]. A membrane-bound VDR may also exist and mediate more immediate, non-genomic actions of 1,25(OH)₂D [44,51,52].

5. Prevalence of Vitamin D Deficiency and Insufficiency

25(OH)D is the vitamin D metabolite that is measured to assess a patient's vitamin D status [7,17]. Vitamin D deficiency is diagnosed when 25(OH)D <20 ng/mL [16,53], vitamin D insufficiency is

defined as 25(OH)D of 21–29 ng/mL, and 25(OH)D >30 ng/mL is considered sufficient, with 40–60 ng/mL being the preferred range [16]. Vitamin D intoxication usually doesn't occur until 25(OH)D >150 ng/mL [7,16,23].

These reference values are in part based on the finding, that the decline of parathyroid hormone (PTH) concentrations with increasing 25(OH)D levels in adults reached its nadir asymptotically at a 25(OH)D of ~30–40 ng/mL in several studies [7,16,23,54–56]. However, a recent cross-sectional analysis of more than 300,000 paired serum PTH and 25(OH)D levels revealed no threshold, even at 25(OH)D levels >60 ng/mL, above which a further increase of the 25(OH)D level failed to further suppress PTH levels. The analysis also showed a strong age-dependency of the PTH-25(OH)D relationship [57].

According to studies in Canada, 30%–50% of children and adults are vitamin D deficient [58–60]. The National Health and Nutrition Examination Surveys 2001–2006 showed a prevalence of vitamin D deficiency of 33% [60,61]. Studies in Indian school children revealed a prevalence of severe vitamin D deficiency (<9 ng/mL) in more than 35% [62] and over 80% of pregnant women in India had 25(OH)D levels <22.5 ng/mL [63]. Also reports from Africa [64], Australia [65], Brazil [66], Middle East [67,68], Mongolia [69], and New Zealand [70] documented a high risk for vitamin D deficiency in both adults and children [60,71].

Based on these findings, it has been estimated that 1 billion people worldwide are vitamin D deficient or insufficient [7,60] (Figure 3A–C).

Figure 3. (A) Prevalence at risk of vitamin D deficiency defined as a 25-hydroxyvitamin D <12–20 ng/mL by age and sex: United States, 2001–2006. (B) Mean intake of vitamin D (IU) from food and food plus dietary supplements from Continuing Survey of Food Intakes by Individuals (CSFII) 1994–1996, 1998 and the Third National Health and Nutrition Examination Survey (NHANES III) 1988–1994. (C) Reported incidence of vitamin D deficiency defined as a 25-hydroxyvitamin D <20 ng/mL around the globe including Australia (AU), Canada (CA), China (CH), India (IN), Korea (KR), Malaysia (MA), Middle East (ME), Mongolia (MO), New Zealand (NZ), North Africa (NA), Northern Europe (NE), United States (USA) [60]. Note: This figure is reproduced with permission from [60], Copyright © 2012 The Endocrine Society.

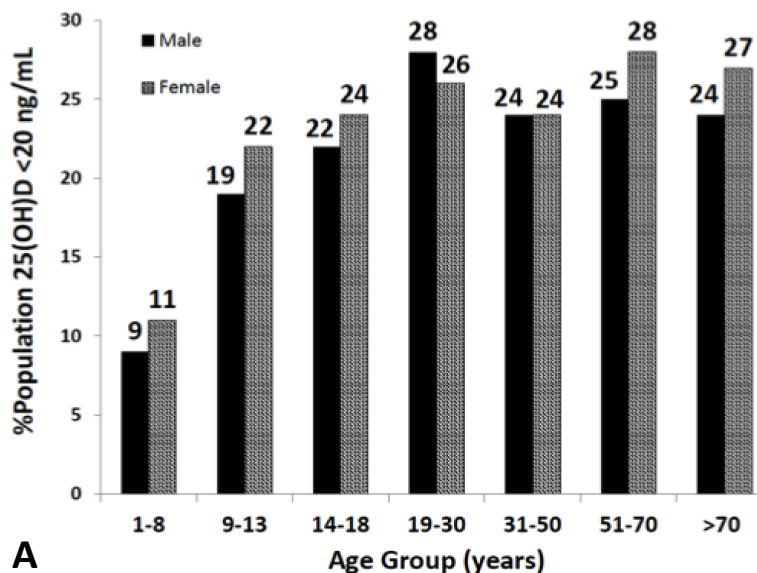
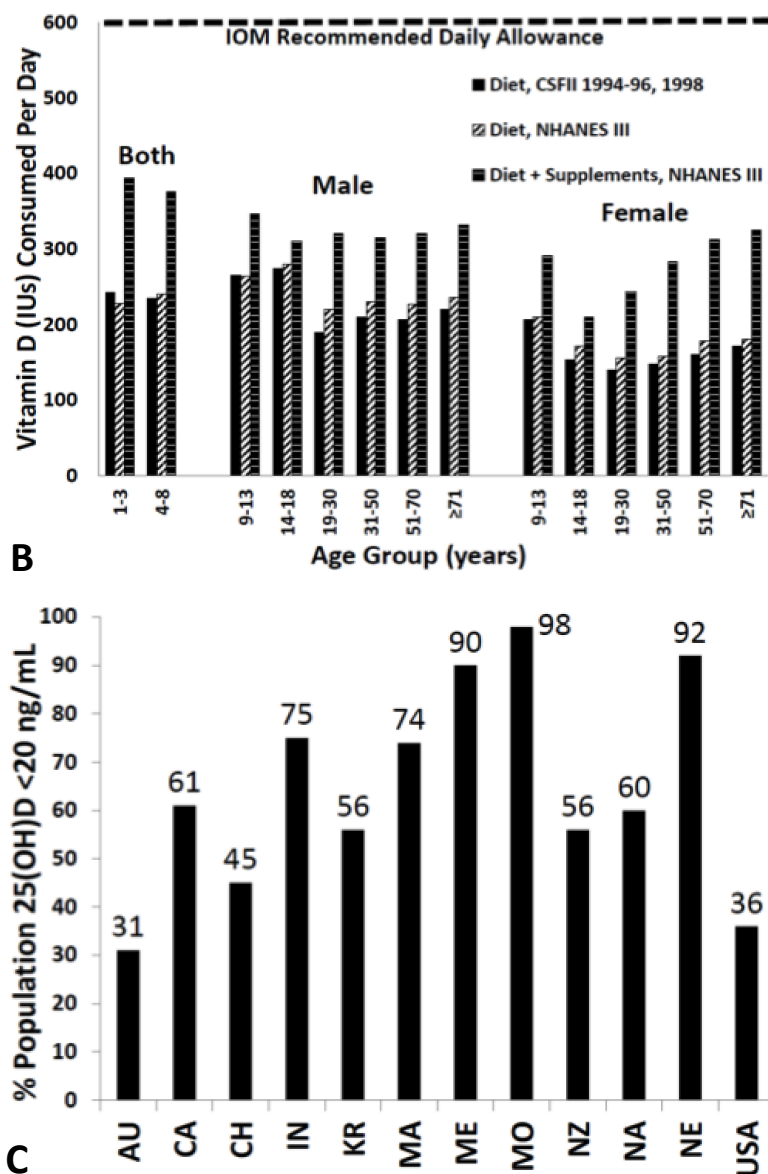


Figure 3. Cont.



6. Vitamin D and Calcium and Phosphorus Metabolism

Vitamin D plays an important role in the calcium and phosphorus metabolism and helps ensure adequate levels of these minerals for metabolic functions and bone mineralization [7]. 1,25(OH)₂D increases the efficiency of intestinal calcium absorption from 10%–15% to 30%–40% by interacting with the VDR-RXR and thereby promoting the expression of an epithelial calcium channel and a calcium-binding protein [7,19,20]. Based on several experiments conducted in rodents [72,73] it has been estimated that 1,25(OH)₂D also increases the intestinal phosphorus absorption from 50%–60% to approximately 80% [7,14].

Vitamin D also mediates indirect effects on calcium and phosphorus by regulating the PTH levels. The parathyroid glands have CYP27B1 activity and the local production of 1,25(OH)₂D using 25(OH)D as substrate could inhibit the synthesis of PTH [74]. However, 25(OH)D could also directly suppress PTH synthesis by directly activating the VDR [75]. Vitamin D deficiency is associated with

lower levels of serum-ionized calcium, a stimulus leading to increased PTH levels. Conversely, higher calcium levels that are associated with higher 25(OH)D levels, suppress the PTH secretion. PTH increases tubular calcium and decreases renal phosphorus reabsorption [14] (Figure 1). PTH also stimulates the production of 1,25(OH)₂D with the above mentioned effects on calcium and phosphorus homeostasis [7,14]. Moreover, both PTH and 1,25(OH)₂D stimulate osteoblasts to mobilize skeletal calcium stores [7,17] (Figure 1). Vitamin D deficiency leads to secondary hyperparathyroidism with PTH-enhanced 1,25(OH)₂D production and is often associated with normal to high 1,25(OH)₂D levels [7].

7. Bone Health

In the mid-1600s most children living in the crowded and polluted industrialized cities in Northern Europe developed a severe bone-deforming disease, rickets, that was characterized by growth retardation, enlargement of the epiphyses of the long bones, deformities of the legs, bending of the spine, knobby projections of the ribcage, and weak and toneless muscles [14,76] (Figure 4). Autopsy studies in children in the Netherlands and Boston in the early 1900s showed a rickets prevalence of 80%–90% [14]. In the 19th and 20th century, the major discoveries regarding the pathogenesis and prevention of rickets were made. In 1822, the importance of sun exposure for the prevention and cure of rickets was recognized by Sniadecki [77]. In 1890, these observations were extended and the recommendation of sun baths to prevent rickets was promoted by Palm [78]. In 1919, Huldschinski [79,80] found that exposing children to UV radiation from a sun quartz lamp (mercury arc lamp) or carbon arc lamp was effective in treating rickets. In 1918, Mellanby *et al.* [81] prevented rickets in puppies with cod liver oil. McCollum *et al.* [82] called this new nutritional factor vitamin D. Hess and Weinstock [83] and Steenbock and Black [84] observed that UV irradiation of various foods and oils imparted antirachitic activity [14].

Vitamin D sufficiency is pivotal for normal skeletal development both *in utero* [7,85] and in childhood [14], and for achieving and maintaining bone health in adults [23]. This is due to the fact that vitamin D sufficiency leads to an adequate calcium-phosphorus product ($\text{Ca}^{2+} \times \text{HPO}_4^{2-}$) resulting in an effective bone mineralization [14]. Maternal vitamin D insufficiency during pregnancy was associated with a significant reduction in bone mineral acquisition in infants [85] that still persisted 9 years after birth [86]. In children whose epiphyseal plates haven't closed, vitamin D deficiency with 25(OH)D levels <15 ng/mL causes chondrocyte disorganization and hypertrophy at the mineralization front as well as skeletal mineralization defects. This results in bone deformities and short stature, the typical signs of vitamin D deficiency rickets [14,87].

In adults low 25(OH)D and high PTH also lead to a low serum calcium \times phosphorus product, resulting in osteomalacia, *i.e.*, a defective mineralization of the collagen matrix causing a reduction of structural support and being associated with an increased risk of fracture [17,28]. Results from the National Health and Nutrition Examination Survey III (NHANES III) showed that bone density in the hip was directly related to the serum 25(OH)D level in both genders of all ethnicities [88,89]. A German study examined 25(OH)D serum levels and transiliac crest bone specimens of 675 individuals mainly in the 6th and 7th decade of life (401 males, mean age 58.7 ± 17 years, and 274 females, mean age: 68.3 ± 17.3 years) dying of unnatural death, such as a motor vehicle accident.

The bone biopsies were taken within 48 h after death as well as the blood samples. Various previous experiments had shown that the 25(OH)D serum levels were stable for at least 10 days postmortem. While there's no uniformly accepted osteoid volume cut-off for the histologic diagnosis of osteomalacia, the study showed a prevalence of osteomalacia of over 25% when using a threshold of >2% osteoid volume/bone volume (OV/BV) for the diagnosis of osteomalacia and a prevalence of >43% when using a threshold of 1.2% OV/BV as described by Delling in 1975 [90]. Osteomalacia was absent in all individuals with 25(OH)D >30 ng/mL, suggesting this as minimum serum level for maintenance of bone health. However, no minimum 25(OH)D level could be determined that was inevitably associated with mineralization defects [91].

One possible explanation is that obtaining a single blood level of 25(OH)D doesn't provide information about the long-term vitamin D status of the individual. It is possible that for example that the subject became ill during the winter and stopped ingesting foods containing vitamin D or decreased sun exposure during the summer that would acutely lower blood levels of 25(OH)D without causing osteomalacia.

Figure 4. Sister (right) and brother (left) ages 4 years and 6.5 years, respectively, demonstrating classic knock-knees and bow legs, growth retardation, and other skeletal deformities [14]. Note: This figure is reproduced with permission from [14], Copyright © 2006 American Society for Clinical Investigation.



8. Osteoporosis and Fractures

As a decrease in 25(OH)D leads to secondary hyperparathyroidism associated with osteoclastogenesis and an increase in bone resorption exceeding osteoblast-mediated bone formation [88], this can precipitate and exacerbate osteopenia and osteoporosis in adults [17,92,93].

Osteoporosis has a prevalence of ~1/3 in women 60–70 years of age and of ~2/3 in women 80 years of age or older [7]. It's estimated that currently 10 million Americans have osteoporosis with

1.5 to 2 million osteoporosis-related fractures annually [94]. An osteoporosis-related fracture will be experienced by one in eight men over age 50 years in their lifetime [95].

Vitamin D promotes bone health by maintaining the PTH levels in a physiologically healthy level, stimulating osteoblastic activity, and promoting bone mineralization as well as reducing risk of falls thereby reducing risk of fracture [93,96].

According to data from the Women's Health Initiative [97], the odds ratio of risk for hip fracture was inversely related to the serum 25(OH)D level [88]. There's evidence that patients with 25(OH)D levels >30 ng/mL have a lower risk of fracture. Several studies have been conducted to evaluate the effect of vitamin D supplementation on the fracture risk, with some studies showing a significant reduction of the risk of fractures while others didn't [98]. One of these showed that the supplementation with calcium (1200 mg) and vitamin D₃ (800 IU/day) decreased the number of hip fractures by 43% ($p = 0.043$) and the total number of nonvertebral fractures by 32% [99]. The RECORD study however, did not show a reduction in fracture risk with supplementation with vitamin D (800 IU/day), or calcium (1000 mg/day), or both [100], but often compliance was poor and serum 25(OH)D levels were not measured at the end of the study in most participants [7,98,100]. A meta-analysis of more than 30,000 participants did show that supplementation with vitamin D (≥ 792 IU/day) led to a significant reduction in the risk of fracture; the risk of hip fracture was reduced by 30%, the risk of any non-vertebral fracture by 14% [98–106].

9. Muscular Health and Falls

Vitamin D exerts multiple effects on muscle health [107]. Its active form 1,25(OH)₂D could be produced locally in muscle cells as suggested by the recent identification of CYP27B1 bioactivity in regenerating mouse muscle and skeletal muscle cells [108], however other studies have failed to detect this enzyme in muscle cells [109]. 1,25(OH)₂D is thought to modulate muscle function via the VDR, which seems to be expressed in skeletal muscles [109–113], by regulating gene transcription and promoting *de-novo* protein synthesis [107]. Also, rapid non-genomic pathways involving a membrane-bound vitamin D receptor could exist and affect the calcium handling involving the sarcoplasmic reticulum and the calcium signaling in muscle cells [109]. Several studies indicate that the muscle function depends on the VDR genotype in the muscle cell [114,115]. The possibility of a direct interaction between 25(OH)D and the VDR has been proposed in CYP27B1^{-/-} cells [109,116]. However, the existence of a VDR in muscle cells is discussed highly controversially, as a more recent study failed to detect the VDR in muscle cells and as the antibodies used for immunocytochemical staining to detect the VDR in previous studies have been shown to be not exclusively specific for the VDR and could explain potentially false-positive results in these previous studies [117].

Vitamin D deficiency is associated with diffuse muscle pain, muscle weakness [7,118], predominantly in the proximal muscle groups [115], and a reduction in performance speed [107,119]. This is caused by muscle atrophy of mainly type II muscle fibers [115]. Proximal muscle weakness in severe vitamin D deficiency could also be caused by secondary hyperparathyroidism and resultant hypophosphatemia [60,106,120].

There is a positive association between 25(OH)D, lower extremity function, proximal muscle strength and physical performance [107,121,122]. Muscle strength [123] and postural and dynamic

balance [124] were increased by vitamin D supplementation [107]. The effect of vitamin D supplementation on the risk of falls was examined in a randomized, controlled multi-dose study, showing that the supplementation of 800 IU/day lowered the adjusted-incidence rate ratio of falls by 72% compared to those taking placebo over 5 months [125]. A meta-analysis of 8 randomized controlled trials ($n = 2426$) showed that supplemental vitamin D of 700–1000 IU/day or a serum 25(OH)D of ≥ 24 ng/mL reduced the risk of falls by 19% and 23% respectively. No benefit was observed with lower supplemental doses or lower serum 25(OH)D concentrations [126].

10. Cancer

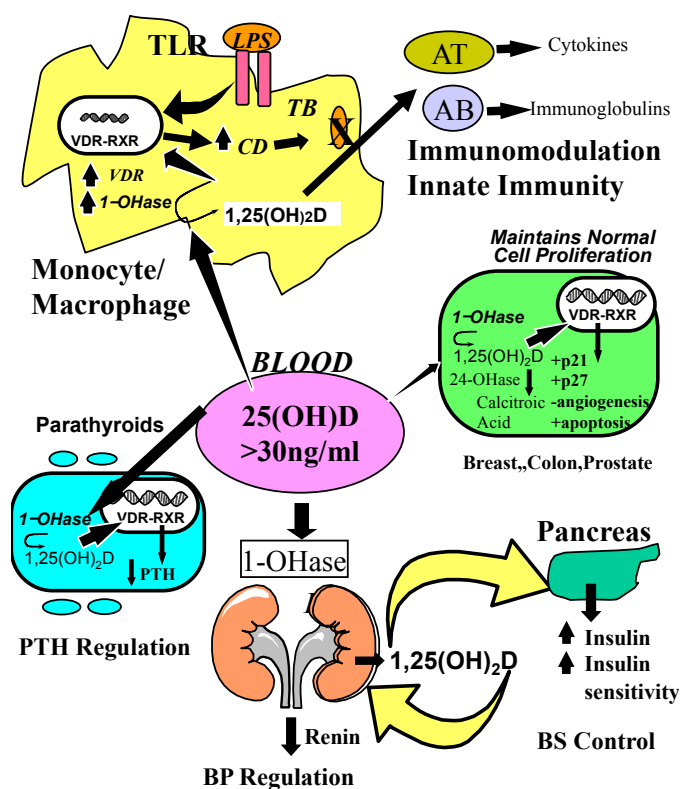
Living at higher latitudes with lower UV exposure and thus lower vitamin D production is associated with an increased risk for the occurrence of a variety of cancers and with an increased likelihood of dying from them, as compared to living at lower latitudes [7,17,127,128]. A recent review of ecological studies associating solar UVB exposure-vitamin D and cancers found strong inverse correlations with solar UVB irradiance for 15 types of cancer: bladder, breast, cervical, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal, renal, and vulvar cancer; and Hodgkin's and non-Hodgkin's lymphoma [129].

