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Vitamin D and cardiovascular disease: Update and outlook

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Abstract
Accumulating evidence suggests that vitamin D may play a role for cardiovascular health. Expression of the vitamin D receptor (VDR) and enzymes for vitamin D metabolism have been identified in the vasculature as well as in the heart. VDR knock-out mice suffer from cardiovascular disease (CVD) and even selective VDR deletion in cardiomyocytes causes myocardial hypertrophy. Many, but not all observational studies showed that vitamin D deficiency is associated with CVD and its risk factors. Low concentrations of 25-hydroxyvitamin D (25(OH)D) are an independent risk factor for cardiovascular events, in particular for strokes and sudden cardiac deaths. Only few randomized controlled trials (RCTs) are available on this topic. These RCTs are frequently limited by the additional supplementation of calcium which may increase the risk of CVD events. RCTs with pure vitamin D supplementation have partially but not consistently shown beneficial effects on cardiovascular risk factors such as arterial hypertension. A number of large RCTs on the impact of vitamin D supplementation on cardiovascular events and mortality have already started but limitations of the study designs such as inclusion of individuals with relatively high 25(OH)D concentrations have to be considered. At present, the evidence is not sufficient for general recommendations to supplement vitamin D in order to prevent and treat CVD. It should, however, be noted that justification for the prevention and treatment of vitamin D deficiency comes from evidence based benefits of vitamin D supplementation on musculoskeletal health.

Key Words: Vitamin D, calcitriol, hypovitaminosis D, atherosclerosis, heart, cerebrovascular, stroke, MI

Introduction
Vitamin D is classically known as a substance that prevents rickets in children and it is therefore recommended to supplement vitamin D in the first year(s) of life [1]. It is established that vitamin D plays a crucial role in the regulation of mineral and bone metabolism [1]. Meta-analyses of randomized controlled trials (RCT) documented that fractures and falls are significantly reduced by vitamin D supplementation [2,3]. As a consequence, vitamin D supplementation became a routine treatment for osteoporosis.

Large epidemiological studies have documented a high prevalence of vitamin D deficiency in general populations [4]. This is most likely attributable to reduced sunlight exposure because UV-B induced vitamin D synthesis in the skin is the main determinant of circulating 25-hydroxyvitamin D (25[OH]D) concentrations that are measured to assess vitamin D status [5]. Hence, vitamin D deficiency can be regarded as a lifestyle related public health problem. This is of growing interest because, beyond musculoskeletal diseases, vitamin D deficiency has also been associated with various extra-skeletal diseases including cancer, infections, autoimmune and cardiovascular diseases (CVD) [6–8]. This makes sense when considering that the vitamin D receptor (VDR) is expressed in almost all human cells and that VDR...
activation regulates approximately three percent of the human genome [9].

In 1981, Robert Scragg [10] hypothesized that the seasonal decrease of CVD in summer might be a consequence of cardiovascular-protective actions of vitamin D. Accumulating evidence over the last three decades indeed indicates a beneficial role of vitamin D for cardiovascular health [8]. Previous in-depth reviews have already summarized the data on the role of vitamin D for CVD and its risk factors, for strokes, heart and renal diseases [8,11–13]. In the present review, we aim to provide a brief overview on experimental, observational and interventional studies on these latter topics with a focus on the most recent data. Given that CVD is the major cause of death in Western countries we also summarize the current evidence relating vitamin D and total mortality. In addition, we want to provide an outlook on the future of vitamin D and CVD by discussing the currently ongoing large RCT’s in this field with particular attention to their strengths and limitations.

**Experimental studies**

Expression of VDR and 1-α-hydroxylase, the enzyme that converts 25(OH)D to the most active vitamin D metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D), has been found in the vessels and the heart [8,9,12,14,15]. This suggests a role of vitamin D in cardiovascular pathophysiology, a notion that is supported by studies of knock-out mice for VDR or 1-α-hydroxylase. These mice suffer from cardiovascular pathologies including arterial hypertension, myocardial hypertrophy and increased thrombogenicity [9,16,17]. Renin overexpression occurs in these models and the molecular pathways mediating renin suppression by VDR activation have already been characterized in detail [9,16,17].

VDR activation has also been shown to prevent myocardial hypertrophy, an effect that may reflect direct vitamin D effects on the heart because even selective deletion of the VDR in cardiac myocytes leads to left ventricular hypertrophy despite normal calcium homeostasis and normal parathyroid hormone (PTH) concentrations [12,18]. Increased expression of various pro-hypertrophic genes such as modulatory calcineurin inhibitory protein 1 (MCIP1) is observed in these cardiomyocyte-specific knock-out mice [12,18]. Myocardial calcium homeostasis, which is crucial for myocardial electrophysiology and contractility, is also partially regulated by vitamin D [12]. Apart from this it has been demonstrated that VDR activation inhibits the development of atherosclerotic lesions by e.g. inhibition of macrophage to foam cell formation or by suppression of vascular smooth muscle cell proliferation [19,20].

