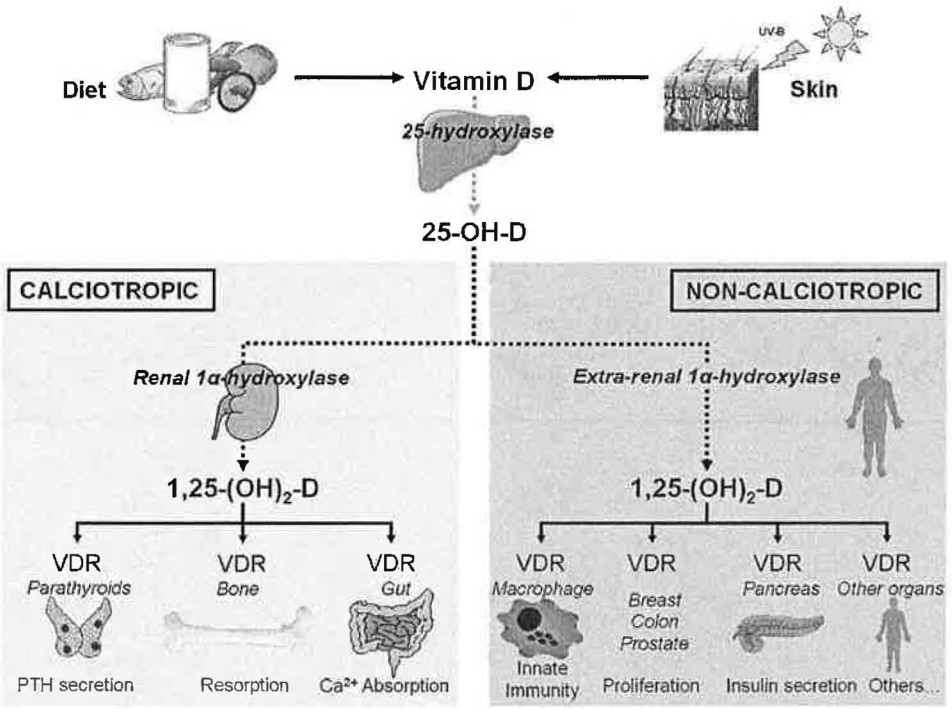
The Non-Calciotropic Actions of Vitamin D

Modulation of Cardiovascular, Immune and Proliferative Disorders



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Internal Medicine Grand Rounds

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This is to acknowledge that Naim Maalouf, M.D. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Maalouf will be discussing off-label uses in this presentation.

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The Non-Calciotropic Actions of Vitamin D:

Modulation of Cardiovascular, Immune and Proliferative Disorders

Vitamin D physiology

Classical metabolism and actions of vitamin D

The first report of vitamin D action came in 1919, when Mellanby reported that he could cure rickets in dogs raised in the absence of sunlight by adding cod-liver oil to their diet (1). Identifying the exact nature of this dietary "antirachitic" factor can be credited to Elmer McCollum (2). This newly discovered substance was fourth in the sequence of discovery of vitamins, and was thus called "vitamin D". Around the same time, Huldchinsky was able to cure rickets in infants exposed to UV radiation using mercury vapor lamp (3). Within a few years, the connection between photosynthesized vitamin D and vitamin D in cod-liver oil was made. This led to the eventual conquest of rickets, and the 1928 Nobel Prize in Chemistry was awarded to Adolf Windaus, who discovered 7-dehydrocholesterol, the vitamin D precursor. It is now well understood that contrary to its original classification as a *vitamin*, vitamin D is in fact a steroid *hormone* whose biological actions are mediated primarily through its effects on gene transcription.

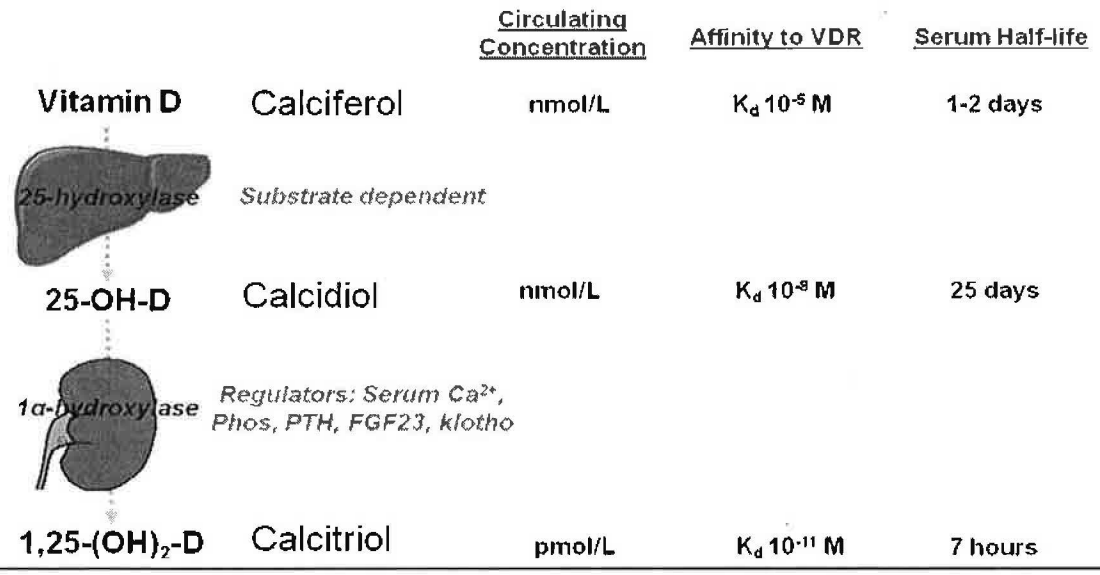
The major sources of vitamin D in humans are cutaneous synthesis, supplements, and diet (fatty fish, fish liver oil, shiitake mushrooms, fortified foods, etc...) (Table 1). Vitamin D from the skin and diet circulates bound to the vitamin D- binding protein (DBP). The initial step in vitamin D metabolism involves its hepatic hydroxylation at the 25 position, generating 25-hydroxyvitamin D (25-OH-D). 25-OH-D is the principal circulating form of vitamin D, has a half-life in circulation of approximately 2 weeks, and is the best biomarker of vitamin D status(4) (Figure 1). 25-OH-D is further hydroxylated in the kidney by the mitochondrial 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) enzyme to form 1,25 dihydroxyvitamin D

Table 1. Determinants of vitamin D status

EXOGENOUS INFLUENCES:	ENDOGENOUS INFLUENCES:
Dietary Sources	Age
Natural foods	Skin pigmentation
Fortified foods	Obesity
Supplements	Malabsorption syndromes
	Liver or Kidney disease
Epidermal Synthesis	Rare genetic defects in CYP27B1 or VDR
Latitude	
Season	
Sunscreen use	
Clothing	

