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Steroid hormone vitamin D: Implications for cardiovascular disease

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Abstract

Understanding of vitamin D physiology is important because about half of the population is being diagnosed with deficiency and treated with supplements. Clinical guidelines were developed based on observational studies showing an association between low serum levels and increased cardiovascular risk. However, new randomized-controlled trials have failed to confirm any cardiovascular benefit from supplementation in the general population. A major concern is that excess vitamin D is known to cause calcific vasculopathy and valvulopathy in animal models. For decades, administration of vitamin D has been used in rodents as a reliable experimental model of vascular calcification. Technically, vitamin D is a misnomer. It is not a true vitamin because it can be synthesized endogenously through ultraviolet exposure of the skin. It is a steroid hormone that comes in three forms that are sequential metabolites produced by hydroxylases. As a fat-soluble hormone, the vitamin D-hormone metabolites must have special mechanisms for delivery in the aqueous blood stream. Importantly, endogenously synthesized forms are carried by a binding protein, whereas dietary forms are carried within lipoprotein particles. This may result in distinct bio-distributions for sunlight-derived vs. supplement-derived vitamin D-hormones. Since the cardiovascular effects of vitamin D-hormones are not straightforward, both toxic and beneficial effects may result from current recommendations.

Keywords

Cholecalciferol; vitamin D; calcitriol; cardiovascular; calcification

On November 30, 2010, at the request of the Canadian and U.S. Governments, the Institute of Medicine provided a report addressing conflicting information on vitamin D.[1] This report took into consideration more than 1,000 studies and reports and considered testimony from scientists and “stakeholders.” Outcomes included bone and cardiovascular diseases as well

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as cancer, diabetes, inflammation, neuropsychological function, physical performance, pre-eclampsia and reproduction. The overall conclusion of this report was that “the majority of Americans and Canadians are receiving adequate amounts of both calcium and vitamin D,” and that “too much of these nutrients may be harmful.” It further noted that “information about health benefits beyond those for bone -- benefits often reported in the media -- were from studies with mixed and inconclusive results that could not be considered reliable.”[2] Even before this report, Towler had noted that the effects of vitamin D on cardiovascular health are complex and biphasic, with direct and indirect actions mediating its vasculotropic actions.[3] There is not yet evidence from a randomized controlled trial showing cardiovascular benefit of vitamin D supplementation.[4]

Until recently, hormonal regulation of calcium-phosphate metabolism by vitamin D metabolites and the parathyroid gland were of little interest to cardiovascular scientists and clinicians. But with new clinical guidelines and media attention, awareness of vitamin D physiology is necessary, especially given that, despite the conclusions of the Institute of Medicine, routine vitamin D testing and supplementation are widely recommended by physicians. As commonly occurs with supplements, it is often used in doses far beyond those directed. Given its extensive actions in human metabolism - both beneficial and harmful - the biochemistry, physiology, and financial motivations surrounding vitamin D warrant attention.

Forms of vitamin D

From a technical standpoint, the term “vitamin D” is a misnomer. It is not a true vitamin because the human body has the capacity to synthesize its own cholecalciferol (D_3), except in rare instances of complete lack of ultraviolet radiation. It is more accurate to view it as a steroid hormone or an oxysterol. The International Union of Pure and Applied Chemistry’s Commission on the Nomenclature of Biological Chemistry defines vitamin D_3 as a steroid or secosteroid. Its chemical name is 9,10-secocholesta-5,7,10(19)-trien-3beta-ol. Six different steroid hormones go by the name “vitamin D,” with varying degrees of activity: the endogenous precursor, cholecalciferol (D_3), which is derived from cholesterol; its hydroxylated derivative, calcidiol [$25(OH)D_3$], which has partial activity; and its hydroxylated derivative, the “active” dihydroxy form, calcitriol [$1,25(OH)_2D_3$]. In addition, there is a plant-derived form, ergocalciferol (D_2), which also has the corresponding monohydroxy and dihydroxy metabolites. Since $25(OH)D_3$ is longer lasting, it is the level of this hormone -- not that of the more active $1,25(OH)_2D_3$ -- that is used to diagnose clinical deficiency. Notably, levels of $25(OH)D_3$ tend to vary inversely with the levels of the active form, possibly due to displacement of the active metabolite from D-binding protein (DBP).[5] In this article, we will use the term “vitamin D-hormones” for all six types of steroid hormones and to emphasize their true physiological nature.

