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REVIEW

Vitamin D and cardiovascular disorders

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Abstract

Vitamin D is necessary for bone health but may also have many extra-skeletal effects. The vitamin D endocrine system has major effects on gene and protein expression in many cells and tissues related to the cardiovascular system. In addition, many preclinical studies in animals with vitamin D deficiency or genetically silenced expression of the vitamin D receptor or vitamin D metabolizing enzymes suggest that the absence of vitamin D action may result in cardiovascular events. This includes dysfunctions of endothelial cells, thereby accelerating the process of atherosclerosis, hypertension or abnormal coagulation, ultimately resulting in higher risks for all major cardiovascular or cerebrovascular events. A wealth of observational studies in different parts of the world have fairly consistently found a strong association between a poor vitamin D status and surrogate markers or hard cardiovascular events. A few Mendelian randomization studies did, however, not find a link between genetically lower serum 25OHD concentrations and cardiovascular events. Finally, many RCTs could not demonstrate a consistent effect on surrogate markers, and a limited number of RCTs did so far not find whatever effect on hard cardiovascular endpoints such as myocardial ischemia or infarction, stroke, or cardiovascular death. In conclusion, preclinical data generated a plausible hypothesis of a link between vitamin D status and extra-skeletal events, including cardiovascular endpoints. Whether the vitamin D endocrine system is redundant for the human vascular system or whether the RCTs have not been optimally designed to answer the research question is thus not yet settled.

Keywords 25-Hydroxyvitamin D · Cardiovascular health · Mendelian randomization studies · Myocardial infarction · Stroke · Vitamin D · Vitamin D receptor

Abbreviations

VDR	Vitamin D receptor
MI	Myocardial infarction
MR	Mendelian randomization
RCT	Randomized controlled trial
25OHD	25-Hydroxyvitamin D
$1,25(OH)_2D$	1a,25-Dihydroxyvitamin D
CYP2R1	Major enzyme responsible for the 25-hydroxylation of vitamin D
CYP27B1	Enzyme responsible for the 1α-hydroxylation of 25OHD
CYP24A1	Enzyme responsible for the degradation of 25OHD or $1,25(OH)_2D$

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Osteoporosis is a frequent disease of the elderly ultimately resulting in major fractures in about a third of all women and somewhat less in men. Cardiovascular events (cardiac ischemia and infarction, stroke, and peripheral arterial diseases) are also affecting a very large percentage of all older men and women. Vitamin D deficiency is also very frequent in old age. Therefore, it is no surprise that these three conditions affect the same elderly subjects. The question is whether this is just because of coincidence or whether one disease or condition is affecting the others. Osteoporosis and cardiovascular diseases may have frequent common pathogenic mechanisms such as oxidative stress [1], sex steroid deficiency, or chronic inflammation. Vitamin D deficiency is also frequently found in both diseases. Therefore, the question whether vitamin D deficiency may accelerate both diseases is important, as prevention of vitamin D deficiency is feasible at low risk and low cost. In this review, I will briefly discuss the association between osteoporosis and cardiovascular diseases and mainly focus on whether vitamin D deficiency (or excess) may accelerate or aggravate cardiovascular diseases.

Several cross-sectional, retrospective, and prospective studies have found an association between osteoporosis and cardiovascular diseases [2]. Indeed, carotid wall thickness [3, 4] or coronary artery calcium burden [5] were higher in patients with osteoporosis compared with controls. Similarly, the risk of coronary artery stenosis was higher in patients with osteoporosis than in controls [6]. Inversely, the presence of osteoporosis significantly increased the risk of coronary artery disease in Caucasians [7] as well as in Koreans [8]. Two independent studies found an inverse correlation between BMD and either ischemic heart disease [9] or silent brain infarction [10]. Males with a previous history of myocardial infarction also had a lower BMD compared to healthy subjects [11]. Similarly, the prevalence of cardiovascular diseases was much higher in women with osteoporosis than in subjects with a normal BMD [12]. The strongest arguments for a link between osteoporosis and cardiovascular diseases come from prospective studies. A selection of relevant studies is shown in Table 1. The overall conclusion is that low BMD, osteopenia, or osteoporosis is associated with higher future risks of cardiovascular events (stroke, myocardial infarction) or that the presence of peripheral arterial disease or aortic calcifications increases future loss of BMD or higher risk of vertebral fractures. A systematic review and meta-analysis of 25 observational and comparative studies of vascular abnormalities and decreased BMD dealing with 10,299 subjects concluded that the OR of atherosclerotic vascular abnormalities was significantly 2.23-fold higher in patients with a low BMD and that the risk was higher if BMD was lower [24].

A poor vitamin D status is associated with increased bone turnover, higher bone loss, and ultimately higher risk of osteoporosis and fractures [25]. Vitamin D deficiency is also associated with many extra-skeletal diseases including vascular risks and events [26–28]. In view of the above-described association between bone loss and cardiovascular diseases, it is logical to evaluate the possible role of vitamin D deficiency as one of the pathogenic mechanisms explaining the joint risk of both diseases in older age.