An inverse association between 25(OH)D and the incidence of several cancers and mortality from these cancers has been shown in case-control studies, prospective and retrospective studies [130–140], especially for cancers of the colon, breast and prostate [7]. Regarding colon cancer, the Nurses' Health cohort study ($n = 32,826$) showed an inverse association of the odds ratios for colorectal cancer with the median 25(OH)D serum levels. At 16.2 ng/mL the odds ratio was 1 and 0.53 at 39.9 ng/mL ($p \leq 0.01$) [7,140].

These associational studies have certain limitations regarding the establishment of a causality between vitamin D status and a reduced risk of cancer, e.g., as low serum 25(OH)D levels are also linked with confounding factors related to higher cancer risk, including obesity (vitamin D is sequestered in adipose tissue), and lack of physical activity (correlated with less time outdoors and less solar exposure) [138]. However, a population-based, double-blind, randomized placebo-controlled trial of 4 years duration with more than thousand postmenopausal women, whose principal secondary outcome was cancer incidence, showed that the supplementation with calcium (1400–1500 mg/day) and vitamin D₃ (1100 IU/day) reduced the relative risk (RR) of cancer by ~60% ($p < 0.01$). The repetition of a cancer free survival analysis after the first 12 months revealed, that the relative risk for the calcium + vitamin D group was reduced by ~77% (confidence interval [CI]: 0.09–0.60; $p < 0.005$). Multiple regression models also showed that both treatment and serum 25(OH)D concentrations were significant, independent predictors of cancer risk [137].

Mounting evidence suggests a biological plausibility for anti-carcinogenic effects of vitamin D, which could explain these results. 1,25(OH)₂D, which has been shown to be produced locally by various cancer cells metabolizing the substrate 25(OH)D [38], inhibits carcinogenesis by several mechanisms [141]. 1,25(OH)₂D exerts anti-proliferative effects on cancer cells by promoting cyclin-dependent kinase (CDK) inhibitor synthesis, and by influencing several growth factors and their signaling pathways including insulin-like growth factor 1 (IGF-1), transforming growth factor β (TGF β), Wnt/ β -catenin, MAP kinase 5 (MAPK5) and nuclear factor κ B (NF- κ B) [142] (Figure 5).

Figure 5. Metabolism of 25-hydroxyvitamin D [25(OH)D] to 1,25 dihydroxyvitamin D 1,25(OH)₂D for non-skeletal functions. When a monocyte/macrophage is stimulated through its toll-like receptor 2/1 (TLR2/1) by an infective agent such as Mycobacterium tuberculosis (TB), or its lipopolysaccharide (LPS) the signal upregulates the expression of vitamin D receptor (VDR) and the 25-hydroxyvitamin D-1-hydroxylase (1-OHase). 25(OH)D levels >30 ng/mL provides adequate substrate for the 1-OHase to convert it to 1,25(OH)₂D. 1,25(OH)₂D returns to the nucleus where it increases the expression of cathelicidin which is a peptide capable of promoting innate immunity and inducing the destruction of infective agents such as TB. It is also likely that the 1,25(OH)₂D produced in the monocytes/macrophage is released to act locally on activated T (AT) and activated B (AB) lymphocytes which regulate cytokine and immunoglobulin synthesis respectively [143–147]. When 25(OH)D levels are ~30 ng/mL, it reduces risk of many common cancers [130–140]. It is believed that the local production of 1,25(OH)₂D in the breast, colon, prostate, and other cells regulates a variety of genes that control proliferation. Once 1,25(OH)₂D completes the task of maintaining normal cellular proliferation and differentiation, it induces the 25-hydroxyvitamin D-24-hydroxylase (24-OHase). The 24-OHase enhances the metabolism of 1,25(OH)₂D to calcitroic acid which is biologically inert [7,18]. Thus, the local production of 1,25(OH)₂D does not enter the circulation and has no influence on calcium metabolism. The parathyroid glands have 1-OHase activity [45] and the local production of 1,25(OH)₂D inhibits the expression and synthesis of PTH [74]. The production of 1,25(OH)₂D in the kidney enters the circulation and is able to downregulate renin production in the kidney [148,149] and to stimulate insulin secretion in the β-islet cells of the pancreas [148,150]. Note: This figure is reproduced with permission from [21], Copyright © 2007 Michael F. Holick.



Apoptosis is characterized as programmed cell death permitting the removal of damaged cells including cancer cells in multicellular organisms without impairing the cellular microenvironment. Defective apoptosis plays a major role in the development and progression of cancer [151]. It has been shown, that both immunobiological mechanisms of cancer immunosurveillance and cancer immunoediting [152], as well as chemotherapeutic agents and radiation, utilize the apoptotic pathway to induce cancer cell death [151,153]. 1,25(OH)₂D₃ might exert anti-carcinogenic effects by promoting various pro-apoptotic mechanisms including the downregulation of the anti-apoptotic gene Bcl-2 [154] and by upregulating of the pro-apoptotic gene Bax [155], 1,25(OH)₂D₃ induces differentiation, partly by reducing the expression of the *c-myc* oncogene [141,156]. It regulates the prostaglandin (PG) metabolism and signaling, thus decreasing PG-mediated promotion of carcinogenesis [141,157]. It suppresses tumor angiogenesis, e.g., mediated by 1,25(OH)₂D's effects on the PG synthesis and by regulating the expression of crucial factors controlling the angiogenesis. 1,25(OH)₂D₃ suppresses tumor invasion and metastasis by various mechanisms [141], e.g., by decreasing the expression and activity of cell invasion-associated serine proteases and metalloproteinases and inducing their inhibitors [158], and by inducing *E*-cadherin expression, contributing to adhesive properties of cells [141,159]. Other effects mediated by 1,25(OH)₂D are thought to be the induction of autophagy as process to trigger the death of cancer cells and to block tumor growth and by inducing enzymes involved in antioxidant defense mechanisms and DNA-repair [142]. 1,25(OH)₂D also regulates androgen and estrogen receptor signaling, thereby inhibiting tumor growth of some sex hormone-dependent tumors such as prostate and breast cancer. It has also been shown to reduce the expression of aromatase, thereby inhibiting breast cancer growth [141].

11. Vitamin D and Cardiovascular Risk

Most epidemiological and prospective studies as well as meta-analyses [148,160–163] suggest a significant inverse association between 25(OH)D serum levels and cardiovascular risk. The prospective Intermountain Heart Collaborative Study with more than 40,000 participants revealed that 25(OH)D <15 ng/mL compared to 25(OH)D >30 ng/mL was associated with highly significant increases in the prevalence of type 2 diabetes mellitus, hypertension, hyperlipidemia, and peripheral vascular disease, coronary artery disease, myocardial infarction, heart failure, and stroke ($p < 0.0001$), as well as with incident death (all-cause mortality was used as primary survival measure), heart failure, coronary artery disease/myocardial infarction ($p < 0.0001$), stroke ($p = 0.003$), and their composite ($p < 0.0001$) [164].

A meta-analysis examining the association between vitamin D status and the risk of cerebrovascular events including >1200 stroke cases found that the pooled relative risk for stroke was 52% higher when comparing 25(OH)D levels ≤ 12.4 ng/mL with 25(OH)D levels >18.8 ng/mL [165].

Many of these associations are well established, causation however is yet to be proven [166]. Individuals spending less time exercising outdoors in the sun, e.g., have a higher risk of developing cardiovascular diseases, and those individuals also will likely have lower 25(OH)D levels coincidentally [166,167]. Also, obesity, a condition associated with cardiovascular disease [168], is associated with a lower vitamin D status due to a sequestration and volumetric dilution of the lipophilic vitamin D in the fat tissue [23,166,169,170], potentially explaining the described correlations [166]. Despite these limitations many studies suggest a biological plausibility for the beneficial effects of vitamin D on cardiovascular risk factors and cardiovascular health.

The vitamin D receptor is present in endothelium, vascular smooth muscle, and cardiomyocytes [162,166] and may protect against atherosclerosis through the inhibition of macrophage cholesterol uptake and foam cell formation, reduced vascular smooth muscle cell proliferation, and reduced expression of adhesion molecules in endothelial cells [166] and through inhibition of cytokine release from lymphocytes [162]. Several meta-analyses indicate an inverse association between vitamin D status and hypertension [171]. Studies showed, that antihypertensive effects were associated with raising 25(OH)D levels with vitamin D supplementation [172–174] or UVB exposure [175].

Mechanistically, this effect could be partly mediated by vitamin D's capability to suppress the levels of PTH, which can cause arrhythmias and lead to myocardial hypertrophy and increased blood pressure [148,176]. 1,25(OH)₂D₃ has also been shown to suppress the levels of renin and could contribute to vitamin D's potential antihypertensive properties [148,149].

A meta-analysis examining the association between vitamin D status or vitamin D supplementation, and incident type 2 diabetes showed that individuals with 25(OH)D levels >25 ng/mL compared to those with 25(OH)D <14 ng/mL had a 43% lower risk of developing type 2 diabetes and that a vitamin D supplementation with >500 IU/day compared to <200 IU/day reduced the risk by 13% [177]. In the Nurses' Health Study >83,000 women were followed-up prospectively and it was shown, that a combined daily intake of >1200 mg calcium and >800 IU vitamin D was associated with a 33% lower risk of type 2 diabetes with RR of 0.67 (CI: 0.49–0.90) compared with an intake of <600 mg calcium and 400 IU vitamin D [178]. A prospective study following-up more than 2000 participants showed, that the risk of progression from prediabetes to diabetes was reduced by 62% when comparing the highest quartile of 25(OH)D levels with the lowest quartile [179,180].

This could be explained by experimental findings indicating that vitamin D exerts various antidiabetic effects. The VDR is expressed in pancreatic beta cells and 1,25(OH)₂D stimulates insulin secretion [148,150]. Improvement in vitamin D status also leads to a improvement of insulin sensitivity, mediated for example by upregulation of insulin receptors [148], and modulates inflammation, which is also thought to play a role in type 2 diabetes [150,179] (Figure 5).

12. Vitamin D's Role in Autoimmune Disease

Ecological studies have shown that the prevalence of certain autoimmune diseases was associated with latitude, suggesting a potential role of sunlight exposure, and thus vitamin D production, on the pathogenesis of type 1 diabetes mellitus, multiple sclerosis and Crohn's disease [181]. The increased prevalence at higher latitudes has been shown for multiple sclerosis (MS) [181,182], inflammatory bowel disease [183], rheumatoid arthritis [184] and type 1 diabetes [181,182,185].

A few case-control studies relate the vitamin D status to the risk of developing these autoimmune diseases [181]. One of them, a prospective, nested case-control study analyzed serum samples and the data of disability databases of more than seven million US military personnel, and showed, that among whites (148 cases, 296 controls), the risk of multiple sclerosis significantly decreased with increasing levels of 25(OH)D (odds ratio for a 20 ng/mL increase in 25(OH)D was 0.59 (95% CI: 0.36–0.97). When comparing the highest quintile of 25(OH)D with the lowest, the odds ratio for developing MS was 0.38 (95% CI: 0.19–0.75; $p = 0.006$), with an particularly strong inverse association for 25(OH)D levels measured before age 20 years [186].

A study addressing vitamin D's effect on multiple sclerosis showed the safety of high-dose vitamin D (~14,000 IU/day). It appeared to have immunomodulatory effects including a persistent reduction in T-cell proliferation and resulted in a trend for fewer relapse events [187]. When examining the association between 25(OH)D serum levels and the relapse rate in MS patients before and after supplementation with ~3000 IU vitamin D per day, a significant strong inverse relationship between the relapse incidence rate and the 25(OH)D level ($p < 0.0001$) was found [188].

An inverse association between maternal 25(OH)D levels and the risk for type 1 diabetes in the offspring has been shown in a population-based, nested cohort study of ~30,000 pregnant women. Compared to the upper quartile of 25(OH)D levels, the odds of type 1 diabetes in the women with the lowest quartile was more than twofold higher [189]. A birth-cohort study with >10,000 children showed, that regular supplementation with 2000 IU vitamin D per day in the first year of life was associated with a 88% reduction of the risk for type 1 diabetes later in life when compared to those without supplementation [190]. However, another study did not show a statistically significant association between taking cod liver oil or other vitamin D supplements in the first year of life and the risk of type 1 diabetes mellitus [191].

Merlino *et al.* [192] showed in a prospective cohort study of 29,368 women of ages 55–69 years without a history of rheumatoid arthritis at study baseline, that greater intake (highest versus lowest tertile) of vitamin D was inversely associated with risk of rheumatoid arthritis (RR 0.67; 95% CI: 0.44–1.00; p for trend =0.05).

These associations indicate a contributory role of vitamin D in the pathophysiology of autoimmune diseases. This is further supported by various experimental findings showing vitamin D's capability to regulate chemokine production, counteracting autoimmune inflammation and to induce differentiation of immune cells in a way that promotes self-tolerance. This involves the enhancement of the innate and the inhibition of the adaptive immune system by regulating the interactions between lymphocytes and antigen presenting cells. By increasing the quantity of Th2 lymphocytes and by inducing proliferation of dendritic cells with tolerance properties, vitamin D exerts anti-inflammatory and immunoregulatory effects [181].

Immune cells possess both the enzymatic machinery to produce 1,25(OH)₂D and a VDR. This could explain, why certain polymorphisms in the VDR gene seem to affect the risk for multiple autoimmune diseases, the time of onset of disease and disease activity [181,193–197].

13. Vitamin D and Infectious Diseases

The plethora of effects of vitamin D on regulating the immune system plays a role in fighting infectious diseases [198]. Vitamin D enhances the innate immunity against various infections [143], especially tuberculosis, influenza and viral upper respiratory tract infections [198].

Historically, cod liver oil (one of only a few natural sources of vitamin D) was given to tuberculosis patients in 19th and 20th century [199–201]. Later in the nineteenth century, tuberculosis patients were treated in sanatoriums with heliotherapy, *i.e.*, sun exposure. In 1903, Niels Ryberg Finsen was awarded the Nobel prize for medicine “in recognition of his contribution to the treatment of diseases, especially lupus vulgaris (tuberculosis of the skin), with concentrated light radiation, whereby he has opened a new avenue for medical science” [199,202]. After vitamin D had been identified as the active

ingredient in cod-liver oil [199,203], vitamin D₂ was used successfully in the treatment of lupus vulgaris in several studies. In 1946 a report in *Proc. R. Soc. Med.* [204] stated that there was no room for doubt that calciferol (vitamin D) in adequate dosage will cure a substantial proportion of cases of lupus vulgaris [199,204]. In 1947 the first reference to successful treatment of pulmonary tuberculosis with vitamin D was published [199,205]. In the wake of the antibiotic era both heliotherapy and vitamin D therapy for treating tuberculosis patients were quickly forgotten [199,206]. However recent studies have suggested that vitamin D may have an important role to play in reducing risk for acquiring one of the most common and deadly infectious diseases that plague third world countries [206].

One case-control study examining the association between vitamin D status and tuberculosis showed, that the mean 25(OH)D levels were statistically significant different ($p < 0.005$) between patients with pulmonary and extrapulmonary tuberculosis (10.7 ng/mL) and controls (19.5 ng/mL) [207]. In another study, 25(OH)D levels <10 ng/mL were significantly associated with active tuberculosis (OR 2.9; 95% CI: 1.3–6.5; $p = 0.008$) [208]. A meta-analysis showed, that low serum 25(OH)D levels were associated with higher risk of active tuberculosis, and that the pooled effect size in random effects meta-analysis was 0.68 (95% CI: 0.43–0.93), representing a medium to large effect [209]. A double-blind, placebo-controlled study in Mongolian school children ($n = 120$) examining the effect of vitamin D supplementation (800 IU/day) on tuberculin skin test conversion to positive showed a trend towards fewer conversions in the vitamin D group ($p = 0.06$), suggesting a potential role of vitamin D in reducing the rate of acquisition of latent tuberculosis infection [210].

Several interventional studies examining the effect of vitamin D supplementation in patients with active tuberculosis have been conducted. Some of them showed an improved immunity against mycobacteria [211], a significantly improved sputum conversion rate and a higher rate of radiological improvement [212], and a significantly hastened sputum culture conversion in participants with the tt genotype of the TaqI vitamin D receptor polymorphism [213]. There was also a higher rate of tuberculosis symptom improvement and a significantly higher weight gain ($p < 0.005$) in children [214]. A prospective, randomized placebo-controlled trial examining the effect of adjunctive vitamin D supplementation in patients receiving antimicrobial therapy showed that vitamin D supplementation led to an accelerated sputum smear conversion and an accelerated resolution of inflammation [215]. Another study however in which three doses of 100,000 IU vitamin D₃ each were given during 8 months did not lead to a reduction in the clinical severity score or mortality [216].

Some studies examined the effect of vitamin D supplementation on the risk of influenza [217,218].

In 1981, R. Edgar Hope-Simpson proposed that a “seasonal stimulus” was intimately associated with solar radiation and explained the remarkable seasonality of epidemic influenza [219,220]. As the vitamin D status changes during the seasons, it has been suggested, that vitamin D could be this “seasonal stimulus” [219]. A randomized trial of vitamin D₃ supplementation (1200 IU/day) in school children ($n = 334$) showed a significantly reduced risk for influenza A as determined by both antibody and sputum testing compared to the placebo group (RR 0.58; 95% CI: 0.34–0.99; $p = 0.04$) [218].

One study using questionnaires to retrospectively determine the occurrence of influenza-like disease in participants of 10 different clinical trials ($n = 569$), receiving 1111–6800 IU/day, however did not show a significant difference in the incidence and severity of influenza-like disease [217].

The NHANES III study ($n > 18$) revealed an inverse association between serum 25(OH)D levels and recent upper respiratory tract infections (URTI). Lower 25(OH)D levels were independently

associated with recent URTI compared with 25(OH)D levels of ≥ 30 ng/mL (OR 1.36; 95% CI: 1.01–1.84 for < 10 ng/mL and OR 1.24; 95% CI: 1.07–1.43 for 10 to < 30 ng/mL). In individuals with asthma or chronic obstructive airway disease this association was stronger (OR of 5.67 in asthma respectively OR of 2.26 in chronic obstructive airway disease) [221]. A study in Finish men ($n = 800$) found a significant association between 25(OH)D serum levels < 16 ng/mL and significantly more days of absence from duty due to respiratory infections ($p = 0.004$) [222]. In Indian children ($n = 150$) vitamin D deficiency has been associated with a significantly higher risk of acute lower respiratory infections [223].

A study with > 200 participants whose primary endpoint was the effect of vitamin D supplementation on bone loss also revealed, that the vitamin D₃ supplementation for 2 years with 800 IU/day and for 1 year with 2000 IU/day was associated with a significantly reduced risk of cold and influenza symptoms, an effect that was magnified with the supplementation of 2000 IU/day [198,224]. Other studies however did not show a statistically significant difference, possibly due to poor compliance [225,226]. Certain VDR polymorphisms were also associated with a significantly increased risk of acute lower respiratory tract infections [227].

