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ABSTRACT

A poor vitamin D status, i.e. low serum levels of 25-hydroxyvitamin D (25[OH]D), is common in the general population. This finding is of concern not only because of the classic vitamin D effects on musculoskeletal outcomes, but also because expression of the vitamin D receptor (VDR) and vitamin D metabolizing enzymes in the heart and blood vessels suggests a role of vitamin D in the cardiovascular system. VDR-knockout mice suffer from cardiovascular disease (CVD) and various experimental studies suggest cardiovascular-protection by vitamin D, including anti-atherosclerotic, anti-inflammatory and direct cardio-protective actions, beneficial effects on classic cardiovascular risk factors as well as suppression of parathyroid hormone (PTH) levels. In epidemiological studies, low levels of 25(OH)D are associated with increased risk of CVD and mortality. Data from randomized controlled trials (RCTs) are sparse and have partially, but not consistently, shown some beneficial effects of vitamin D supplementation on cardiovascular risk factors (e.g. arterial hypertension). We have insufficient data on vitamin D effects on cardiovascular events, but meta-analyses of RCTs indicate that vitamin D may modestly reduce all-cause mortality. Despite accumulating data suggesting that a sufficient vitamin D status may protect against CVD, we still must wait for results of large-scale RCTs before raising general recommendations for vitamin D in the prevention and treatment of CVD. In current clinical practice the overall risks and costs of vitamin D supplementation should be weighed against the potential adverse consequences of untreated vitamin D deficiency.

INTRODUCTION

Vitamin D is classically known for its crucial role in calcium and bone metabolism.[1,2] Recent advances in research provoked enormous public health interest in vitamin D: (i) the vitamin D receptor (VDR) was identified in almost all human cells,[2] (ii) vitamin D deficiency was shown to be highly prevalent among general populations[3] and (iii) vitamin D deficiency has been associated with various chronic diseases including cardiovascular diseases (CVDs), which are the leading causes of death.[1,2,4] In this review we summarise and discuss current knowledge on the association of vitamin D with CVD and mortality. We start with an overview of vitamin D metabolism, vitamin D`s history in science, and the current prevalence of vitamin D deficiency. Then, we summarise mechanistic and clinical data on the role of vitamin D for the cardiovascular system and for CVD risk factors. Finally, we present epidemiological and interventional data of vitamin D administration, CVD, and mortality and offer some guidance for treating vitamin D deficiency.

BASIC VITAMIN D METABOLISM

Sunlight induced vitamin D synthesis in the skin accounts for about 80% of obtained vitamin D.[5] Specifically, ultraviolet-B (UV-B) radiation induces the conversion of 7-dehydrocholesterol to previtamin D which spontaneously isomerises to vitamin D.[6] This vitamin D production by sunlight exposure is particularly efficient in individuals with low levels of skin melanin. Therefore, an intriguing hypothesis suggests that in human evolution, those individuals migrating to northern regions developed a fair skin to efficiently synthesize vitamin D under conditions of less UV-B exposure, whereas those individuals residing in sunny regions have a high melanin content of the skin, which protects against sunlight-induced damage.[7,8] Diet makes a relatively small contribution to vitamin D status.[1,5] Vitamin D can be obtained from natural foods (e.g. oily

fish, eggs, or UV irradiated and sun dried mushrooms), vitamin D-fortified food (e.g. vitamin D-fortified milk and orange juice in the US) or vitamin D supplements.[1] Two major forms of vitamin D exist: vitamin D3 (cholecalciferol), the main vitamin D form derived mainly from synthesis in the skin and from animal sources, and vitamin D2 (ergocalciferol), the plant- and yeast-derived form. Unless otherwise stated, we do not differentiate between these two isoforms in this review and usually refer to vitamin D (meaning both vitamin D2 and D3) in general.

Vitamin D as a precursor compound exerts no significant biological activity. Two hydroxylation steps are required to produce the most active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25[OH]2D).[6] First, vitamin D from any source is hydroxylated to 25-hydroxyvitamin D (25[OH]D). This is generally a substrate-dependent process that occurs mainly in the liver. Then, the enzyme 1α-hydroxylase converts 25(OH)D to 1,25(OH)2D. The kidney is the major site for production of circulating 1,25(OH)2D. Serum levels of 1,25(OH)2D are therefore significantly determined by renal 1α-hydroxylase activity, which is tightly regulated by factors related to calcium and phosphorus metabolism (e.g. stimulation by parathyroid hormone [PTH] or inhibition by fibroblast-growth factor 23). Correlations of serum level of 25(OH)D and 1,25(OH)2D are usually not very strong and patients with low 25(OH)D levels frequently have relatively high 1,25(OH)2D levels as a consequence of PTH-induced stimulation of renal 1α-hydroxylase activity. Many extra-renal tissues also express 1α-hydroxylase, which can produce significant tissue levels of 1,25(OH)2D.[6] In contrast to renal 1α-hydroxylase activity such local production of 1,25(OH)2D seems to be significantly dependent on the substrate availability of 25(OH)D, but in addition is also regulated by other parameters such as cytokines or growth factors.[6] Hence, a widely accepted hypothesis is that serum levels of 25(OH)D are the main determinant of 1,25(OH)2D tissue levels in various organs. Further 24-hydroxylation of 25(OH)D or 1,25(OH)2D is considered the main degradation process and produces vitamin D metabolites (24,25[OH]2D or 1,24,25[OH]3D) which are converted to water