Preclinical data linking the vitamin D endocrine system with cardiovascular system

There are several excellent reviews dealing with various aspects of the action of the vitamin D endocrine system on the

Table 1 Association of bone mass and cardiovascular diseases

Study	Country	Subjects/*n*	Follow-up (years)	Original disease	Later events
Zhou [13]	China	PMW/1724	5 years	± Aortic calcifications	Vertebral fractures: 12.2 vs 4.5% *
Szulc [14]	USA	Men > 50 years/744	7.5 years	Lowest quartile BMD: RR CV events × 2	Or highest quartile bone resorption
Wiklund [15]	Sweden	M + W/6872	5.7 years	Low hip BMD OR myocardial infarction: M 1.74 * F 1.33 *	
Browner [16]	USA	W < 65 years/9704	2.8 years	Low radius BMD	Fatal stroke RR 1.74 *
Zhou [17]	China	PMW/5136	5 years	Femoral neck BMD	Stroke OR 2.24 *
Collins [18]	USA	M > 65 years/5781	4.6 years	Peripheral arterial disease**	Yearly loss of hip BMD × 2 and RR of non-vertebral fractures 1.47*
Von Muhlen [19]	USA	Healthy adults/1332	4 years	Peripheral arterial disease**	Greater bone loss but no difference in fracture incidence
Kiel [20]	USA	W364 + M190 Framingham adults	25 years	Greater loss of metacarpal bone associated with greater increase in aortic calcification	
Hak [21]	NL (R'dam)	PMW/236	9 years	Greater metacarpal bone loss associated with greater increase in atherosclerotic calcifications (*)	
Schulz [22]	USA	PMW/2348	2.1 years	Greatest gain in aortic calcifications associated with 4× greater yearly bone loss	
Barzilay [23]	USA	3.385	12.1 years		Carotid artery intimal medial thickness (cIMT) was associated with increased risk of hip fracture [(HR 1.18 [1.04, 1.35]), even though cIMT was positively associated with BMD

*Significant difference

**Measured by low ankle-branchial index (< 0.9)

cardiovascular system, and the implications thereof for cardiovascular risk factors and events [26, 29–32].

Vitamin D and endothelial cells

The vitamin D receptor and the enzymes necessary for the activation of 25OHD into $1,25(OH)_2D$ or their inactivation into 24R-hydroxylated metabolites are expressed in several cells of the vascular wall such as the endothelial cells and vascular smooth muscle cells [33]. In addition, these cells respond to the presence of $1,25(OH)_2D$ by regulation of a number of crucial genes with a favorable overall effect (Table 2). The mechanisms involved include the local production of a potent vasodilator, nitric oxide, or NO, by stimulation of the endothelial inducible NO synthetase [34]. At the same time, $1,25(OH)_2D$ decreases the production of vasoconstrictors by inhibition of COX-1 [35]. $1,25(OH)_2D$ also decreases the production of superoxides in the vascular wall by inhibition of p22(phox) and NADPH oxidase subunit, thereby decreasing the production of H_2O_2 [36]. Sirtuin-1 is also stimulated by $1,25(OH)_2D$, or at least $1,25(OH)_2D$ can block the down-regulation of Sir-1 by H_2O_2. This strengthens its inhibitory effects on oxidative stress and atherogenesis (Table 2). Finally, $1,25(OH)_2D$ inhibits apoptosis of endothelial cells in vitro, by inhibition of oxidative stress, inhibition of release of cytochrome C from mitochondria, inhibition of caspase activity, and apoptosis/autophagy-related genes [37] (Table 2).

All these in vitro effects are difficult to reproduce in vivo in animals or humans, but one study demonstrated that $1,25(OH)_2D$ can promote the re-endothelialization of the carotic artery after experimental injury in diabetic mice [35]. Selective deletion of Vdr in endothelial cells increased the sensitivity of the vascular wall to angiotensin-2, thereby increasing systemic blood pressure [40].

Vitamin D and cardiac muscle/heart

Cardiomyocytes are responsive to $1,25(OH)_2D$ as shown in vitro by increased calcium uptake and increased contractility and improved relaxation [47, 48] (Table 3). Older ex vivo studies in hearts from vitamin D-deficient chicks showed more rapid myocardial dysfunction and decreased ATP storage compared with hearts from vitamin D replete chickens [54] (Table 3). Similarly, hearts from vitamin D-deficient rats showed cardiomegaly but not due to larger cardiac muscle but due to expansion of the extracellular fluid and collagen content [55]. This is in line with reduced systolic function found in mice with systemic Cyp27b1 deletion [53] (Table 3).

$1,25(OH)_2D$ also suppressed Rcan1 expression in neonatal cardiomycytes. This gene codes for a calcineurin inhibitory protein, calcipressin-1. This action on the calcineurin/NFAT/Rcan1 pathway may explain its antihypertrophic effects [56]. Indeed, Vdr deletion in cardiomyocytes causes myocardial hypertrophy and fibrosis [56]. These hearts also show a prolonged fetal gene program with increased atrial natriuretic peptide and actin expression, very similar to the prolonged immature gene profile found in skeletal muscle of global Vdr null mice [57].

Vitamin D and systemic effects related to the cardiovascular system

Vdr or Cyp27b1 mice develop systemic hypertension mainly mediated by activation of the renin-angiotensin (RAS) system [52, 58, 59]. These mice have higher (renal) renin expression compared to wild-type mice with therefore higher serum concentrations of angiotensin II and aldosterone. These effects can be reversed in cyp27b1 null mice by administration of $1,25(OH)_2D$. In Vdr null mice, an angiotensin blocker can correct the systemic hypertension [52]. This phenotype cannot be corrected by a rescue diet normalizing serum calcium homeostasis, indicating that it is the direct consequence of the failure of the vitamin D endocrine system. In vitro studies have clearly shown that the renin gene is under the negative control of VDR by blocking the activity of the cAMP responsive element in the renin promotor [60]. Vitamin D-deficient rats also develop hypertension, independent of hypocalcemia and correctable by $1,25(OH)_2D$ or a low calcemic vitamin D analog [61].

Vdr null mice display increased (stimulated) thrombogenicity and impaired fibrinolysis [62]. The in vitro data on thrombogenicity were largely confirmed in normocalcemic vdr null mice, raised on a rescue diet [62]. These mice indeed showed increased platelet aggregation, reduced NO synthetase expression, and decreased liver expression of antithrombin and decreased expression of thrombomodulin in several tissues. After lipopolysaccharide injection, the vdr null mice showed exacerbated multi-organ thrombus formation [62].