Several mechanisms could explain vitamin D's potentially beneficial effects on infectious diseases. Monocytes and macrophages can sense pathogen-associated molecular patterns (PAMPs) of, e.g., tuberculosis by utilizing their toll-like receptors (TLRs). This induces both VDR and CYP27B1, which increases the local production of 1,25(OH)₂D that is dependent on the serum 25(OH)D concentration [145,228]. 1,25(OH)₂D enhances the innate immune system by inducing the production of antimicrobial peptides like cathelicidin, reactive oxygen species by the (reduced) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and potentially reactive nitrogen species by inducible nitric oxide synthase (iNOS), and by inducing autophagy [143–147] (Figure 4).

14. Vitamin D and Respiratory Diseases

Although some studies did not find a consistent association between 25(OH)D levels in cord blood, maternal vitamin D intake or status during pregnancy and the risk for asthma in childhood [229–236], in children with asthma, 25(OH)D levels seem to correlate positively with asthma control [237] and lung function [238], and inversely with corticosteroid use [239]. A few interventional studies examining vitamin D's effect on asthma exist [229]. One of them showed as secondary outcome that vitamin D₃ supplementation (1200 IU/day) in school children was associated with a significant 83% reduced risk for asthma exacerbations [218]. Presumably vitamin D's immunomodulatory and pulmonary effects could play a role [229].

15. Prevention and Treatment of Vitamin D Deficiency

According to the Endocrine Society Practice Guidelines a screening for vitamin D deficiency by measuring the 25(OH)D serum level is only recommended for individuals at risk (the most important risk factors are listed in Figure 6), and not for the general population [16]. To prevent vitamin D deficiency, the Institute of Medicine (IOM) recommends, that infants should immediately receive a daily supplementation of vitamin D of 400 IUs during the first year of life. Individuals between 1 and 70 years should receive 600 IU of vitamin D daily and adults > 70 years should receive a daily dose of 800 IU vitamin D [53] (Table 2). The serum 25(OH)D level increases for every 100 IU/day by

~0.6–1.0 ng/mL [29,37,240,241]. The doses recommended by IOM will likely increase the 25(OH)D level to 20 ng/mL, which they considered to be adequate for bone health, but not to levels >30 ng/mL, as recommended by the Endocrine Society.

That’s why the Endocrine Society recommended in its Practice Guidelines that infants during their first year of life receive a daily supplementation of 400–1000 IU (up to 2000 IU is safe), children and adolescents between 1 and 18 years a daily supplementation of 600–1000 IU (up to 4000 IU is safe), and adults >18 years a daily supplementation of 1500–2000 IU (up to 10,000 IU is safe) for the prevention of vitamin D deficiency [16,53] (Table 2).

Figure 6. A Schematic representation of the major causes for vitamin D deficiency and potential health consequences. Note: This figure is reproduced with permission from [21], Copyright © 2007 Michael F. Holick.

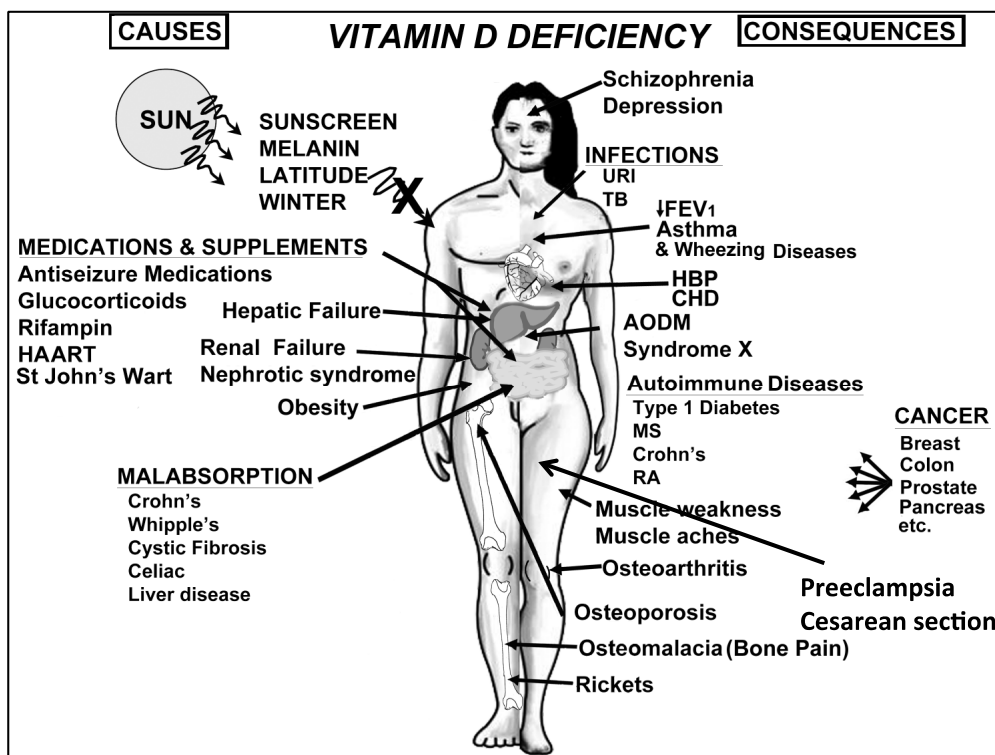


Table 2. Recommendations of the Institute of Medicine and the Endocrine Society Practice Guidelines for daily vitamin D supplementation to prevent vitamin D deficiency. This table is reproduced with permission from [16], Copyright © 2011 The Endocrine Society.

Life Stage Group	IOM Recommendations				Endocrine Society's Recommendations	
	AI	EAR	RDA	UL	Daily Allowance (IU/day)	UL (IU)
<i>Infants</i>						
0 to 6 months	400 IU (10 µg)			1000 IU (25 µg)	400–1000	2000
6 to 12 months	400 IU (10 µg)			1500 IU (38 µg)	400–1000	2000
<i>Children</i>						
1–3 years		400 IU (10 µg)	600 IU (15 µg)	2500 IU (63 µg)	600–1000	4000
4–8 years		400 IU (10 µg)	600 IU (15 µg)	3000 IU (75 µg)	600–1000	4000

Table 2. Cont.

<i>Males</i>						
9–13 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
14–18 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
19–30 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
31–50 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
51–70 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
>70 years	400 IU (10 µg)	800 IU (20 µg)	4000 IU (100 µg)	1500–2000	10,000	
<i>Females</i>						
9–13 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
14–18 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
19–30 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
31–50 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
51–70 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
>70 years	400 IU (10 µg)	800 IU (20 µg)	4000 IU (100 µg)	1500–2000	10,000	
<i>Pregnancy</i>						
14–18 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
19–30 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
31–50 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
<i>Lactation *</i>						
14–18 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	600–1000	4000	
19–30 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	
31–50 years	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000	10,000	

* Mother's requirement 4000–6000 (mother's intake for infant's requirement if infant is not receiving 400 IU/day); AI = Adequate Intake; EAR = Estimated Average Requirement; IU = International Units; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

However, obese individuals, patients with malabsorption syndromes, and patients on glucocorticoids, anti-seizure and AIDS medications may require higher doses of vitamin D than individuals without these conditions [16]. The Endocrine Society's Clinical Practice Guidelines also recommended sensible sun exposure, which for most individuals is the main physiological source of vitamin D, and provided a list of the foods rich in vitamin D, and encouraged taking a daily vitamin D supplement to ensure adequate 25(OH)D levels.

The Endocrine Society's Practice Guidelines also recommended treatment strategies for patients with vitamin D deficiency depending on age and underlying medical conditions. For vitamin D deficient infants 0–1 years old, a treatment with 2000 IU/day of vitamin D₂ or vitamin D₃ or with 50,000 IU of vitamin D₂ or vitamin D₃ once weekly for 6 weeks was suggested, followed by maintenance therapy of 400–1000 IU/day. For vitamin D deficient children aged 1–18 years who are vitamin D deficient, treatment with 2000 IU/day of vitamin D₂ or vitamin D₃ or with 50,000 IU of vitamin D₂ once a week, both for at least 6 weeks, was suggested, followed by maintenance therapy of 600–1000 IU/day. Vitamin D deficient adults should be treated with 50,000 IU of vitamin D₂ or vitamin D₃ once a week for 8 weeks or with ~6000 IU/day of vitamin D₂ or vitamin D₃, followed by maintenance therapy of 1500–2000 IU/day. In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, two to three times higher doses are

(at least 6000–10,000 IU/day) of vitamin D to treat vitamin D deficiency are recommended, followed by maintenance therapy of at least 3000–6000 IU/day [16]. This strategy of giving 50,000 IU of vitamin D twice monthly to treat or prevent recurrence of vitamin D deficiency or insufficiency was without any toxicity for up to six years [242] (Figure 7).

Figure 7. (A) Mean serum 25-hydroxyvitamin D [25(OH)D] levels in all patients: includes patients treated with 50,000 IU vitamin D₂ every 2 weeks (maintenance therapy, $n = 81$), including those patients with vitamin D insufficiency who were initially treated with 8 weeks of 50,000 IU vitamin D₂ weekly prior to maintenance therapy ($n = 39$). Error bars represent standard error of the mean, mean result over 5 years shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6 month intervals. When mean 25(OH)D in each 6 month group was compared to mean initial 25(OH)D, a significant difference was shown with $p < 0.001$ up until month 43 and $p < 0.001$ when all remaining values after month 43 were compared to mean initial 25(OH)D. (B) Mean serum 25(OH)D levels in patients receiving maintenance therapy only: Levels for 37 patients who were vitamin D insufficient (25(OH)D levels < 30 ng/mL) and 5 patients who were vitamin D sufficient (25(OH)D levels ≥ 30 ng/mL) who were treated with maintenance therapy of 50,000 IU vitamin D₂ every two weeks. Error bars represent standard error of the mean, mean result over 5 years shown. Time 0 is initiation of treatment, results shown as mean values averaged for 6 month intervals. When mean 25(OH)D in each 6 month group were compared to mean initial 25(OH)D, a significant difference was shown with $p < 0.001$ up until month 37 and $p < 0.001$ when all remaining values after month 43 were compared to mean initial 25(OH)D. (C) Serum calcium levels: Results for all 81 patients who were treated with 50,000 IU of vitamin D₂. Error bars represent standard error of the mean. Time 0 is initiation of treatment, results shown as mean values averaged for 6 month intervals. Normal serum calcium: 8.5–10.2 mg/dL. Note: This figure is reproduced with permission from [242], Copyright © 2009 American Medical Association.

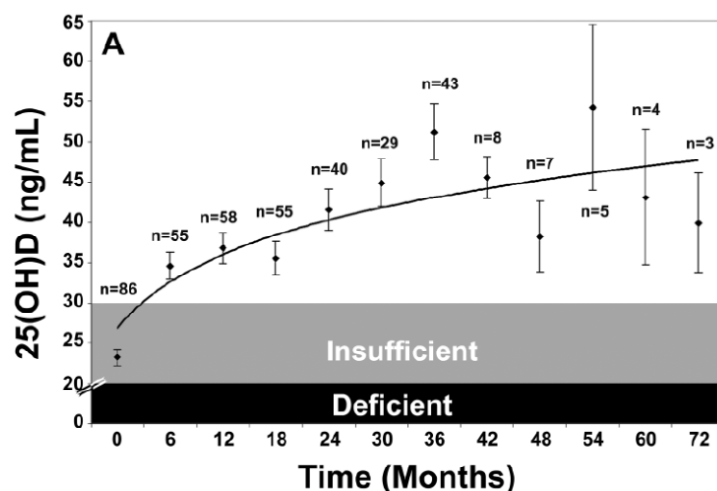
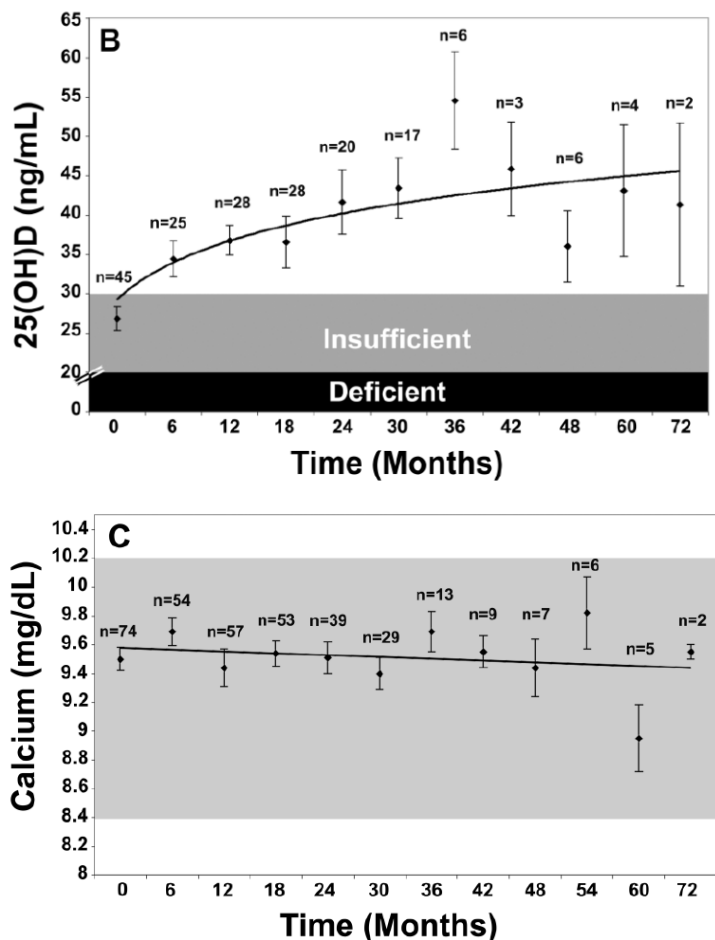


Figure 7. Cont.



However, certain conditions like granulomatous conditions [243], genetic disorders [244] or rare polymorphisms of enzymes involved in vitamin D metabolism [245] are associated with an increased risk for vitamin D toxicity.

16. Conclusion

What continues to be needed are randomized controlled interventional studies with high power and using sufficiently high doses of vitamin D examining vitamin D’s effects on various health outcomes.

However, the present body of evidence of experimental findings, ecological, case-control, retro- and prospective observational and interventional studies is substantial and suggests a pivotal role of vitamin D for a plethora of physiological functions and health outcomes including neuropsychiatric disorders [246], justifying the recommendation to enhance children’s and adults’ vitamin D status by following recommendations for sensible sun exposure, ingesting foods that contain vitamin D and vitamin D supplementation. Increasing the vitamin D status worldwide in the general adult and children population without rare conditions associated with an increased risk for vitamin D toxicity will help improve their overall health and well-being (Figure 6).

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Conflict of Interest

The authors declare no conflict of interest.

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Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline

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Objective: The objective was to provide guidelines to clinicians for the evaluation, treatment, and prevention of vitamin D deficiency with an emphasis on the care of patients who are at risk for deficiency.

Participants: The Task Force was composed of a Chair, six additional experts, and a methodologist. The Task Force received no corporate funding or remuneration.

Consensus Process: Consensus was guided by systematic reviews of evidence and discussions during several conference calls and e-mail communications. The draft prepared by the Task Force was reviewed successively by The Endocrine Society's Clinical Guidelines Subcommittee, Clinical Affairs Core Committee, and cosponsoring associations, and it was posted on The Endocrine Society web site for member review. At each stage of review, the Task Force received written comments and incorporated needed changes.

Conclusions: Considering that vitamin D deficiency is very common in all age groups and that few foods contain vitamin D, the Task Force recommended supplementation at suggested daily intake and tolerable upper limit levels, depending on age and clinical circumstances. The Task Force also suggested the measurement of serum 25-hydroxyvitamin D level by a reliable assay as the initial diagnostic test in patients at risk for deficiency. Treatment with either vitamin D₂ or vitamin D₃ was recommended for deficient patients. At the present time, there is not sufficient evidence to recommend screening individuals who are not at risk for deficiency or to prescribe vitamin D to attain the noncalcemic benefit for cardiovascular protection. (*J Clin Endocrinol Metab* 96: 1911–1930, 2011)

Summary of Recommendations

1.0 Diagnostic procedure

1.1 We recommend screening for vitamin D deficiency in individuals at risk for deficiency. We do not recommend population screening for vitamin D deficiency in individuals who are not at risk (1|⊕⊕⊕⊕).

1.2 We recommend using the serum circulating 25-hydroxyvitamin D [25(OH)D] level, measured by a reliable

assay, to evaluate vitamin D status in patients who are at risk for vitamin D deficiency. Vitamin D deficiency is defined as a 25(OH)D below 20 ng/ml (50 nmol/liter), and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (525–725 nmol/liter). We recommend against using the serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] assay for this purpose and are in favor of using it only in monitoring certain conditions, such as acquired and inherited disorders of vitamin D and phosphate metabolism (1|⊕⊕⊕⊕).

2.0 Recommended dietary intakes of vitamin D for patients at risk for vitamin D deficiency

2.1 We suggest that infants and children aged 0–1 yr require at least 400 IU/d (IU = 25 ng) of vitamin D and children 1 yr and older require at least 600 IU/d to maximize bone health. Whether 400 and 600 IU/d for children aged 0–1 yr and 1–18 yr, respectively, are enough to provide all the potential nonskeletal health benefits associated with vitamin D to maximize bone health and muscle function is not known at this time. However, to raise the blood level of 25(OH)D consistently above 30 ng/ml (75 nmol/liter) may require at least 1000 IU/d of vitamin D (2|⊕⊕⊕⊕).

2.2 We suggest that adults aged 19–50 yr require at least 600 IU/d of vitamin D to maximize bone health and muscle function. It is unknown whether 600 IU/d is enough to provide all the potential nonskeletal health benefits associated with vitamin D. However, to raise the blood level of 25(OH)D consistently above 30 ng/ml may require at least 1500–2000 IU/d of vitamin D (2|⊕⊕⊕⊕).

2.3 We suggest that all adults aged 50–70 and 70+ yr require at least 600 and 800 IU/d, respectively, of vitamin D. Whether 600 and 800 IU/d of vitamin D are enough to provide all of the potential nonskeletal health benefits associated with vitamin D is not known at this time. However, to raise the blood level of 25(OH)D above 30 ng/ml may require at least 1500–2000 IU/d of supplemental vitamin D (2|⊕⊕⊕⊕).

2.4 We suggest that pregnant and lactating women require at least 600 IU/d of vitamin D and recognize that at least 1500–2000 IU/d of vitamin D may be needed to maintain a blood level of 25(OH)D above 30 ng/ml (2|⊕⊕⊕⊕).

2.5 We suggest that obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for AIDS be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement (2|⊕⊕⊕⊕).

2.6 We suggest that the maintenance tolerable upper limits (UL) of vitamin D, which is not to be exceeded without medical supervision, should be 1000 IU/d for infants up to 6 months, 1500 IU/d for infants from 6 months to 1 yr, at least 2500 IU/d for children aged 1–3 yr, 3000 IU/d for children aged 4–8 yr, and 4000 IU/d for everyone over 8 yr. However, higher levels of 2000 IU/d for children 0–1 yr, 4000 IU/d for children 1–18 yr, and 10,000 IU/d for children and adults 19 yr and older may be needed to correct vitamin D deficiency (2|⊕⊕⊕⊕).

