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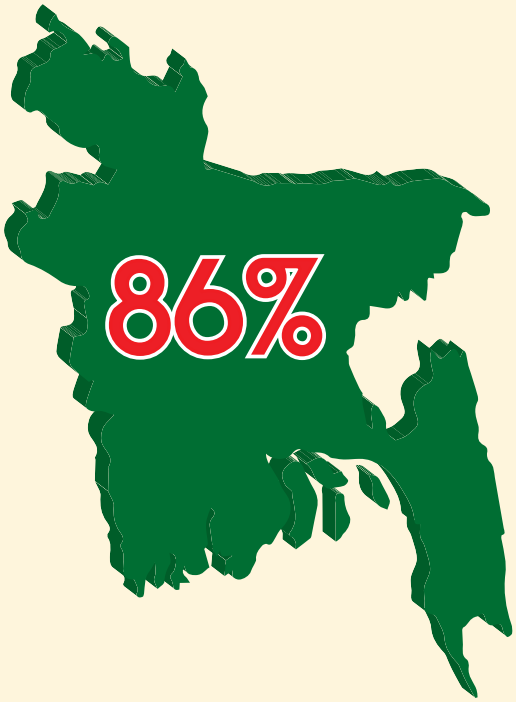
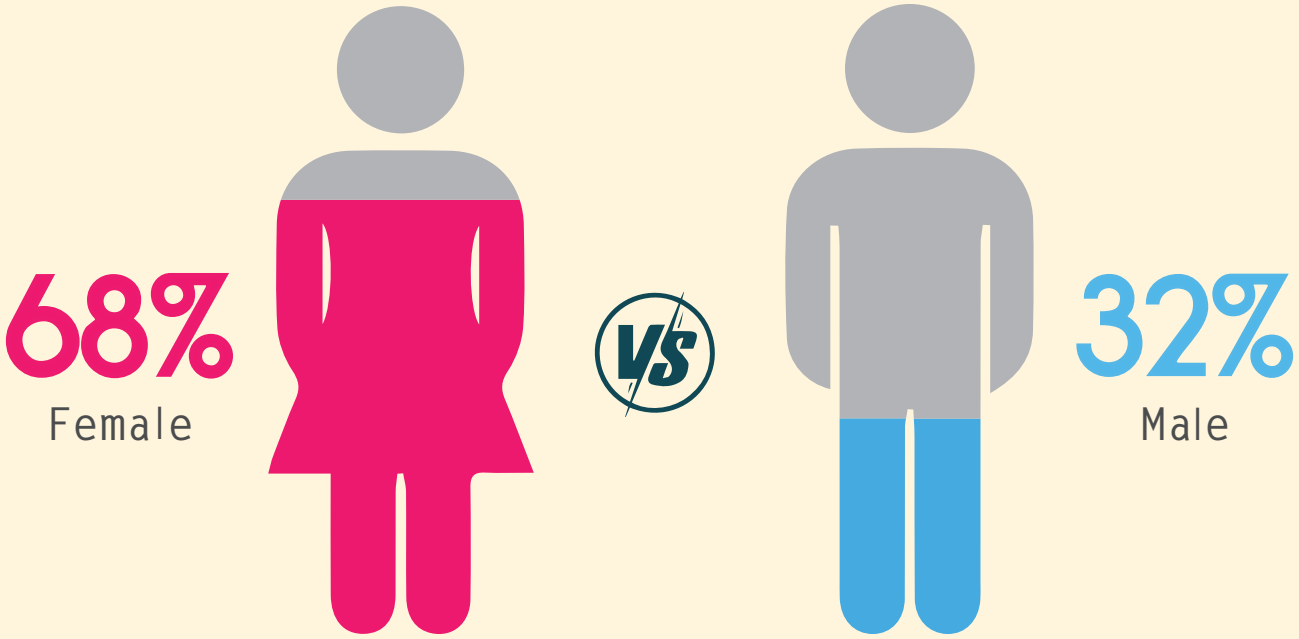