soluble inactive calcitroic acid. VDR activation induces this 24-hydroxylase, resulting in a regulatory loop.[6] This process seems to be important for overall vitamin D metabolism because recent data suggest that the decline of circulating 1,25(OH)2D in chronic kidney disease (CKD) might be a consequence of increased inactivation (24-hydroxylation) rather than of reduced production (1α-hydroxylation) of 1,25(OH)2D.[9] Plasma 1,25(OH)2D concentrations are usually not measured as part of the clinical routine except in rare cases (e.g. VDR dependent rickets and granuloma-forming diseases such as sarcoidosis).[1] Low 1,25(OH)2D concentrations are associated with adverse health outcomes including CVD.[10] Furthermore, studies on treatment with 1,25(OH)2D or selective VDR activators (e.g. paricalcitol) documented various beneficial effects on CVD as well as improved overall survival in CKD patients.[11] A detailed discussion of this issue is, however, beyond the scope of this review.

Vitamin D metabolites circulate in serum mainly bound to vitamin D binding protein (DBP). Concentrations of 25(OH)D are up to 1000-fold higher than plasma 1,25(OH)2D concentrations. 25(OH)D also has a significantly longer half-life than that of 1,25(OH)2D (2-3 weeks versus 4-6 hours). DBP is important not only for transport of vitamin D metabolites in the bloodstream, but also for uptake of 25(OH)D or 1,25(OH)2D by target cells.[6] However, our understanding on the cellular uptake of vitamin D metabolites in many target tissues is still in its infancy.[6]

Vitamin D metabolites exert their effects by binding to the almost ubiquitously expressed cytoplasmatic VDR.[2] After ligand binding this receptor forms a heterodimer with retinoid X receptor (RXR) and translocates to the nucleus. There, this complex interacts with specific DNA regions, called vitamin D-responsive elements. By additional interactions with coregulatory proteins the VDR-RXR complex regulates approximately 3% of the human genome.[2]

HISTORICAL PERSPECTIVE

Historically, vitamin D (alphabetically named "D" as the fourth known vitamin) was discovered by McCollum *et al.* in 1922 as the substance that cured rickets, a bone disease in children characterized by bone pain and skeletal deformities.[12] Even before McCollum identified vitamin D, it has already been known that sunlight was effective in preventing rickets.[12] The chemical structures of vitamin D2 and D3 have been elucidated in the 1930s. In the late 1960s and early 1970s, the structures of 25(OH)D and 1,25(OH)2D were characterized, respectively.[2,12] Around the mid-20th century reports on vitamin D overdosing, in particular in children, shed some negative light on vitamin D and until the 1970s people believed that vitamin D may even cause CVD. [13] In 1981, Robert Scragg raised the hypothesis that the increased CVD incidence in winter may be a consequence of low UV-B irradiation, with correspondingly low vitamin D status in that season.[14] The discovery of the VDR in the rat heart by Robert Simpson in 1983 further stimulated research on vitamin D and CVD.[15] Over the last three decades and several reports have described vitamin D´s effects on the cardiovascular system.

PREVALENCE OF VITAMIN D DEFICIENCY

Serum concentrations of 25(OH)D are the best parameter to assess whole-body vitamin D status. Therefore, such measures are used to assess vitamin D deficiency.[16] However, no general consensus has yet emerged on 25(OH)D cut-off levels for vitamin D status and debate on how to classify vitamin D status is ongoing. Cut-off levels were initially based on vitamin D´s effects on calcium metabolism. A poor vitamin D status is associated with decreased intestinal calcium resorption, leading to low serum calcium levels. This condition in turn stimulates PTH secretion. The resulting effects of increased PTH levels on the intestines (enhanced 1,25(OH)2D production), bones (calcium mobilisation), and kidneys (e.g. reduced calcium loss) ensure adequate physiologic serum calcium levels, albeit at the expense of several deleterious effects of

this secondary hyperparathyroidism. Based on this consideration the 25(OH)D level below which PTH levels start to rise (~75 nmol/L; to convert 25[OH]D levels from ng/mL to nmol/L multiply by 2.496) is frequently used for the definition of a sufficient vitamin D status (≥75 nmol/L).[1] This supposition is also in line with histomorphometric analyses of bone biopsies that showed pathologic mineralisation defects in patients with 25(OH)D serum levels below 75 nmol/L.[17] A proposed vitamin D status classification, which we support, differentiates between the following groups: vitamin D sufficiency (≥ 75 nmol/L), vitamin D insufficiency (50 to 74 nmol/L) and vitamin D deficiency < 50 nmol/L) (see Figure 1).[1] Some authors use lower thresholds for vitamin D deficiency, such as cut-offs of <37.5 nmol/L or < 25 nmol/L.[18] The Institute of Medicine (IOM) in the US classified vitamin D sufficiency as 25(OH)D levels ranging from 50 to 125 nmol/L.[19] More recently, classifications of vitamin D status have also been based on 25(OH)D levels at which the risk of adverse health outcomes increases significantly; a level of ~ 75 to 100 nmol/L (=optimal range) is associated with the best outcomes for multiple diseases.[4,20] This optimal range, which lies exactly in the middle of the IOM vitamin D sufficiency range, is however based only on observational data including achieved 25(OH)D concentrations from meta-analyses of RCTs because no published interventional studies exist on, for example, CVD or mortality outcomes with specific target concentrations of 25(OH)D.[4] Therefore, any vitamin D status classification, including the one we propose (see Figure 1), is debatable unless sufficient data from RCTs are available to support that targeting certain 25(OH)D ranges reduces hard endpoints such as CVD or mortality. 25(OH)D levels of 250 nmol/L can be considered as safe and are used by several reference laboratories as the safe upper limit of normal. This safe upper limit of normal is based mainly on the evidence level of an expert opinion and was chosen because 25(OH)D concentrations of 250 nmol/L can be achieved but are rarely exceeded by healthy subjects who have spent prolonged periods in a sunny environment.[4,21] However, only limited clinical data are available on the long-