3.0 Treatment and prevention strategies

3.1 We suggest using either vitamin D₂ or vitamin D₃ for the treatment and prevention of vitamin D deficiency (2|⊕⊕⊕⊕).

3.2 For infants and toddlers aged 0–1 yr who are vitamin D deficient, we suggest treatment with 2000 IU/d of

vitamin D₂ or vitamin D₃, or with 50,000 IU of vitamin D₂ or vitamin D₃ once weekly for 6 wk to achieve a blood level of 25(OH)D above 30 ng/ml, followed by maintenance therapy of 400–1000 IU/d (2|⊕⊕⊕⊕).

3.3 For children aged 1–18 yr who are vitamin D deficient, we suggest treatment with 2000 IU/d of vitamin D₂ or vitamin D₃ for at least 6 wk or with 50,000 IU of vitamin D₂ once a week for at least 6 wk to achieve a blood level of 25(OH)D above 30 ng/ml, followed by maintenance therapy of 600–1000 IU/d (2|⊕⊕⊕⊕).

3.4 We suggest that all adults who are vitamin D deficient be treated with 50,000 IU of vitamin D₂ or vitamin D₃ once a week for 8 wk or its equivalent of 6000 IU of vitamin D₂ or vitamin D₃ daily to achieve a blood level of 25(OH)D above 30 ng/ml, followed by maintenance therapy of 1500–2000 IU/d (2|⊕⊕⊕⊕).

3.5 In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, we suggest a higher dose (two to three times higher; at least 6000–10,000 IU/d) of vitamin D to treat vitamin D deficiency to maintain a 25(OH)D level above 30 ng/ml, followed by maintenance therapy of 3000–6000 IU/d (2|⊕⊕⊕⊕).

3.6 In patients with extrarenal production of 1,25(OH)₂D, we suggest serial monitoring of 25(OH)D levels and serum calcium levels during treatment with vitamin D to prevent hypercalcemia (2|⊕⊕⊕⊕).

3.7 For patients with primary hyperparathyroidism and vitamin D deficiency, we suggest treatment with vitamin D as needed. Serum calcium levels should be monitored (2|⊕⊕⊕⊕).

4.0 Noncalcemic benefits of vitamin D

4.1 We recommend prescribing vitamin D supplementation for fall prevention. We do not recommend prescribing vitamin D supplementation beyond recommended daily needs for the purpose of preventing cardiovascular disease or death or improving quality of life (2|⊕⊕⊕⊕).

Method of Development of Evidence-Based Clinical Practice Guidelines

The Task Force commissioned the conduct of two systematic reviews of the literature to inform its key recommendations. The Task Force used consistent language and geographical descriptions of both the strength of recommendation and the quality of evidence using the recommendations of the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system.

The Clinical Guidelines Subcommittee of The Endocrine Society deemed vitamin D deficiency a priority area in need of practice guidelines and appointed a Task Force

to formulate evidence-based recommendations. The Task Force followed the approach recommended by the GRADE group, an international group with expertise in development and implementation of evidence-based guidelines (1). A detailed description of the grading scheme has been published elsewhere (2). The Task Force used the best available research evidence to develop some of the recommendations. The Task Force commissioned the conduct of two systemic reviews of the literature to inform its key recommendations.

The Task Force also used consistent language and graphical descriptions of both the strength of a recommendation and the quality of evidence. In terms of the strength of the recommendation, strong recommendations use the phrase “we recommend” and the number 1, and weak recommendations use the phrase “we suggest” and the number 2. *Cross-filled circles* indicate the quality of the evidence, such that ⊕○○○ denotes very low quality evidence; ⊕⊕○○, low quality; ⊕⊕⊕○, moderate quality; and ⊕⊕⊕⊕, high quality. The Task Force has confidence that persons who receive care according to the strong recommendations will derive, on average, more good than harm. Weak recommendations require more careful consideration of the person’s circumstances, values, and preferences to determine the best course of action. Linked to each *recommendation* is a description of the *evidence* and the *values* that panelists considered in making the recommendation; in some instances, there are *remarks*, a section in which panelists offer technical suggestions for testing conditions, dosing, and monitoring. These technical comments reflect the best available evidence applied to a typical person being treated. Often this evidence comes from the unsystematic observations of the panelists and their values and preferences; therefore, these remarks should be considered suggestions.

Vitamin D Photobiology, Metabolism, Physiology, and Biological Functions

Vitamin D is unique among hormones because it can be made in the skin from exposure to sunlight (3–7). Vitamin D comes in two forms. Vitamin D₂ is obtained from the UV irradiation of the yeast sterol ergosterol and is found naturally in sun-exposed mushrooms. Vitamin D₃ is synthesized in the skin and is present in oil-rich fish such as salmon, mackerel, and herring; commercially available vitamin D₃ is synthesized from the cholesterol precursor 7-dehydrocholesterol naturally present in the skin or obtained from lanolin (3). Both vitamin D₂ and vitamin D₃ are used for food fortification and in vitamin D supplements. Vitamin D (D represents D₂, or D₃, or both) that is

ingested is incorporated into chylomicrons, which are absorbed into the lymphatic system and enter the venous blood. Vitamin D that comes from the skin or diet is biologically inert and requires its first hydroxylation in the liver by the vitamin D-25-hydroxylase (25-OHase) to 25(OH)D (3, 8). However, 25(OH)D requires a further hydroxylation in the kidneys by the 25(OH)D-1 α -OHase (CYP27B1) to form the biologically active form of vitamin D 1,25(OH)₂D (3, 8). 1,25(OH)₂D interacts with its vitamin D nuclear receptor, which is present in the small intestine, kidneys, and other tissues (3, 8). 1,25(OH)₂D stimulates intestinal calcium absorption (9). Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus are absorbed. Vitamin D sufficiency enhances calcium and phosphorus absorption by 30–40% and 80%, respectively (3, 10). 1,25(OH)₂D interacts with its vitamin D receptor in the osteoblast to stimulate the expression of receptor activator of nuclear factor κ B ligand; this, in turn, interacts with receptor activator of nuclear factor κ B to induce immature monocytes to become mature osteoclasts, which dissolve the matrix and mobilize calcium and other minerals from the skeleton. In the kidney, 1,25(OH)₂D stimulates calcium reabsorption from the glomerular filtrate (3, 11).

The vitamin D receptor is present in most tissues and cells in the body (3, 12). 1,25(OH)₂D has a wide range of biological actions, including inhibiting cellular proliferation and inducing terminal differentiation, inhibiting angiogenesis, stimulating insulin production, inhibiting renin production, and stimulating macrophage cathelicidin production (3, 12–14). In addition, 1,25(OH)₂D stimulates its own destruction by enhancing the expression of the 25-hydroxyvitamin D-24-OHase (CYP24R) to metabolize 25(OH)D and 1,25(OH)₂D into water-soluble inactive forms. There are several tissues and cells that possess 1-OHase activity (3, 7, 12, 13). The local production of 1,25(OH)₂D may be responsible for regulating up to 200 genes (15) that may facilitate many of the pleiotropic health benefits that have been reported for vitamin D (3–7, 12).

Prevalence of Vitamin D Deficiency

Vitamin D deficiency has been historically defined and recently recommended by the Institute of Medicine (IOM) as a 25(OH)D of less than 20 ng/ml. Vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/ml (3, 10, 16–20). In accordance with these definitions, it has been estimated that 20–100% of U.S., Canadian, and European elderly men and women still living in the community are vitamin D deficient (3, 21–25). Children and young and middle-aged adults are at equally high risk for

vitamin D deficiency and insufficiency worldwide. Vitamin D deficiency is common in Australia, the Middle East, India, Africa, and South America (3, 26, 27). In the United States, more than 50% of Hispanic and African-American adolescents in Boston (28) and 48% of white preadolescent girls in Maine had 25(OH)D below 20 ng/ml (29). In addition, 42% of African-American girls and women aged 15–49 yr throughout the United States had a blood level of 25(OH)D below 15 ng/ml at the end of the winter (30), and 32% of healthy students and physicians at a Boston hospital had 25(OH)D below 20 ng/ml (31). Pregnant and lactating women who take a prenatal vitamin and a calcium supplement with vitamin D remain at high risk for vitamin D deficiency (32–34).

Causes of Vitamin D Deficiency

The major source of vitamin D for children and adults is exposure to natural sunlight (3, 7, 35–37). Very few foods naturally contain or are fortified with vitamin D. Thus, the major cause of vitamin D deficiency is inadequate exposure to sunlight (5–7, 38). Wearing a sunscreen with a sun protection factor of 30 reduces vitamin D synthesis in the skin by more than 95% (39). People with a naturally dark skin tone have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin D as a person with a white skin tone (40, 41). There is an inverse association of serum 25(OH)D and body mass index (BMI) greater than 30 kg/m², and thus, obesity is associated with vitamin D deficiency (42). There are several other causes for vitamin D deficiency (3, 38). Patients with one of the fat malabsorption syndromes and bariatric patients are often unable to absorb the fat-soluble vitamin D, and patients with nephrotic syndrome lose 25(OH)D bound to the vitamin D-binding protein in the urine (3). Patients on a wide variety of medications, including anticonvulsants and medications to treat AIDS/HIV, are at risk because these drugs enhance the catabolism of 25(OH)D and 1,25(OH)₂D (43). Patients with chronic granuloma-forming disorders, some lymphomas, and primary hyperparathyroidism who have increased metabolism of 25(OH)D to 1,25(OH)₂D are also at high risk for vitamin D deficiency (44, 45).

Consequences of Vitamin D Deficiency

Vitamin D deficiency results in abnormalities in calcium, phosphorus, and bone metabolism. Specifically, vitamin D deficiency causes a decrease in the efficiency of intestinal calcium and phosphorus absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels (3,

10, 22, 23). Secondary hyperparathyroidism maintains serum calcium in the normal range at the expense of mobilizing calcium from the skeleton and increasing phosphorus wasting in the kidneys. The PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalized decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. Phosphaturia caused by secondary hyperparathyroidism results in a low normal or low serum phosphorus level. This results in an inadequate calcium-phosphorus product, causing a mineralization defect in the skeleton (3, 46). In young children who have little mineral in their skeleton, this defect results in a variety of skeletal deformities classically known as rickets (24, 47). In adults, the epiphyseal plates are closed, and there is enough mineral in the skeleton to prevent skeletal deformities so that this mineralization defect, known as an osteomalacia, often goes undetected. However, osteomalacia causes a decrease in BMD and is associated with isolated or generalized aches and pains in bones and muscles (48, 49). Vitamin D deficiency also causes muscle weakness; affected children have difficulty standing and walking (47, 50), whereas the elderly have increasing sway and more frequent falls (51, 52), thereby increasing their risk of fracture.

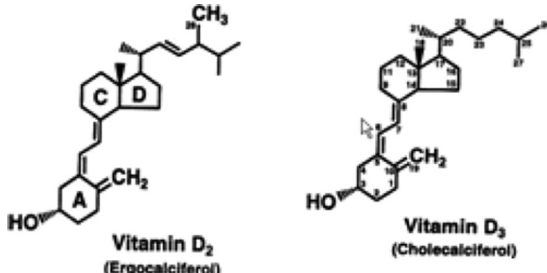
Sources of Vitamin D

A major source of vitamin D for most humans comes from exposure of the skin to sunlight typically between 1000 h and 1500 h in the spring, summer, and fall (3–5, 7). Vitamin D produced in the skin may last at least twice as long in the blood compared with ingested vitamin D (53). When an adult wearing a bathing suit is exposed to one minimal erythemal dose of UV radiation (a slight pinkness to the skin 24 h after exposure), the amount of vitamin D produced is equivalent to ingesting between 10,000 and 25,000 IU (5). A variety of factors reduce the skin's production of vitamin D₃, including increased skin pigmentation, aging, and the topical application of a sunscreen (3, 39, 40). An alteration in the zenith angle of the sun caused by a change in latitude, season of the year, or time of day dramatically influences the skin's production of vitamin D₃ (3, 5). Above and below latitudes of approximately 33°, vitamin D₃ synthesis in the skin is very low or absent during most of the winter.

Few foods naturally contain vitamin D₂ or vitamin D₃ (Table 1).

In the United States and Canada, milk is fortified with vitamin D, as are some bread products, orange juices, cereals, yogurts, and cheeses (3). In Europe, most countries do not fortify milk with vitamin D because in the 1950s,

TABLE 1. Sources of vitamin D₂ and vitamin D₃

Source	Vitamin D content
Natural sources	
	
	<p>Vitamin D₂ (Ergocalciferol)</p> <p>Vitamin D₃ (Cholecalciferol)</p>
Cod liver oil	~400–1,000 IU/teaspoon vitamin D ₃
Salmon, fresh wild caught	~600–1,000 IU/3.5 oz vitamin D ₃
Salmon, fresh farmed	~100–250 IU/3.5 oz vitamin D ₃ , vitamin D ₂
Salmon, canned	~300–600 IU/3.5 oz vitamin D ₃
Sardines, canned	~300 IU/3.5 oz vitamin D ₃
Mackerel, canned	~250 IU/3.5 oz vitamin D ₃
Tuna, canned	236 IU/3.5 oz vitamin D ₃
Shiitake mushrooms, fresh	~100 IU/3.5 oz vitamin D ₂
Shiitake mushrooms, sun-dried	~1,600 IU/3.5 oz vitamin D ₂
Egg yolk	~20 IU/yolk vitamin D ₃ or D ₂
Sunlight/UVB radiation	~20,000 IU equivalent to exposure to 1 minimal erythral dose (MED) in a bathing suit. Thus, exposure of arms and legs to 0.5 MED is equivalent to ingesting ~3,000 IU vitamin D ₃ .
Fortified foods	
Fortified milk	100 IU/8 oz, usually vitamin D ₃
Fortified orange juice	100 IU/8 oz vitamin D ₃
Infant formulas	100 IU/8 oz vitamin D ₃
Fortified yogurts	100 IU/8 oz, usually vitamin D ₃
Fortified butter	56 IU/3.5 oz, usually vitamin D ₃
Fortified margarine	429 IU/3.5 oz, usually vitamin D ₃
Fortified cheeses	100 IU/3 oz, usually vitamin D ₃
Fortified breakfast cereals	~100 IU/serving, usually vitamin D ₃
Pharmaceutical sources in the United States	
Vitamin D ₂ (ergocalciferol)	50,000 IU/capsule
Drisdol (vitamin D ₂) liquid	8,000 IU/cc
Supplemental sources	
Multivitamin	400, 500, 1,000 IU vitamin D ₃ or vitamin D ₂
Vitamin D ₃	400, 800, 1,000, 2,000, 5,000, 10,000, and 50,000 IU

IU = 25 ng. [Reproduced with permission from M. F. Holick: *N Engl J Med* 357:266–281, 2007 (3). © Massachusetts Medical Society.]

there was an outbreak of vitamin D intoxication in young children, resulting in laws that forbade the fortification of foods with vitamin D. However, Sweden and Finland now fortify milk, and many European countries add vitamin D to cereals, breads, and margarine (3).

Multivitamin preparations contain 400-1000 IU of vitamin D₂ or vitamin D₃, whereas pharmaceutical preparations in the United States contain only vitamin D₂ (Table 1) (3).

1.0 Diagnostic Procedure

Recommendation

1.1 We recommend screening for vitamin D deficiency in individuals at risk for deficiency. We do not recommend

population screening for vitamin D deficiency in individuals who are not at risk (1|⊕⊕⊕⊕).

1.1 Evidence

There is no evidence demonstrating benefits of screening for vitamin D deficiency at a population level. Such evidence would require demonstration of the feasibility and cost-effectiveness of such a screening strategy, as well as benefits in terms of important health outcomes. In the absence of this evidence, it is premature to recommend screening at large at this time.

Currently, 25(OH)D measurement is reasonable in groups of people at high risk for vitamin D deficiency and in whom a prompt response to optimization of vitamin D status could be expected (Table 2) (3, 25, 52, 54–56).

TABLE 2. Indications for 25(OH)D measurement (candidates for screening)

Rickets
Osteomalacia
Osteoporosis
Chronic kidney disease
Hepatic failure
Malabsorption syndromes
Cystic fibrosis
Inflammatory bowel disease
Crohn's disease
Bariatric surgery
Radiation enteritis
Hyperparathyroidism
Medications
Antiseizure medications
Glucocorticoids
AIDS medications
Antifungals, e.g. ketoconazole
Cholestyramine
African-American and Hispanic children and adults
Pregnant and lactating women
Older adults with history of falls
Older adults with history of nontraumatic fractures
Obese children and adults (BMI > 30 kg/m ²)
Granuloma-forming disorders
Sarcoidosis
Tuberculosis
Histoplasmosis
Coccidiomycosis
Berylliosis
Some lymphomas

Recommendation

1.2 We recommend using the serum circulating 25(OH)D level, measured by a reliable assay, to evaluate vitamin D status in patients who are at risk for vitamin D deficiency. Vitamin D deficiency is defined as a 25(OH)D below 20 ng/ml (50 nmol/liter), and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (525–725 nmol/liter). We recommend against using the serum 1,25(OH)₂D assay for this purpose and are in favor of using it only in monitoring certain conditions, such as acquired and inherited disorders of vitamin D and phosphate metabolism (1|⊕⊕⊕⊕).

1.2 Evidence

25(OH)D is the major circulating form of vitamin D, with a circulating half-life of 2–3 wk, and it is the best indicator to monitor for vitamin D status (3, 8, 25, 54, 56). The circulating half-life of 1,25(OH)₂D is approximately 4 h. It circulates at 1000 times lower concentration than 25(OH)D, and the blood level is tightly regulated by serum levels of PTH, calcium, and phosphate. Serum 1,25(OH)₂D does not reflect vitamin D reserves, and measurement of 1,25(OH)₂D is not useful for monitoring the vitamin D status of patients. Serum 1,25(OH)₂D is frequently either normal or even elevated in those with vitamin D deficiency, due to secondary hyperparathyroidism. Thus, 1,25(OH)₂D measurement does not reflect vitamin

D status. Measurement of 1,25(OH)₂D is useful in acquired and inherited disorders in the metabolism of 25(OH)D and phosphate, including chronic kidney disease, hereditary phosphate-losing disorders, oncogenic osteomalacia, pseudovitamin D-deficiency rickets, vitamin D-resistant rickets, as well as chronic granuloma-forming disorders such as sarcoidosis and some lymphomas (3, 11, 50, 57, 58).

1.2 Remarks

All clinical assays, including 25(OH)D measurements, are subject to variability. Such variability confounds attempts to define a single “cut point” value as indicating low vitamin D status. Multiple methodologies for 25(OH)D measurement exist, including RIA, HPLC, and liquid chromatography tandem mass spectroscopy (3, 54, 59). For clinical care, it appears that all current methodologies are adequate if one targets a 25(OH)D value higher than current cut points; for example, a value of 40 ng/ml is without toxicity and virtually ensures that the individual's “true” value is greater than 30 ng/ml. A clinical approach of targeting a higher 25(OH)D value seems prudent in that improving vitamin D status should reduce multiple adverse consequences of vitamin D deficiency at extremely low cost with minimal toxicity risk. Finally, the comparability of 25(OH)D results seems likely to improve as uniform standards available through the National Institute of Standards and Technology become widely implemented.