The vitamin D endocrine system also has important anti-inflammatory and immune effects with indirect repercussions on the cardiovascular system. In vitro studies of dendritic cells, macrophages, or immune cells in general have shown that $1,25(OH)_2D$ decreases the expression of pro-inflammatory genes (e.g., IL1, IL6, IL23, TNFα, and IFNγ), while upregulating anti-inflammatory genes (e.g., IL4 and IL10) [63–65]. In addition, $1,25(OH)_2D$ is a potent inhibitor of Th1–17 cells and modifies the phenotype of dendritic and related cells into more tolerogenic pathways, including an increase in T-reg cells [65], thereby probably decreasing the risk of atherosclerosis [31].

The effects of the vitamin D system on serum lipids are more complex. Rats with transgenic generalized overexpression of Cyp24a1 develop a remarkable phenotype of high serum lipids (all lipids) and accelerated atherosclerotic

Table 2 Molecular and cellular effects of $1,25(OH)_2D$ on cells of the vascular wall

Cells or tissue/ref	Genes	Effect
Endothelial cells		
Andrukhova [34]	Endothelial NO synthetase (+) (eNOS)	NO production and vasodilatation
Wong [35]	Cyclooxygenase 1 (−) or COX-1	decrease in vasoconstrictors
Hirata [36]	p22(Phox)(−) and NADPH oxidase subunit (−)	Decreased H_2O_2 production (superoxide)
	Sirtuin-1 (SirT-1) (+) blocks downregulation of SirT-1 by H_2O_2	Inhibition of oxidative stress and atherogenesis
	Inhibition of superoxide production	Maintenance of mitochondrial function
Uberti [37]	Inhibition of cytochrome C release Inhibition of caspase activity Inhibition of apoptosis-related genes Activation of pro-autophagy pathways (mediated by phosphorylation of ERK1/2 and Akt)	Inhibition of apoptosis Stimulation of autophagy
Wong [35] Wong [38]	Activation of Hypoxia-inducible factor	Promote re-endothelialization of Carotic artery after injury in diabetic mice
Norman [31]	Endothelin receptor type B (EDNRB) (+) Oxytocin receptor (OXTR) (−)	Vascular relaxation
Norman [31]	NFkB (−) IL 6 (−) and other inflammatory cytokines Tissue Factor (F3) (−) Thrombomodulin (+)	Anti-inflammatory action Reduced thrombogenicity
Macrophages/foam cells		
Oh [39] Yin [40]		Inhibition of foam cell formation Increased cholesterol efflux Reduced uptake of oxidized LDL
Vascular smooth muscle cells		
Norman [31] Torremade [41]	Expression of osteoblast like genes	Transdifferention into osteoblast-like cells and vascular calcification
Wu-Wong [42]	Thrombogenic genes (−) Fibrinolytic genes (+) Vasodilatory genes (+)	Anti-atherosclerotic actions
Wakasugi [43]	Prostacyclin (+)	Vasodilatation
Bukoski [44]	Oxytocin receptor (−) Type B endothelin receptor (+)	NO release and vasodilatation and less hypertension
Wu-Wong [45] Cardus [46]	VEGF (+), PAI1 (+) Thrombospondin1 (+) Thrombomodulin (+)	Improved endothelial repair Decreased thrombogenicity

lesions in the aorta. This phenotype can be greatly enhanced by a high-fat high-cholesterol diet. These rats also have marked albuminuria [66]. The pathogenic mechanism is unknown, but serum $1,25(OH)_2D$ was not altered and despite the transgenic expression of Cyp24a1, serum $24,25(OH)_2D$ concentration was lower than in controls. Other studies linking the vitamin D system with serum lipids and adipocyte development generated variable results. Mice with deletion of vdr or cyp27b1 are lean and resistant to diet-induced obesity due to enhanced energy expenditure, oxygen consumption, and higher than normal uncoupling protein expression [67]. In humans, however, a low vitamin D status is fairly consistently associated with obesity and this species difference remains unexplained. In vitro effects of $1,25(OH)_2D$ on fat cell metabolism and development also generated inconsistent results [67].

In a cross-sectional study of healthy adults, a strong positive correlation was found between serum 25OHD and HDL or apolipoprotein A-1 ($p < 0.001$), whereby variations in serum 25OHD may explain up to 10% of the variation of these lipid concentrations. Similarly, a 6-week treatment with intravenous $1,25(OH)_2D$ of patients with chronic renal failure resulted in a significant increase in serum apoprotein A1 [68]. This is in strong contrast with a study reporting a negative regulation of the apolipoprotein A-1 by $1,25(OH)_2D$ in a human hepatoma cell line [69].

From this overview, it is clear that the vitamin D endocrine system has an effect on many genes and proteins, cells and tissues involved in cardiovascular physiology. Overall, the VDR action seems to be beneficial for optimal cardiovascular function (Fig. 1). $1,25(OH)_2D$ stimulates in vascular smooth muscle cells the production of matrix gla

Table 3 Molecular and cellular effects of $1,25(OH)_2D$ on cardiac muscle and heart

In vitro effects on cardiomyocytes		
Walters [47]; Green [48]	Calcium uptake (+)	Enhanced contractility Improved relaxation
Rahman [49]	Tissue inhibitor or matrix metalloproteiase-1 and 2 (−) Matrix metalloproteiase-2 and 9	Decreased cardiac hypertrophy
O'Connell [50]	Myosin expression (+) Sarcomere function (+)	Cardiac contractility (+) and improved coronary perfusion
Li [51]	Atrial natriuretic peptide (−) Type 1 natriuretic peptide receptor (+)	Prevention of cardiac hypertrophy
In vivo effects on heart		
VDR null mice (systemic); Li [52]	Renin-mediated activation of RAS	Systemic hypertension and cardiac hypertrophy
Cardiomyocyte specific vdr deletion; Gardner [53]		Cardiac hypertrophy and fibrosis
Vitamin D-deficient chicks; Hochhauser [54]	Ex vivo studies	Enhanced rate of decline of ATP More rapid decline in myocardial contractility
Vitamin D-deficient rats; Weishaar [55]	Cardiomegaly due to extracellular fluid and collagen accumulation and decreased myofibrillar area	

protein, a potent inhibitor of extraskeletal calcification, and thus could potentially have a favorable effect [70]. In vivo, however, excess vitamin D (whether too high $1,25(OH)_2D$ or large excess of 25OHD) may stimulate the transdifferentation of smooth muscle cells into osteoblast like cells, and accelerate vascular calcification and atheromatosis [71]. This is in line with the observation that calcitriol treatment significantly increased aortic expression of the calcification genes Runx2 and Pit-1 [72]. High-dose vitamin D has been used frequently to induce severe aortic calcifications in rats and to test preventive interventions [73].