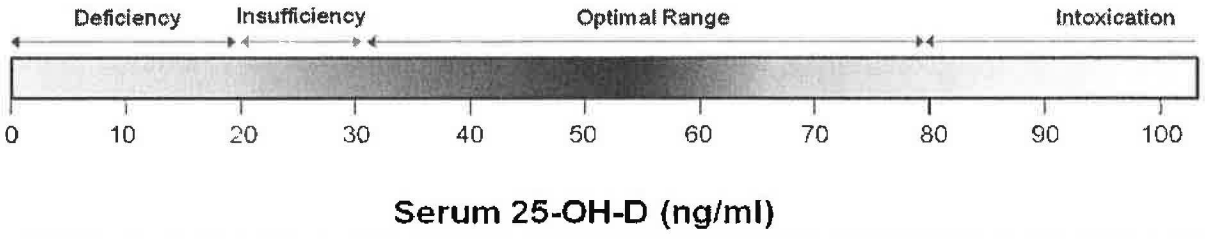
(1,25-$(OH)_2$-D), the physiologically active form of vitamin D. The *classical actions* of 1,25-$(OH)_2$-D include enhancement of intestinal calcium and phosphorus absorption, suppression of parathyroid hormone (PTH) secretion, and stimulation of bone resorption. These *classical actions* occur via interaction of 1,25-$(OH)_2$-D with the vitamin D receptor (VDR), a nuclear receptor in small intestinal cells, parathyroid cells, and osteoblasts (which activate osteoclastic bone resorption via secretion of RANKL). 1,25-$(OH)_2$-D also induces the expression of the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24), which catabolizes both 25-OH-D and 1,25-$(OH)_2$-D into biologically inactive, water-soluble calcitroic acid. The renal production of 1,25-$(OH)_2$-D is tightly regulated by circulating 1,25-$(OH)_2$-D, 24,25-$(OH)_2$-D calcium, phosphorus, PTH, and fibroblast growth factor 23 levels (Figure 1).

Figure 1. Properties of the major vitamin D metabolites and regulation of key enzymes involved in vitamin D metabolism



The "normal range" for serum 25-OH-D is a debatable question (5;6), and depends on the method used to estimate this range. Nevertheless, a number of authorities in the field of vitamin D have proposed the optimal range to be 30-80 ng/mL (Figure 2) (7).

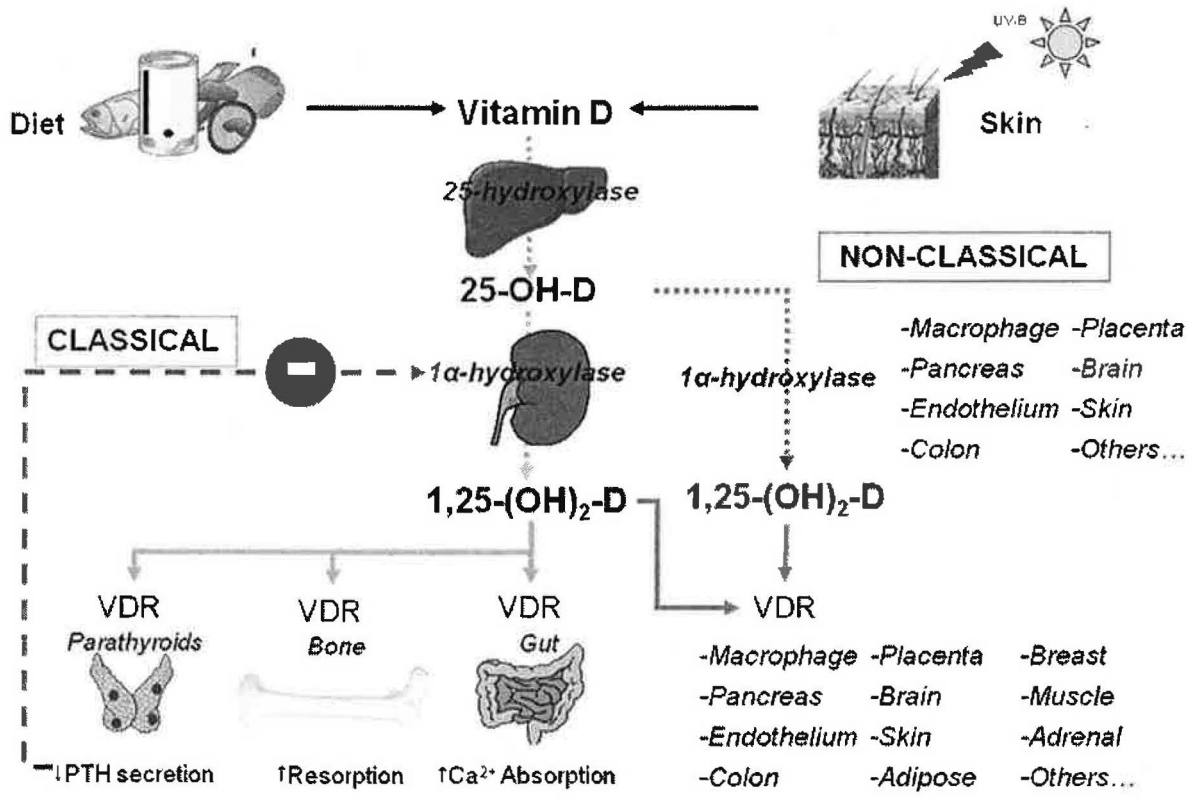
Figure 2. Proposed classification of vitamin D status- The optimal range for serum 25-OH-D is 30-80 ng/mL



Non-classical metabolism of vitamin D

The classical function of 1,25-$(OH)_2$-D is the physiological regulation of calcium transport and bone mineralization. However, the observation that the vitamin D receptor (VDR) is present in cells other than those of the intestine, bone, kidney, and parathyroid gland led to the recognition of non-calcemic actions of 1,25-$(OH)_2$-D (Figure 3).

Figure 3. Classical vs. Non-classical vitamin D metabolism.



Extra-renal 1-alpha-hydroxylase

While the kidney is the major source of circulating 1,25-$(OH)_2$-D, synthesis of 1,25-$(OH)_2$-D can occur in several other tissues. This was initially suspected after a nephrectomized patient with sarcoidosis presented with elevated serum 1,25-$(OH)_2$-D (8), and demonstrated in cultured alveolar macrophages from lungs of patients with sarcoidosis (9). Additional tissues expressing CYP27B1 include the skin, prostate, breast, colon, pancreas, brain, and placenta (10). The extra-renal production of 1,25-$(OH)_2$-D is largely substrate dependant, highlighting the importance of adequate circulating serum 25-OH-D for 1,25-$(OH)_2$-D production within extrarenal tissues. In

contrast to the renal enzyme, the activity of extra-renal 1α-hydroxylase does not appear to be regulated by circulating 1,25-$(OH)_2$-D, PTH, or calcium concentrations (11;12).