In rat models, it was shown that early life vitamin D deficiency is associated with impaired vascular endothelial and smooth muscle cell function [21]. This is in line with a study on umbilical vein endothelial cells that showed significantly increased endothelial nitric oxide (NO) production after stimulation with 1,25(OH)₂D [22]. In addition, there exists evidence from experimental studies suggesting that VDR activation may exert anti-oxidative properties [23,24].

Beyond direct effects on the cardiovascular system there is compelling experimental evidence that vitamin D may exert beneficial effects on common cardiovascular risk factors such as arterial hypertension, kidney dysfunction, inflammation, dyslipidemia, obesity or diabetes mellitus [25–28]. Beyond suppressive effects on the renin angiotensin aldosterone system (RAAS), vitamin D has been shown to exert various other antihypertensive effects like modulation of vascular function including improved endothelial function [21,22,25].

VDR activation has also been shown to be nephroprotective by e.g. antiproteinuric effects that may be partially mediated by protective effects on podocytes [13]. VDR activation induces renal megalin expression which is required for tubular protein reabsorption [29]. Inhibition of Tumor Necrosis Factor-α (TNF-α)/Epidermal Growth Factor Receptor (EGFR) signalling pathways including inhibition of the TNF-α converting enzyme (TACE) may also reduce renal parenchymal lesions and proteinuria [29].

Moreover, vitamin D plays a crucial role in immune regulation. Vitamin D has several anti-inflammatory properties that are mediated by e.g. suppression of nuclear factor-κB (NF-κB) as well as by inhibition of pro-inflammatory and stimulation of anti-inflammatory cytokines [30,31]. It has been concluded that vitamin D causes a switch from a Th1/Th17 response to a Th2/Treg profile that may in turn protect against overwhelming inflammation and autoimmunity [28].

Vitamin D, which is itself formed from 7-dehydrocholesterol in the human skin, is also involved in lipid metabolism and may e.g. lower triglyceride concentrations by decreasing hepatic triglyceride formation or by modulating hepatic calcium homeostasis [32]. Mechanistic studies suggest that VDR activation may increase HDL-cholesterol and its main protein component apolipoprotein A-1 [27]. Apart from this, it has been documented that vitamin D sequestration occurs in the adipose tissue so that obesity may cause vitamin D deficiency [9].

Vitamin D may also be important for glucose homeostasis by directly modulating beta cell functions including calcium dependent processes that are involved in insulin secretion or by enhancing insulin signalling via up-regulation of the insulin-receptor [9].

**Observational studies on vitamin D, CVD and its risk factors**

Numerous epidemiological studies have evaluated the associations of 25(OH)D with CVD events and its risk factors [8,11–13,25–28]. Regardless, CVD risks...
factors it has been shown in a meta-analysis that low concentrations of 25(OH)D are associated with an increased risk of prevalent and incident arterial hypertension [33]. In this context, it has also been reported that low 25(OH)D concentrations are associated with increased activity of the RAAS [34].

Prospective studies showed that individuals with 25(OH)D concentrations above 62.5 nmol/L (divide by 2.496 to convert nmol/L to ng/mL) had a 43% lower risk of developing type 2 diabetes mellitus compared to individuals with 25(OH)D concentrations below 35 nmol/L [35]. Inverse correlations of 25(OH)D and HbA1c have been found in some but not all studies addressing this issue [36]. Observational data suggest that the association of vitamin D deficiency and increased risk of type 2 diabetes mellitus may be partially mediated by subclinical inflammation [37].

An association of low 25(OH)D and inflammation (i.e., C-reactive protein) has been observed in the continuous National Health and Nutrition Examination Survey (NHANES), but not all studies found such associations [7,8,28,38].

Mixed results are available on the association of vitamin D status and dyslipidemia but most studies in this field reported an inverse association of 25(OH)D and serum triglycerides and positive correlations of 25(OH)D with HDL-cholesterol and apolipoprotein A-1 [26,27,32]. It should, however, be considered that associations of vitamin D status with metabolic and inflammatory parameters may be confounded by the link of vitamin D and obesity [9]. This is of particular importance because it is becoming increasingly clear that vitamin D deficiency is not the cause but the consequence of obesity [39].