term prognosis of individuals with 25(OH)D levels > 125 to 150 nmol/L.[4] Vitamin D toxicity begins at 25(OH)D levels >375 to 500 nmol/L (see Figure 1).[1,4]

Mithal *et al.* aimed to review the global vitamin D status.[3] Reduced 25(OH)D levels (<75 nmol/L) are highly prevalent in almost every region of the world.[3] Risk factors for low 25(OH)D levels were advanced age, female sex, winter season, darker skin pigmentation, less sunlight exposure and low intake of vitamin D by diet including absence of vitamin D fortification of food items. In Europe or the US, the majority of the general population has 25(OH)D serum concentrations below 75 nmol/L. Data from the US indicating that the prevalence of low 25(OH)D concentrations is still increasing.[22,23] Specifically, 25(OH)D levels in the US population declined from 1988-1994 to 2000-2004, although the worsening of vitamin D status may be partially attributed to assay differences.[22,23] Seasonal variation of vitamin D status, the amplitude of which increases with distance from the equator, must be considered. In the UK, the values at the end of summer are ~50% higher compared to the end of winter and even in South Florida a significant 14% increase of 25(OH)D levels was observed during summertime.[5,24] Genetics also influence circulating 25(OH)D concentrations.[25] Wang *et al.* found three genetic loci that were significantly related to 25(OH)D levels.[25] One gene is involved in metabolism of the vitamin D precursor 7-dehydrocholesterol, another one encodes an enzyme with 25-hydroxylase activity and the third one is the DBP.[25]

One should also consider different characteristics of 25(OH)D assays when determining vitamin D status.[26] Whereas data on cut-off levels for vitamin D status are generally based on the DiaSorin radioimmunoassay, the increasing request for 25(OH)D measurements has prompted the development of various automated immunoassays.[26]

VITAMIN D AND CARDIOVASCULAR RISK FACTORS

In the following we summarise clinical and mechanistic evidence on the effect of vitamin D status on cardiovascular risk factors (see Figure 2).

PTH levels are inversely correlated with 25(OH)D concentrations and epidemiological studies demonstrated that elevated and high-normal PTH levels are associated with an increased risk of cardiovascular events and mortality.[27] Mechanistically, PTH increases blood pressure and exerts various effects on the heart including myocardial hypertrophy and pro-arrhythmic actions.[28] PTH suppression by vitamin D supplementation might therefore reduce cardiovascular risk.

Low 25(OH)D levels are an independent risk factor for prevalent and incident hypertension.[29] Moreover, meta-analyses of randomized controlled trials (RCTs) documented that vitamin D supplementation lowers systolic blood pressure by 2 to 6 mm Hg. [30-32] This latter effect was statistically significant in two of three meta-analyses.[30-32] Proposed antihypertensive effects of vitamin D include PTH suppression, reno- and vasculo-protective properties, and anti-inflammatory and anti-diabetic actions. In addition, 1,25(OH)2D has been shown to suppress renin transcription.[2,33] Molecular pathways for this effect have already been clarified, and this finding is in line with observations of VDR and 1α-hydroxylase-knockout mice who display increased activity of the renin-angiotensin aldosterone system (RAAS).[2,33]

CKD patients are particularly prone to low 25(OH)D levels.[34] This outcome can be attributed to morbidity-related limitations in sunlight exposure, impaired vitamin D synthesis in the skin, malnutrition, and disturbances in vitamin D metabolism, such as loss of protein-bound vitamin D metabolites via the urine in proteinuric patients.[34] Interestingly, a poor vitamin D status is a risk factor for decline in renal function.[34] This may be explained by several reno-protective effects of vitamin D such as anti-proteinuric (e.g. vitamin D might increase megalin-mediated tubular protein reabsoprtion) and antihypertensive properties, suppression of the RAAS, and anti-inflammatory and anti-autoimmunological actions.[5,34]

A poor vitamin D status is partially, but not consistently, associated with a higher prevalence and incidence of type 2 diabetes mellitus.[35] Vitamin D metabolites stimulate insulin secretion and improve insulin sensitivity, for example, by upregulating the insulin-receptor.[35] Some, but not all, interventional studies have shown that vitamin D supplementation improves glucose metabolism for example, by reducing insulin resistance.[35] Vitamin D may also be important for preventing type 1 diabetes mellitus. Low vitamin D intake during childhood and low solar UV-B radiation are associated with an increased risk of developing type 1 diabetes mellitus.[35,36] In addition, variants of genes involved in vitamin D metabolism and related to 25(OH)D levels are associated with risk of type 1 diabetes.[37] Vitamin D affects autoimmunological processes by increasing regulatory T cells (Tregs), which protect against autoimmunity.[38] Whether vitamin D supplementation is definitely useful to prevent or treat type 1 and type 2 diabetes mellitus remains to be proven in RCTs.