Vitamin D receptor distribution

The vitamin D receptor (VDR) is distributed in various tissues including keratinocytes, vascular smooth muscle and endothelial cells, prostate, breast, intestinal, and pancreatic cells, monocyte-macrophage cells, as well as activated activated T and B lymphocytes (Table 2). This suggests that 1,25-$(OH)_2$-D could regulate a number of biologic processes besides the classical actions of vitamin D in bone, gut, and kidney. The vitamin D receptor (VDR) is a ligand-dependent transcription factor that modulates the expression of more than 200 vitamin D-responsive genes, including genes responsible for the regulation of cellular proliferation, differentiation, and apoptosis. Recent studies have revealed new insights into regulation of the vitamin D receptor and new targets for its action.

Table 2. Cellular and Tissue Distribution of Vitamin D Receptor

Endocrine	Parathyroid gland, Thyroid, Pituitary, Adrenal
Cardiovascular	Cardiac muscle
Respiratory	Alveolar cells
Gastrointestinal	Esophagus, Stomach, Small and Large Intestine, Hepatocytes
Renal / Genitourinary	Kidney, Urethra, Prostate
Reproductive	Testis, Ovary, Uterus, Placenta
Musculoskeletal	Osteoblasts, Osteocytes, Chondrocytes, Fibroblasts, Striated Muscle
Epidermis	Skin, Hair Follicle, Breast
CNS	Brain Neurons
Immune	Thymus, Bone Marrow, T-cells, B-cells, Macrophage

Immunomodulatory effects of vitamin D

A major non-classical function of vitamin D is its role in the regulation of the immune system. Recent studies have unraveled some of the underlying mechanisms, and the therapeutic role of vitamin D, its metabolites, and its analogues for infectious and auto-immune disorders in humans are currently under study.

Vitamin D and infectious disorders

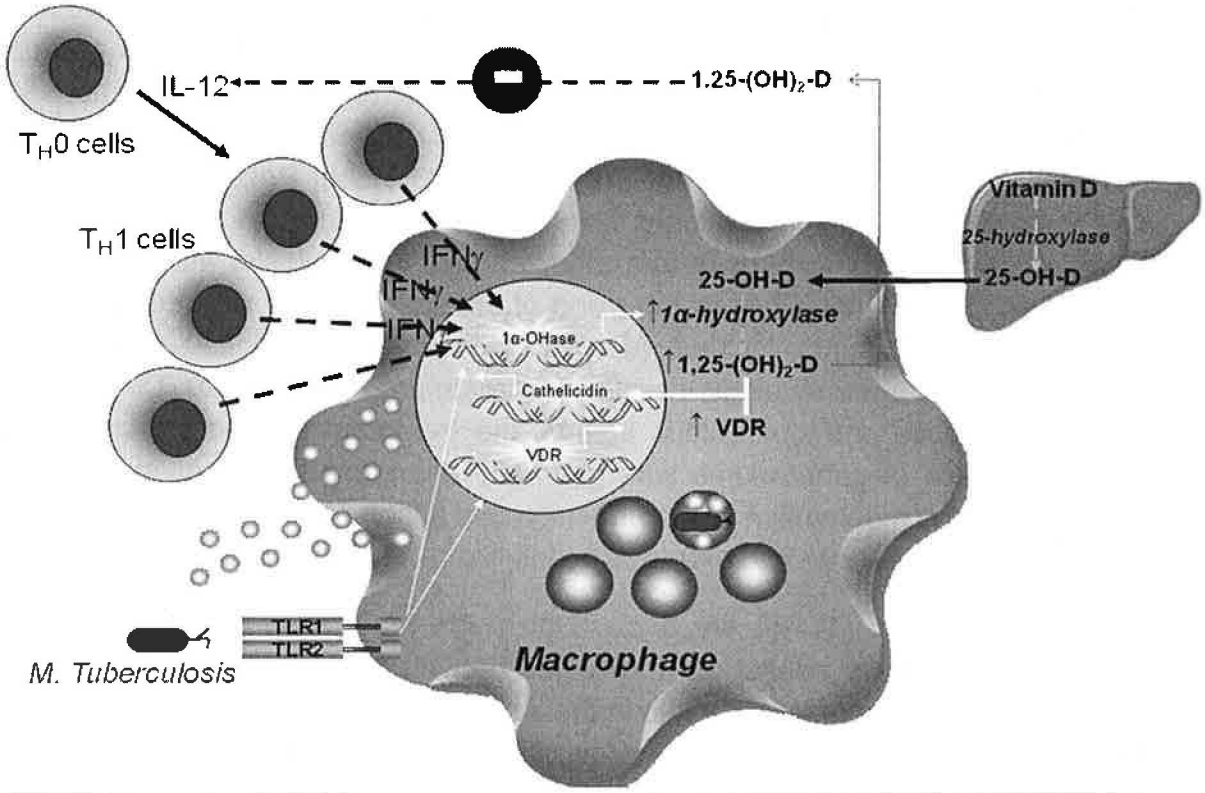
Patients with rickets have impaired macrophage phagocytic function(13), which could be reversed by 1,25-$(OH)_2$-D repletion(14) In mice, targeted ablation of the 25-hydroxyvitamin D 1α-hydroxylase enzyme resulted in enlarged lymph nodes and a reduction in CD4- and CD8- positive peripheral T lymphocytes(15) These findings suggest that vitamin D plays an essential role in the ability of immune system to fight infections.

Vitamin D, Tuberculosis, and Cathelicidin

The use of vitamin D for the treatment of tuberculosis dates back to 1849 when Williams reported results of administering fish liver oil to 234 patients with tuberculosis. He noted that "even in a few days ... the appetite, flesh and strength were gradually improved" concluding that "the pure fresh oil from the liver of the cod is more beneficial in the treatment of pulmonary consumption than any agent, medicinal, dietetic, or regiminal, that has yet been employed" (16). The 1903 Nobel Prize in Physiology or Medicine was awarded to Niels Ryberg Finsen, a Danish scientist who successfully treated patients with *lupus vulgaris* (a form of cutaneous tuberculosis) with light from an electric arc lamp. This finding began the trend of sending patients with tuberculosis to sanatoriums housed in sunny locales. After the isolation and synthesis of vitamin D, Charpy pioneered its pharmacologic use for the treatment of cutaneous TB (17). With the advent of effective anti-tuberculous agents, the treatment of tuberculosis evolved rapidly, and the use of sanatoriums was gradually abandoned. Recent studies have shed new light on the mechanism of the anti-microbial action of vitamin D, a mechanism that involves recognition of *M. Tuberculosis* by toll-like receptors.