Suggested 25(OH)D levels

Vitamin D deficiency in children and adults is a clinical syndrome caused by a low circulating level of 25(OH)D (3, 10, 25, 47, 50). The blood level of 25(OH)D that is defined as vitamin D deficiency remains somewhat controversial. A provocative study in adults who received 50,000 IU of vitamin D₂ once a week for 8 wk along with calcium supplementation demonstrated a significant reduction in their PTH levels when their initial 25(OH)D was below 20 ng/ml (16). Several, but not all, studies have reported that PTH levels are inversely associated with 25(OH)D and begin to plateau in adults who have blood levels of 25(OH)D between 30 and 40 ng/ml (20–22, 60); these findings are consistent with the threshold for hip and nonvertebral fracture prevention from a recent meta-analysis of double-blind randomized controlled trials (RCT) with oral vitamin D (56). When postmenopausal women who had an average blood level of 25(OH)D of 20 ng/ml increased their level to 32 ng/ml, they increased the efficiency of intestinal calcium absorption by 45–65% (17). Thus, based on these and other studies, it has been suggested that vitamin D deficiency be defined as a 25(OH)D

below 20 ng/ml, insufficiency as a 25(OH)D of 21–29 ng/ml, and sufficiency as a 25(OH)D of 30–100 ng/ml (3). The IOM report (20) also concluded, based in part on the PTH data, that vitamin D deficiency was defined as 25(OH)D below 20 ng/ml. They dismissed the calcium absorption study by Heaney *et al.* (17) as being a single study that did not directly measure calcium absorption and noted studies such as Hansen *et al.* (18), which showed no increase in intestinal calcium absorption across a broad range of serum 25(OH)D levels. However, the Heaney *et al.* (17) study was strengthened by the fact that they investigated a change in intestinal calcium absorption in the same women who had a blood level of 25(OH)D of approximately 20 ng/ml that was raised to an average of 32 ng/ml. The normalization of PTH at certain levels of 25(OH)D indirectly implies that these values can be suggested to define deficiency and insufficiency and indirectly informs treatment decisions. Studies of vitamin D replacement and treatment showing changes in patient-important outcomes (61) at certain levels of 25(OH)D are needed and would provide higher quality evidence that would lead to stronger recommendations.

2.0 Recommended Dietary Intakes of Vitamin D for Patients at Risk for Vitamin D Deficiency

Several recent studies have suggested that the recommended dietary allowances (RDA) of the IOM (20) may be inadequate, especially for patients who have underlying conditions or are receiving medications that put them at risk for vitamin D deficiency. The studies were reviewed, and Table 3 summarizes what the present RDA recommendations are and what we believe should be the recommended dietary intakes, especially for patients who are at risk based on the most current literature. These recommendations are often based on lower quality evidence (expert opinion, consensus, inference from basic science experiments, noncomparative or comparative observational studies); therefore, they should be considered as suggestions for patient care.

Recommendation

2.1 We suggest that infants and children aged 0–1 yr require at least 400 IU/d (IU = 25 ng) of vitamin D, and

TABLE 3. Vitamin D intakes recommended by the IOM and the Endocrine Practice Guidelines Committee

Life stage group	IOM recommendations				Committee recommendations for patients at risk for vitamin D deficiency	
	AI	EAR	RDA	UL	Daily requirement	UL
Infants						
0 to 6 months	400 IU (10 µg)			1,000 IU (25 µg)	400–1,000 IU	2,000 IU
6 to 12 months	400 IU (10 µg)			1,500 IU (38 µg)	400–1,000 IU	2,000 IU
Children						
1–3 yr		400 IU (10 µg)	600 IU (15 µg)	2,500 IU (63 µg)	600–1,000 IU	4,000 IU
4–8 yr		400 IU (10 µg)	600 IU (15 µg)	3,000 IU (75 µg)	600–1,000 IU	4,000 IU
Males						
9–13 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
14–18 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
51–70 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
>70 yr		400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
Females						
9–13 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
14–18 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
51–70 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
>70 yr		400 IU (10 µg)	800 IU (20 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
Pregnancy						
14–18 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
Lactation ^a						
14–18 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	600–1,000 IU	4,000 IU
19–30 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU
31–50 yr		400 IU (10 µg)	600 IU (15 µg)	4,000 IU (100 µg)	1,500–2,000 IU	10,000 IU

AI, Adequate intake; EAR, estimated average requirement; UL, tolerable upper intake level.

^a Mother’s requirement, 4,000–6,000 IU/d (mother’s intake for infant’s requirement if infant is not receiving 400 IU/d).

children 1 yr and older require at least 600 IU/d to maximize bone health. Whether 400 and 600 IU/d for children 0–1 yr and 1–18 yr, respectively, are enough to provide all the potential nonskeletal health benefits associated with vitamin D is not known at this time. However, to raise the blood level of 25(OH)D consistently above 30 ng/ml may require at least 1000 IU/d of vitamin D (2|⊕⊕⊕⊕).

2.1 Evidence

Birth to 18 yr

Risk factors for vitamin D deficiency and rickets in an infant include breast-feeding without vitamin D supplementation, dark skin pigmentation, and maternal vitamin D deficiency (38, 50, 62–68). *In utero*, the fetus is wholly dependent on the mother for vitamin D. The 25(OH)D passes from the placenta into the blood stream of the fetus. Because the half-life for 25(OH)D is approximately 2–3 wk, the infant can remain vitamin D sufficient for several weeks after birth, as long as the mother was vitamin D sufficient. However, most pregnant women are vitamin D deficient or insufficient (33–35). In a study of 40 mother-infant pairs, Lee *et al.* (33) reported that 76% of mothers and 81% of newborns had a 25(OH)D below 20 ng/ml at the time of birth, despite the fact that during pregnancy, the mothers ingested about 600 IU/d of vitamin D from a prenatal supplement and consumption of two glasses of milk.

Infants depend on either sunlight exposure or dietary vitamin D to meet their requirement from birth. Human breast milk and unfortified cow's milk have very little vitamin D (32). Thus, infants who are fed only human breast milk are prone to developing vitamin D deficiency, especially during the winter when neither they nor their mothers can obtain vitamin D from sunlight. Conservative estimates suggest that to maintain serum 25(OH)D concentrations above 20 ng/ml, an infant in the Midwest fed human milk must be exposed to sunlight in the summer about 30 min/wk while wearing just a diaper (69, 70).

Human milk and colostrum contain low amounts of vitamin D, on average 15.9 ± 8.6 IU/liter (32). There is a direct relationship between vitamin D intake and vitamin D content in human milk. However, even when women were consuming between 600 and 700 IU/d of vitamin D, the vitamin D content in their milk was only between 5 and 136 IU/liter (71). Preliminary data suggest that only after lactating women were given 4000–6000 IU/d of vitamin D was enough vitamin D transferred in breast milk to satisfy her infant's requirement (32).

Vitamin D intakes between 340 and 600 IU/d have been reported to have the maximum effect on linear growth of infants (72, 73). When Chinese infants were given 100, 200, or 400 IU/d of vitamin D, none demonstrated any

evidence of rickets (74). This observation is consistent with what Jeans (75) observed in 1950, and it was the basis for recommending that children only need 200 IU/d of vitamin D. However, Markestad and Elzouki (76) reported that Norwegian infants fed formula containing 300 IU/d obtained blood levels of 25(OH)D above 11 ng/ml, which at the time was considered the lower limit of normal. However, the IOM report says that the blood level should be at least 20 ng/ml, which implies that consuming even 300 IU/d is not adequate for infants (20, 47, 77).

Pediatric health care providers need to be aware of the deleterious effects of rickets on growth and bone development, including potential effects on bone density and development of peak bone mass (78). Musculoskeletal signs of rickets are well-described (47, 50, 66, 79, 80).

The American Academy of Pediatrics and the Canadian Pediatric Association (77) both recommended 400 IU/d. The IOM (20) recommended that the adequate intake and RDA for children 0–1 and 1–18 yr should be 400 and 600 IU/d, respectively. Whether 400 and 600 IU/d for these children is enough to provide all the health benefits associated with vitamin D is not known at this time.

Infants who received at least 2000 IU/d of vitamin D during the first year of life in Finland reduced their risk of developing type 1 diabetes in the ensuing 31 yr by 88%, without any reports of toxicity (81). Japanese children who received 1200 IU/d of vitamin D from December through March compared with placebo reduced their risk of influenza A by 42% (82). African-American normotensive children (16.3 ± 1.4 yr) who received 2000 IU/d compared with 400 IU/d for 16 wk in a randomized controlled trial had significantly higher serum 25(OH)D levels (36 ± 14 vs. 24 ± 7 ng/ml) and significantly lower arterial wall stiffness (83).

In the past, children of all races obtained most of their vitamin D from exposure to sunlight and drinking vitamin D-fortified milk, and therefore, they did not need to take a vitamin D supplement (3, 84). However, children are spending more time indoors now, and when they go outside, they often wear sun protection that limits their ability to make vitamin D in their skin. Children and adolescents are also drinking less vitamin D-fortified milk (28, 29, 85–90). There are reports that children of all ages are at high risk for vitamin D deficiency and insufficiency and its insidious health consequences (91–93), but with the cutoff of 20 ng/ml set by the IOM (20), the prevalence of vitamin D deficiency should be reevaluated. There are no data on how much vitamin D is required to prevent vitamin D deficiency in children aged 1–9 yr. A few studies have shown that during the pubertal years, children maintained a serum 25(OH)D above 11 ng/ml with dietary vitamin D intakes of 2.5–10 μ g/d (100–400 IU/d) (94). When in-

takes were less than 2.5 $\mu\text{g}/\text{d}$, Turkish children aged 12–17 yr had 25(OH)D levels consistent with vitamin D deficiency, *i.e.* below 11 ng/ml (95). A 2008 study by Maalouf *et al.* (91) suggests that this age group needs 2000 IU/d vitamin D to maintain a blood level above 30 ng/ml. Another study, by El-Hajj Fuleihan (96), provides an insight into the vitamin D requirement for children aged 10–17 yr (who were presumably exposed to an adequate amount of sun-mediated vitamin D because they lived in Lebanon) who ingested weekly doses of either 1,400 or 14,000 IU vitamin D₃ for 1 yr. Those who received 1400 IU/wk increased their blood level of 25(OH)D from 14 ± 8 to 17 ± 6 ng/ml, whereas the children who received 14,000 IU/wk for 1 yr increased their blood levels from 14 ± 8 to 38 ± 31 ng/ml. No signs of intoxication (hypercalcemia) were noted in the group receiving 14,000 IU/wk, although three subjects had a high 25(OH)D at the end of the study (103, 161, and 195 ng/ml) (96).

Children aged 9–18 yr have a rapid growth spurt characterized by a marked increase in their requirement of calcium and phosphorus to maximize skeletal mineralization. During puberty, the metabolism of 25(OH)D to 1,25(OH)₂D increases. In turn, the increased blood levels of 1,25(OH)₂D enhance the efficiency of the intestine to absorb dietary calcium and phosphorus to satisfy the growing skeleton's requirement for these minerals during its rapid growth phase. However, although production of 1,25(OH)₂D is increased, there is no scientific evidence to date demonstrating an increased requirement for vitamin D in this age group, possibly because circulating concentrations of 1,25(OH)₂D are approximately 500–1000 times lower than those of 25(OH)D (*i.e.* 15–60 pg/ml *vs.* 20–100 ng/ml, respectively) (97).

Recommendation

2.2 We suggest that adults aged 19–50 yr require at least 600 IU/d of vitamin D to maximize bone health and muscle function. It is unknown whether 600 IU/d is enough to provide all the potential nonskeletal health benefits associated with vitamin D. However, to raise the blood level of 25(OH)D consistently above 30 ng/ml may require at least 1500–2000 IU/d of vitamin D (2|⊕⊕⊕⊕).

2.2 Evidence

Ages 19–50 yr

This age group is at risk for vitamin D deficiency because of decreased outdoor activities and aggressive sun protection. Available data have not sufficiently explored the relationship between total vitamin D intake *per se* and health outcomes, nor have data shown that a dose-response relationship between vitamin D intake and bone health is lacking (20).

Very few studies have evaluated this age group's vitamin D requirement. However, in the large Third National Health and Nutrition Examination Survey (NHANES III) population-based study, a threshold for optimal 25(OH)D and hip bone density has been addressed among 13,432 younger (20–49 yr) and older (50+ yr) individuals with different ethnic and racial background (98). Compared with the lowest quintile of 25(OH)D, the highest quintile had higher mean bone density by 4.1% in younger whites (test for trend; $P < 0.0001$), by 1.8% in younger Mexican-Americans ($P = 0.004$), and by 1.2% in younger blacks ($P = 0.08$). In the regression plots, higher serum 25(OH)D levels were associated with higher BMD throughout the reference range of 10 to 38 ng/ml in all subgroups. In younger whites and younger Mexican-Americans, higher 25(OH)D was associated with higher BMD, even beyond 40 ng/ml. An evaluation of 67 white and 70 black premenopausal women ingesting 138 ± 84 and 145 ± 73 IU/d, respectively, revealed that serum 25(OH)D levels were in the insufficient or deficient range (circulating concentrations of 21.4 ± 4 and 18.3 ± 5 ng/ml, respectively) (99).

During the winter months (November through May) in Omaha, Nebraska, 6% of young women aged 25–35 yr ($n = 52$) maintained serum concentrations of 25(OH)D above 20 ng/ml but below 30 ng/ml when estimated daily vitamin D intake was between 131 and 135 IU/d (100). Healthy adults aged 18–84 yr who received 1000 IU/d vitamin D₃ for 3 months during the winter increased their 25(OH)D from 19.6 ± 11.1 to 28.9 ± 7.7 ng/ml (101).

A dose-ranging study reported that men who received 10,000 IU/d of vitamin D₃ for 5 months did not experience any alteration in either serum calcium or urinary calcium excretion (127). Adults older than 18 yr who received 50,000 IU vitamin D₂ every 2 wk (which is equivalent to 3000 IU/d) for up to 6 yr had a normal serum calcium and no evidence of toxicity (102).

Recommendation

2.3 We suggest that all adults aged 50–70 and 70+ yr require at least 600 and 800 IU/d, respectively, of vitamin D to maximize bone health and muscle function. Whether 600 and 800 IU/d of vitamin D are enough to provide all of the potential nonskeletal health benefits associated with vitamin D is not known at this time. (Among those age 65 and older we recommend 800 IU/d for the prevention of falls and fractures.) However, to raise the blood level of 25(OH)D above 30 ng/ml may require at least 1500–2000 IU/d of supplemental vitamin D (2|⊕⊕⊕⊕).

2.3 Evidence

Men and women older than 51 yr depend on sunlight for most of their vitamin D requirement. Increased use of

clothing and sunscreen over sun-exposed areas and decreased consumption of vitamin D-fortified milk increases the risk for vitamin D deficiency (3, 31, 39, 103). In addition, age decreases the capacity of the skin to produce vitamin D₃ (3). Although it has been suggested that aging may decrease the ability of the intestine to absorb dietary vitamin D, studies have revealed that aging does not alter the absorption of physiological or pharmacological doses of vitamin D (101, 104–106).

The IOM report (20) suggests that 25(OH)D levels need to be at least 20 ng/ml to maintain skeletal health. Prior estimates have ranged from as little as 12 to as high as 40 ng/ml (107). Recently, Priemel *et al.* (108) examined 675 iliac crest biopsies from male and female German adults (401 males, mean age, 58.2 yr; and 270 females, mean age, 68.2 yr) for structural histomorphometric parameters including osteoid indices. They reported that although they could not establish a minimum 25(OH)D level that was inevitably associated with mineralization defects, they did not find pathological accumulation of osteoid in any patients with circulating 25(OH)D above 30 ng/ml. They concluded that in conjunction with sufficient calcium intake, the dose of vitamin D supplementation should ensure that circulating levels of 25(OH)D reach a minimum threshold of 30 ng/ml to maintain skeletal health. In contrast, the IOM (20) concluded from the same study that a level of 25(OH)D of 20 ng/ml was adequate to prevent osteomalacia in at least 97.5% of the population and therefore recommended a threshold of 20 ng/ml to maintain skeletal health in 97.5% of the adult population.

Many studies have evaluated the influence of dietary vitamin D supplementation on serum 25(OH)D, PTH, and bone health as measured by BMD and fracture risks in older men and women. Several randomized, double-blind clinical trials of senior men and women who had an intake of 400 IU/d showed insufficient 25(OH)D levels (25, 55, 80, 109–112). When men and women received

supplements of 400–1000 IU/d, they had a significant reduction in bone resorption. In a randomized, placebo-controlled trial of elderly French women, those given calcium and 800 IU/d of vitamin D had significantly fewer vertebral and nonvertebral fractures (113). A similar observation was made in free-living men and women aged 65 yr and older who received 500 mg of calcium and 700 IU/d of vitamin D (114).

A threshold for optimal 25(OH)D and hip BMD has been addressed among 13,432 individuals studied in the NHANES III, including both younger (20–49 yr) and older (>50 yr) individuals with different ethnic and racial backgrounds (98). In the regression plots, higher hip BMD was associated with higher serum 25(OH)D levels throughout the reference range of 9–37 ng/ml in all subgroups.

A 2005 meta-analysis of high-quality primary prevention RCT of vitamin D and fracture risk consistently found that antifracture efficacy of vitamin D increases with a higher achieved level of 25(OH)D (Fig. 1) (51). Antifracture efficacy started at 25(OH)D levels of at least 30 ng/ml. This level was reached only in trials that gave 700–800 IU/d vitamin D₃ (high-quality trials with oral vitamin D₂ were not available at the time).

The most up-to-date meta-analysis focused on antifracture efficacy from high-quality double-blind RCT (55). The higher received dose (treatment dose*adherence) of 482–770 IU/d vitamin D reduced nonvertebral fractures in community-dwelling (–29%) and institutionalized (–15%) older individuals, and its effect was independent of additional calcium supplementation (–21% with additional calcium supplementation; –21% for the main effect of vitamin D). As with the 2005 meta-analysis, antifracture efficacy started at 25(OH)D levels of at least 30 ng/ml (75 nmol/liter).

Muscle weakness is a prominent feature of the clinical syndrome of severe vitamin D deficiency. Clinical findings in vitamin D-deficiency myopathy include proximal muscle weakness, diffuse muscle pain, and gait impairments such as a waddling way of walking (115, 116). Double-blind RCT demonstrated that 800 IU/d vitamin D₃ resulted in a 4–11% gain in lower extremity strength or function (80, 117), an up to 28% improvement in body sway (117, 118), and an up to 72% reduction in the rate of falling (119) in adults older than 65 yr after 5 months of treatment.