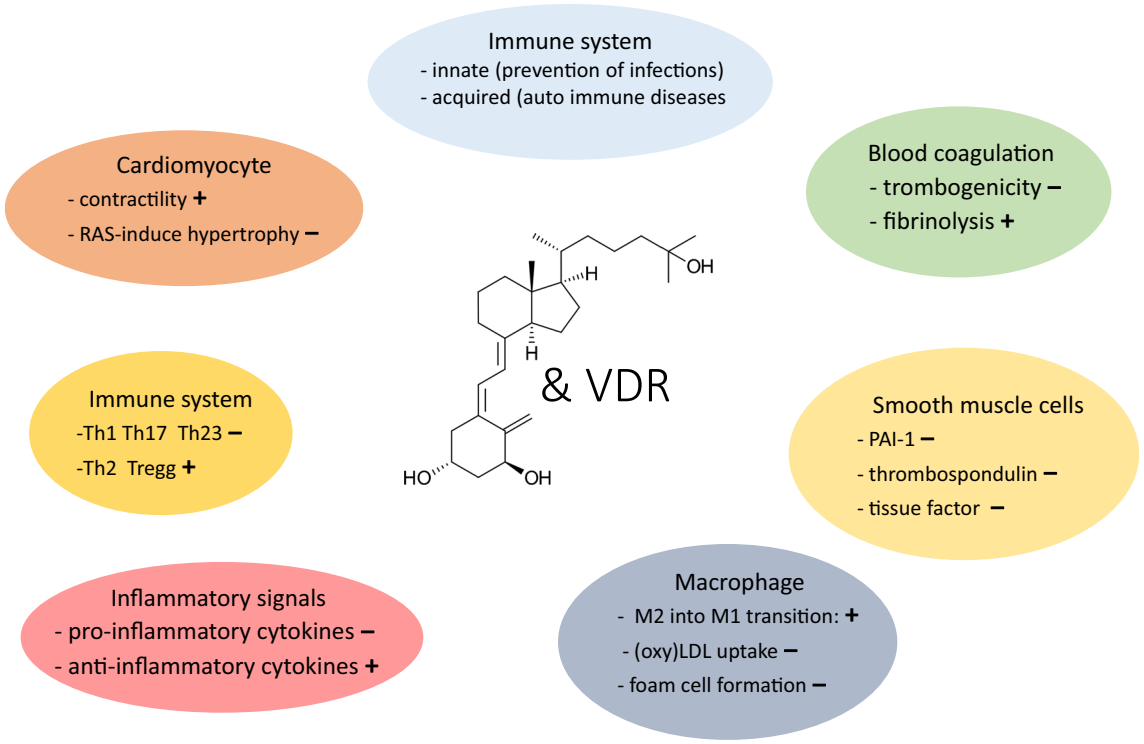

Fig. 1 The vitamin D endocrine system and potential targets in the cardiovascular system

The majority of the symptoms due to vitamin D toxicity are related to hypercalcemia (neurological and gastrointestinal symptoms) and acute impairment of kidney function, but many children nevertheless also show signs of extraskeletal vascular calcifications [74].

Clinical data linking the vitamin D endocrine system with cardiovascular risks and events

Observational studies

A large number of observational studies found a fairly consistent association between a low vitamin D status and cardiovascular risks, hypertension, and cardiovascular events, including ischemic cardiac events, cardiomyopathy, congestive heart failure, stroke, and cardiovascular mortality [29, 30, 75–78]. Therefore, I will only discuss the main messages here without detailed overview of all individual studies. The major and best known studies are summarized in Table 4, and the major meta-analyses dealing with CV endpoints or CV mortality are summarized in Table 5.

In the Framingham cohort of 1739 subjects followed for 5.4 years, serum 25OHD concentrations below 15 ng/ml were associated with a 62% higher risk for cardiovascular diseases compared to subjects with better vitamin D status (even after correction for identifiable other risk factors) [79]. Very similar results were generated in the Copenhagen City Heart study as subjects with the lowest vitamin D status (1–4th percentile versus those in percentile 50–100) had a 64% higher risk of myocardial infarction or fatal CV event during a 29-years follow-up [82]. Similar results on the risk of myocardial infarction were found in the Health Professional Follow up Study of more than 18,000 subjects: Those with serum 25OHD below 15 ng/ml (measured or calculated) versus > 30 ng/ml had a 2.09-fold higher risk [81]. In the NHANES III study, subjects with serum 25OHD below 12 ng/ml, followed for 18 years, had a 36% higher risk of CV mortality compared with subjects with serum 25OHD between 20 and 30 ng/ml [83]. Also incident hypertension is higher in men as well as in women with baseline normotension and serum 25OHD below 15 ng/ml compared with the ones with serum 25OHD above 30 ng/ml. The overall RR of hypertension during a 4–18 years follow-up period associated with low serum 25OHD was 3.18 and highly significant [80] (Table 4). In a multi-ethnic study, a poor vitamin D status was associated with higher risks of all types of CV diseases in Whites and Chinese but not in Blacks and Hispanics [85]. Using the large Intermountain Healthcare system of a general healthcare population, Anderson et al. [78] found a strong association between serum 25OHD and cardiovascular risks and events (Table 4). This was highly significant whether vitamin D deficiency was defined as serum 25OHD below 30 ng/ml or as serum 25OHD between 16 and 30 ng/ml. Based on NHANES III data on more than 15,000 US adults followed for 9 years, the subjects with the lowest quartile of serum 25OHD (< 14 ng/ml) displayed a higher risk of cardiovascular death in comparison with all others subjects (Black or whites) [88]. Kerstenbaum looked at a lower number of subjects without cardiovascular disease at baseline; over a follow-up of 14 years, those with lower 25OHD concentrations (< 10 ng/ml) had a higher risk for cardiovascular death and myocardial infarction [89] (Table 4).