Toll-like receptors (TLRs) are a class of single membrane-spanning receptors that recognize structurally conserved molecules derived from microbes, and activate immune responses. TLRs play a key role in the innate immune system, and activation of TLR by a variety of microbial-derived ligands, including bacterial lipopeptides, triggers direct antimicrobial activity against intracellular bacteria. Toll-like receptors (TLRs), particularly TLR2, contribute to innate immunity against *M. Tuberculosis* (18). Compared with control animals, TLR2-deficient mice display reduced reduced survival, a defective granulomatous response, and develop chronic pneumonia after aerosol infection with live mycobacteria, highlighting the importance of TLR2 in the immune response against mycobacterial infections (19). A recent study investigating the response of human immune cells to TLR activation identified the 1α-hydroxylase and VDR as two genes that are uniquely expressed in monocytes/macrophages activated by TLR 2/1 (20). This expression leads to a vitamin D–dependent induction of the antimicrobial peptide cathelicidin and enhanced killing of intracellular *Mycobacterium tuberculosis* (Figure 4). Sera from African-American individuals had low 25-hydroxyvitamin D concentrations and were inefficient in supporting cathelicidin messenger RNA induction (20). This may be the basis for the observation that African-Americans have increased susceptibility to tuberculosis (21). While it appears that 1,25-$(OH)_2$-D plays a cardinal role in antimycobacterial immunity *in vitro*, most existing studies investigating the effects of vitamin D in TB treatment *in vivo* are methodologically flawed (22). The only study examining the role of vitamin D added to quadruple anti-mycobacterial therapy was a randomized placebo-controlled trial in 67 Indonesian patients with pulmonary tuberculosis (23). Compared with placebo, provision

Figure 4. The interrelationship between TLR1/2, *M. Tuberculosis* and vitamin D



of 10,000 IU vitamin D daily for 6 weeks was associated with a higher rate of sputum conversion and a greater frequency of radiologic improvement, with no cases of hypercalcemia observed (23). A recent double-blind randomized controlled trial randomized 192 healthy adult TB contacts in London to receive a single oral dose of 2.5 mg vitamin D or placebo and subjects were followed up at 6 weeks (24). Vitamin D supplementation significantly enhanced the ability of participants' whole blood to restrict BCG-*lux* luminescence *in vitro* compared with placebo suggesting greater ability to restrict growth of recombinant reporter mycobacteria *in vitro*. Whether this will translate into fewer TB infections in exposed individuals remains to be shown, and further randomized studies are clearly needed to assess whether vitamin D repletion alters the course of TB infection or the recurrence of latent TB.

Other infections

Cathelicidin exhibits a broad spectrum of antimicrobial activity against a number of bacteria, fungi, and viral pathogens (25). Since induction of cathelicidin expression was recent shown to be vitamin D-dependent (20), vitamin D could potentially enhance host defence against infections other than tuberculosis. Case control studies have found

associations between vitamin D deficiency and various infections (26;27). Data from randomized trials is extremely limited. Intramuscular 1,25-$(OH)_2$-D co-administration with influenza vaccine in a randomized fashion did not appear to enhance humoral immunity in healthy young volunteers (28). The frequency of self-reported infections and antibiotic use were assessed as part of the RECORD trial, a large randomized, placebo-controlled trial of oral vitamin D3 and/or calcium supplementation for the secondary prevention of osteoporotic fractures (29). 17.2 % of respondents randomized to vitamin D reported an infection, compared with 18.8% on placebo (adjusted odds ratio 0.90, 95% confidence interval 0.76 to 1.07, P = 0.23). 6.4 % of vitamin D treated patients reported antibiotic use compared with 7.5% on placebo (adjusted odds ratio 0.84, 95% CI 0.64 to 1.09, $P = 0.18$). Thus, although there was a tendency toward vitamin D reducing the risk of infection, the results were not statistically significant. At this time, there is no good evidence regarding the effectiveness of vitamin D against infections.

Vitamin D and auto-immune disorders

Besides promoting innate immunity, the principal effect of 1,25-$(OH)_2$-D on the immune system is generation of tolerance and angergy rather than immune activation. In the presence of 1,25-$(OH)_2$-D, dendritic cells exhibit reduced expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules necessary for full T-cell stimulation. Furthermore, 1,25-$(OH)_2$-D suppresses adaptive immunity by shifting the balance of helper T-cells from Th1 to Th2 and T-regulatory cells through inhibition of interleukin 12 production via interference with the nuclear factor kB pathway(30) (Figure 4). Epidemiologic studies have correlated limited sunlight exposure, reduced dietary vitamin D intake and/or 25-OH-D levels with a number of auto-immune disorders such as multiple sclerosis, systemic lupus erythematosis (SLE) and type 1 diabetes mellitus. Animal studies have shown effectiveness of 1,25-$(OH)_2$-D and its analogs in a variety of animal models of these autoimmune disorders, but prospective clinical trials in humans have not yet been performed. Exploiting the immunomodulatory effects of 1,25-$(OH)_2$-D in humans is in part limited by the development of hypercalcemia. Novel analogues have been developed that are more potent in T-cell modulation and less calcemic, thus allowing higher doses to target the immune system. These analogues are being analyzed for their therapeutic potential.

Multiple sclerosis

The prevalence of multiple sclerosis (MS) is well known to increase with latitude both north and south of the equator from a low of 1–2 cases per 10,000 individuals near the equator to a high of over 200 cases per 10,000 people at latitudes higher than 50° (31;32). Although genetic predisposition likely plays a role in this variation (33), the role of an environmental factor is suggested by the changing risk of multiple sclerosis with

migration in individuals with common ancestry (34). The role of sunshine was proposed several decades ago (35), and recent epidemiologic and experimental studies have provided evidence that high levels of vitamin D may decrease the risk of multiple sclerosis. In the Nurses' Health Study and Nurses' Health Study II, intake of vitamin D from diet and from supplements was inversely associated with risk of MS (36). The relative risk comparing women with intake of >400 IU/day with women with no supplemental vitamin D intake was 0.59 (95% CI: 0.38 to 0.91; *p* for trend = 0.006), while the relative risk in women in the highest quintile of total vitamin D intake at baseline compared to those in the lowest quintile was 0.67 (95% CI = 0.40 to 1.12; *p* for trend = 0.03), suggesting a protective effect of vitamin D intake on risk of developing MS. In a nested case-control study among > 7 million US military personnel who had serum samples stored in the Department of Defense Serum Repository, the risk of multiple sclerosis significantly decreased with increasing levels of 25-hydroxyvitamin D (37). Studies in experimental allergic encephalomyelitis (EAE), a widely used animal model for human MS disease, have provided some insights into the protective role of vitamin D in MS. When EAE was induced in mice after immunization with myelin basic protein, administration of 1,25-$(OH)_2$-D prevented the induction of the disease (38) by inhibiting the mitogen-induced differentiation of neonatal CD4+ cells into T helper 1 (Th1) cells which play a cardinal role in the induction of multiple sclerosis (39). 1,25-$(OH)_2$-D also ameliorated the disease when the treatment was administered at the appearance of the first symptoms of disability, while its withdrawal resulted in the resumption of EAE. Furthermore, female mice fed diets high in vitamin D had significantly fewer clinical and pathological signs of EAE than mice fed a vitamin D–deficient diet (40). No clinical trials of vitamin D or its analogs in human multiple sclerosis have been reported to date.