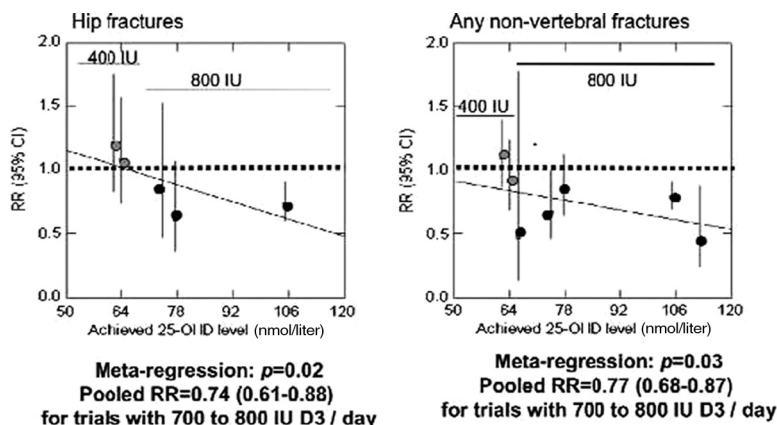


FIG. 1. Fracture efficacy by achieved 25(OH)D levels. To convert nmol/liter to ng/ml, divide by 2.496. [Reproduced with permission from H. A. Bischoff-Ferrari *et al.*: JAMA 293:2257–2264, 2005 (51). © American Medical Association.]

Several systematic reviews and meta-analyses have demonstrated a reduction in falls associated with interventions to raise 25(OH)D levels. Murad *et al.* (120) demonstrated that such interventions were associated with statis-

tically significant reduction in the risk of falls [odds ratio (OR) = 0.84; 95% confidence interval (CI), 0.76–0.93; inconsistency (I^2) = 61%; 23 studies). This effect was more prominent in patients who were vitamin D deficient at baseline. Results of other reviews were consistent. A meta-analysis of only five high-quality double-blind RCT (n = 1237) found that vitamin D reduced the falling risk by 22% (pooled corrected OR = 0.78; 95% CI, 0.64–0.92) compared with calcium or placebo (116). For two trials with a total of 259 subjects using 800 IU/d of vitamin D₃ over 2 to 3 months (117, 121), the corrected pooled OR was 0.65 (95% CI, 0.40–1.00) (116), whereas 400 IU/d was insufficient to reduce falls (122). The importance of dose of supplemental vitamin D in minimizing risk of falls was confirmed by a multidose double-blind RCT among 124 nursing home residents receiving 200, 400, 600, or 800 IU/d vitamin D or placebo over 5 months (119) and by a 2009 meta-analysis (52). Participants receiving 800 IU/d had a 72% lower rate of falls than those taking placebo or a lower dose of vitamin D (rate ratio = 0.28; 95% CI, 0.11–0.75).

In the 2009 meta-analysis for supplemental vitamin D, eight high-quality RCT (n = 2426) were identified, and heterogeneity was observed for dose of vitamin D (low dose, <700 IU/d, *vs.* higher dose, 700 to 1000 IU/d; P = 0.02) and achieved 25(OH)D level (<24 ng/ml *vs.* 24 ng/ml; P = 0.005). Higher dose supplemental vitamin D reduced fall risk by 19% [pooled relative risk (RR) = 0.81; 95% CI, 0.71–0.92; n = 1921 from seven trials]. Falls were not reduced by low-dose supplemental vitamin D (pooled RR = 1.10; 95% CI, 0.89–1.35 from two trials) or by achieved serum 25(OH)D concentrations below 24 ng/ml (pooled RR = 1.35; 95% CI, 0.98–1.84). At the higher dose of vitamin D, the meta-analysis documented a 38% reduction in the risk of falling with treatment duration of 2 to 5 months and a sustained effect of 17% fall reduction with treatment duration of 12 to 36 months (52). Most recently, the IOM did a very thorough review on the effect of vitamin D on fall prevention (20). Their synopsis is that the evidence of vitamin D on fall prevention is inconsistent, which is in contrast to the 2010 assessment by the International Osteoporosis Foundation and the 2011 assessment of the Agency for Healthcare Research and Quality for the U.S. Preventive Services Task Force (123), both of which identified vitamin D as an effective intervention to prevent falling in older adults.

Recommendation

2.4 We suggest that pregnant and lactating women require at least 600 IU/d of vitamin D and recognize that at least 1500–2000 IU/d of vitamin D may be needed to

maintain a blood level of 25(OH)D above 30 ng/ml (2|⊕⊕⊕⊕).

2.4 Evidence

Pregnancy and lactation

During the first and second trimesters, the fetus is developing most of its organ systems and laying down the collagen matrix for its skeleton. During the last trimester, the fetus begins to calcify the skeleton, thereby increasing maternal demand for calcium. This demand is met by increased production of 1,25(OH)₂D by the mother's kidneys and placenta. Circulating concentrations of 1,25(OH)₂D gradually increase during the first and second trimesters, owing to an increase in vitamin D-binding protein concentrations in the maternal circulation. However, the free levels of 1,25(OH)₂D, which are responsible for enhancing intestinal calcium absorption, are only increased during the third trimester. Pregnant women are at high risk for vitamin D deficiency, which increases the risk of preeclampsia (34) and cesarean section (124). Daily doses of 600 IU do not prevent vitamin D deficiency in pregnant women (34, 124). Their daily regimen should at least include a prenatal vitamin containing 400 IU vitamin D with a supplement that contains at least 1000 IU vitamin D.

During lactation, the mother needs to increase the efficiency of dietary absorption of calcium to ensure adequate calcium content in her milk. The metabolism of 25(OH)D to 1,25(OH)₂D is enhanced in response to this new demand. However, because circulating concentrations of 1,25(OH)₂D are 500–1000 times less than 25(OH)D, the increased metabolism probably does not significantly alter the daily requirement for vitamin D. To satisfy their requirement to maintain a 25(OH)D above 30 ng/ml, lactating women should take at least a multivitamin containing 400 IU vitamin D along with at least 1000 IU vitamin D supplement every day. To satisfy the requirements of an infant who is fed only breast milk, the mother requires 4000 to 6000 IU/d to transfer enough vitamin D into her milk (32). Thus, at a minimum, lactating women may need to take 1400–1500 IU/d, and to satisfy their infant's requirement, they may need 4000–6000 IU/d if they choose not to give the infant a vitamin D supplement.

Recommendation

2.5 We suggest that obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungals such as ketoconazole, and medications for AIDS be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement (2|⊕⊕⊕⊕).

2.5 Evidence

Obesity and medications

Obese adults (BMI > 30 kg/m²) are at high risk for vitamin D deficiency because the body fat sequesters the fat-soluble vitamin. When obese and nonobese adults were exposed to simulated sunlight or received an oral dose of 50,000 IU of vitamin D₂, they were able to raise their blood levels of vitamin D by no more than 50% compared with nonobese adults. Patients on multiple anticonvulsant medications, glucocorticoids, or AIDS treatment are at increased risk for vitamin D deficiency because these medications increase the catabolism of 25(OH)D (3, 42, 43).

Recommendation

2.6 We suggest that the maintenance tolerable UL of vitamin D, which is not to be exceeded without medical supervision, should be 1000 IU/d for infants up to 6 months, 1500 IU/d for infants from 6 months to 1 yr, at least 2500 IU/d for children aged 1–3 yr, 3000 IU/d for children aged 4–8 yr, and 4000 IU/d for everyone over 8 yr. However, higher levels of 2000 IU/d for children 0–1 yr, 4000 IU/d for children 1–18 yr, and 10,000 IU/d for children and adults 19 yr and older may be needed to correct vitamin D deficiency (2|⊕⊕⊕⊕).

2.6 Evidence

Vitamin D is a fat-soluble vitamin and is stored in the body's fat. Thus, there is concern about the potential toxicity of vitamin D. Bariatric patients who were found to have vitamin D in their fat (4–320 ng/g) showed no significant change in their serum 25(OH)D levels 3, 6, and 12 months after surgery (125). Limited human data (125, 126) show relatively low levels of vitamin D storage in fat at prevailing inputs. Neonates who were given at least 2000 IU/d of vitamin D for 1 yr in Finland not only did not experience any untoward side effect but also had the benefit of reducing their risk of developing type 1 diabetes by 88% in later life (81).

Preteen and teen girls who received an equivalent of 2000 IU/d of vitamin D for 1 yr showed improvement in muscle mass without any untoward side effects (96). A dose-ranging study reported that 10,000 IU/d of vitamin D₃ for 5 months in men did not alter either urinary calcium excretion or their serum calcium (127). A 6-yr study of men and women aged 18–84 yr who received an equivalent of 3000 IU/d of vitamin D₂ reported no change in serum calcium levels or increased risk of kidney stones (102). However, long-term dose-ranging studies in children are lacking.

Based on all of the available literature, the panel concluded that vitamin D toxicity is a rare event caused by

inadvertent or intentional ingestion of excessively high amounts of vitamin D. Although it is not known what the safe upper value for 25(OH)D is for avoiding hypercalcemia, most studies in children and adults have suggested that the blood levels need to be above 150 ng/ml before there is any concern. Therefore, an UL of 100 ng/ml provides a safety margin in reducing risk of hypercalcemia (3, 96). The IOM report (20) recommended that the tolerable UL for vitamin D should be 1000 IU/d for children 0–6 months, 1500 IU/d for children 6 months to 1 yr, 2500 IU/d for children 1–3 yr, and 3000 IU/d for children 4–8 yr. For children 9 yr and older and all adults, they recommend that the UL be 4000 IU/d. These recommendations were based on a variety of observations dating back to the 1940s. They also recognized that high intakes of calcium along with high intakes of vitamin D exacerbate the risk for hypercalcemia. Hyppönen *et al.* (81) observed that children during their first year of life received 2000 IU/d of vitamin D without any untoward toxicity. To prevent rickets, children during their first year of life received as much as 250,000 IU of vitamin D as a single im injection without any reported toxicity. Therefore, it is reasonable for the UL to be 2000 IU/d for children 0–1 yr of age. Toddlers who received 2000 IU/d of vitamin D for 6 wk raised their blood level from 17 to 36 ng/ml without any reported toxicity (47). Although no long-term studies have examined these higher doses of vitamin D on serum calcium levels, there are no reported cases of vitamin D intoxication in the literature to suggest that intakes of up to 4000 IU/d of vitamin D cause hypercalcemia. In healthy adults, 5 months of ingesting 10,000 IU/d of vitamin D neither caused hypercalcemia nor increased urinary calcium excretion, which is the most sensitive indicator for potential vitamin D intoxication (127). Therefore, a UL of 10,000 IU/d of vitamin D for adults is reasonable.

Hence, vitamin D supplementation should not be a major concern except in certain populations who may be more sensitive to it. Patients who have chronic granuloma-forming disorders including sarcoidosis or tuberculosis, or chronic fungal infections, and some patients with lymphoma have activated macrophages that produce 1,25(OH)₂D in an unregulated fashion (3, 44). These patients exhibit an increase in the efficiency of intestinal calcium absorption and mobilization of calcium from the skeleton that can cause hypercalciuria and hypercalcemia. Thus, their 25(OH)D and calcium levels should be monitored carefully. Hypercalciuria and hypercalcemia are usually observed only in patients with granuloma-forming disorders when the 25(OH)D is above 30 ng/ml (44).

3.0 Treatment and Prevention Strategies

Recommendation

3.1 We suggest using either vitamin D₂ or vitamin D₃ for the treatment and prevention of vitamin D deficiency (2|⊕⊕⊕⊕).

3.1 Evidence

Some (47, 101, 128) but not all (129–131) studies have shown that both vitamin D₂ and vitamin D₃ are effective in maintaining serum 25(OH)D levels. Two meta-analyses of double-blind RCT suggested reduction in falls and non-vertebral fractures with vitamin D₂ compared with vitamin D₃ (52, 56).

Several studies using vitamin D₂ and vitamin D₃ as an intervention have recorded changes in serum 25(OH)D after up to 6 yr of treatment (47, 96, 102), and dose-ranging studies extending out to 5 months of continuous therapy produced data with respect to the steady-state inputs needed to produce and sustain a specified level of 25(OH)D (127). Results of these studies converge on a rate of rise in serum 25(OH)D at approximately 0.4 ng/ml/μg/d, which means that ingesting 100 IU/d of vitamin D increases serum 25(OH)D by less than 1 ng/ml approximately (101, 127). For example, a typical patient with a serum 25(OH)D level of 15 ng/ml would require an additional daily input of about 1500 IU of vitamin D₂ or vitamin D₃ to reach and sustain a level of 30 ng/ml. Most of these studies have been conducted in adults. Similar changes in 25(OH)D have been observed in children (47, 96); two to three times as much vitamin D, however, is required to achieve this same increase in serum 25(OH)D levels in patients who are obese (3, 38, 42).

Vitamin D can be taken on an empty stomach or with a meal. It does not require dietary fat for absorption. Vitamin D given three times a year, once a week, or once a day can be effective in maintaining serum 25(OH)D levels in both children and adults (23, 47, 61, 96, 102).

Recommendation

3.2 For infants and toddlers aged 0–1 yr who are vitamin D deficient, we suggest treatment with 2000 IU/d of vitamin D₂ or vitamin D₃, or with 50,000 IU of vitamin D₂ or vitamin D₃ once weekly for 6 wk to achieve a blood level of 25(OH)D above 30 ng/ml followed by maintenance therapy of 400–1000 IU/d (2|⊕⊕⊕⊕).

3.2 Evidence

Vitamin D-deficient infants and toddlers who received either 2000 IU of vitamin D₂ or vitamin D₃ daily or 50,000 IU of vitamin D₂ weekly for 6 wk demonstrated equivalent increases in their serum 25(OH)D levels (47). No signs of

vitamin D intoxication were seen with any of the three regimens studied.

Children with rickets have been successfully treated with 600,000 IU of vitamin D either orally or im once a year (47, 50). In the United States, there are two pharmaceutical formulations of vitamin D. For the pediatric population, vitamin D₂ is available in a liquid form at a concentration of 8000 IU/ml, and for older children and adults, a gelatin capsule containing 50,000 IU of vitamin D₂ is available.

Recommendation

3.3 For children aged 1–18 yr who are vitamin D deficient, we suggest treatment with 2000 IU/d of vitamin D₂ or vitamin D₃ for at least 6 wk or with 50,000 IU of vitamin D₂ once a week for at least 6 wk to achieve a blood level of 25(OH)D above 30 ng/ml followed by maintenance therapy of 600–1000 IU/d (2|⊕⊕⊕⊕).

3.3 Evidence

Children of all ages are at risk for vitamin D deficiency and insufficiency (3, 29, 47, 77, 84–90), with the caveat that at present we do not know optimal serum 25(OH)D levels for any functional outcome. Vitamin D-deficient infants and toddlers who received either 2000 IU of vitamin D₂ or vitamin D₃ daily or 50,000 IU of vitamin D₂ weekly for 6 wk demonstrated equivalent increases in their serum 25(OH)D levels (47). There are sparse data to guide pediatric clinicians in the treatment of young children with vitamin D deficiency. One study showed that infants with vitamin D deficiency who receive doses of ergocalciferol exceeding 300,000 IU as a one-time dose were at high risk for hypercalcemia (132). Therefore, most pediatric providers use lower dose daily or weekly regimens. Caution also needs to be shown in children with Williams syndrome or other conditions predisposing to hypercalcemia (133).

Some studies indicate that children who receive adult doses of vitamin D experience changes in 25(OH)D similar to those seen in adults (47, 96). In accordance with the findings of Maalouf *et al.* (91), this age group needs 2000 IU/d vitamin D to maintain a blood level above 30 ng/ml. Children who received 1400 IU/wk increased their blood level of 25(OH)D from 14 ± 9 to 17 ± 6 ng/ml, whereas children who received 14,000 IU/wk for 1 yr increased their blood levels from 14 ± 8 to 38 ± 31 ng/ml.

Recommendation

3.4 We suggest that all adults who are vitamin D deficient be treated with 50,000 IU of vitamin D₂ or vitamin D₃ once a week for 8 wk or its equivalent of 6000 IU/d of vitamin D₂ or vitamin D₃ to achieve a blood level of

25(OH)D above 30 ng/ml, followed by maintenance therapy of 1500–2000 IU/d (2|⊕⊕⊕⊕).

3.4 Evidence

A dose of 50,000 IU of vitamin D₂ once a week for 8 wk is often effective in correcting vitamin D deficiency in adults (3, 16). Patients who do not show an increase in their blood level of 25(OH)D should be worked up for celiac disease or occult cystic fibrosis, assuming that they were compliant with treatment. To prevent recurrence of vitamin D deficiency, 50,000 IU of vitamin D₂ once every other week was effective in maintaining blood levels of 25(OH)D between 35 and 50 ng/ml without any untoward toxicity (102). Obese adults need at least two to three times more vitamin D to treat and prevent vitamin D deficiency (38, 42).

Alternative strategies for nursing home residents include 50,000 IU of vitamin D₂ three times per week for 1 month (134) or 100,000 IU of vitamin D every 4 months (61).

Recommendation

3.5 In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, we suggest a higher dose (two to three times higher; at least 6000–10,000 IU/d) of vitamin D to treat vitamin D deficiency to maintain a 25(OH)D level above 30 ng/ml, followed by maintenance therapy of at least 3000–6000 IU/d (2|⊕⊕⊕⊕).

3.5 Evidence

Obese adults need at least two to three times more vitamin D (at least 6000–10,000 IU/d) to treat and prevent vitamin D deficiency (42, 135). Patients receiving anticonvulsant medications, glucocorticoids, and a wide variety of other medications that enhance the activation of the steroid xenobiotic receptor that results in the destruction of 25(OH)D and 1,25(OH)₂D often require at least two to three times more vitamin D (at least 6000–10,000 IU/d) to treat and prevent vitamin D deficiency (3, 43). In both groups, the serum 25(OH)D level should be monitored and vitamin D dosage adjusted to achieve a 25(OH)D level above 30 ng/ml.

Recommendation

3.6 In patients with extrarenal production of 1,25(OH)₂D, we suggest serial monitoring of 25(OH)D levels and serum calcium levels during treatment with vitamin D to prevent hypercalcemia (2|⊕⊕⊕⊕).

3.6 Evidence

Patients who suffer from chronic granuloma-forming disorders including sarcoidosis, tuberculosis, and chronic fungal infections and some patients with lymphoma have activated macrophages that produce 1,25(OH)₂D in an

unregulated fashion (3, 44). This results in an increase in the efficiency of intestinal calcium absorption and mobilization of calcium from the skeleton that can cause hypercalciuria and hypercalcemia. These patients may require vitamin D treatment to raise their blood level of 25(OH)D to approximately 20–30 ng/ml to prevent vitamin D-deficiency metabolic bone disease while mitigating hypercalciuria and hypercalcemia.

The 25(OH)D levels need to be carefully monitored for these patients. Hypercalciuria and hypercalcemia are usually observed when the 25(OH)D is above 30 ng/ml (44).

Recommendation

3.7 For patients with primary hyperparathyroidism and vitamin D deficiency, we suggest treatment with vitamin D as needed. Serum calcium levels should be monitored (2|⊕⊕⊕⊕).

3.7 Evidence

Patients with primary hyperparathyroidism and hypercalcemia are often vitamin D deficient. It is important to correct their vitamin D deficiency and maintain sufficiency. Most patients will not increase their serum calcium level, and serum PTH may even decrease (45). Their serum calcium should be monitored.

4.0 Noncalcemic Benefits of Vitamin D

Recommendation

4.1 We recommend prescribing vitamin D supplementation for fall prevention. We do not recommend prescribing vitamin D supplementation beyond recommended daily needs for the purpose of preventing cardiovascular disease or death or improving quality of life (2|⊕⊕⊕⊕).