Numerous other studies, mostly cited in the meta-analyses (Table 5), also reported similar results. Of course, a few studies also generated null results [96, 97]. The main recent meta-analyses on the association of vitamin D status and cardiovascular events are summarized in Table 5. Most analyses dealt with a very large number of subjects as described in the original studies. None of the meta-analyses reported an analysis of individual patient data (only mean study data). Overall, the data are simple to summarize: Subjects with the lowest vitamin D status (whether expressed as percentiles or quartiles or on a more linear basis) have the highest risk for whatever cardiovascular endpoint—either overall cardiovascular disease, myocardial infarction, ischemic heart diseases, ischemic stroke, hypertension, or cardiovascular mortality. The effect size is relatively large as RR in subjects with the lowest vitamin D status is frequently 40% higher than in the ones with a higher serum 25OHD concentration (Table 5). The highest risks are found in subjects with serum 25OHD below 10 ng/ml, and there is frequently a linear trend with higher serum 25OHD concentration. Several studies, however, also suggested that serum 25OHD concentrations above ~ 25 ng/ml do not further improve the outcome (e.g., as described in [77, 94]). Such observational data, however, are not suitable to define an absolute threshold above which no further risk reductions can be expected. In addition except for one study [90], all studies used serum 25OHD assays that were not standardized according to NIST or NIST equivalent absolute concentrations.

Conclusion Most of the very large number of observational studies (whether cross-sectional or long-term prospective) conclude that subjects with a low vitamin D status have a higher risk of cardiovascular events. The risk increases with lower 25OHD status, but there seems to be a plateau of about 25 ng/ml above which the risks do not change significantly. As cardiovascular events are frequent and the relative risk potentially attributable to low 25OHD is high, potential gain from vitamin D supplementation, if proven, might be very substantial.

Mendelian randomization studies

Observational studies are usually considered as hypothesis generating, whereas randomized controlled trials should

Table 4 Selection of major prospective observational studies on vitamin D and cardiovascular events

Study	No. of subjects/duration of follow-up/study	25OHD status	Outcome
Wang [79]	1.739/5.4 years/Framingham study	< 15 ng/ml versus	All CVD + 62%*
Forman [80]		Measured or predicted 25OHD < 15 versus > 30 ng/ml	RR hypertension: -Men 6.13 * -Women 2.67* -Pooled 3.18*
Giovannucci [81]	18.225 men/10 years/Health professionals follow-up study	Serum 25OHD < 15 versus > 30 ng/ml (graded effect from low to high 25OHD)	RR MI 2.42*
Brondum-Jacobson [82]	10.170/29 years/Copenhagen city heart study	1–4% percentile versus 50–100 percentile	MI 64%* Fatal CV event + 81%*
Al-Khalidi [83]	7.958/17.9 years/NHANES III	25OHD < 12 ng/ml versus 20–30 ng/ml	CV mortality + 36%*
Melamed [84]	4.839/NM/NHANES III	Q1 vs Q4 25OHD (< 18 versus > 29 ng/ml)	Peripheral arterial disease RR 1.8*
Robinson [85]	6.436/8.5 years/multi-ethnic study of atherosclerosis	Per 16 ng/ml lower 25OHD	RR for CVD: -Overall 1.15* -Whites 1.26* -Chinese 1.67* -Blacks 0.93 (NS) -Hispanics 1.01 (NS)
Perna [86]	7.709/6.5 years/ESTHER study	25OHD < 12 versus > 20 ng/ml	HR CVD 1.24*
Kuhn [87]	2.132/7.6 years/EPIC study	25OHD < 10 versus > 20 ng/ml	HR 1.53*
Anderson [78]	41.504/1.3 years /	25OHD < vs > 30 ng/ml	Vitamin D deficiency associated ($p < 0.001$) with -Hypertension -Hyperlipidemia -Peripheral vascular disease -Myocardial infarction -Coronary artery disease -Heart failure -Stroke
Fiscella [88]	15.363 adults/9 years/NHANES III	25OHD lowest vs Q2–Q4	25OHD < 14 ng/ml associated with HR of CV death 1.4*
Kestenbaum [89]	2312/14 years/healthy adults	25OHD (continuous)	10 ng/ml lower 25OHD: HR of CV death + 9% HR of myocardial infarction + 25%
Gaksch [90]	26.916/10.5 years/from 8 prospective studies	25OHD 50–75 nmol/l vs > 75–100 nmol/l 40–50 nmol/l vs > 75–100 nmol/l 30–40 nmol/l vs > 75–100 nmol/l < 30 nmol/ vs 75–100 nmol/l	relative risk for CV mortality 1.37* 1.65* 1.61* 2.21* Significantly different even after correction for many confounding factors

* = statistically significant at $p < 0.05$

provide the definitive proof of causality and define strategies for prevention of diseases. However, long-term prospective studies may study a very large number of subjects (100,000) for a very long time (several decades), exceeding by far the potential scope of RCTs but still are potentially confounded by hard to correct confounding factors. Mendelian randomization studies can bring together the combination of very large study subjects and very long (~lifetime) follow-up studies while avoiding interfering other factors. Indeed, serum 25OHD concentrations are under strong hereditary control as concluded by twin studies and a large number of GWAS studies have so far identified about 7–8 genes that predispose subjects to higher or lower lifetime serum 25OHD concentrations [98]. Based on up to a limited number of these genes, related to vitamin D metabolism or transport, one can predict about 5% of the variation in serum 25OHD concentrations in