Systemic Lupus Erythematosus

The threefold greater incidence of systemic lupus erythematosus (SLE) in African-Americans and increased morbidity and mortality compared to Caucasians (41) has been attributed to lower serum vitamin D concentrations (42). In support of this hypothesis, 25-OH-D levels were significantly lower in recently diagnosed SLE patients compared to controls, and severe deficiency was associated with the presence of renal disease and photosensitivity (43). Furthermore, in a murine model of lupus, the MRL/lpr mouse, treatment with 22-oxa-1,25-dihydroxyvitamin D3, an analog of 1,25-$(OH)_2$-D significantly improved longevity and reduced proteinuria (44). On the other hand, vitamin D intake from food and supplements was not associated with risk of SLE in two large cohorts, the Nurses' Health Study and Nurses' Health Study II (186,389 women) (45). Thus, the role of vitamin D deficiency in the pathogenesis of SLE is still not entirely proven, and whether vitamin D supplementation can improve the course of the disease is the subject of an ongoing study (ClinicalTrials.gov Identifier: NCT00418587).

Vitamin D and Cardiovascular disease

Vitamin D and Cardiovascular Risk Factors

In small case-control studies, vitamin D deficiency is associated with a greater incidence of myocardial infarction (46;47). This association may be in part related to the association of lower vitamin D levels with a number of cardiovascular risk factors including obesity, hypertension, hyperglycemia and hypertriglyceridemia (48) as well as other confounders such as lower advanced age, socioeconomic class, and decreased physical activity. Vitamin D also influences pro- and anti-inflammatory cytokines linked to cardiovascular disease such as TNF-α and IL-10 (49). The association between obesity and lower serum 25-OH-D levels is unlikely to be a direct effect of vitamin D, and more likely to be due to its reduced bioavailability due to sequestration in adipose tissue, as obese individuals exhibit a blunted rise in serum vitamin D in response to sun light and to orally supplemented vitamin D (50). While reduced sun exposure related to a more sedentary lifestyle is another postulated mechanism linking obesity to vitamin D deficiency, this theory was refuted in a recent study (51).

Hypertension and vitamin D

The relationship between vitamin D and blood pressure was first described over two decades ago (52). Rostand suggested that differences in ultra-violet (UV) light exposure contribute to the geographic variability in blood pressure across various countries (53). In elegant studies in VDR knock-out mice, 1,25-$(OH)_2$-D was shown to negatively regulate the renin-angiotensin-aldosterone system (RAAS) (54). This work is supported by few small studies examining the change in blood pressure in response to vitamin D repletion. Full body UVB radiation significantly lowered systolic and diastolic blood pressure in untreated patients with mild essential hypertension (55), while an 8-week course of calcium and vitamin D supplementation significantly lowered systolic and diastolic blood pressure (56). However, calcium and vitamin D

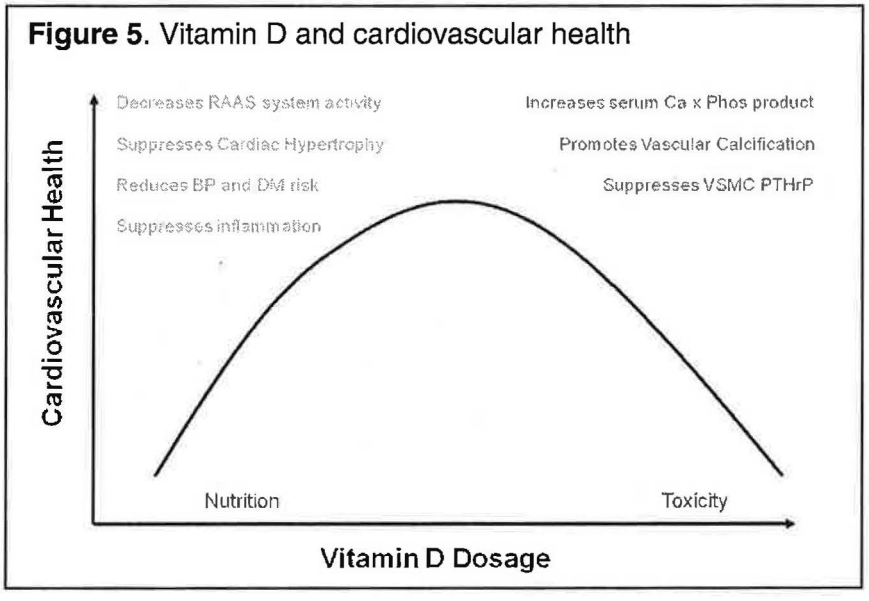


Figure 5. Vitamin D and cardiovascular health

supplementation was associated with a slightly greater *increase* in blood pressure in the Women's Health Initiative, the largest randomized controlled trial of calcium and vitamin D in postmenopausal women (57). At present, data from controlled clinical trials are too limited to determine whether vitamin D supplementation will be effective in lowering blood pressure or preventing hypertension.

In the Women's Health Initiative, calcium/vitamin D supplementation did not alter coronary or cerebrovascular risk (57). The lack of vitamin D effect may be due to poor compliance among study participants, or may be due to the low dose of vitamin D used (400 IU per day). On the other hand, the results dissipate some of the concerns regarding vitamin D raising cardiovascular risk by increasing vascular calcification (58) (Figure 5).

Vitamin D and Diabetes Mellitus

Type 1 diabetes

Type 1 diabetes mellitus (T1DM) is another disorder that may be modulated by vitamin D (59). In non-obese diabetic (NOD) mice, a mouse model of human T1DM, vitamin D deficiency increased the incidence of diabetes two-fold (60), and oral administration of 1,25-$(OH)_2$-D prevented disease onset (61). Low serum 25-OH-D levels have been reported in patients with T1DM from several countries (62-64). In a 30 year follow-up study from Finland, vitamin D supplementation was associated with a decreased risk of T1DM: Children who regularly took the recommended dose of vitamin D (2,000 IU daily) had a relative risk of 0.22 (95% C.I. 0.05-0.89) compared with those who regularly received less than the recommended amount (65). In the same study, children suspected of having rickets during the first year of life had a RR of 3.0 (1.0-9.0) for type 1 diabetes compared with those without such a suspicion. A large multicentre European trial also found that vitamin D supplementation in infancy was associated with a significantly the risk for type 1 diabetes

Table 3. Vitamin D and diabetes mellitus

	Type 1 diabetes	Type 2 diabetes
Pre-clinical	VDR and 1α-OHase in β-cells VDRE in insulin gene promoter NOD mice: Vit.D def. ↑DM risk ↑ NOD mice: Calcitriol ↓ DM risk ↓ NOD mice: Calcitriol ↓ DM reversal	Vit.D regulates Ca^{2+} flux in β-cells Vit.D deficiency in rats: ↓ glucose-mediated insulin secretion in β-cells Vit.D ↑ expression of IR and insulin-mediated glucose transport *in vitro*
Clinical	↓ 25-OH-D in T1DM patients Vit.D in pregnancy: ↓ IAA in infants Cod liver oil: ↓ T1DM risk Vit.D supplementation : ↓ T1DM risk Ongoing RCT in at risk individuals	Observational studies: Vit. D deficiency ↑↑ T2DM risk Interventional studies: Combined vit.D+Ca may ↓ T2DM risk in high risk populations

(66). Use of cod liver oil during the first year of life was also associated with lower risk of type 1 diabetes in a large Norwegian population-based, case–control study (67). In line with these findings, increased vitamin D intake during pregnancy significantly reduced β-cell autoimmunity in offspring as detected by islet auto-antibodies (68). Since vitamin D deficiency appears to be a risk factor for T1DM, provision of vitamin D may be a reasonable protection against type 1 diabetes in later life. An ongoing randomized controlled trial is currently recruiting individuals at risk for T1DM in Manitoba, Canada to address this issue (69).