4.1 Evidence

Because most tissues and cells in the body have a vitamin D receptor and 1,25(OH)₂D influences the expression levels along with other factors of up to one third of the human genome, it is not at all unexpected that a numerous of studies has demonstrated an association of vitamin D deficiency with increased risk of more than a dozen cancers, including colon, prostate, breast, and pancreas; autoimmune diseases, including both type 1 and type 2 diabetes, rheumatoid arthritis, Crohn's disease, and multiple sclerosis; infectious diseases; and cardiovascular disease. There are, however, very few RCT with a dosing range adequate to provide level I evidence for the benefit of vitamin D in reducing the risk of these chronic diseases (20). In the cancer prevention study by Lappe *et al.* (136), postmenopausal women who received 1100 IU of vitamin D₃ daily along with calcium supplementation reduced their

overall risk of all cancers by more than 60%. This was associated with an increase in mean serum 25(OH)D levels from 29–39 ng/ml. Several observational studies have reported that colon cancer risk became progressively lower as serum 25(OH)D increased up to 30–32 ng/ml. However, because population values above 30–32 ng/ml are uncommon, most observational studies do not extend much beyond this level of repletion, and thus, observational data are largely silent about the optimal 25(OH)D levels.

Several studies found associations between 25(OH)D levels and hypertension, coronary artery calcification, as well as prevalent and incident heart disease (137–140). Prevalent myocardial infarction (MI) was found to be inversely associated with plasma 25(OH)D levels. The RR of MI for subjects with levels at the median or above was 0.43 (95% CI, 0.27–0.69), compared with subjects below the median. Similarly, individuals with levels below 15 ng/ml had a multivariable-adjusted hazard ratio of 1.62 (95% CI, 1.11–2.36) for incident cardiovascular events compared with those with levels above 15 ng/ml (137). Furthermore, although vitamin D deficiency is documented in long-term stroke survivors and is associated with post-stroke hip fractures, recent reports demonstrated low levels of 25(OH)D in patients presenting with acute strokes, suggesting that this deficiency had likely preceded the stroke and may be a potential risk factor for it (141).

Therefore, two systematic reviews were conducted as well as meta-analyses to summarize the best available research evidence regarding the effect of vitamin D-raising interventions on functional outcomes (falls, pain, quality of life) and cardiovascular outcomes (death, stroke, MI, cardiometabolic risk factors) (120, 142).

Vitamin D-raising interventions were associated with a not significant and potentially trivial reduction in mortality that was consistent across studies (RR = 0.96; 95% CI, 0.93–1.00; $P = 0.08$; $I^2 = 0\%$). There was no significant effect on MI (RR = 1.02; 95% CI, 0.93–1.13; $P = 0.64$; $I^2 = 0\%$), stroke (RR = 1.05; 95% CI, 0.88–1.25; $P = 0.59$; $I^2 = 15\%$), lipid fractions, glucose, or blood pressure; blood pressure results were inconsistent across studies, and the pooled estimates were trivial in absolute terms (142). In terms of functional outcomes, there was a clear reduction in the risk of falls as mentioned earlier, but no effect on pain or quality of life. The evidence supporting the latter outcomes was sparse, inconsistent, and of lower quality.

4.1 Values

The Task Force acknowledges the overall low-quality evidence in this area (20) and the fact that many of their recommendations are based on understanding of the biology of vitamin D pharmacokinetics, bone and minerals, basic science experiments, and epidemiological studies.

Nevertheless, in making recommendations, the panel placed the highest value on preserving musculoskeletal health and preventing childhood rickets and adult bone disease, and less value on vitamin D cost and potential for toxicity. Vitamin D supplementation/treatment is likely inexpensive and would be cost-effective, particularly in treating entities such as osteoporosis, rickets, and osteomalacia. Cost and resource utilization in other preventive indications are less known. Ample evidence provided the panel with a high level of confidence that toxicity of vitamin D at the recommended dosages is quite unlikely. The Task Force also acknowledges that science is changing rapidly in this field and that recommendations will likely need to be revised as future evidence accumulates.

Future Directions

There needs to be an appreciation that unprotected sun exposure is the major source of vitamin D for both children and adults and that in the absence of sun exposure it is difficult, if not impossible, to obtain an adequate amount of vitamin D from dietary sources without supplementation to satisfy the body's requirement. Concerns about melanoma and other types of skin cancer necessitate avoidance of excessive exposure to midday sun. These observations strengthen the arguments for supplementation, especially for people living above 33° latitude (143). All available evidence suggests that children and adults should maintain a blood level of 25(OH)D above 20 ng/ml to prevent rickets and osteomalacia, respectively. However, to maximize vitamin D's effect on calcium, bone, and muscle metabolism, the 25(OH)D blood level should be above 30 ng/ml. Numerous epidemiological studies have suggested that a 25(OH)D blood level above 30 ng/ml may have additional health benefits in reducing the risk of common cancers, autoimmune diseases, type 2 diabetes, cardiovascular disease, and infectious diseases.

Few RCT have used an amount of vitamin D that raises the blood level above 30 ng/ml, and thus there remains appropriate skepticism about the potential noncalcemic benefits of vitamin D for health. Concern was also raised by the IOM report (20) that some studies have suggested that all-cause mortality increased when blood levels of 25(OH)D were greater than approximately 50 ng/ml. RCT that evaluate the effects of vitamin D doses in the range of 2000–5000 IU/d on noncalcemic health outcomes are desperately needed. There is no evidence that there is a downside to increasing vitamin D intake in children and adults, except for those who have a chronic granuloma-forming disorder or lymphoma.

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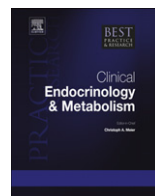


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Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/ml)

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The Institutes of Medicine (IOM) recently revised the recommended dietary allowances (RDA) for vitamin D, to maintain serum 25-hydroxyvitamin D (25(OH)D) at or above 50 nmol/L, to sustain bone density, calcium absorption, and to minimize risk of osteomalacia and rickets. However there are compelling reasons why 25(OH)D should preferably exceed 75 nmol/L: (A) Scrutiny of actual data specified by the IOM relating 25(OH)D to bone density and osteomalacia shows the desirable minimum 25(OH)D to be 75 nmol/L (30 ng/mL). (B) Humans are primates, optimized through evolution to inhabit tropical latitudes, with serum 25(OH)D over 100 nmol/L. (C) Epidemiologic relationships show health benefits if 25(OH)D levels exceed 70 nmol/L; these include fewer falls, better tooth attachment, less colorectal cancer, improved depression and wellbeing. Some studies of populations at high-latitude relate higher 25(OH)D to risk of prostate cancer, pancreatic cancer or mortality. Those relationships are attributable to the dynamic fluctuations in 25(OH)D specific to high latitudes, and which can be corrected by maintaining 25(OH)D at steady, high levels throughout the year, the way they are in the tropics. (D) There are now many clinical trials that show benefits and/or no adversity with doses of vitamin D that raise serum 25(OH)D to levels beyond 75 nmol/L. Together, the evidence makes it very unlikely that further research will change the conclusion that risk of disease with serum 25(OH)D higher than 75 nmol/L is lower than the risk of disease if the serum 25(OH)D is approximately 53 nmol/L.

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Introduction

The National Academy of Sciences (NAS) is an arm's-length scientific advisory body to the United States government. The Institute of Medicine (IOM) is a division of the NAS. Recently, the Canadian and United States governments supported the IOM to conduct a thorough review of all available evidence, and if necessary, to revise dietary guidelines for vitamin D and for calcium.¹ For calcium, the recommendations underwent minimal change. However, for vitamin D, the recommendations went from what had previously been referred to as an "adequate intake" – essentially a preliminary approximation – into what is now a recommended dietary allowance (RDA), which is considered more scientifically credible. The basis for the RDA was the opinion of a carefully selected panel of experts, that a serum 25(OH)D concentration as low as 50 nmol/L is enough to maintain healthy bones for most of the population.^{1,2} From this, the RDA for vitamin D was determined as the daily amount of oral vitamin D intake that assures 97.5% the population will sustain a serum 25(OH)D concentration higher than 50 nmol/L. Compared to the advice the IOM published in 1997,³ the dietary recommended intakes for vitamin D tripled for most of the population: from 200 IU up to 600 IU daily. Furthermore, the intake that can be safely consumed by adults doubled, from 2000 IU daily up to 4000 IU daily. Despite these substantial increases in dietary recommendations, should we feel comfortable with a serum 25(OH)D level of 53 nmol/L as Rosen contends?²

Vitamin D does not fit the conventional paradigms attributed to a nutrient, and it needs to be approached with a different way of thinking. Except for communities in the far north, where fish consumption is traditionally a major component of the diet, most humans rely upon sun exposure as their major source of vitamin D. The consumption of fish as well as casual sunshine exposure can provide adults with the equivalent of 3000 IU of vitamin D daily.^{4,5} In the context of the amounts of vitamin D acquired naturally, even the new RDA values are quite modest.

Only once the IOM committee was convinced by what it described as "compelling" evidence about the efficacy of vitamin D for bone health, mainly based on placebo control clinical trials (RCT's), did it consider it appropriate to select a minimum level of serum 25(OH)D against which to calibrate its intake recommendations for the nutrient.¹ For the rest of this paper I will discuss the issue of whether the 50 nmol/L minimum level selected by the IOM committee was appropriate, and address the minimum serum 25(OH)D concentration that a knowledgeable person would be comfortable with, in order to maintain health and to prevent disease?

Bone health

Let us accept for the time being, the position of the IOM panel, that bone health is the only criterion for which the evidence is compelling enough to justify a dietary recommendation.¹ To justify its recommendation for serum 25(OH)D, the IOM report presents what is reprinted here as Fig. 1. The Figure leads to the unambiguous conclusion that a serum 25(OH)D level of 50 nmol/L is more than adequate for virtually all of the population, and that beyond 50 nmol/L, there is no further health benefit. Although it is a subjective decision as to where along a continuous scale of 25(OH)D levels, the point of adequacy should be placed, it is appropriate to examine the rationale of the committee more carefully.

As "conceptualized" in Fig. 1, the relationship between serum 25(OH)D levels and bone mineral density (BMD) reaches its maximum well before 50 nmol/L. The actual data that would have led to these conceptualized graphs are not specified in the IOM report. It is safe to say that for BMD, Table 8 in Appendix C of the IOM report is the only place that contains data pertinent to the BMD representation in Fig. 1.¹ What is not mentioned in the IOM report, are the published data that are, to my knowledge, most similar to the IOM's conceptualized curve. Bischoff-Ferrari et al. presented cross-sectional data for 13,432 adults of all ages, sampled from the National Health and Nutritional Examination Survey (NHANES) cohort from across the USA.⁶ The preliminary "Ottawa report" that had been prepared for the IOM committee to summarize the pertinent knowledge on bone health, stated in reference to Bischoff-Ferrari, that "the association between serum 25(OH)D concentrations and BMD had a steep positive slope in the reference range, reaching a plateau at a concentration of 90–100 nmol/L in an

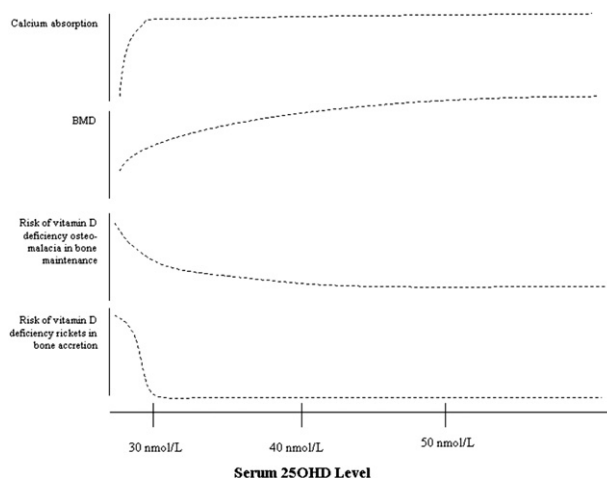


Fig. 1. The horizontal axis ranges from 30 to 50 nmol/L (12–20 ng/mL), and the curves represent summary approximations (from top to bottom) of efficiency of intestinal calcium absorption, bone mineral density, the risk of osteomalacia, and the risk of rickets. Reprint of Figure 5-1 of the IOM report with its caption, “Conceptualization of integrated bone health outcomes and vitamin D exposure.”

older Caucasian population.”⁷ In its full document, the IOM did not mention this work¹; instead, it presented in its Appendix C, Table 8, data from 13 smaller studies that had a combined total sample size of 3298 subjects. Because of their smaller sample sizes, the studies summarized in IOM Table 8 had low statistical power, and it should be expected that the 13 studies pertinent to vitamin D and BMD exhibited inconsistent occurrences of statistical significance. In contrast, the regression plots of Bischoff-Ferrari et al. (Fig. 2) represent a sample size that was four times bigger than the total number of subjects in all of the 13 studies that the IOM committee took into consideration.^{1,6} The evidence in Fig. 2 is the strongest data that we have on this topic, and the lines that summarize actual BMD data differ dramatically from the conceptual BMD illustration presented by the IOM committee to justify its target of 50 nmol/L (Fig. 1). Furthermore, Bischoff-Ferrari et al. reported that all of the progressive trends in bone mineral density among quintiles in 25(OH)D were statistically significant when appropriately stratified by Race/Ethnicity and Age. These data for the United States NHANES population are strong evidence of substantial benefits to BMD beyond 50 nmol/L.

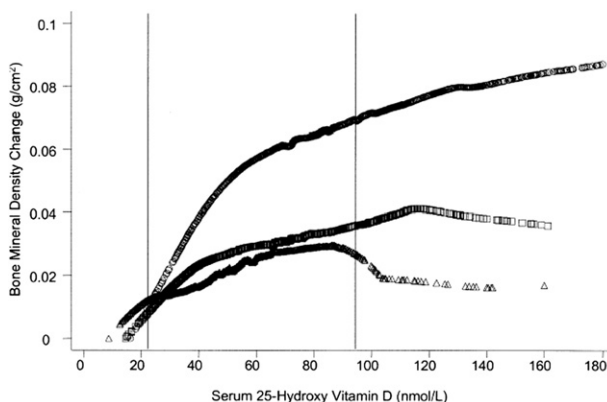


Fig. 2. Bone mineral density data for Americans, from the NHANES Study of the US Population, for White, African American, and Spanish Americans (top to bottom). The lines are non-parametric curve fits, similar to running averages of BMD for subjects having the 25(OH)D levels along the horizontal axis. These lines show bone densities for men and women < age 50. BMD at the origin is set to 0 to cancel out density differences among the groups.

To prevent the classic vitamin D-deficiency disease of osteomalacia, the IOM committee report placed substantial emphasis on data from Priemel et al.⁸ “to support a serum 25(OH)D level of 50 nmol/L as providing coverage for at least 97.5 percent of the population.”¹ In Fig. 1, the line that represents the relationship between 25(OH)D levels and the risk of osteomalacia reflects the conceptualization of IOM committee, about the available data on osteomalacia in relation to serum 25(OH)D. The conceptualization is not consistent with the actual histomorphometric data published by Priemel et al. that are reproduced here in Fig. 3. If one focuses on the data for people whose serum 25(OH)D was in the range 50–75 nmol/L, then there is evidence of osteomalacia in the bones of 18–39% of them. The data by Priemel et al. are critical because they are singled out in the IOM committee report to support its decisions about serum 25(OH)D.¹ However, as Priemel et al. have stated repeatedly, their data clearly point to a desirable minimum of 75 nmol/L⁸ – in contrast, the IOM committee minimum is 50 nmol/L for osteomalacia (Fig. 1). When a specific data set is identified as a key criterion, then the objective analysis of the actual numbers takes precedence over the subjective “conceptualization” that was presented by the IOM.

Human biology

“Evidence-based medicine” is not limited by what can be known from RCT’s. The originators of the concept of evidence-based medicine made it clear that evidence encompasses the breadth of science in the human context.^{9,10} Any decision about what an optimal level of serum 25(OH)D might be, needs to start from the context of evolution. Evolution is a process of virtual design that, through trial-and-error, optimizes the suitability of an organism for its environment. The concept of what is “normal” for the human should start from the perspective that we are a primate species, optimized to inhabit tropical environments. Contemporary outdoor workers and inhabitants of sunny environments often possess serum 25(OH)D concentrations much higher than 100 nmol/L, despite minimal dietary vitamin D.⁴ Most members of modern society rarely expose much of their skin to the vitamin D-forming

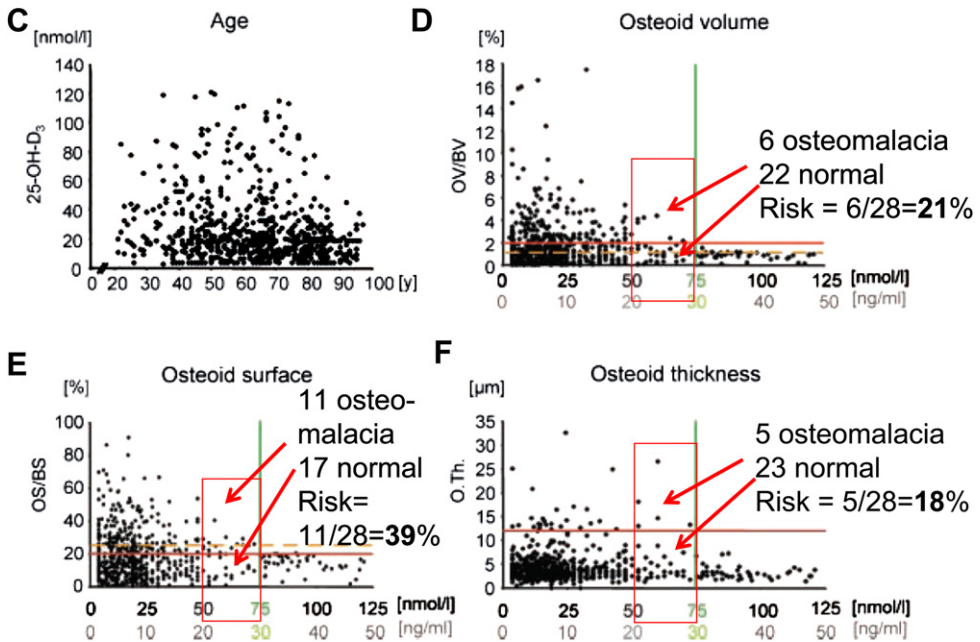


Fig. 3. The data from Priemel et al., 2010, annotated with counts of actual data (as confirmed by the authors). The boxes around the values 50–75 nmol/L are drawn by me, and the figure is annotated by me (RV). The IOM Report states that, based on the data of Priemel et al., adults with 25(OH)D of at least 50 nmol/L are at <2.5% risk of osteomalacia.

ultraviolet B (UVB) rays of sunshine, and their diets contain little vitamin D. The resulting, endemic levels of serum 25(OH)D in modern societies are not logical as a starting point from which to decide as to what the optimal level might be for human health. Nonetheless, endemic 25(OH)D levels have been the starting point for the deliberations of both the IOM and the International Agency for Research on Cancer (IARC).^{1,11} Both contend that any beneficial relationship beyond currently prevalent 25(OH)D levels should require a standard of evidence achievable only with placebo controlled clinical trials (RCT's).