Some RCTs have already documented anti-infectious and anti-inflammatory actions of vitamin D such as reduction of the inflammation marker tumor necrosis factor-α (TNF-α) and increases of the anti-inflammatory cytokine interleukin-10 (IL-10).[2,39-41]

Vitamin D may also affect other cardiovascular risk factors such as blood lipids or coagulation parameters but currently available data are insufficient to draw final conclusions.[42,43]

VITAMIN D EFFECTS ON HEART AND BLOOD VESSELS

Evidence is accumulating, that vitamin D may also exert various direct effects on the cardiovascular system (see Figure 3). Heart and blood vessels are target tissues for vitamin D and express both VDR and 1α-hydroxylase.[2,44-47] In the following, we summarise experimental and observational clinical studies on the role of vitamin D for the heart and blood vessels.

VDR-knockout and 1α-hydroxylase-knockout mice develop heart failure despite normalised calcium levels.[2,45,48,49] Increased activation of the RAAS seems to be the mediating pathway

because RAAS blockade with, for example, the ACE-inhibitor captopril reverses cardiac abnormalities in these mouse models.[2,48,49] Preliminary data published in abstract form suggest that even cardiomyocyte-specific VDR-knockout mice develop myocardial damage.[50] The crucial role of vitamin D for myocardial health is further supported by increased VDR expression in myocardial hypertrophy.[45,46] VDR expression increased in cardiac myocytes and fibroblasts after treatment with the pro-hypertrophic vasoactive peptide endothelin.[46] Experimental studies documented anti-hypertrophic and anti-proliferative actions of vitamin D metabolites, which downregulate several genes involved in the development of myocardial hypertrophy.[45,51] VDR activation modulates cardiac calcium flux and thereby induces an accelerated relaxation of cardiomyocytes, which may improve diastolic function of the heart.[45,52] Vitamin D-mediated regulation of cardiac extracellular matrix (ECM) turnover may also be important to maintain cardiac health.[45,53] In this context, a study of 171 healthy British Bangladeshi adults, showed that matrix metalloproteinase-9 (MMP-9) is elevated in vitamin D deficient individuals but is significantly decreased after vitamin D supplementation.[53] Several clinical studies confirmed that heart failure patients have a poor vitamin D status but whether vitamin D deficiency is only the consequence of heart failure or possibly contribute to myocardial diseases is unclear.[45] Interventional trials produced inconsistent results on the effect of vitamin D on parameters of myocardial structure and function warranting further large-scale RCTs.[40,45]

Vitamin D may also protect against atherosclerosis, vascular calcification and endothelial dysfunction.[44] Anti-atherosclerotic vitamin D effects may include (i) inhibition of macrophage cholesterol uptake and foam cell formation, (ii) downregulation of vascular smooth muscle cell (VSMC) proliferation and migration, and (iii) suppression of inflammation triggered endothelial activation and expression of endothelial adhesion molecules.[44,54-56] Vitamin D effects may also protect against endothelial dysfunction, for example, by anti-oxidative actions and by inhibiting

lipid peroxidation.[44,57] Finally, vitamin D may reduce vascular calcification, for example, by inhibiting bone morphogenic proteins, but data on this topic are somewhat controversial.[44,58] This could be attributed to the fact that both a poor vitamin D status as well as vitamin D intoxication may contribute to vascular calcification, although it should be noted that the largest study on vitamin D plus calcium supplementation found no effect on coronary artery calcification.[54,59]

Observational and interventional studies showed inconsistent results regarding the association of vitamin D with vessel diseases (e.g. inconclusive data on 25[OH]D and carotid intima-media thickness).[44,60,61] Several, but not all, interventional studies showed that vitamin D supplementation improves endothelial function.[54,62] Hence, we need further RCTs before we can draw final conclusions on the effect of vitamin D on the vasculature.

VITAMIN D AND CARDIOVASCULAR DISEASES

Observational data

Accumulating evidence suggests that CVD is associated with vitamin D deficiency. Various epidemiological studies have reported reduced 25(OH)D concentrations in patients with previous and prevalent cardiovascular or cerebrovascular diseases.[44,45,63-66] Several prospective studies have addressed this issue, including the Intermountain Heart Collaborative (IHC) Study, which included >40,000 individuals from a general health care population.[65] In this study, individuals with low serum concentrations of 25(OH)D were at significantly increased risk for future CVD, in particular for heart failure and cerebrovascular events.[65] Consistent with this finding, population-based studies have partially, but not consistently, documented that poor vitamin D status is associated with an increased risk of cardiovascular events and cardiovascular mortality (see Table 1).[66-80] Significant associations of low 25(OH)D levels and increased risk of fatal cardiovascular events, in particular, sudden cardiac death, were also observed in patients referred

to coronary angiography, as well as in diabetic dialysis patients.[81,82] Regarding specific cardiovascular events, a significant association of low 25(OH)D and fatal strokes was reported.[64] This finding may also have clinical relevance because post-stroke patients with such increased risk of vitamin D deficiency may be more prone to develop musculoskeletal complications.[64] Associations of vitamin D deficiency with incident CVD seem particularly strong in patients with CVD or at high risk of CVD and some indication exists for a nonlinear relationship with pronounced CVD risk increase at 25(OH)D concentrations below ~37.5 nmol/L.[65-82] Meta-analyses performed so far on this topic support the notion that low 25(OH)D concentrations are associated with incident CVD.[63,66] In this context, Grandi *et al.* found that the risk of cardiovascular mortality was increased by 83% (hazard ratio 1.83; 95% CI: 1.19-2.80) in individuals with low 25(OH)D levels, that is, patients with 25(OH)D levels below a cut-off ranging from ~25 to 50 nmol/L.[66]