Given that low testosterone concentrations have been associated with increased cardiovascular risk it should be acknowledged that low 25(OH)D concentrations have been associated with low testosterone concentrations in epidemiological studies [40,41].

A poor vitamin D status has also been associated with lower glomerular filtration rate (GFR) and has been identified as a risk factor for rapid GFR loss in prospective analyses of the Cardiovascular Health Study [13,29,42].

Regarding cross-sectional associations of vitamin D status with manifest vascular diseases there exist conflicting data with either an inverse or no association of 25(OH)D concentrations with coronary artery disease, coronary artery calcification and carotid intima-media thickness [8,43,44]. Concerning vascular calcification it should be acknowledged that there might be an U-shaped association with increased risk of vascular calcification at both low 25(OH)D concentrations and vitamin D toxicity [44]. Some but not all studies found an association of low 25(OH)D with endothelial dysfunction and arterial stiffness [8,45,46], and numerous studies have almost consistently shown that low 25(OH)D concentrations are a risk factor for prevalent and incident heart failure [12]. Myocardial infarction has also been linked to vitamin D deficiency in some but not all studies and epidemiological data showed an inverse association of 25(OH)D concentration and matrix metalloproteinase-9 (MMP-9), a marker of myocardial remodelling and inflammation [47–49]. Most but not all studies showed that low 25(OH)D concentrations are associated with cerebrovascular events including strokes and a particular strong association has repeatedly been reported for low 25(OH)D and increased risk of sudden cardiac death [50–56]. In general, it can be concluded that low 25(OH)D concentrations are a risk factor for cardiovascular events, a notion that is supported by meta-analyses of observational studies [55–57]. Apart from this, it is also important to note that PTH, which increases as a consequence of vitamin D deficiency, can also be regarded as a cardiovascular risk factor and has been shown to predict cardiovascular events and mortality [58–60].

When interpreting the above mentioned observational studies it is difficult to differentiate whether the observed association between 25(OH)D concentrations and CVD reflects a “true” pathophysiologic relationship or whether it is confounded by the fact that patients suffering from CVD or risk conditions for CVD have reduced sunlight exposure due to their physical impairments or their risk factor related social behaviour. The situation is even more complex when considering the reciprocal influences of vitamin D deficiency, CVD and cardiovascular risk factors (see Figure 1). For example, low 25(OH)D concentrations may hypothetically contribute to myocardial dysfunction but heart failure associated limitations in mobility may reduce outdoor activities and sunlight exposure thus contributing to low 25(OH)D concentrations. On the other hand, heart failure may also cause wasting with weight loss and subsequently beneficial effects on metabolic risk factors (e.g. improved glucose homeostasis) but also higher 25(OH)D concentrations due to lower BMI.

Figure 1. Reciprocal influences of vitamin D deficiency, CVD and cardiovascular risk factors.
Studies on vitamin D supplementation, cardiovascular risk factors and CVD events

In contrast to many observational studies on vitamin D status and CVD there are only few data on vitamin D supplementation and CVD and its risk factors. Meta-analyses on vitamin D treatment and blood pressure suggest that natural vitamin D may reduce systolic blood pressure in a range of approximately 2 to 6 mm Hg, but this result was not significant in all meta-analyses [56,61,62]. UV-B exposure has previously been shown to reduce systolic and diastolic blood pressure compared to UV-A exposure, but this could not be replicated in a recent study by Scragg et al. [63,64]. By contrast, a study in 20 postmenopausal women showed that 25(OH)D supplementation is associated with a significant reduction in systolic blood pressure [65]. Regarding diabetes mellitus, a meta-analysis by Mitri et al. concluded that vitamin D supplementation had no significant effect on glycemic outcomes in patients with normal glucose tolerance as well as in type 2 diabetics [35]. Studies among patients with glucose intolerance, however, showed that vitamin D supplementation improved insulin resistance [35]. Furthermore, a recent original study among adults at high risk of diabetes showed that vitamin D supplementation improved beta-cell function and there was a strong non-significant trend for an attenuated rise in HbA1c concentrations in individuals on vitamin D treatment [66]. In line with this, another study showed that a vitamin D-fortified yogurt drink improved glycemic status in patients with type 2 diabetes mellitus [67].