of the population

have low level of ***Vitamin D***

Among them



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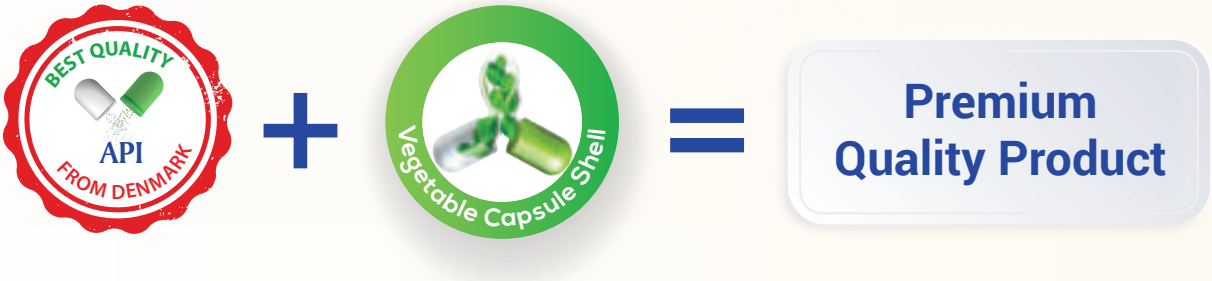

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Original Article

Vitamin D status in Bangladeshi subjects: a laboratory based study

Islam AKMM[a], Hasan MN[b], Rahman KM[c], Asaduzzaman M[d], Rahim MA[e], Zaman S[f], Islam MR[g], Jesmin H[h], Yeasmin L[i]

Abstract

Background: *Vitamin D plays important role in normal functioning of multiple organs of the body. Hypovitaminosis D is known to be prevalent worldwide including the tropical countries. The present study was carried out to evaluate the vitamin D status in Bangladeshi patients undergoing laboratory investigation for vitamin D.*

Methods: *This was a laboratory-based study. Data were extracted from the database of a diagnostic centre of Dhaka city and were analysed. Vitamin D status was defined as follows: deficiency 0 to <20 ng/ml, insufficiency 20 to <30 ng/ml, sufficiency 30-100 ng/ml and potential toxicity >100 ng/ml.*

Results: *A total of 793 plasma vitamin D level reports were analysed. Out of 793 subjects, 269 (33.9%) were male and 524 (66.1%) were female. Majority (62.0%) were between 21 and 60 years of age. Mean (+/- standard deviation) vitamin D level of the study subjects was 21.66 (+/- 18.63) ng/ml. Eighty-six percent had hypovitaminosis D; 61.4% had deficiency and 24.1% had insufficiency. Vitamin D level was found sufficient in 13.1% subjects. Among the deficient subjects, 31.6% were male and 68.4% were female; among the insufficient subjects, 35.1% were male and 64.9% were female. Sixty-eight percent of the deficient subjects belonged to the 21 to 60 year age group, whereas 57.1% of the insufficient subjects were between 21 and 60 years.*

Conclusion: *Hypovitaminosis D is common among the real-world clinical subjects undergoing vitamin D estimation in Bangladesh. Middle-aged females are more likely to be affected.*

Keywords: *Bangladesh, cholecalciferol, prevalence, vitamin D deficiency.*

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Introduction

Vitamin D, also known as the sunshine vitamin, is an important molecule which plays crucial role in human body. Beyond its well-recognized effects on musculoskeletal system, this vitamin is now known to exert gene-mediated pleotropic effect on a wide range of extra-skeletal tissues. In fact, vitamin D receptors are present in the nuclei of almost all types of nucleated cells to which calcitriol, the active form of vitamin D binds and gets involved in regulation of gene activity.[1] Observational studies have suggested an inverse association between vitamin D status and risk of developing a number of diseases including type 1 diabetes mellitus, cardiovascular disease, certain cancers, cognitive decline, depression, pregnancy complications, autoimmunity, allergy and even frailty.[2-5] Results from randomized controlled trials (RCTs) and meta-analyses of RCTs do, however, only provide limited support for such effects.[6] Vitamin D deficiency is pandemic, affecting both temperate and tropical countries; almost half of the world's population has got hypovitaminosis D.[7-9] Like elsewhere, vitamin D deficiency is highly prevalent in south Asian countries.[10]

According to a recently published review, the prevalence of vitamin D deficiency in India ranged from 40% to 99%, with most of the studies reporting a prevalence of 80%-90%.[11] Data regarding vitamin D status in Bangladesh are scarce and is derived from small cross-sectional studies involving specific class of people e.g., diabetic patients, women and doctors in a tertiary care hospital of Dhaka City.[12-18]The National Micronutrient Survey 2011-12 provided nationally representative data on vitamin D status, but only in pre-school children, school-age children and non-pregnant, non-lactating women.[19]All these data lack generalizability. The present study was planned to determine the vitamin D level among the subjects for whom laboratory analysis of serum vitamin D level was sought.