Sources of vitamin D-hormones and biodistribution

Sources of vitamin D-hormones include exposure to ultraviolet light, certain foods, and dietary supplements. As one of the four fat-soluble vitamins (A, D, E, and K), its lipophilicity requires special mechanisms to pass through the aqueous environment of blood

to reach tissues and cells. Separate mechanisms are used for the endogenous D_3 synthesized in sun-exposed skin vs. exogenous D_3 obtained from diet or supplements. This may result in distinct pharmacokinetic volumes and targets of distribution (Figure 1).

Endogenous vitamin D-hormone synthesis, transport and activation

Endogenous vitamin D–hormone synthesis occurs by ultraviolet light exposure of 7-dehydrocholesterol within the microvessels of the skin resulting in its conversion into cholecalciferol (D_3). But, as a fat-soluble oxysterol, D_3 must be carried in the blood by DBP, a liver-derived apoprotein and a member of the albumin gene family.[6] For light-skinned individuals, sun exposure of the face and arms for just 15 minutes per week may produce tens of thousands of units of cholecalciferol. This endogenous production from sun exposure had been the major source for most humans for centuries. The fact that sun-derived D_3 is carried on DBP is a key difference from exogenous D_3, because of its potential influence on bio-distribution.

Exogenous vitamin D-hormone sources and delivery

Exogenous sources of vitamin D-hormones include diet (eggs, fish, liver, and marine mammal fat) and supplements. A cup of milk provides about 100 international units (IU) and a serving of salmon contains about 400 IU of D_3. While the dietary sources may be in the D_3 or D_2 form, supplements typically derive from the plant-derived hormone, ergocalciferol (D_2). A key feature of dietary or supplemental sources is that D_3 taken orally is absorbed from the intestinal tract via chylomicrons,[7] which pass into the lymphatic circulation before returning to the central venous circulation via the thoracic duct. Eventually, about 35% of ingested D_3 is carried in lipoproteins,[8] rather than DBP.

Activation by sequential hydroxylation of D_3

For both endogenous and exogenous sources, the D_3 carried in the bloodstream on either DBP or lipoproteins undergoes a two-step sequential hydroxylation to active metabolites. First, it is converted by 25-hydroxylase to the monohydroxy- derivative, $25(OH)D_3$, the metabolite that is measured for “vitamin D levels.” This occurs primarily in the liver, but may take place in other tissues as well. Next, $25(OH)D_3$ is further hydroxylated by 1-alpha hydroxylase to the active, dihydroxy- form, $1,25(OH)_2D_3$.[8] This occurs primarily in the capillaries surrounding the proximal convoluted tubules of kidney, but, importantly, the enzyme producing the active form is also found in vascular cells and monocytes among other tissues and cells (Table).

Guidelines for vitamin D-hormone assessment and supplementation

Different criteria for vitamin D deficiency have been proposed by the Endocrine Society, Osteoporosis Society, and Institute of Medicine. Normal reference values shown by individual clinical laboratories are not standardized. Conservative definitions define vitamin D deficiency as levels of $25(OH)D_3 < 20$ ng/ml (< 50 nmol/L), and vitamin D insufficiency as 20 – 30 ng/ml (50 – 75 nmol/L).[9] The Institute of Medicine chooses cut-off values of < 12 ng/ml and > 50 ng/ml as levels with increased risk of deficiency and excess, respectively.[1] The Institute of Medicine does not recommend specific doses, but, based on bone health

indicators, their analysis suggests that the daily use of vitamin D is 600 IU for individuals from 1 to 70 years of age, and 800 IU for individuals 71 and older, some or all of which may be achieved by ordinary sun exposure.[1] They further suggest a “safe upper limit” of dietary vitamin D intake as 4000 IU daily, a level at which risk for toxicity begins to increase. Yet, the Institute of Medicine emphasizes that this upper limit “should not be misunderstood as amounts people need or should strive to consume.”[1]