In contrast to their principles that demand the highest possible level of evidence to support a benefit, both the IARC, and IOM committees have been indiscriminate in accepting without hesitation, any suggestion that a relationship with higher 25(OH)D might be adverse. The source of most concern for the IARC, and the IOM committees and other conservative commentators have been the instances where prostate cancer, pancreatic cancer, or increased mortality were associated with the highest serum 25(OH)D concentrations in certain population groups.^{1,11–13} What appears to have been overlooked by those who focus on the rare suggestions of adversity, is that those instances of U-shaped risk curves with serum 25(OH)D have been specific to the most northerly of populations.^{12,14–17} To my knowledge, none of the U-shaped risk relationships have been reported for populations of sunnier, southern regions where serum 25(OH)D levels should range to levels higher than they do in the north. The most likely mechanism for adversity with higher 25(OH)D levels within the physiologically achievable range involves the annual rises and falls in serum 25(OH)D that occur at latitudes away from the equator.^{17–19} Higher summertime 25(OH)D levels result in sharper serum 25(OH)D declines through winter. Those persons with the highest wintertime 25(OH)D levels are also the ones whose 25(OH)D levels were highest during summer, and who exhibit the largest declines into winter.^{20,21} Temperate latitudes not only deliver less UVB throughout the year than do the equatorial regions, but at temperate latitudes, the intensity of UVB fluctuates dramatically throughout the year.²² Consequently, in the context of the tropical environment into which they evolved, humans are biologically optimized for serum 25(OH)D levels that are both higher and more stable than those seen in the contemporary cultures. Health is harmed in subtle ways by environments in which 25(OH)D levels go through annual cycles of ups and downs. For example, in Australia, where falls and fractures cannot be attributable to ice and snow, both falls and fractures increase during the annually declining phases in serum 25(OH)D.²³ Likewise, a recent New Zealand clinical trial of large once-a-year doses of vitamin D caused annual cycles of increased falls and fractures during the first three months after each dose.²⁴ The patterns of falls and fractures in both the Australian and the New Zealand studies cannot be attributed to low bone density per se, because the intervals of adversity were too brief to have much effect on bone. More likely, actively declining 25(OH)D levels adversely affected muscle function and balance to the point of causing falls and fractures. If the hypothesis of adversity caused by fluctuating 25(OH)D levels is true as I contend, then the preventive strategy would be to increase vitamin D intakes in order to lessen the effect of seasonality on serum 25(OH)D concentrations.¹⁷ Paradoxically, by keeping their dietary advice for vitamin D as low as possible, the IOM and IARC are, in my view, perpetuating the very U-shaped risk phenomena they wish to avoid.

Anthropologic considerations point to “biologically normal” 25(OH)D levels that firstly, exceed 75 nmol/L (30 ng/mL), ranging to 225 nmol/L (90 ng/mL), and that secondly, fluctuate minimally throughout the year. Regrettably, the biology of the human being is something that guideline committees have ignored. I have been told that this is because the biology of early humans is regarded as theoretical and not amenable to testing with an RCT.

Epidemiology

There are now many cross-sectional and case-control epidemiological studies of relationships between health and the level of 25(OH)D. Most of these have been reviewed by AHRQ and IOM. Bischoff-Ferrari presented a very nice summary of why a desirable serum 25(OH)D level would appear to be one that exceeds 75 nmol/L (Fig. 4).²⁵ Most of the statistically significant health relationships with serum 25(OH)D show trends for improved health for levels that are higher than 50 nmol/L.^{25,26} Nonetheless, a recent review under the auspices of the World Health Organization concludes “...no compound should be recommended for cancer chemoprevention if its efficacy and side effects have not

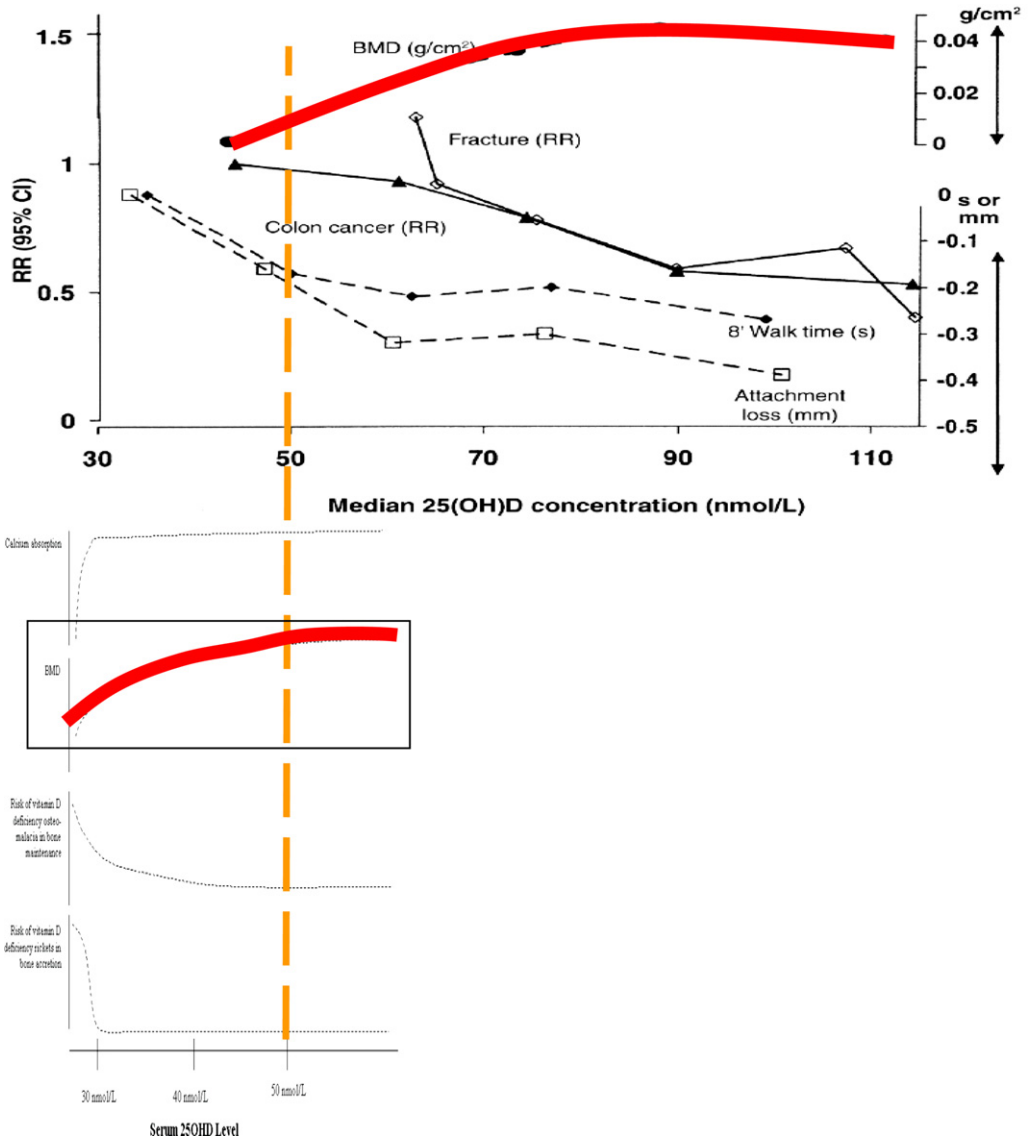


Fig. 4. Health and disease relationships with serum 25(OH)D as summarized by Bischoff-Ferrari et al.²⁵ From top to bottom, the curves represent BMD, Fracture risk from a meta-analysis of RCT's, Case-control studies of colon cancer, NHANES data for 8-foot walking time test, and depth of tooth pockets in Americans. Inset below the figure is the conceptualized figure from the IOM, adjusted so that the units line up with the units of the figure by Bischoff-Ferrari et al. In both figures, the lines representing BMD are traced in bold.

been evaluated in large, randomized trials. Ideally, these trials should be double-blind and placebo controlled.”¹¹ There are many reasons why it is not realistic to anticipate that long-term, placebo controlled clinical trials can provide evidence for the primary prevention of disease across society.^{27,28}

The need for RCT's as a criterion before doing anything about vitamin D is clear from the deliberations of the most recent IOM committee.¹ The common criticism of health benefits epidemiologically related to 25(OH)D levels is that vitamin D nutritional status is a covariate that coincides with, and which is attributable to a healthy lifestyle. Compared to bone health, there is not a lot of evidence that

the actual dietary intake of vitamin D will bring about the desirable changes suggested by epidemiologic studies.

Unless epidemiology suggests an adverse relationship, the IOM and IARC have tended to downplay the discipline. The committee's conservatism may have been stimulated by the Helzlsouer et al., who summarized the publications of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.¹² All of the cancer relationships they summarized were negative ones (no relationships), except for a single data point, in relation to pancreatic cancer risk. Because of that data point, the report by Helzlsouer et al. along with an accompanying editorial were highly negative about vitamin D.^{12,29} They pointed out that in the light of ongoing clinical trials involving vitamin D, as listed at the Clinical Trials Registry maintained by the National Institutes of Health (<http://clinicaltrials.gov/>), it is best to wait before raising any guidelines for vitamin D. In particular, they cited the Vitamin D and Omega-3 Trial (referred to as the "VITAL Study"), which is studying 20,000 adults and randomized 2000 IU per day of vitamin D (<http://www.vitalstudy.org/>). The aim is to find out whether vitamin D and/or omega-3 fatty acids reduce the risk of developing cancer, heart disease, and stroke. Like most of the ongoing trials, it will be at least another 5 years before the results are reported, and even then, because women younger than age 60 are excluded, the results will not be applicable to most of the population. Furthermore, a meta-analysis of the currently ongoing clinical trials will likely take 5 years beyond that. At the current rate, it will take another decade before another IOM committee can address these things again.

Placebo controlled clinical trials

For the sake of argument, let us accept again the position of the IOM panel and IARC, that health relationships for vitamin D are only valid if they are supported by controlled clinical trials. It is appropriate then, to look at the existing clinical trial data that the IOM committee dismissed as "uninformative" because individual trials rarely use multiple dose levels. In effect, efficacy of clinical trials using higher doses of vitamin D was disregarded because the committee considered it possible that the same effect of a higher dose could have been achieved with a lower, intermediate dose. If 25(OH)D levels in the control arm of clinical trials are already at or above the 50 nmol/L desired by the IOM, then the clinical trials are certainly appropriate to address the question of whether there are further health benefits beyond 50 nmol/L.

As 2011, there are at least 13 published clinical trials of doses vitamin D higher than the osteoporosis dose of 800 IU/day (Table 1). What makes any summary analysis of the clinical trials particularly complex is the variety of health outcomes that were statistically significant. With vitamin D₃ at an average daily dose rate of approximately 4000 IU, the benefits for adults included general well being,⁴³ lower depression scores,³⁵ and improved insulin responsiveness.⁴⁴ The 25(OH)D levels associated with benefit in the clinical trials summarized in Table 1 are never less than 70 nmol/L. It is also important to note that, none of the 13 trials of higher-dose vitamin D have reported a significant adverse event attributable to the vitamin D.

For premenarcheal girls, daily average vitamin D intakes of 2000 IU (14000 IU weekly) improved the gain in bone density and muscle mass.³³ In another randomized trial in children during winter, supplementation with 1200 IU daily lowered the risk of influenza A and the incidence of asthmatic attacks.⁴² Burton et al. reported an RCT involving patients with multiple sclerosis and showed that up to 40,000 IU of vitamin D are safe, and with evidence of benefit in lowering relapse rates and severity scores of multiple sclerosis.³² The Burton RCT was a tolerability study, where a series of vitamin D₃ loading doses were given to accelerate attainment of super-physiological levels of serum 25(OH)D, to test the safety of those levels; the RCT was not an assessment of therapeutic maintenance doses.

The clinical trial of Lappe et al. demonstrated a reduction in cancer diagnosis in the group of women for whom serum 25(OH)D had been raised from an initial control-group mean of 71 nmol/L, to a mean of 96 nmol/L³⁶ (Table 1). Even though cancer was not the primary, fracture-preventive outcome of the trial, the Lappe publication represents a higher level of evidence than epidemiological data. Since the serum 25(OH)D in the control group was already at 71 nmol/L – essentially the same level defined as optimal in the meta-analysis of Bischoff-Ferrari et al. for bone health⁴⁵ – the Lappe clinical trial stands as a strong justification that 25(OH)D levels higher than 71 nmol/L can provide further health benefits.

Table 1

Clinical Trials of Vitamin D3 at Doses >800 IU/day (>20 mcg/day) and Effects of the Attained 25(OH)D Levels.

Clinical trial	Study subjects	Treatment dose and Duration	Primary Outcome	Treatment effect (a secondary outcome if different from the primary one)	Type of Control (double-blind unless stated otherwise)	Control 25(OH)D nmol/L	Treatment 25(OH)D nmol/L	Primary outcome improved with vitamin D	A Secondary outcome improved	Adverse Effect of Treatment
Aloia 2010 ³⁰	Men and women ages 20–80	4000 IU/day for 4 months with or without 1200 mg calcium	Characterize interaction of calcium and vitamin D on bone turnover markers	No effect of vitamin D on PTH or bone turnover markers. Calcium alone lowered these.	Double placebo, without calcium or vitamin D	66 (24)	112 (30) [active D, placebo Ca group]	No	No	No
Amir 2010 ³¹	Women with metastatic breast cancer	10,000 IU/day for 4 months	Overall pain scores	No effect on overall pain or bone turnover markers, but significantly fewer pain sites	Single-arm trial	70 (19–169)	162 (74–226)	No	Yes	No
Burton 2010 ³²	Patients with multiple sclerosis	Average 14,000 IU/day over 12 months (doses ranged up to 40,000 IU/day)	Safety and exploratory outcomes	Lower risk of progression of disability score	Open label untreated group (many took up to 4000 IU/day vitamin D anyway)	83 (27)	179 (76), at 12 months (varied through study)	NA since Phase 1–2 clinical trial.	Yes	No
El-Haj Fuleihan 2006 ³³	Pre-menarcheal girls	2000 IU/day for 12 months	Bone density	Greater gains in hip and vertebral bone density, and lean body mass	Placebo	27 (15)	70 (23)	Yes	Yes	No
Hitz 2007 ³⁴	Hip-fracture patients	1400 IU/day plus 1200 mg calcium for 12 months	Bone density	Improved lumbar spine BMD	200 IU vitamin D only	53 (17)	82 (19)	Yes	No	No
Hitz 2007 ³⁴	Patients with lower-extremity fracture	1400 IU/day plus 1200 mg calcium for 12 months	Bone density	Improved lumbar spine BMD	200 IU vitamin D only	77 (18)	90 (24)	Yes	No	No
Jorde 2008 ³⁵	Adults with BMI>28	Weekly 20,000 IU or 40,000 IU	Weight loss	Lower (improved) Beck Depression Index	Placebo group	50.0 (20–100)	88 (51–162) and 112 (47–193)	NA	Yes	No

Table 1 (continued)

Clinical trial	Study subjects	Treatment dose and Duration	Primary Outcome	Treatment effect (a secondary outcome if different from the primary one)	Type of Control (double-blind unless stated otherwise)	Control 25(OH)D nmol/L	Treatment 25(OH)D nmol/L	Primary outcome improved with vitamin D	A Secondary outcome improved	Adverse Effect of Treatment
Lappe 2007 ³⁶	Women over age 65	1100 IU/day, plus 1000 mg/day calcium, 4 yrs	Bone density	Lower rates of new cancer diagnosis	Placebo	71 (20)	96 (21)	NA	Yes	No
Martineau 2011 ³⁷	Tuberculosis		Time to sputum conversion					Yes (for a subgroup)	No	No
Mocanu 2009 ³⁸	Nursing-home residents	5000 IU/day for 12 months, single-arm study	Bone density	Increase in bone density at both in hip and vertebral spine	Baseline of same group	29 (20–36)	125 (104–150)	Yes	No	No
Nursyam 2006 ³⁹	Pulmonary tuberculosis patients	10,000 IU/day for 6 weeks	TB Sputum Clearance	Radiological improvement	Placebo group	Not measured	Not measured	Yes	Yes	No
Smith 2009 ⁴⁰	Men and women through the South Pole winter	2000 IU/day or 1000 IU/day for 5 months	Effect on serum 25(OH)D	NA	Group receiving 400 IU/day	57 (18)	71 (23)	NA	NA	No
Stubbs 2010 ⁴¹	End-stage renal disease	50,000 IU/wk for 8 wk but adjusted so 25(OH)D was 112–150 nM	Cytokine concentrations	Lower inflammatory cytokines	Single-arm trial	35	135	Yes		no
Urashima 2010 ⁴²	School children 6–15 y	1200 IU/day for 4 months through winter	Incidence of influenza A	Lower risk of influenza A and lower risk of asthma attacks	Placebo	Not measured	Not measured	Yes	Yes	No
Vieth 2004 ⁴³	Well thyroid clinic outpatients	4000 IU/day for 3 months and 15 months	Wellbeing and mood in February	Wellbeing scores improved into winter	Lower dose group, 600 IU/d	79 (30)	112 (41)	Yes	No	No
von Hurst 2009 ⁴⁴	Insulin resistant Asian women	4000 IU/day vs 600 IU/day for 6 months	Insulin responseiveness	Improved insulin responsiveness vs placebo	Placebo group	21 (11–40)	75 (50–84)	Yes	No	No

Conclusion

Dr. Bouillon argues elsewhere in this journal for the conservative perspective, that a lower desirable level – 50 nmol/L (20 ng/mL) for 25(OH)D – is justified unless and until much stronger RCT evidence can support the efficacy and the safety of widespread use of vitamin D intakes beyond 800 IU/day (see *Why modest but widespread improvement of the vitamin D status is the best strategy* in this issue). Since most modern populations exhibit 25(OH)D levels that are on average, higher than 50 nmol/L,^{46,47} the conservative perspective adheres to the notion that endemic levels of 25(OH)D are inherently the healthiest ones. The conservative approach to vitamin D recommendations does not take into account all of the evidence available, because it downplays the usefulness of existing knowledge of human biology, epidemiology, and even the existing evidence from clinical trials. In at least 7 clinical trials the 25(OH)D level in the control group were at or above the IOM value of 50 nmol/L, yet additional vitamin D produced significant health effects.^{32–37,43} To paraphrase the highest level criterion for the GRADE evidence based approach,¹⁰ “Is further research likely to alter the conclusion that risk of disease is lower if serum 25(OH)D exceeds 75 nmol/L than if the level is at 51 nmol/L?” A desirable minimum 25(OH)D level of 75 nmol/L is readily justifiable now, based on the very criteria used by the IOM, as well as by the basic biology of humans, by epidemiologic relationships and by existing evidence from randomized clinical trials. There will always be further ongoing studies to await, but the conservative perspective begs the question, “Exactly how much evidence is needed before the IOM and the IARC accept that a minimally desirable serum 25(OH)D of 75 nmol/L is preferable to 50 nmol/L?” The conservative view that 50 nmol/L is appropriate is not scientifically defensible. Modest scrutiny of the key criteria that the IOM committee specified to justify its conclusion shows that healthy bones require a 25(OH)D level substantially higher than what they claim.

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