Table 5 Meta-analyses of vitamin D status (observational data) and cardiovascular events

Study	Number of subjects/study	25OHD status	Outcome
Brondum-Jacobson [82]	*n* = 82,982 18 studies	Q1 versus Q4 25OHD	RR ischemic heart disease + 38%* RR early death +46%*
Wang [76]	*n* = 65,994	Lowest versus highest serum 25OHD category	RR for: - All CVD 1.42* - CV mortality 1.38* - Ischemic heart disease 1.64* - Stroke 1.64*
Tomson [91]	*n* = 42,565 12 studies	Q4 25OHD versus Q1	CV mortality − 21% *
Autier [92]	*n* > 100,000 3–17 studies per endpoint	RR highest versus lowest quartile serum 25OHD	MI 0.65* stroke 0.66* hypertension 0.57* CVD ~ 0.60* CV mortality 0.70*
Theodoratou [93]	*n* > 100,000 3–17 studies per endpoint	RR highest vs lowest quartile of serum 25OHD	CV disease 0.66* CV mortality 0.55* Hypertension 0.70* Ischemic heart disease 0.72* (Ischemic) stroke 0.60*
Gaksch [90]	*n* = 26,916	RR CV mortality versus subjects with 25OHD > 30 ng/ml	< 12 ng/ml: RR 3.2* 12–20 ng/ml: RR 1.8* 20–30 ng/ml: RR 1.3*
Zhang [94]	*n* = 180,667 34 studies	Pooled RR per 10 ng/ml difference in serum 25OHD (no significant difference once serum 25OHD > 25 ng/ml)	CV events 0.9* CV mortality 0.88*
Zhou [95]	*n* > 100,000 subjects	RR ischemic stroke Lowest vs highest 25OHD	RR 1.60*

* = statistically significant at $p < 0.05$

Caucasians and Chinese populations [98]. Several large consortia have combined their data on gene polymorphisms involved in serum 25OHD concentrations and a variety of outcomes including cardiovascular events [28].

Two large MR studies looked at a link between genetically low serum 25OHD concentrations and cardiovascular diseases in general. A large Canadian study [99] dealing with more than 80,000 subjects and using 4 SNPs identified by the Sunlight consortium found no relationship. Similarly, in a very large European study dealing with more than 90,000 subjects and using SNPs in 2 genes could not reveal a link with CV diseases. This study is remarkable because measured serum 25OHD was linked with ischemic heart diseases: The HR for this outcome was significantly increased (1.82) in subjects with the lowest quartile of serum 25OHD when compared with the highest quartile [100]. A much smaller Canadian study using only SNPs in one gene also did not find a causal link but that may well be due to underpowering of the study [101].

One MR study revealed a positive result. Vimaleswaran [102] reported that a 10% genetically higher serum 25OHD is associated with 0.3 mmHg lower diastolic and systolic blood pressure and lower risk for hypertension. The study dealt with more than 140,000 Danish subjects and looked at only 2SNPs located in the DHCR7 and CYP2R1 genes, which code for key enzymes responsible for the metabolism of vitamin D. The study is important because of its large size in a homogenous population. The effect size is, however, small, and 10% difference in serum 25OHD is beyond the effect size of the SNPs; thus the study should be considered as supportive but not as a final proof of causality. An extension of this study as to include other SNPs with greater impact on serum 25OHD is desirable. If confirmed, it may have major implications for vitamin D supplementation policy at a population level.

One MR study describes a rather mysterious effect of fillagrin gene polymorphism on higher serum 25OHD concentrations and a better serum lipid profile [103]. They did not study known SNPs related to 25OHD status but found that fillagrin mutation or polymorphism resulted in 10% higher measured serum 25OHD (possibly related to higher UVB induced efficacy in vitamin D production) and better lipid profile (higher HDL, lower LDL and lower VLDL, and triglycerides). This effect of fillagrin on vitamin D synthesis or 25OHD status was not described before nor detected in several very large GWAS studies. Therefore, this study should be interpreted as a potentially novel pathway explaining

the large variability of serum 25OHD concentrations and its relationship with serum lipids.

Finally, one large MR study also addressed a novel potential mechanism linking vitamin D status with CV events. The authors studied more than 100,000 Danish subject in search for genes explaining the variation in non-fasting remnant cholesterol and LDL/HDL concentrations [104]. They found that the polymorphisms in genes predisposing to higher cholesterol remnants were associated with lower (measured) serum 25OHD. A doubling of the cholesterol remnant concentrations implied a 15% lower serum 25OHD concentration. The study also reported that measured serum remnant cholesterol concentration (rather than geneticaly predicted) was negatively associated with serum 25OHD. The same genes predisposing for higher remnant cholesterol concentrations were already known to be associated with higher cardiovascular events and low-grade chronic inflammation [104]. Inversely, the genes known to predispose to lower serum 25OHD had no significant effect on serum lipid concentrations. If confirmed in other studies, this may imply that some genes increase cholesterol remnants, a well-known risk factor for cardiovascular diseases, and for unknown reasons, also decrease serum 25OHD, thereby creating a (potentially non-causal) link between vitamin D status and CV diseases [104]. This may also imply that lower serum 25OHD is just a surrogate marker of higher remnant cholesterol concentrations and higher CV risks without being directly and causally linked with these diseases.

Randomized controlled trials

Several randomized controlled trials have evaluated the effects of vitamin D supplementation (with our without calcium) on surrogate cardiovascular endpoints such as vascular stiffness, hypertension (diastolic and systolic blood pressure), and hard endpoints such as myocardial infarction, stroke, coronary revascularisation, cardiovascular, or cerebrovascular death.