The use of the terms “daily” and “per day” in these recommendations may give the false impression that a day without sunshine requires a dose of supplement. Even though adults may use a given amount of cholecalciferol each day, such daily use does not necessarily require daily replacement. 25(OH)D_3 has a half-life of 2 weeks[10] to 3 months,[11] and is stored primarily in adipose tissue[12, 13] and, to a lesser extent, in the liver.[14] Presumably, this stored source of vitamin D is available for release back into the plasma, as indicated by a long-term study in Norwegians.[15] Moreover, cholecalciferol recycles in the enterohepatic circulation.[16] Thus, vitamin D-hormones may not require daily, weekly or even monthly replenishment. Summer sun exposure may provide enough for the winter.[17] Major institutions have used dosing schedules as infrequent as once every 1–4 months.[18, 19] It may be more correct to refer to a monthly requirement, and this requirement may vary depending on age (< 70 years or > 70 years) or season (i.e., summer vs. winter).[17]

Personalized approach to vitamin D-hormones

A personalized medicine approach is important in considering vitamin D-hormone supplementation because of the influence of differences in body composition, environmental factors, and genetic variations in D binding protein as well as variations in the intracellular vitamin D receptor. Although darkly pigmented individuals are believed to require more sun exposure to generate the same amount of vitamin D-hormones, they have genetic polymorphisms of the vitamin D binding protein,[20] which change the bioavailability of vitamin D, counteracting the decrease in synthesis.[21] The half-life of 25(OH)D_3 in the bloodstream is influenced by genotype of the DBP. Based on in vitro studies, the intracellular vitamin D receptor (VDR), which binds and translocates 1,25$(OH)_2D_3$ to the nucleus, has approximately 7% readthrough efficiencies, producing VDR proteoforms that have reduced binding.[22] This phenomenon may vary among individuals. High body fat content may decrease availability of fat-soluble 25(OH)D_3 due to sequestration in adipose tissue.[23] Conversely, high skeletal muscle content also modulates vitamin D-hormone availability; muscle cells internalize D-binding protein and expose it, allowing extensive intracellular uptake and retention of 25(OH)D_3.[24] The elderly may have lower levels due to less outdoor activity and sun exposure.[17] Polymorphisms arising in the Inuit Eskimo background limit intestinal D_3 uptake protect against excess D_3 ingestion from the ancestral diet of whale blubber rich in vitamin D hormones. These genetic variations, phenotypic differences, and environmental influences underscore the importance of tailoring any recommendations for vitamin D supplementation to individualized needs.

Vitamin D-hormones and cardiovascular health

Evidence for cardiovascular protection by vitamin D-hormones was almost entirely inferred from observational studies. Confounders that may adversely affect results of these studies include obesity, which alters the storage of vitamin D, skeletal muscle content, physical exercise, which corresponds with time outdoors in the sun, and illness, which corresponds with time indoors.

Widespread supplementation became a guideline[25] based on observational studies without adequate randomized controlled trials.[2] Although it is widely cited as showing an inverse relation between $25(OH)D_3$ levels and cardiovascular risk, a close look at data in the Offspring Cohort of the Framingham Heart Study[26] shows a U-shaped relationship between $25(OH)D_3$ levels and cardiovascular risk (Figure 2[26]). The apparent minimum risk occurs at a serum level of approximately 20 ng/ml, far below the level considered sufficient. A U-shaped curve was also found for the relationship between $25(OH)D_3$ and all-cause mortality in the NHANES III population.[27] Preclinical studies support this relationship with both deficiency and excess of $25(OH)D_3$ increasing atherosclerotic calcification.[28] Yet, public education campaigns continue to describe the relationship as inverse.