However, one must interpret all these above-mentioned observational studies in the light that sun exposure habits, which may be an important confounder for the association of 25(OH)D and CVD, significantly determine vitamin D status. Furthermore, nutritional intake of vitamin D is usually related to other micronutrients (e.g. vitamins D and A in cod liver oil), which interferes with the ability to evaluate how vitamin D status affects CVD. Apart from this, vitamin D-independent effects of sunlight exposure might partially drive the association of vitamin D status with CVD. Cardiovascular risk factors and CVD may also have a reciprocal effect on vitamin D status (e.g. vitamin D may affect myocardial function, but impaired myocardial function may cause vitamin D deficiency owing to morbidity-associated limitation of sunlight exposure). Single-nucleotide polymorphisms (SNPs) of the VDR and of key enzymes for vitamin D metabolism have also been associated with left ventricular hypertrophy, heart failure and coronary artery calcification.[83-85] Even though some questions on the functionality of these SNPs

remain unresolved, these latter results may further support a possible causal relationship between vitamin D metabolism and CVD .[83-85]

Data from interventional studies

Results of RCTs designed primarily to assess effects of vitamin D supplementation on CVD are still missing. Data on vitamin D and CVD are derived mainly from studies designed to evaluate vitamin D´s effect on musculoskeletal outcomes.[86] Most of those studies evaluated the impact of combined calcium plus vitamin D supplementation.[86] This approach limits the ability to differentiate the effects of vitamin D from those of calcium. This factor may be important because calcium per se might even be harmful in terms of cardiovascular outcomes.[86,87] A systematic review identified two RCTs with exclusive vitamin D supplementation that reported on CVD events as a secondary outcome.[86] These studies, with vitamin D doses of approximately 1000 IU per day, found a moderate, but statistically nonsignificant, reduction in CVD risk (pooled relative risk, 0.90 [95% CI, 0.77 to 1.05]) in the vitamin D group.[86] Further large studies have been initiated to definitively prove whether vitamin D supplementation reduces CVD in the general population, but these studies will still take several years (e.g. the VITAL; see www.vitalstudy.org).

VITAMIN D AND TOTAL MORTALITY

Beyond CVD, vitamin D deficiency is associated with increased risk of total mortality. Most, but not all, studies on this topic documented increased mortality in patients with low 25(OH)D concentrations.[68,70-82] A meta-analysis among 6853 CKD patients showed that mortality risk decreases by 14% (relative risk 0.86 [95% CI 0.82-0.91]) per 25 nmol/L increase in 25(OH)D levels.[88] This finding is in line with the results of a meta-analysis of RCTs including mainly studies on frail, elderly patients.[89] In this report of Autier and Gandini, vitamin D

supplementation was associated with a significant 7% decrease in total mortality (summary relative risk 0.93 [95% CI 0.87-0.99]).[89]Another meta-analysis on vitamin D and its analogues showed less significant results and reported a significant mortality reduction only in patients with the combined treatment of vitamin D plus calcium (relative risk 0.94 [95% CI 0.89-0.99].[90]

VITAMIN D TREATMENT GUIDANCE

Vitamin D treatment can be done by either vitamin D supplementation or UV-B exposure. Increased vitamin D intake by natural or vitamin D-fortified food is usually not sufficient to correct vitamin D deficiency.[1]

Vitamin D supplementation is relatively easy, cheap and safe. Supplementation can be done with vitamin D3, which is almost exclusively used in Europe, or with vitamin D2. Several studies addressed the question whether vitamin D3 is superior to vitamin D2 in raising and maintaining 25(OH)D serum concentrations but study results are inconsistent so that it is still not clear whether vitamin D3 should be preferred over vitamin D2. [91,92] According to a rule of thumb, a daily intake of 1,000 IU of vitamin D increases 25(OH)D concentration by approximately 25 nmol/L (15 to 25 nmol/L).[91] Daily, weekly, or monthly dosing regimens can be applied because they produce comparable increases of 25(OH)D levels. Some authors recommend loading doses at the beginning of vitamin D supplementation (i.e. 50,000 IU of vitamin D weekly for 8 weeks).[1] High-dose vitamin D supplementation only once or just a few times per year, however, can currently not be recommended because no sufficient long-term outcome data exist on doses of >100,000 IU. Such unphysiologic high doses may even be harmful.[93] Therefore, we should be cautious with high dose vitamin D supplementation also when considering that some study results suggest a J-shaped association of 25(OH)D and mortality.[70] Individual variations in response to vitamin D supplementation should be considered, and in particular, obese individuals require higher doses as a result of vitamin D depletion in the adipose tissue.[4] In patients with

vitamin D insufficiency or deficiency it has been shown that the lower the baseline 25(OH)D levels, the greater is the increase of 25(OH)D after vitamin D supplementation.[94] Re-testing of 25(OH)D concentrations may therefore be reasonable, also in an effort to improve compliance, but this should be done only 3 months after initiation of vitamin D supplementation which is the time necessary to reach a plateau.[4] No adverse effects have been observed with daily vitamin D intakes of up to 10,000 IU.[95] Potential side effects of vitamin D (i.e. vitamin D intoxication) are seen at 25(OH)D serum levels > 375 nmol/L to 500 nmol/L and are related to symptoms of hypercalcemia with the risk of calcifications (e.g. of the vessels or the kidney) and hypercalciuria with dehydration and renal failure.[95] No convincing evidence exists that vitamin D supplementation alone increases the risk of kidney stones, but the combined supplementation of calcium plus vitamin D may be associated with significantly increased incidence of kidney stones.[95]