Surrogate end points

Endothelial dysfunction can be used as a surrogate marker for more complex cardiovascular endpoints. One meta-analysis looking at flow-mediated dilation as measured by post-occlusion hyperemia did not detect a positive effect of prior vitamin D supplementation [105]. A more recent meta-analysis looking at 16 publications dealing with 1177 participants also concluded that vitamin D supplementation did not significantly improve endothelial function (except for a minor benefit in diabetic patients) [106].

Motivated by the role of the vitamin D endocrine system on renin-angiotensin, many (more than 40) RCTs have looked at the effects of vitamin D supplementation on blood pressure using very different study designs. The interpretations of several meta-analyses are not uniform. An early meta-analysis of Witham et al. [107] concluded, based on 11 RCTs, that vitamin D supplementation of subjects with mild hypertension generated a modest but significant reduction in systolic blood pressure. No effect was seen in subject who were normotensive at baseline. Similarly, Wu et al. [108] concluded, based on the results of 4 RCTs involving 429 participants, that vitamin D supplementation generated a modest reduction in systolic (− 2.5 mmHg) but not in diastolic blood pressure. Pittas [109] concluded in a meta-analyses of 10 trials that supplementation nonsignificantly reduced systolic blood pressure (weighted mean difference [WMD] − 1.9; 95% CI − 4.2, 0.4 mmHg) and did not affect diastolic blood pressure (WMD − 0.1; 95% CI − 0.7, 0.5 mmHg). Beveridge [110] evaluated 46 trial in a trial-level meta-analysis and found no effect of vitamin D supplementation on systolic or diastolic blood pressure (effect size between 0 and 0.1 mmHg). The same group also evaluated 27 trials for which individual patient ($n = 3092$) data were available and came to the same conclusion: no effect on blood pressure overall nor in subgroups [110].

Hard end points

Table 6 summarizes the main RCTs evaluating directly the effects of vitamin D supplementation on hard endpoints. The large Women's Health Initiative (WHI) trial has been evaluated twice. First, including all subjects whether or not they were taking already calcium supplementation at baseline, no effect was found on the hazard ratio for myocardial infarction, death from coronary heart disease, or stroke [111]. A reanalysis of the data using only results from women randomized to either vitamin D plus calcium or double placebo excluding those that did already take such supplements at baseline revealed a significant increase of myocardial infarction, coronary revascularization, stroke, or all such cardiovascular events in women assigned to vitamin D and calcium supplements [112] (Table 6). The RECORD trial was designed to study effects on bone but also studied cardiovascular endpoints [115]. Supplementation with 800 IU of vitamin D_3 (twice the amount of the WHI study) plus calcium (1 g/day) significantly decreased the risk of heart failure (HR 0.75) but without effect on myocardial infarction or stroke (Table 6). Serum 25OHD was not measured at baseline, nor in the WHI nor in the RECORD trial, and therefore the possible effects of supplementation on subjects with the most severe deficiency could not be evaluated. The most recent VITA trial studied the effect of monthly doses of 100,000 IU of vitamin D_3 during 3.3 years in New Zealand adults (after a single loading dose of 200,000 IU). No effect was observed on the hazard ratio of all cardiovascular diseases (Table 6). Whether this negative effect is due to lack of effect of vitamin D in general or due to high intermittent dosing or due to the relatively good vitamin D status at baseline cannot be defined. All these studies have several or divergent limitations, such as including a

Table 6 Major RCTs of vitamin D supplementation and cardiovascular events

Ref	Study, number of subjects	Follow-up	Treatment	Outcome
Hsia [111]	original WHI trial *n* = 36.282	7 years	400 IU D3 + 1 g calcium	HR for -MI or coronary heart disease death: 1.04 -Stroke: 0.95
Bolland [112]	WHI reanalysis *n* = 16.718 women not taking calcium supplements at baseline	7 years	400 IU D3 + 1 g calcium	HR (all NS) for: -MI: 1.2 -coronary revascularization: 1.15 -Stroke: 1.17 -All CV events: 1.13
Ford [115]	Record trial *n* = 5.292	9 years	800 IU D3 + 1 g calcium	HR for -cardiac failure: 0.75* -MI: 0.97 -Stroke: 1.06
Scragg [110]	VIDA trial (New Zealand) *n* = 5.108	3.3 years	100,000 IU D3 per month (baseline mean 25OHD: 24 ng/ml)	HR for -all CV diseases: 1.02

*Statistically significant

limited number of subjects with poor vitamin D status at baseline, lack of measurement of serum 25OHD in some, or even all subjects, and many studies used methods for serum 25OHD that lack validation of accuracy.

Several authors have tried repeatedly to evaluate the effects of vitamin D (with or without calcium) supplementation on cardiovascular endpoint by analyzing all existing data by systematic meta-analysis (Table 7).

Pittas [109] concluded that 4 RCTs separately or combined could not demonstrate an effect on cardiovascular outcome. Bolland summarized the results of 3 RCTs [112] and, 3 years later, again of 40 RCTs [114]. From the first meta-analysis, they concluded that vitamin D and calcium negatively influenced major vascular events (myocardial infarction and stroke), but the larger meta-analysis did not show positive nor negative effects (Table 7). Four other meta-analyses [113, 115–117] (Table 7) selected different numbers of RCTs, but all concluded that vitamin D supplementation (with or without calcium) did not significantly influence several vascular events.

General discussion and conclusions

As for other extra-skeletal effects, preclinical data strongly suggest that the vitamin D endocrine system may have effects on cells and tissues belonging to the cardiovascular system (Fig. 1). These data include direct effects of $1,25(OH)_2D$ on several genes that regulate endothelial function or blood pressure and hemostasis. In line with these cellular in vitro data, animals with severe vitamin D deficiency or vitamin D resistance display systemic hypertension, cardiac hypertrophy, increased thrombogenicity, and decreased fibrolysis. An important remark, however, is that such data were generated by studying situations of (near) total absence of vitamin D action or in situations of exposure to high concentrations of $1,25(OH)_2D$ or its analogs.