Although observational studies show associations between $25(OH)D_3$ levels and cardiovascular risk, the few randomized controlled trials available have failed to confirm any cardiovascular benefit of supplementation[29] with the exception of patients with chronic kidney disease, where the kidney's 1alpha-hydroxylase activity and capacity to produce active $1,25(OH)_2D_3$ are greatly diminished. One study found no reduction in cardiovascular risk factors in patients randomized to supplementation.[30] Another study of over 5000 patients found no reduction in cardiovascular mortality in patients randomized to supplementation, even though the treatment increased $25(OH)D_3$ levels by an average of 20 ng/ml.[18] In contrast, patients with renal insufficiency and/or dialysis, where vitamin D-hormone deficiency is prevalent,[31] vitamin D-hormone supplementation improved vascular function[32–34] without affecting plasma levels of calcium and phosphate.[35, 36] Overall, only the observational studies showed reductions in all-cause and cardiovascular mortality.[37–39]

Accumulation and activation of LDL-associated vitamin D-hormones in the artery wall

Both mono- and dihydroxy- forms of vitamin D-hormones are delivered to cells either by DBP,[40] where entry is mediated by the endocytic receptors, cubilin/megalin,[41] or by lipoproteins,[8] where entry is mediated by the low-density lipoprotein (LDL) receptor.[42] However, during pathogenesis of atherosclerosis, vitamin D-hormones that are consumed in the diet may accompany LDL into the subendothelial space of the artery wall where atherosclerotic lesions form.[43] In peripheral tissues that express lipoprotein lipase, the chylomicron metabolizing enzyme, a fraction of vitamin D-hormones can be taken up by the tissues. Since 1-alpha hydroxylase is present in tissues and cells, including vessel walls and monocyte-derived cells, the active form may be produced locally within the artery wall and, conceivably, within monocyte-laden atherosclerotic plaque.[44]

Targets of vitamin D-hormones

Cellular and molecular effects of vitamin D-hormones are extensive. In addition to homodimerization, VDR heterodimerizes with the retinoid X receptor to activate transcription of a wide range of genes. As steroid hormones, they are related to estrogen, testosterone, mineralocorticoids, and glucocorticoids. Even our limited search of the literature (Table) reveals hundreds of diverse genomic and non-genomic targets of vitamin D-hormones, affecting a vast array of physiological functions. Adding further complexity, vitamin D-hormones have significant cross-talk with steroid and nuclear hormones and their receptors.[45] For instance, vitamin D_3 may affect actions of glucocorticoids.[46,47] Conversely, steroid and xenobiotic receptors[48] as well as peroxisome proliferator-activated receptor gamma[49] inhibit VDR-mediated CYP24 (24-hydroxylase) promoter activity.

Effects of vitamin D-hormones in the vasculature

Given that diet-derived 25(OH)D_3 is carried in lipoproteins, and that lipoproteins accumulate in the subendothelial space of arteries leading to atherosclerotic lesions, it is likely that diet-derived 25(OH)D_3 also accumulates in the neointima artery wall and atherosclerotic plaque. Given that vascular smooth muscle cells and monocytes both produce 1-alpha hydroxylase, it follows that 1,25$(OH)_2D_3$ may also accumulate in artery walls and atherosclerosis. Potential effects of this accumulation remain to be determined. One possibility is acceleration of both atherosclerosis and cardiovascular calcification, based on studies showing that vitamin D-receptor deficiency significantly reduces calcific atherosclerosis in hyperlipidemic mice.[50] Vitamin D-hormones are known to stimulate smooth muscle cell proliferation[51] and induce expression of fibroblast growth factor-23 (FGF-23), high levels of which are linked to adverse cardiovascular events.[52] With respect to mineralization, effects of vitamin D-hormones are double-edged. Although there is convincing evidence that supplements increase bone density,[53] any benefit to bone may be at the cost of cardiovascular morbidity and mortality due to calcific vasculopathy and valvulopathy. Cardiovascular calcification has been shown to occur by many of the same cellular and molecular processes as bone mineralization,[54] including induction of osteogenic factors by vitamin D-hormones.[55] Indeed, high dose vitamin D supplements used for several decades as an experimental model reproducibly induces severe aortic calcification, acutely and chronically, over a wide range of conditions in a variety of species in the hands of many different investigators.[56,57] The dramatic vascular calcification seen in patients with chronic kidney disease may be due in part to local induction of 1-alpha hydroxylase in the artery wall.[58] The extensive immunomodulatory effects of vitamin D have been reviewed elsewhere.[59]