Methods

This retrospective, laboratory-based, observational study was carried out from January 2015 to May 2017 in the Department of Pathology of a diagnostic centre of Dhaka City. Data were extracted from the database. All the patients who were referred to the centre for vitamin D estimation during the study period were purposively included. During the study period, 793 subjects presented for vitamin D investigation. For each subject, three ml blood was collected in gel tubes and serum was separated via centrifugation at 4,000 rpm for 5 minutes. The immunodiagnostic enzyme linked immunosorbent assay (ELISA) was used for quantitative determination of the 25(OH)D in serum and plasma. The assay utilizes a competitive ELISA technique with a selected monoclonal antibody recognizing 25(OH)D. Vitamin D status was defined as follows: deficiency 0 to <20 ng/ml, insufficiency 20-<30 ng/ml, sufficiency 30-100 ng/ml, potential toxicity >100 ng/ml.

Data were analyzed by using statistical package for social scientists (SPSS) (version 16) for Windows (spss16-t2). Descriptive statistics was applied to calculate frequency and percentages from categorical variables and mean and standard deviation were measured from continuous numerical variables. For comparing proportions between different groups, chi-square tests of significance were done.

Results

A total of 793 plasma vitamin D level reports were analysed. Out of 793 subjects, 269 (33.9%) were male and 524 (66.1%) were female (Table I).

Table I Distribution of subjects by sex, age and serum 25(OH)D status (N=793)

Variable	Frequency	Percentage
Gender		
Male	269	33.9
Female	524	66.1
Total	793	100.0
Age (years)		
0-20	138	17.4
21-40	236	29.8
41-60	255	32.2
>60	164	20.7
Total	793	100.0
S. 25(OH)D status		
Deficient	487	61.4
Insufficient	191	24.1
Sufficient	104	13.1
Potentially toxic	11	1.4
Total	793	100.0

[25(OH)D=25 hydroxy cholecalciferol]

Majority (62.0%) were between 21 and 60 years of age. Mean vitamin D level of the study subjects was 21.66 ng/ml (standard deviation 18.63). Eighty-six percent had hypovitaminosis D, 61.4% had deficiency and 24.1% had insufficiency. Vitamin D level was found sufficient in 13.1% subjects. Eleven persons out of 793 were having potential toxic level of serum 25(OH)D (>100 ng/mL). Among the deficient subjects, 31.6%were male and 68.4% were female; among the insufficient subjects, 35.1% were male and 64.9% were female (Table II). There was no statistically significant difference of the distribution of sex across different categories of vitamin D status (p=0.207).In terms of age distribution by vitamin D status, 68.2% of the deficient subjects belonged to the 21 to 60 year age group, whereas 57.1% of the insufficient subjects aged between 21 and 60 years (Table III).

Table II Distribution of subjects by sex and vitamin D status (N=793)

			Vitamin D status				Total	P value
			Deficient	Insufficient	Sufficient	Potential toxicity		
sex	Male	Count	154	67	44	4	269	0.207
		% within sex	31.6%	35.1%	42.3%	36.3%	33.9%	
	Female	Count	333	124	60	7	524	
		% within sex	68.4%	64.9%	57.7%	63.6%	66.1%	
	Total	Count	487	191	104	11	793	
		% within sex	100.0%	100.0%	100.0%	100.0%	100.0%	

Table III Distribution of subjects by age group and vitamin D status (N=793)

Age(years)	Vitamin D status (ng/ml)				Total
	Deficient	Insufficient	Sufficient	Potentially toxic	
0-20	76	33	24	5	138
	15.6%	17.3%	23.1%	45.5%	17.4%
21-40	171	41	22	2	236
	35.1%	21.5%	21.2%	18.2%	29.8%
41-60	161	68	24	2	255
	33.1%	35.6%	23.1%	18.2%	32.2%
>60	79	49	34	2	164
	16.2%	25.7%	32.7%	18.2%	20.7%
Total	487	191	104	11	793
	100.0%	100.0%	100.0%	100.0%	100.0%

Discussion

The present study was a retrospective analysis of data involving people from different socio-economic background and of different age groups and both sexes. Similar lab data-based study was carried out in neighbouring countries like India, Pakistan and Saudi Arabia.[20-23] Prevalence of hypovitaminosis D [serum 25(OH)D <30 ng/mL] was 85.5% in the present study; 61.4% had deficiency (serum 25(OH)D <20 ng/mL) whereas 24.1% had insufficiency (serum 25(OH)D 20-29.9 ng/mL). Vitamin D level was found sufficient in 13.1% subjects only.