Interventional studies on vitamin D and blood lipids produced mixed results. Zittermann et al. showed that vitamin D supplementation significantly decreased serum triglycerides but also caused an increase in LDL-cholesterol [26,27,32,68]. RCTs also produced inconsistent results regarding the effects of vitamin D supplementation on inflammation [7,28,69–72]. Some studies showed that vitamin D supplementation increases the anti-inflammatory cytokine interleukin-10 (IL-10) and decreases TNF-α [70–72]. Circulating regulatory T cells (Tregs), that are considered to protect against autoimmunity including type 1 diabetes mellitus, can be increased by vitamin D supplementation [73]. This is in line with observations by Hyppönen et al., who found a significantly decreased risk of type 1 diabetes mellitus in children receiving recommended vitamin D supplementation during their first years of life [74]. In addition, genetic variants affecting 25(OH)D concentrations have also been linked to type 1 diabetes mellitus [75,76].

Limited and inconclusive data exist on the effect of vitamin D supplementation on markers of heart failure and some, but not all RCTs have shown that vitamin D supplementation improves endothelial function [77–81]. Interestingly, paricalcitol, an active vitamin D analogue, was shown to reduce albuminuria in patients with diabetic nephropathy [82].

RCTs on vitamin D supplementation and CVD events are sparse and a major problem with the interpretation of these studies is that vitamin D supplementation has often been combined with calcium supplementation [83]. Considering that calcium supplementation may be associated with increased risk of cardiovascular events, as reported by a meta-analysis, we cannot draw adequate conclusions regarding the effect of vitamin D on CVD events when looking at studies with combined calcium plus vitamin D supplementation [83]. When including studies with pure vitamin D supplementation, Wang et al. found a non-significant trend for reduced risk of CVD events (pooled relative risk, 0.90 [95% CI, 0.77 to 1.05]) in patients randomized to vitamin D supplementation [84]. Similar results i.e. a non-significant reduction of CVD events (hazard ratio = 0.91; 95% CI = 0.79–1.05) were also reported by Avenell et al., who analyzed 5,292 older patients from the RECORD trial [85]. In addition, it has been observed that chronic kidney disease (CKD) patients who were sufficiently treated with vitamin D experienced significantly less CVD events compared to untreated patients [86]. Hence, limited evidence suggests that vitamin D supplementation might reduce CVD events in specific subgroups but this is not proven and has to be evaluated in further RCTs.

Vitamin D and total mortality

Given that CVDs are the major cause of death in Western countries it is of interest to evaluate whether vitamin D status or vitamin D supplementation is associated with total mortality. Prospective observational studies have largely, but not consistently shown, that low 25(OH)D concentrations are associated with increased risk of total mortality [87,88]. A meta-analysis of studies among the general population confirmed this notion and found a non-linear association of 25(OH)D and mortality with the lowest risk at 25(OH)D concentrations ranging from 75 to 87.5 nmol/L [88]. Highest mortality risk was found in individuals with the lowest 25(OH)D concentrations [88]. Among specific patient groups it has been observed that in CKD patients low 25(OH)D concentrations are also a risk factor for mortality [89–91]. Importantly, recent observational data showed that vitamin D supplementation was associated with improved survival [92]. This is in line with meta-analyses of RCTs that found significantly reduced mortality in patients randomized to vitamin D supplementation [93]. In detail, a Cochrane review by Bjelakovic et al. reported a statistically significant 6% reduction of mortality by vitamin D3 supplementation compared to placebo [94]. It was calculated that when treating 161 individuals with vitamin D3, one additional death can be prevented [94].
**Future outlook**