Vitamin D-hormone toxicity and benefits

Overuse of vitamin D-hormone supplements carries significant risks that have been known for decades, and these risks have traditionally been associated with those of the resulting hypercalcemia that can occur at 25(OH)D_3 plasma concentrations of > 150 ng/ml (> 375 nmol/L). Thus, the traditional clinical manifestations of vitamin D-hormone toxicity are those of hypercalcemia, which include generalized (fatigue, weakness), neurological (altered mental status, irritability, coma), gastrointestinal (nausea, vomiting, constipation), and

endocrinological (polyuria, polydipsia) symptoms. Additionally, renal injury as well as the development of kidney stones may occur. As such, studies evaluating the safety of various dosing regimens typically use measurements of serum and urinary calcium to monitor the safety of the administered doses.[60, 61]

However, given the number of cell types and tissues that possess 25-hydroxylase, vitamin D-hormones may have effects on these systems without necessarily affecting the serum or urinary calcium levels, and all of the biological processes listed in the Table may be deranged by excess intake. A daily intake of 25(OH)D_3 up to 4,000 IU is deemed to be the upper limit of safety,[62] as the risk of harm appears to increase above this level. Yet as discussed above, variations in vitamin D-hormone production and metabolism may depend significantly on individual genotype, phenotype, and environmental conditions; thus, a universal upper limit of safety and a universal lower limit of sufficiency for all patients may not necessarily be accurate. Additionally, excess vitamin D-hormone supplements also displace the active form from binding sites, making it more available even when not appropriate.[5] Further, given the cross-talk with other steroid hormone receptors, vitamin D-hormones in excess may have physiological effects similar to those of glucocorticoids, estrogen, or even those of anabolic steroids.[63] Nonetheless, in general, it is difficult to categorize any one of the numerous effects of vitamin D as necessarily beneficial or toxic, given the dependence on location as well as physiological and pathological contexts. For instance, osteoblastogenesis may be beneficial in osteoporosis but hazardous in calcific vasculopathy and valvulopathy.

Concluding remarks

Widely used guidelines for monitoring and supplementing vitamin D_3 hormones resembles the ill-fated call, years ago, for widespread use of another steroid hormone, estradiol, for post-menopausal women based on observational studies. The impact of confounding environmental factors was not recognized. Even after the Women's Health Initiative showed increased cardiovascular risk in postmenopausal women randomized to hormone replacement therapy,[19] recommendations were slow to change. Observational studies are not sufficient to recommend widespread hormonal supplementation, and the same applies to vitamin D-hormones. The ongoing randomized clinical trial, Vitamin D and Omega-3 Trial (VITAL), will be helpful in determining whether vitamin D-hormone supplementation provides any benefit in the primary prevention of cancer and cardiovascular disease.[64]

Decades ago, the pioneering Johns Hopkins cardiologist, Dr. Helen Taussig, anticipated the need for a personalized approach to D_3 supplements: "As is so common, the popular belief was that 'if some is good, more is better.' The result was the overdosing with vitamin D and adding it to various foods. Then came the recognition of vitamin D intoxication... we are coming to appreciate that there exists an inborn variation in man's ability to metabolize vitamin D and that some individuals may be injured by doses of vitamin D which are safe for others."[65] For health reasons, many Americans pay extra for bread free of preservatives (such as antioxidants) and meats that are free of steroid hormones. In the next aisle of the store, they buy bottles of antioxidant preservatives and steroid hormones in pill form, labeled as nutritional supplements, including D_3 hormones. Scientists need to use their knowledge

of molecular, cellular, and integrative physiology to advocate for rational use of vitamin D-hormone supplements to prevent adverse consequences to cardiovascular health by overenthusiastic guidelines followed by well-meaning physicians.