Increased sunlight exposure of the skin can also significantly increase 25(OH)D serum concentrations. Sunlight induced vitamin D synthesis in the skin can be equivalent to a daily vitamin D supplementation of up to 10,000 to 20,000 IU.[1] However, the skin´s capacity to produce vitamin D decreases with age, sunscreen use and increasing skin pigmentation. Hence, significant interindividual differences in cutaneous vitamin D synthesis exist, but it seems that ~ 15 to 30 minutes of sunlight exposure of the skin (legs and arms) between 10 am and 3 pm for at least three times per week is usually enough for a sufficient vitamin D synthesis.[1,12,96] Interestingly, excessive sunbathing is not associated with very high 25(OH)D concentrations because excess previtamin D3 and vitamin D3 are photodegraded.[12] This finding explains why there are no known cases of sunlight induced vitamin D intoxication. It should also be considered that at northern and southern latitudes above and below ~33° sunlight exposure during wintertime is not sufficient for vitamin D synthesis in the skin. However, excessive sunlight exposure increases the risk of non-melanoma skin cancer. By contrast, accumulating

evidence suggests that vitamin D may be beneficial for various other types of cancers.[1,2,96] Dermatologists have therefore started to change their "no sun policy" towards recommendations for moderate sun exposure, which may even protect against melanomas.[96]

VITAMIN D TREATMENT RECOMMENDATIONS

None of the major Cardiology Societies has recently proposed specific recommendations regarding vitamin D. We therefore briefly discuss recommendations from various other societies or expert panels that may be interesting for those who consider vitamin D supplementation in patients with CVD or at high risk of CVD.

Based on positive vitamin D effects on "bone health" the 2011 report from the IOM recommends a 25(OH)D level of at least 50 nmol/L.[19] The report asserts that the daily vitamin D intake should be 600 IU for individuals up to the age of 70 years and 800 IU for older adults of the general population corresponding to the Recommended Daily Allowance (RDA, covering requirements of $\geq 97.5\%$ of the population).[19] These RDAs of the IOM report are based on an assumption of minimal or no sun exposure. Given that in general populations the average daily vitamin D intake by diet (nutrition) is usually not higher than ~200 IU, this would mean that vitamin D supplementation may be indicated in most individuals of the general population. Tolerable Upper Intake Levels (UL) were set at 4,000 IU per day in the 2011 IOM statement.[19] Moreover, the 2009 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines include a recommendation to test for and treat vitamin D deficiency and insufficiency (25[OH]D levels) in CKD patients stages 3-5D (glomerular filtration rate [GFR] below 60 ml/min/1.73m²).[97] This recommendation is based on a low evidence level but impaired vitamin D metabolism may have deleterious consequences on renal function, proteinuria, and outcome in CKD patients. [34,82,85,98] Further expert panels have recommended supplementing 800 IU of vitamin D in all individuals older than 65 years, and one expert panel recommended testing for and treating vitamin D

deficiency in all patients with or at high risk of CVD.[4,99] We also draw attention to the fact that vitamin D supplementation is generally recommended in the first year of life (usually 400 IU per day) and that vitamin D supplementation of at least 800 IU per day is a standard treatment for osteoporosis patients.[4,99]

CONCLUSIONS

Vitamin D deficiency is common and the cardiovascular system is a target tissue for vitamin D. Experimental studies showed beneficial vitamin D effects on cardiovascular risk factors, the heart, and the blood vessels. Clinical studies have largely, but not consistently, indicated that CVD and mortality are associated with vitamin D deficiency. Data from RCTs, however, are sparse, and some, but not all, studies showed beneficial effects of vitamin D supplementation on cardiovascular risk factors (e.g. arterial hypertension). Available data are insufficient to draw final conclusions on the effect of vitamin D supplementation on CVD events, but results from meta-analyses suggest that vitamin D treatment may modestly reduce all-cause mortality. The above-mentioned promising data on cardiovascular-protective properties of vitamin D have stimulated the initiation of large-scale RCTs to evaluate the effect of vitamin D on cardiovascular outcomes. These studies will, however, not be finished within the next few years, leaving us with the question of how to handle vitamin D testing and treating in the clinical routine. On the basis of current evidence, we cannot justify proposing general recommendations for vitamin D supplementation for the treatment and prevention of CVD. However, we also cannot ignore the available knowledge on vitamin D. Consequently, we are of the opinion that overall risks and cost of vitamin D supplementation should be weighed against potential adverse consequences of untreated vitamin D deficiency. In this context, the proposed multiple health benefits of vitamin D and the relatively easy, cheap, and safe manner of its supplementation should be considered. [1,2,4,89,95,99] Currently available data suggest that 25[OH]D levels of ~75 to

100 nmol/L are associated with the best health outcomes and we therefore believe that aiming for this "optimal range" of vitamin D status is reasonable. However, we should be aware that these data on the optimal vitamin D status are based on relatively low evidence levels, are controversially discussed, and need to be further evaluated in large-scale RCTs.