A wealth of observational studies fairly consistently concluded that subjects or patients with poor vitamin D status have a higher risk of cardiovascular risks or events, spanning from endothelial dysfunction, hypertension, ischemic heart diseases, or its clinical consequences, stroke, or cardiovascular death (Table 4). These observational data remain highly significant when subjected to critical meta-analysis, and the effect is not only highly statistically significant but suggest a major effect size of very frequent causes of morbidity or mortality. Of course, all of these cardiovascular diseases have multifactorial origin or pathogenesis, and observational studies may not be able to eliminate or correct for possible confounding factors such as age, gender, physical activity, and exposure to sunlight, obesity, or metabolic syndrome or lipid profile.

The final proof of causality largely depends on the results of Mendelian Randomization or randomized controlled trials. Surprisingly, these MR studies could not detect an association of lifetime genetically lower serum 25OHD and whatever cardiovascular outcome. However, the number of these studies is still limited and the predicted differences in serum 25OHD are small (about 5% difference in serum 25OHD). Larger studies and especially finding additional genetic reasons for differences in serum 25OHD concentrations are needed to validate the conclusion. A fairly large number of intervention studies did not consistently reveal an effect on blood pressure, whether systolic or diastolic. Similarly, RCTs could not reveal an effect on other surrogate markers or even the major cardiovascular events (coronary ischemia, myocardial infarction, stroke, or cardiovascular or cerebrovascular mortality.

What is now the best interpretation of the existing data?

It may well be that the vitamin D endocrine system is just redundant for humans and that the associations are just a

Table 7 Overview of major meta-analyses of vitamin D supplementation and CV diseases

Ref	Studies, number of	Follow-up	Treatment	Outcome
Pittas [109]	4 RCTs $n = 2.646 + 192 + 36.282 + 302$	1–7 yrs	Vitamin D vs placebo	No significant effect on CV events
Wang [116]	2 RCTs $n = 2.686 + 302$	1–5 yrs	Vitamin D_2/D_3 vs placebo	RR all CV diseases 0.9
	2 RCTs $n = 192 + 36.282$	1–7 yrs	Vitamin D + calcium vs placebo	RR all CV diseases 1.04
Bolland [112]	3 RCTs $n = 20.090$	up to 10 yrs	Vitamin D + calcium vs placebo	HR for -MI 1.21* -Stroke 1.20 -MI or stroke 1.16*
Elamin [113]	51 RCTs $n = 31.155$	?	Vitamin D + calcium vs placebo	RR stroke: NS
Mao [92]	11 RCTs $n = 50.252$	?	Vitamin D or calcium vs placebo**	HR for -Major CV events 1.03 -Stroke 1.01
Bolland [114]	40 RCTs $n = 81.173$	??	Vitamin D + calcium vs placebo	RR CV events: NS Cerebrovascular events: NS
Ford [115]	21 RCTs $n = 13.033$	1–8 yrs	Vitamin D ± calcium vs placebo	HR (all NS): -Cardiac failure 0.82 -MI 0.96 -Stroke 1.07
Bjelakovic [93]	10 RCTs $n = 47.267$	0.3–6 yrs	Vitamin D ± calcium vs placebo	RR for CV mortality: 0.98

*Statistically significant

**In this Mao et al. meta-analysis, 5 out of the 11 RCTs used only calcium and no vitamin D. Calcium supplementation alone increased non-significantly the risk of cardiovascular events

reflection of "remaining confounding factors." This would imply that improvements in vitamin D status would be futile for the prevention of cardiovascular events. In view of the strong preclinical and observational data, such interpretation is rather unlikely.

Another option could be that the very severe vitamin D deficiency as used in preclinical studies that generated the hypothesis of plausible interactions between VDR-$1,25(OH)_2D$ and cardiovascular events is not reproduced in humans because such extreme vitamin D deficiency is so rare in humans. Similarly, the beneficial effects of high doses of $1,25(OH)_2D$ or its analogs in preclinical models may not be reproduced in human trials because such very high doses of the active hormone were not reached systemically nor locally. If this option is correct, improvement of the overall vitamin D status is unlikely to reduce the risks of cardiovascular events, unless only subjects with extremely low vitamin D status at baseline are studied or treated for a sufficiently long time.

Finally, it may well be that the duration of the supplementation with vitamin D in the published RCTs is too short to demonstrate an effect. Indeed, the origin of most cardiovascular events has a long trajectory of events such as intimal lesions, exposure to oxidative stress or high pressure, or to inflammatory signals before the clinical onset of symptoms or events. It may thus well be that decades of exposure to low vitamin D status is necessary to promote such long processes of cardiovascular damage and that only living with a better vitamin D status for many decades is effective to prevent its consequences for the cardiovascular system. Mendelian randomization studies should be able to compensate for this long time window of exposure to lower/better vitamin D status, but unfortunately, the present gene polymorphisms are not able to detect more than a 5% difference in serum 25OHD and this difference may well be too small to generate major or detectable differences in outcome.

There are many ongoing studies [118, 119] trying to evaluate the effects of vitamin D supplementation on extra-skeletal effects, including cardiovascular and cerebrovascular events. The design of these studies differs from study to study, as some RCTs include a very large number of subjects and will look at a wide variety of outcomes, whereas other RCTs are much smaller but focus on well-defined outcomes in selected subjects. It is not sure that the design of most ongoing studies can overcome the limitations described above with regard to the selection of the study population, dosage and dosage regimens, and duration of treatment that we think are needed to fully answer the questions and thereby guide clinical treatment options.

Compliance with ethical standards

Conflict of interest The author declares to have received lecture fees (last 2 years) from Abiogen, FAES, l'Oreal and Frisenius. He is also co-owner of a university patent on vitamin D analogs licensed to Hybrigenics (France).

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