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Nonstandard Abbreviations and Acronyms:

25(OH)D_3	Calcidiol
1,25$(OH)_2D_3$	Calcitriol
DBP	(Vitamin) D-binding protein
IU	International unit
D_2	Ergocalciferol
VDR	Vitamin D receptor
LDL	Low-density lipoprotein
VLDL	Very low-density lipoprotein CYP24 (24-hydroxylase)
FGF-23	Fibroblast growth factor-23
SMC	Smooth muscle cell
VSMC	Vascular smooth muscle cell
Hox-8	Muscle segment homeobox-containing gene Msx-2
UV	Ultraviolet
NFAT1	Nuclear factor of activated T cells 1
MAPK	Mitogen-activated protein kinase
c-myc	Cellular homolog of the oncogene of avian myelocytomatosis virus strain 29
Runx2	Runt related transcription factor-2

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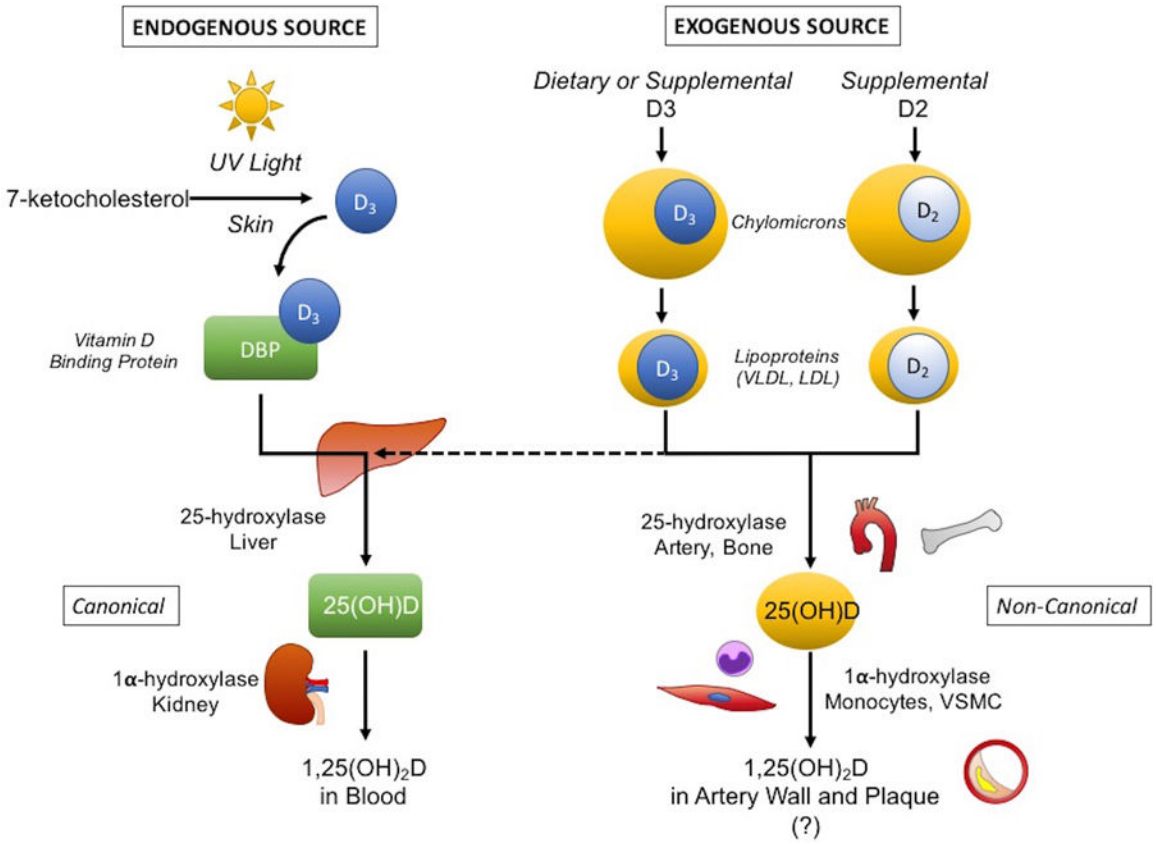

Figure 1. Vitamin D-hormone metabolism, carriers and distribution.