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Table 1. Population-based studies on the association of 25(OH)D with cardiovascular (CV) events and mortality

First author (ref.)	Country	Age, yrs	Males (%)	No. of subjects	Follow-up, yrs	Event type	No. of events	Main analysis (25[OH]D in nmol/L)	Adjusted relative risk (95% CI)
Wang[67]	USA	59	45	1,739	5.4	CV events	120	<37.5 vs. ≥37.5	1.66 (1.13-2.43)
Giovannucci[69]	USA	64	100	1,345	10	Myocardial infarction	454	≤37.5 vs. ≥37.5	2.09 (1.24-3.54)
Melamed[70]*	USA	45	47	13,331	8.7	CV mortality	777	<44.4 vs >80.1	1.20 (0.87-1.64)
Semba[76]	Italy	74	75	1,006	6.5	CV mortality	107	<26.2 vs. >63.9	2.64 (1.68-2.19)
Pilz[68]	Netherlands	70	49	614	6.2	CV mortality	20	first vs. highest three quartiles	5.33 (1.97-14.45)
Kilkkinen[71]	Finland	49	45	6,219	27.1	CV mortality	933	≥62 vs. ≤28	0.76 (0.61-0.95)
Bolland[74]	New Zealand	74	0	1,471	4	CV events	110	<50 vs ≥50	1.2 (0.8-1.8)
Hutchinson[77]†	Norway	59	38 41	4,751 2,410	11.8 11.4	CV mortality	325 188	first vs. fourth quartile	1.08 (0.79-1.48) 0.93 (0.61-1.44)
Fiscella[78]*	USA	44	48	15,363	9	CV mortality	933	>79.9 vs. <44.9	0.79 (0.62-1.01)
Cawthon[72]	USA	74	100	1,490	7.3	CV mortality	110	<50 vs ≥75	1.51 (0.83-2.80)
Jassal[75]	USA	74	38	1,073	6.4	CV mortality	111	per SD=35	1.07 (0.86-1.33)
Michaëlsson[73]‡	Sweden	71	100	1,194	12.7	CV mortality	177	<46 vs. 46-93	1.53 (0.97-2.41)
Virtanen[79]	Finland	62	49	1,136	9.1	CV mortality	35	≤34.0 vs. ≥34.1	2.70 (1.31-5.56)
Messenger[80]‡	USA	76	100	813	4.4	CV events	140	≤50.2 vs. ≥75.1	1.18 (0.69-2.03)

*Both reports on the same study: Third National Health and Nutrition Examination Survey (NHANES-III)

†Separate reports for non-smokers (upper line) and smokers (lower line)