Although there exist accumulating data that vitamin D may be beneficial for CVD and its risk factors, evidence is still not sufficient to establish general recommendations to supplement vitamin D for the prevention and treatment of CVD. Therefore, well-designed large-scale RCTs are urgently needed. Such RCTs designed to evaluate vitamin D effects on CVD events and mortality have recently been started and include e.g. a study among 1,000 heart failure patients in Germany by Zittermann et al. (EVITA study) and a study among 5,100 older individuals in New Zealand by Scragg et al. (Vitamin D Assessment Study, ViDA). The largest study in this field is the VITAL Study (VTitamin D and Omega-3 TriaL) among 20,000 study participants in the US by Manson et al. [95]. Strengths of these studies are e.g. the large number of study participants and the relatively long follow-up periods. However, it will take a few years from now until we can expect the published results of these trials between ~2015 to 2017 [95]. We should critically discuss whether the results of e.g. the VITAL Study can definitely answer the question whether vitamin D supplementation is effective for the prevention and treatment of CVD. In general, the VITAL Study includes study participants representing the older population in the US. Study participants are included regardless of their 25(OH)D concentrations at baseline and apart from the study medication (= 2,000 IU vitamin D per day or placebo) an intake of up to 800 IU vitamin D per day is allowed [95]. Results of the VITAL study might therefore be limited by relatively high 25(OH)D concentrations in the placebo group. Although the results of the VITAL Study are of great importance because they allow drawing conclusions regarding general vitamin D supplementation in the older population the study is not mainly designed to address vitamin D benefits in individuals suffering from vitamin D deficiency. In this context, it should be considered that many observational studies showed a significantly increased risk of CVD events only in patients with 25(OH)D concentrations below ~37.5 nmol/L [8,55–57]. Furthermore, beneficial outcomes are mostly observed in individuals with 25(OH)D concentrations ranging from ~75 to 100 nmol/L and some study results indicate a slightly increasing risk for adverse events at very high 25(OH)D concentrations [88,95]. In our opinion, important vitamin D RCTs should therefore aim to study high-risk participants who are expected to be very sensitive to vitamin D supplementation (e.g. severely vitamin D deficient patients with high PTH concentrations and at high CVD risk) and should include an optimal vitamin D dosage regime (e.g. with the aim to reach the 25(OH)D target concentrations of ~75 to 100 nmol/L). The time window for funding of such trials will likewise close within the next few years after the above mentioned RCTs have been published and we can only hope that the vitamin D story will not be similar to vitamin E [96]. Vitamin E exerts anti-oxidative actions but large RCTs including relatively unselected study participants with vitamin E concentrations mainly in the sufficiency interval failed to prove significant benefits with suboptimal dosages of vitamin E [96]. Therefore, the open question still remains whether there are relevant effects in vitamin E sensitive individuals with e.g. low vitamin E concentrations and/or high oxidative stress [96,97].

**Conclusions**

Experimental studies suggest that vitamin D plays a crucial role for the maintenance of cardiovascular health. This notion is supported by large epidemiological studies which highlight vitamin D deficiency as an independent cardiovascular risk factor. Data from interventional studies on vitamin D treatment and cardiovascular risk are, however, sparse and inconclusive and are often limited by the concomitant supplementation of vitamin D plus calcium. Large-scale RCTs on vitamin D and CVD risk have already started but their limitations such as inclusion of study participants regardless of their 25(OH)D concentrations may reduce the ability to detect beneficial vitamin D effects on cardiovascular morbidity and mortality in high-risk vitamin D deficient individuals. At present, the evidence is not sufficient for general recommendations to supplement vitamin D in order to prevent and treat CVD. It should, however, be noted that justification for the prevention and treatment of vitamin D deficiency needs only one proven benefit of vitamin D supplementation and this are (at least) the beneficial effects on musculoskeletal health [1–3,98].

**Questions and Answers**

**M Kaelin**, Switzerland

Are you confident that there will be randomised controlled trials that will give better results than those for vitamin E? When you mention that there are so many factors influencing CVD or strokes, there are always multifunctional cases and diseases. Will it therefore ever be possible to have comparable groups taking into account all these factors?

**S Pilz**

I hope that on-going trials will provide definite answers, but have severe limitations. These include that study participants are included irrespective of their serum 25(OH)D concentration. Quite a significant vitamin D intake is permitted so that may be a problem.

**M Fukagawa**, Japan

You did not mention FGF23. Did vitamin D supplementation increase the serum concentration of FGF23?
S Pilz
I have not looked at this.

M Fukagawa
I ask because, at least in CKD patients, treatment with active vitamin D sterols increases serum FGF23 concentrations and it is known that this therapy improves survival and reduces cardiac events. However, the increased FGF23 is another risk factor for cardiac function.

S Pilz
Yes, but for CKD I agree with what has been said by RVieth; that we should consider the 25(OH)D concentration. If it is normal, we should think of prescribing active vitamin D. Sometimes there are difficulties in metabolism. If it is normal, we should think of prescribing active vitamin D sterols increases serum FGF23 concentrations and it is known that this therapy improves survival and reduces cardiac events. However, the increased FGF23 is another risk factor for cardiac function. The issue is, was this the right therapy? It may be that the cardiomyocytes need more then 25(OH)D. If just a small amount of 25(OH)D is intracellularly converted to the active form then a much higher local concentration can be achieved than with systemic therapy with 1,25(OH)2D3 which may be harmful or have a very narrow therapeutic window.

R Jorde, Norway
I think we focus too much on what is the 'sufficient' level of intake. We should also consider what is too much. We have done several intervention studies and have given up to 6,000 U/day. We found that there is a small but significant increase in systolic blood pressure and a slight significant decrease in glucose tolerance, so we should be careful. The serum concentrations were 120–150 nmol/L.

Disclosure summary
Nothing to disclose.

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Vitamin D and cardiovascular disease


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Vitamin D and cardiovascular disease

91


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