Due to its lipophilic nature, endogenously produced D3 is carried in the aqueous environment of blood by D-binding protein (DBP), whereas exogenous (dietary and supplemental) D3 and D2, absorbed from the intestines, are transported within chylomicrons, which are further processed to lipoproteins (e.g., VLDL and LDL), many of which continue to carry the exogenous D. The conventional sites of vitamin D-hormone metabolism are the liver and proximal tubules of the kidney, where hydroxylases convert it to its active form. But hydroxylase activity is also found in parenchymal and immune cells, including VSMC and monocytes, in other tissues. This raises the potential for accumulation of vitamin D-hormone within LDL in the subendothelial space, where it may undergo activation in atherosclerotic plaque and possibly influence ectopic differentiation and calcification. Abbreviations: LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; VSMC, vascular smooth muscle cells; UV, ultraviolet.

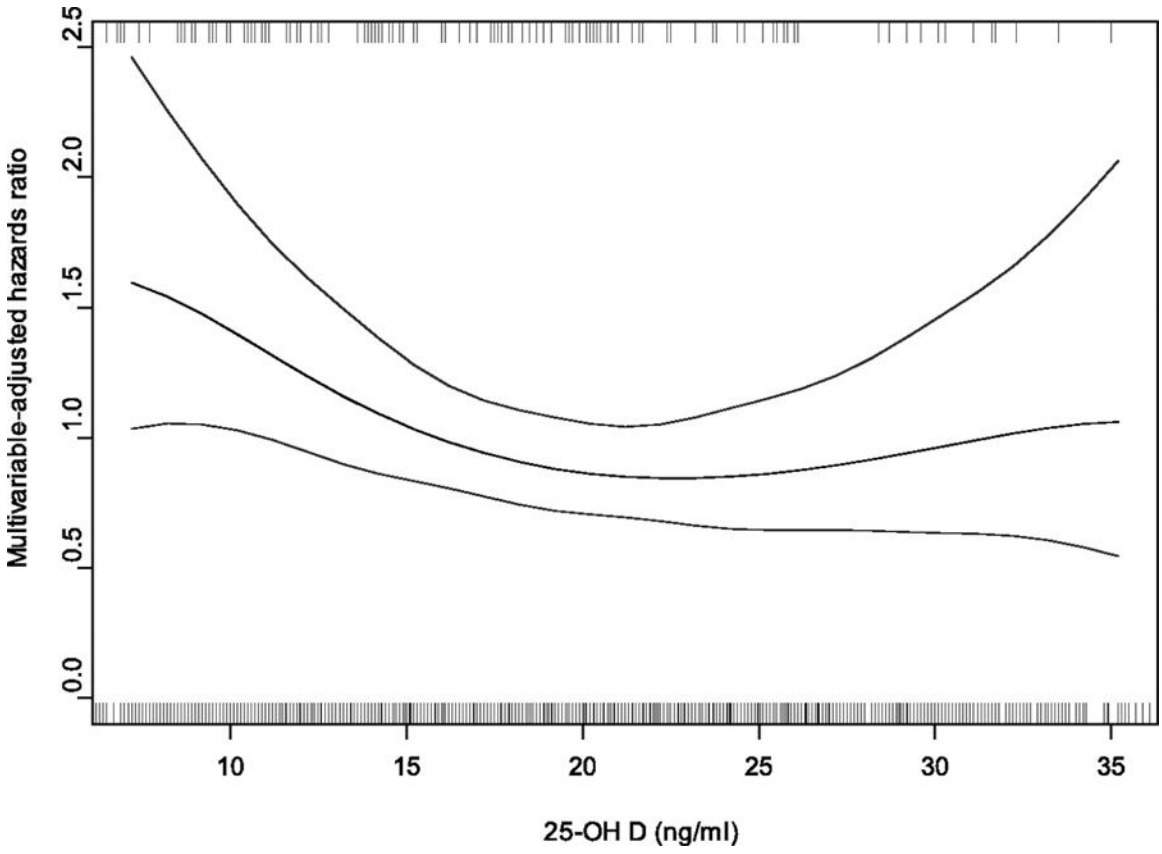

Figure 2.