The study involving 26,346 subjects coming for executive health check-up in Gurgaon, India revealed hypovitaminosis D, defined as serum 25(OH)D <40 ng/mL in 93% of the study subjects; vitamin D deficiency (serum 25(OH)D <20 ng/mL) was found in 59%.[20] Similar large study from Pakistan involving 60,937 specimens revealed prevalence of vitamin D deficiency, defined as serum 25(OH)D <20 ng/mL to be 66.1%.[21] Smaller study with identical design found the prevalence of vitamin D deficiency (<20 ng/mL) and insufficiency (20-29.9 ng/mL) to be 60% and 27.6%, respectively.[22] So, the prevalence of hypovitaminosis D is more or less similar in the three Asian countries i.e., Bangladesh, India and Pakistan.

In the present study, mean serum 25(OH)D level of the study subjects was 21.66 ng/mL which is almost similar to the mean value of 21.4 ng/mL in the Indian study.[20] On the other hand, the median 25(OH)D level was 13.5 ng/mL in the study from Pakistan which is lower than the values obtained in the present study.[21]

In the present study, females were more severely affected by hypovitaminosis D than the males, however the differences were not statistically significant. In the Indian study, there was significant differences in mean serum 25(OH)D levels between male and female subjects.[20] However, Kiani et al. did not find such differences.[22]

Despite paucity of data, such high prevalence of vitamin D deficiency was found in previous studies in Bangladesh. In 2001, hypovitaminosis D, defined as serum 25(OH)D ≤15 ng/mL was observed in 50% of subjects in low socio-economic group and 38% of subjects in high socio-economic group, respectively.[13] In another study, the prevalence of hypovitaminosis D, defined as serum 25(OH)D <16 ng/mL was 78% in young women, 83% in veiled women and 76% in diabetic women.[14] The mean serum 25(OH)D was 14.68 ng/mL in a cross-sectional study involving 200 female garment workers, the value is lower than the value of mean serum 25(OH)D found in the present study.[15] In a more recent study, 89.8% of the physicians working in a tertiary care hospital of Dhaka City had vitamin D deficiency (serum 25(OH)D <20 ng/mL).[16] Among the newly diagnosed type 2 diabetic patients, the mean serum 25(OH)D level was 27.91±2.58 ng/mL; 30% had vitamin D deficiency defined as serum 25(OH)D ≤20 ng/mL, whereas 36% had vitamin D insufficiency with serum 25(OH)D >20 to 29.9 ng/mL, indicating better vitamin D status in these subjects in comparison to those in the present study.[12]

The study has got some important limitations. This was a retrospective study, based on laboratory reports only, thereby lacking details. There might be some confounding physiological and pathological factors influencing the serum vitamin D status like exposure to sun, skin complexion, dietary habits, medications. It was not possible to investigate whether patients, who were having toxic levels, were taking vitamin D or not. So, the results, in true sense, cannot be generalized. Despite these limitations, the study gives an insight regarding the high prevalence of hypovitaminosis D in a heterogenous population of Bangladeshi context. Moreover, the present study warrants carrying out well-designed cross-sectional study and nationwide survey to generate representative data on vitamin D deficiency in Bangladesh which will be an aid to formulate appropriate public health policy in future.

Conflicts of interest: Nothing to declare.

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SCIENTIFIC REPORTS

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Vitamin D both facilitates and attenuates the cellular response to lipopolysaccharide

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Vitamin D has a range of non-skeletal health effects and has been implicated in the response to respiratory infections. The aim of this study was to assess the effect of vitamin D on the response of epithelial cells, neutrophils and macrophages to lipopolysaccharide (LPS) stimulation. BEAS-2B cells (airway epithelial cell line) and primary neutrophils and macrophages isolated from blood samples were cultured and exposed to LPS with and without vitamin D ($1,25(OH)_2D$). The production of IL-6, IL-8, IL-1β and TNF-α of all cells and the phagocytic capacity of neutrophils and macrophages to *E. coli* were assessed. Vitamin D had no effect on BEAS-2B cells but enhanced the production of IL-8 in neutrophils ($p = 0.007$) and IL-1β in macrophages ($p = 0.007$) in response to LPS. Both vitamin D ($p = 0.019$) and LPS ($p < 0.001$) reduced the phagocytic capacity of macrophages. These data suggest that the impact of vitamin D on responses to infection are complex and that the net effect will depend on the cells that respond, the key response that is necessary for resolution of infection (cytokine production or phagocytosis) and whether there is pre-existing inflammation.