Type 2 diabetes

Hypovitaminosis D has been linked to greater risk for type 2 diabetes and worse glycemic control in a number of case control studies (70). In NHANES III, lower serum 25-OH-D levels was associated with a greater degree of insulin resistance as measured by HOMA (Homeostasis Model for Assessment of insulin resistance), and a higher prevalence of diabetes mellitus among Caucasians and Hispanics (71). Potential mechanisms underlying this association include the direct influence of vitamin D on insulin secretion. Glucose-induced insulin secretion from pancreas of vitamin D deficient rats was significantly blunted in comparison with that of control rats (72). The molecular mechanism is likely via vitamin D mediated intracellular calcium influx in pancreatic beta-cells, which in turn determines insulin secretion (73). In addition to modulating insulin secretion, vitamin D may also influence insulin action in peripheral tissues. 1,25-(OH)2-D alters insulin-induced glucose uptake at the level of adipocytes (74) and stimulates insulin receptor expression and insulin responsiveness for glucose transport *in vitro* (75). While pre-clinical studies suggest that vitamin D influences pancreatic beta-cell function, and peripheral insulin sensitivity and observational studies have linked hypovitaminosis D to insulin resistance and type 2 diabetes, evidence from interventional studies with vitamin D in humans is not entirely conclusive (70). Short-term interventional studies with a number of vitamin D metabolites did not show changes in fasting serum glucose or insulin (76;77). On the other hand, compared with placebo, combined vitamin D and calcium supplementation for 3 years was associated with a smaller rise in fasting serum glucose in subjects with impaired fasting glucose (78). Overall, while vitamin D may be beneficial in optimizing glucose metabolism, definitive evidence for recommending its use specifically for this purpose is still lacking.

Anti-proliferative properties of vitamin D

A major non-classical action of 1,25-$(OH)_2$-D is its modulation of benign and malignant hyperproliferative conditions by regulating cellular proliferation, differentiation, apoptosis, and angiogenesis. Recent epidemiologic studies have described inverse associations between the risk of various malignancies and biomarkers of sunlight exposure, dairy products and/or dietary vitamin D. A variety of experimental animal

models and *in vitro* studies have confirmed these observations, and a number of prospective trials testing various vitamin D analogs in humans are currently ongoing.

Vitamin D and psoriasis

The first practical application for the use of 1,25-$(OH)_2$-D and its analogues for the treatment of a proliferative disorder has been in psoriasis. Psoriasis, a recurrent inflammatory skin disorder, is characterized by immune-cell infiltration into the epidermis and dermis, along with keratinocyte hyperproliferation and abnormal differentiation. It is typified by $CD8^+$ lymphocytes infiltration into the epidermis and $CD4^+$ lymphocytes into the dermis. At the molecular level, the expression of normal suprabasal keratins K1 and K10 is inhibited and replaced by the expression of the hyperproliferative keratins K6 and K16. The first clinical evidence to support the use of vitamin D analogs in psoriasis was in a patient treated with oral calcitriol for osteoporosis who had a remarkable remission of psoriatic lesions (79). Oral and topic calcitriol showed remarkable results in clinical studies, but hypercalciuria was noted at higher doses (80). Calcipotriol (calcipotriene or Dovonex®), a synthetic 1,25-(OH)2-D analog, was chemically engineered to be metabolized quickly in systemic circulation, providing a much less calcemic compound that has become first-line therapy for plaque psoriasis, either as monotherapy or in combination (81). The major mode of action of vitamin D analogs in psoriasis appears to be inhibiting the proliferation of basal keratinocytes in psoriatic epidermis and promoting their differentiation (82;83). An immunomodulatory effect on T-cell subsets has also been suggested, although this appears to play only a minor role (84).

Vitamin D and cancer

Table 4. Evidence linking vitamin D and cancer

Epidemiology

- Inverse associations between risk of various malignancies and biomarkers of sunlight exposure, dairy products and/or dietary vitamin D

- Low serum 25-OH-D is associated with greater cancer risk and mortality

Cellular Systems

- VDR expressed and regulated in a number of normal and cancerous tissues

- Alteration in vitamin D receptor expression, and in the synthesis and catabolism of vitamin D metabolites in various tumors

-The anti-neoplastic effects of 1,25-(OH)2-D involves regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis

Animal models

- 1,25-(OH)2-D inhibits cellular proliferation and carcinogen induced pre-neoplastic lesions

- Enhanced proliferation and reduced apoptosis in organs of VDR KO mice

An inverse relationship between sun exposure and cancer incidence has been described in several recent studies (Table 4)(85-88). In Caucasians, solar UV-B exposure was inversely correlated with incidence and mortality for ten cancers: bladder, colon, Hodgkin lymphoma, myeloma, other biliary, prostate, rectum, stomach, uterus, and vulva (89). Vitamin D is widely believed to underlie this association (90). Most recently, in data from the National Health and Nutrition Examination Study (NHANES) III, serum 25-OH-D levels was not associated with total cancer mortality (91)). However, in the same study, individuals with higher 25-OH-D levels had significantly lower risk of colon cancer mortality. This review will focus on the relationship between vitamin D and this malignancy. The interested reader is referred to other studies on vitamin D and prostate cancer (92) breast cancer (93), lung cancer (94;95), and pancreatic cancer (96).