U-shape of the multivariable-adjusted relation between baseline serum 25(OH)D_3 levels and incident cardiovascular events reported by Wang et al. (from *Circulation* 117:503–511, with permission [**pending**]). Solid lines show the estimated relation of adjusted hazard ratios (with 95% confidence limits) and 25(OH)D_3 levels when time to cardiovascular event is modeled as a function of penalized regression splines of 25(OH)D_3 levels with adjustment for all other covariates. Hatched lines on the horizontal axis represent cardiovascular events (top axis) and individuals (bottom axis). This relationship suggests increased cardiovascular risk at both low and high levels.

Table.

Partial literature summary of vitamin D-hormone actions

Vitamin D-hormone effects	Targets
Cells that metabolize D3	• Hepatocytes,[66] renal cells,[67] endothelial cells,[68] smooth muscle cells,[69] monocytes,[70] skeletal muscle cells[71]
Pathophysiology affected	• Osteomalacia,[72] vascular calcification,[56, 57,73,28] renal and myocardial calcification,[73] atherosclerosis,[74] heart failure,[75] thrombosis[76]
Tissue content and function affected	• Calcium balance,[77] aortic elastin,[78] bile acid synthesis,[79] vasoconstrictor response to norepinephrine,[80] lipogenesis,[81] insulin sensitivity,[82] endothelial-dependent contraction[83]
Cellular functions	• Osteoblastogenesis,[84] osteoclastogenesis,[85] chondrogenesis[86] • Myogenesis,[87]adipogenesis,[88] hematopoiesis,[89] nerve growth,[90] • Smooth muscle cell (SMC) dedifferentiation,[91] SMC migration,[92] SMC contraction,[93] • Tumor cell differentiation[94] • Macrophage cholesterol uptake,[95] T-lymphocytes[96] • Apoptosis,[97] DNA synthesis,[98] arachidonic acid turnover[99] • Oscillation of inositol phosphate 3 and diacylglycerol production[100] • Prostaglandin production,[101, 102] superoxide anion production[103] • Nitric oxide synthase[104]
Signaling pathways	• Protein kinase C-alpha,[105] cyclic AMP,[106] p38 MAPK,[107] c-myc,[108] c-fos,[85] NFAT1,[109] Wnt,[110] nuclear factor-kappa B[111]
Known interactions	• Retinoic acid receptors,[112] retinoid X receptors,[113] glucocortoid,[114] • Runx2,[115] transforming growth factor-beta,[116] insulin-like growth factor binding protein-5,[117] vitamin K,[118] estrogen,[119]
Protein synthesis and serum levels regulated	•Low-density lipoprotein (LDL),[120] prostaglandin synthesis,[101] endothelin receptor[91]
Gene expression	• Bone morphogenetic protein −2,[121] alkaline phosphatase,[122] bone sialoprotein,[123] collagen I,[124] osteopontin,[124] osteocalcin[115] tissue plasminogen activator,[125] parathyroid hormone,[126] fibroblast growth factor-23[127] • Receptor activator of nuclear factor kappa B ligand[128] • Muscle segment homeobox-containing gene Msx-2 (Hox-8),[129] • Peroxisome proliferator activated receptor-gamma[130] • Integrins,[131] fibronectin,[132] laminin receptor[133] • Collagenase,[134] matrix metalloproteinase[135] • Granulocyte-macrophage colony stimulating factor,[109, 114] macrophage-colony stimulating factor[136] tumor necrosis factor-alpha,[137] interleukin 8[138] • Insulin receptor[139] • Vascular endothelial growth factor,[140] nerve growth factor,[141] • Type A natriuretic peptide receptor[142] • Aromatase[143] • Nephrin[112] • CYP7A1,[144] CYP19A1,[143] CYP24A1[145] • p450 cytochrome,[146] Mullerian-inhibiting substance[147]