Vitamin D is a secosteroid hormone which is well-known of its role in mineral and skeletal homeostasis[1]. A plethora of studies have suggested that vitamin D has a range of roles beyond the regulation of bone metabolism and that it plays a critical role in modulating the immune response; including the response to respiratory infections[2]. Respiratory tract infections (RTI), which are highly prevalent, are responsible for significant morbidity and mortality, and are associated with the onset, progression and exacerbation of chronic lung diseases[3,4].

Epidemiological studies have demonstrated strong associations between serum vitamin D levels and the incidence of RTIs[5] and it has been suggested that the seasonal variations in vitamin D levels could explain the increased prevalence of RTIs in winter[6], when vitamin D synthesis is low[7]. However, clinical trials with vitamin D supplementation have reported varying effects of on responses to respiratory infections[8,9] such that current evidence linking vitamin D status and RTIs is equivocal.

Airway epithelial cells, as the first defensive barrier in the airway tract, play an important role in orchestrating neutrophil and macrophage recruitment to clear invading pathogens[10]. Neutrophils and macrophages play an important role in clearing pathogens through their phagocytic capacity by producing oxidants to kill engulfed microorganisms[11]. It is believed that the local production of 1α, 25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D, exerts protective effects during infections by upregulating the expression of cathelicidin and β defensin 2 in phagocytes[12,13] and epithelial cells[14]. The clearance of pathogens and apoptotic cells by phagocytosis plays an important role in resolving inflammatory responses, and any impairment of these processes can lead to a chronic inflammatory state[15].

While vitamin D is thought to be important in mediating the immune response to infection, as discussed above, the role of vitamin D in modulating cytokines production is unclear. Previous studies have shown conflicting effects (anti-inflammatory and pro-inflammatory) of vitamin D on the production of IL (interleukin) -1β, IL-6 and TNF (tumor necrosis factor) -α in response to a variety of insults[16–18]. IL-1β and TNF-α are essential for the activation of the epithelium and downstream inflammatory responses[19]. In addition, IL-1β, IL-6, IL-8 and TNF-α are involved in the innate immune responses by neutrophils and macrophages[20] and may be influenced by vitamin D[16–18,21].

Given the inconsistencies in the *in vitro* and clinical trial data, we aimed to determine the effect of exogenous vitamin D on the inflammatory response in key cells involved in the response to RTIs including epithelial cells

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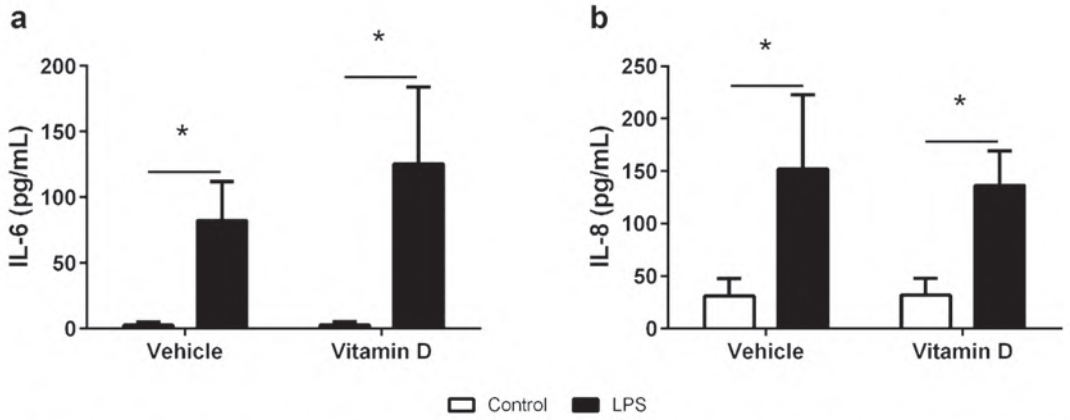

Figure 1. Production of IL-6 (**a**) and IL-8 (**b**) in the supernatant of BEAS-2B cells with (black bars) and without (white bars) LPS in the presence of vehicle or vitamin D ($1,25(OH)_2D_3$). Data are represented as mean (SD), *indicates $p < 0.05$, n = 4/group.