Colon cancer

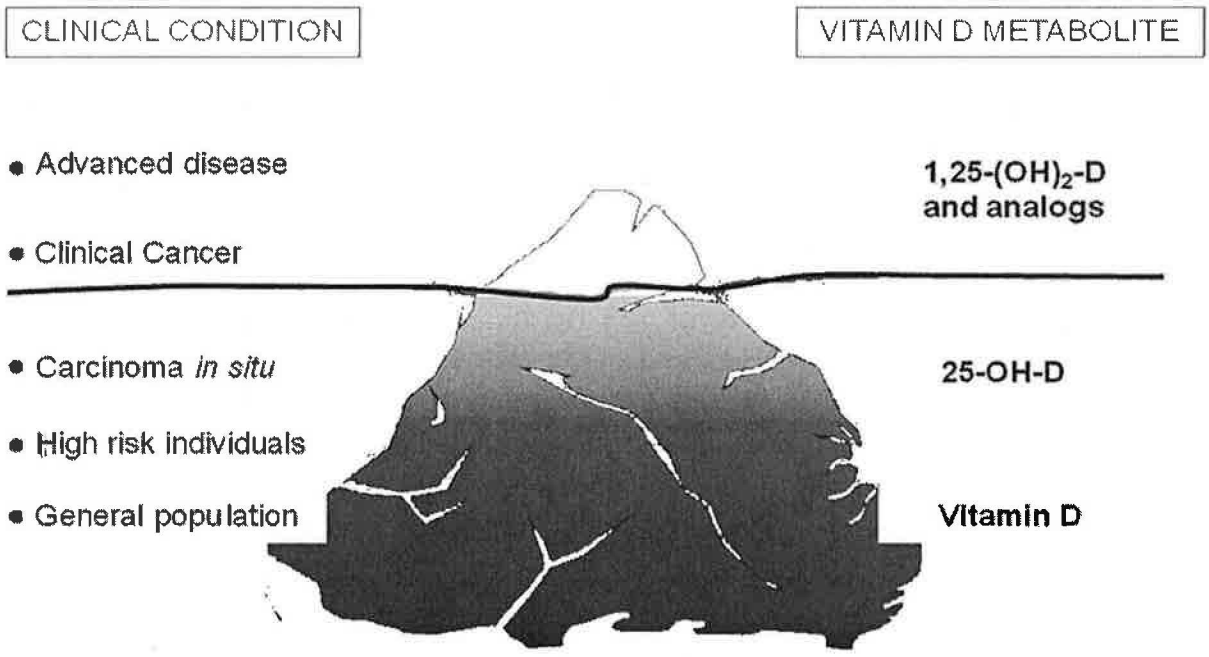
Several lines of evidence, including epidemiologic, *in vitro*, and animal studies suggest a role for vitamin D in the prevention of colon cancer. Lower 25-OH-D has been linked to increased risk of colon cancer in a number of large cohort studies, including the Health Professional Follow-Up Study and the Nurses' Health Study (97) and NHANES III (91), with a meta-analysis showing that a 50% lower risk of colorectal cancer with a serum 25-OH-D level >33 ng/mL, compared to <12 ng/mL (98). The association between low 25-OH-D and colon cancer may be coincidental rather than causal: A number nutritional factors associated with vitamin D deficiency have also been linked to colorectal cancer (including high intake of alcohol and animal fat, and low intake of vegetables, fiber and calcium). Furthermore, low 25-OH-D levels have been associated with obesity (51;99), which is in turn associated with colon cancer (100). Finally, it is possible that low 25-OH-D is a consequence rather than a cause of colorectal cancer, as cancer patients may have less sun exposure due to their underlying malignancy.

Plausible biological mechanisms for a role for vitamin D in the prevention of colon cancer invoke the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis by 1,25-$(OH)_2$-D. The 1-α-hydroxylase enzyme is expressed in normal colon, allowing for local conversion of 25-OH-D to 1,25-$(OH)_2$-D (10). Importantly, increased *CYP27B1* expression is observed in well-to-moderately differentiated colon cancer (101), but decreased expression has been described in poorly differentiated colon carcinomas (102). These cells also express the VDR and thus can respond to 1,25-$(OH)_2$-D in an autocrine and paracrine fashion. At the same time, 24-hydroxylase mRNA expression is upregulated in high-grade colon carcinomas (103), and may thus locally decrease 1,25-$(OH)_2$-D levels and reduce its anti-proliferative activity.

Prevention and treatment of cancer with vitamin D

While the association between hypovitaminosis D and the incidence of colon cancer and other malignancies is relatively well-established, the role of vitamin D supplementation in the prevention and treatment of cancer is still not entirely clear. Some authors have proposed that clinical cancer is only "the tip of the iceberg" in this association, and suggest that treatment with vitamin D metabolites will depend on the stage of the disease (Figure 6). In such a scheme, vitamin D supplementation would be useful in preventing cancer in the general population and at-risk indiviudals, while 1,25-$(OH)_2$-D and its metabolites is reserved for established cancer and advanced disease.

Figure 6. Potential interventions with vitamin D and its metabolites in cancer- Adapted from Vitamin D, 2nd edition, 2005, Elsevier



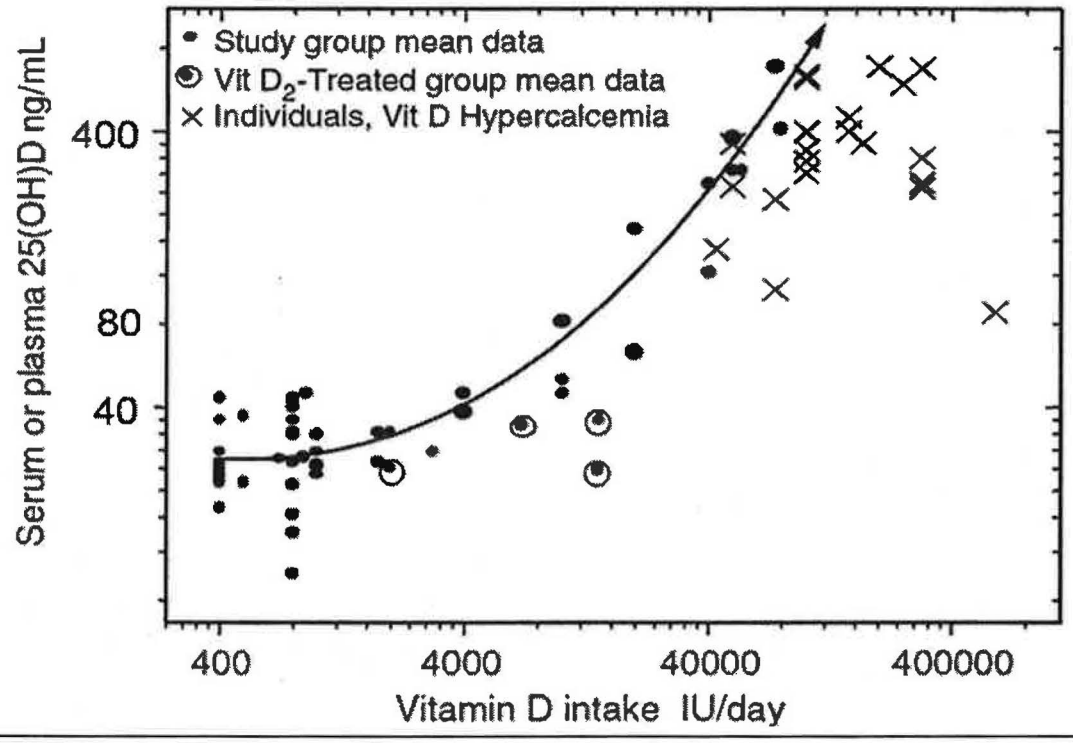
In a widely publicized 4-year study of 1179 community-dwelling postmenopausal women randomly assigned to calcium and vitamin D supplementation, calcium supplementation alone, or placebo, cancer incidence was significantly lower in the Ca+D women than in the placebo control subjects ($P< 0.03$), and serum 25-OH-D level was a significant independent predictor of cancer risk (104). The results of this study are limited in view of the small number of actual malignancies observed (50), and because incidence of cancer was a secondary end-point, but also suggest that calcium itself may play a big role beyond that of vitamin D. The largest randomized placebo-controlled study of vitamin D and the risk of colon cancer is the Women's Health Initiative, in which over 36,000 women were randomized to receive 400 IU vitamin D along with 1,000 mg elemental calcium or placebo daily (105). The incidence of invasive colorectal cancer