‡Both reports on the same study: Osteoporotic Fractures in Men (MrOS) study

FIGURE LEGENDS

Figure 1: Proposed vitamin D status classification

Figure 2: Associations of vitamin D deficiency with cardiovascular risk factors

Figure 3: Proposed vitamin D effects on heart and blood vessels

Figure 1

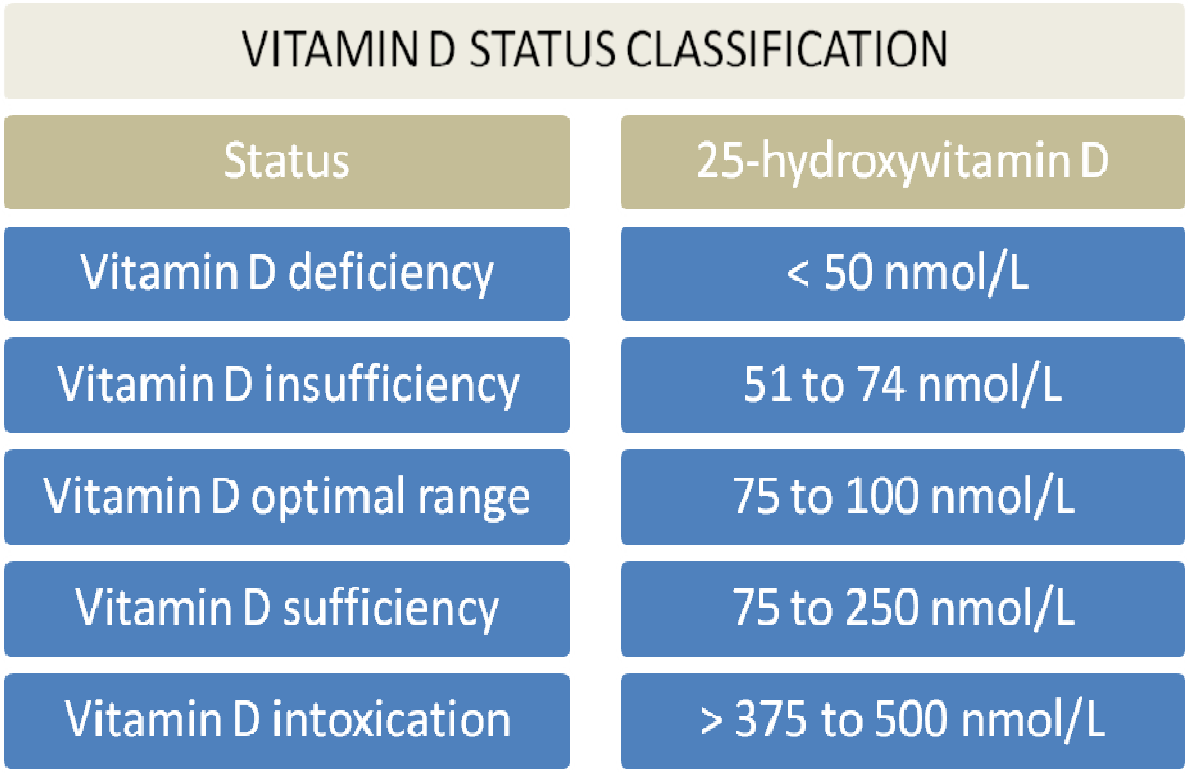


Figure 2

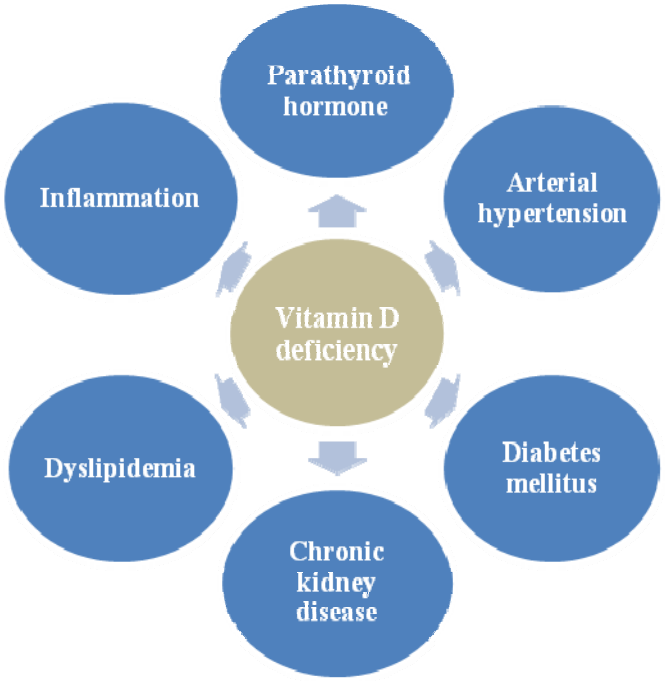
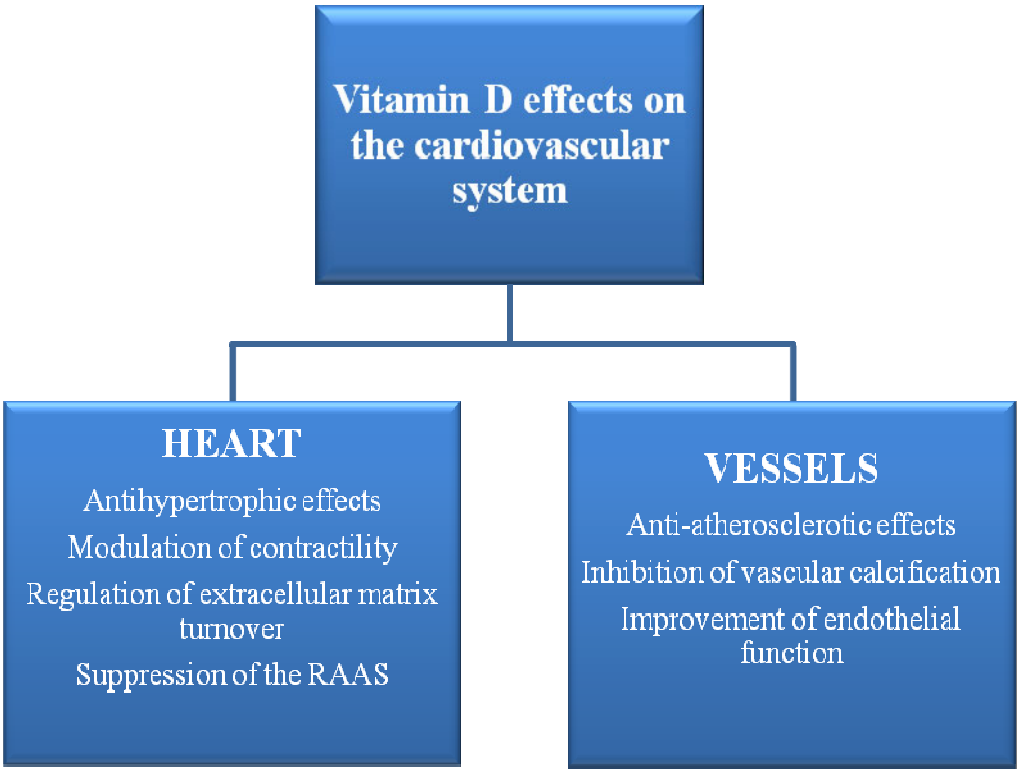

Parathyroid hormone
Arterial hypertension
Diabetes mellitus
Chronic kidney disease
Dyslipidemia
Inflammation
Vitamin D deficiency

Figure 3


Vitamin D effects on the cardiovascular system
HEART
Antihypertrophic effects
Modulation of contractility
Regulation of extracellular matrix turnover
Suppression of the RAAS
VESSELS
Anti-atherosclerotic effects
Inhibition of vascular calcification
Improvement of endothelial function