after 7 years did not differ significantly between women assigned to calcium / vitamin D supplementation and those assigned to placebo (hazard ratio, 1.08; 95 % CI: 0.86 to 1.34; p = 0.51). In a nested case–control study, the risk of colorectal cancer was significantly higher in women with lower baseline serum 25-OH-D. It is plausible that the dose of vitamin D supplement used was too small to show an effect, and poor compliance and vitamin D use by placebo treated patients may have masked a potential protective effect. In terms of established cancer, a single published trial in men with advanced, androgen-insensitive prostate cancer treatment showed that use of DN-101, a calcitriol analog, was associated with improved survival, although DN-101 did not produce a statistically significant improvement in the PSA response rate, the primary end point of the study (106). The major side effect of vitamin D and calcitriol that limits their expanded use and clinical development as anti-neoplastic agents is hypercalcemia. Consequently, vitamin D analogs with less hypercalcemic liability have been /are being developed as preventative and therapeutic agents (107), and certain compounds are currently in various stages of clinical investigation (108).

Vitamin D supplementation

While rickets is no longer a major health problem in the United States, vitamin D deficiency remains quite prevalent. Recommendations for optimal vitamin D intake are limited, as the Institute of Medicine has determined that there is insufficient scientific

Figure 7. Vitamin D supplementation and serum 25-OH-D- Reproduced from Vitamin D, 2nd edition, 2005, Elsevier



information to establish a recommended daily allowance (RDA) for vitamin D. Instead, the recommended intake is listed as an Adequate Intake (AI), which represents the daily vitamin D intake that should *maintain* bone health in healthy individuals (109). The adequate intake in adults age 18-50 years is 200 IU/day, age between 51-70 years 400 IU/day, and age over 71 years 600 IU/day. On the other hand, the National Osteoporosis Foundation (NOF) modified its recommendation in July 2007, stating that adults age 50 and older need 800 – 1,000 IU of vitamin D_3 daily. A group of experts in the field have also suggested higher intake than recommended by the Institute of Medicine (110). While the Food and Nutrition Board of the Institute of Medicine has set the tolerable upper intake level for vitamin D at 2,000 IU for adults, true vitamin D toxicity presenting with symptomatic hypercalcemia has not been described with vitamin D supplements under 10,000 IU/day (Figure 7).

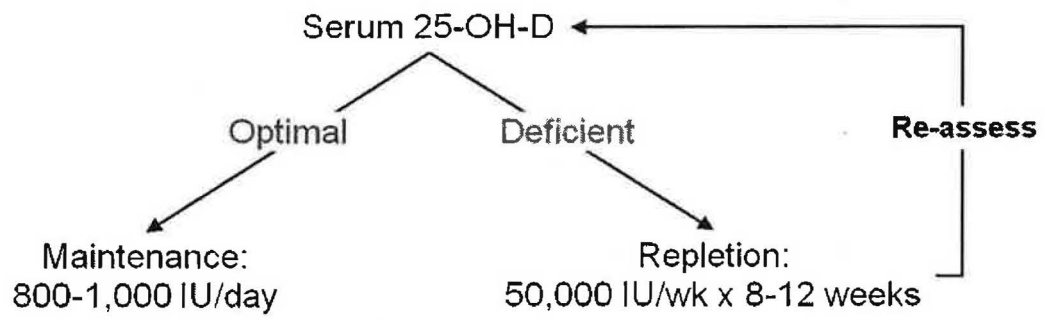
While the optimal intake is debated, food consumption data suggest that the median intake of vitamin D for U.S. adult women is below current recommendations (109). This is because only a few commonly consumed foods are good sources of vitamin D. While sun exposure is the most important source of vitamin D, age, skin pigmentation, season, geographic latitude, time of day, and sunscreen affect UV ray exposure and vitamin D synthesis (Table 1). Consequently, the vast majority of elderly individuals, and a good proportion of young adults with limited sun exposure are likely to require vitamin D supplements (Table 5). Available preparations in the United States include vitamin D_3 (Cholecalciferol) found in 400-1,000 IU of vitamin D, but 400 IU is the most commonly available dose. Vitamin D_3 is also found in calcium and multivitamin supplements. Larger doses that may be needed for vitamin D repletion in deficient individuals are only found in the form of vitamin D_2 (ergocalciferol, Drisdol ®), which may be less potent that vitamin D_3. Calcitriol (Rocaltrol ®), the active form of vitamin D, is typically reserved to individuals who cannot synthesize 1,25-$(OH)_2$-D such patients with chronic kidney disease or hypoparathyroidism.

Table 5. Vitamin D preparations in the United States

Preparation	Commercial name	Forms	Remarks
Cholecalciferol (vit. D3)	--	400-1000 u supplement	Found in multivitamins or with calcium preparations
Ergocalciferol (vit. D2)	Drisdol	50,000 u capsule	Inexpensive, long half-life
Calcitriol (1,25$(OH)_2$-D)	Rocaltrol	0.25, 0.5 mcg caps	Expensive, short half-life Hypoparathyroidism, CKD

Patients with adequate vitamin D stores are likely to require 1,000 IU per day to maintain vitamin D sufficiency (111). On the other hand, patients with vitamin D deficiency, repletion can be achieved with ergocalciferol 50,000 IU given weekly for 8-12 weeks. Serum 25-OH-D should be re-checked at the end of the ergocalciferol course, with maintenance doses of vitamin D given to those whose stores are repleted, and a repeat ergocalciferol course given to those who are still insufficient (Figure 8). Patients with bowel disease, cystic fibrosis or post-bariatric surgery are likely to require long-term high doses of vitamin D.

Figure 8. Proposed algorithm for vitamin D supplementation



Conclusions

The field of vitamin D is moving fast, with newly discovered targets of vitamin D action, and better understanding of mechanisms. The wide tissue distribution of the 1α-hydroxylase enzyme and the VDR establish a role for vitamin D beyond its function in calcium homeostasis. Accumulating evidence suggest that adequate vitamin D levels are required for prevention of malignancy and optimal function of the immune and cardiovascular systems. While vitamin D and its analogs have a potential therapeutic role for a range of clinical conditions, clinical studies to date have not conclusively demonstrated efficacy. Until such evidence becomes available, the basics of maintaining good vitamin D nutrition remain essential, not just for maintaining strong bones.

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