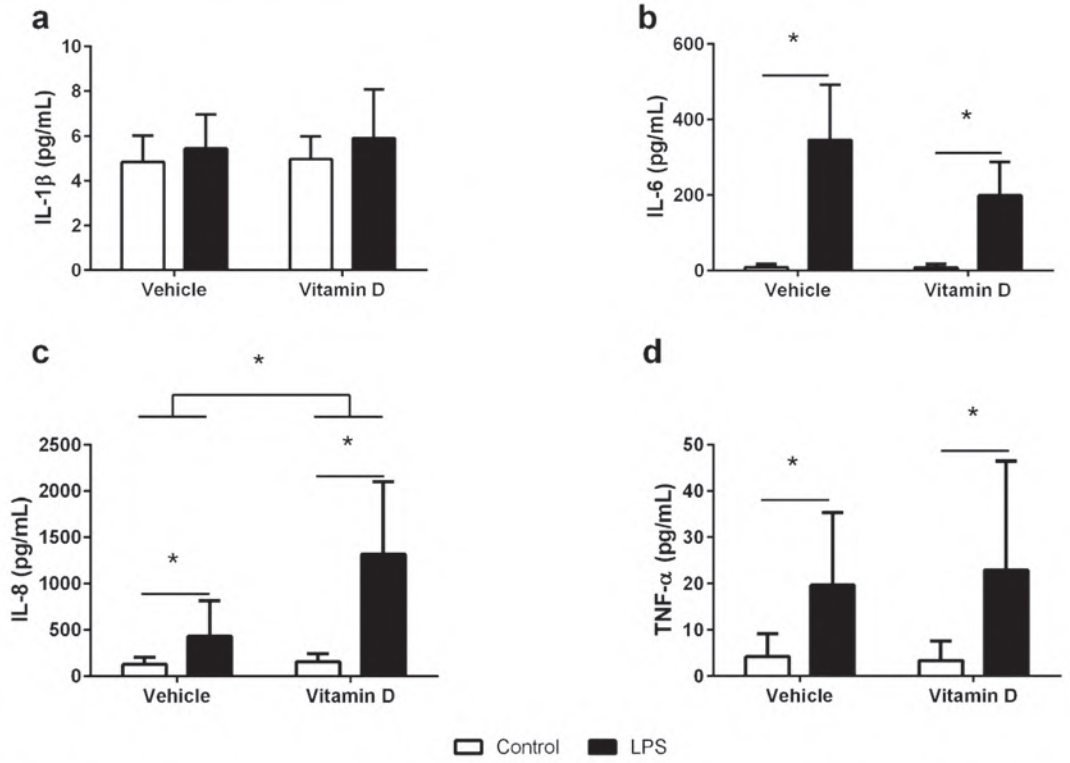

Figure 2. Production of IL-1β (**a**), IL-6 (**b**), IL-8 (**c**) and TNF-α (**d**) in the supernatant of neutrophils with (black bars) and without (white bars) LPS in the presence of vehicle or vitamin D ($1,25(OH)_2D_3$). Data are represented as mean (SD), *indicates $p < 0.05$, n = 9/group for IL-1β testing, n = 5/group for IL-6 testing, n = 8/group for IL-8 testing, and n = 9/group for TNF-α testing.

macrophages and neutrophils. In order to achieve this, we examined whether vitamin D modulated the response to bacterial lipopolysaccharide (LPS).

Results

Cytokine production by BEAS-2B cells. 24 hours after exposure to LPS, the production of IL-6 (Fig. 1a, $p < 0.001$) and IL-8 (Fig. 1b, $p < 0.001$) was increased in BEAS-2B cells compared to controls. However, the magnitude of the response was not altered by the presence of $1,25(OH)_2D_3$ (IL-6, $p = 0.552$; IL-8, $P = 0.994$). IL-1β and TNF-α were not detectable by ELISA in any of the supernatants (*data not shown*).

Cytokine production by neutrophils. 24 hours after exposure to LPS, the production of IL-6 (Fig. 2b, $p < 0.001$), IL-8 (Fig. 2c, $p < 0.001$) and TNF-α (Fig. 2d, $p = 0.002$) was increased in neutrophils compared

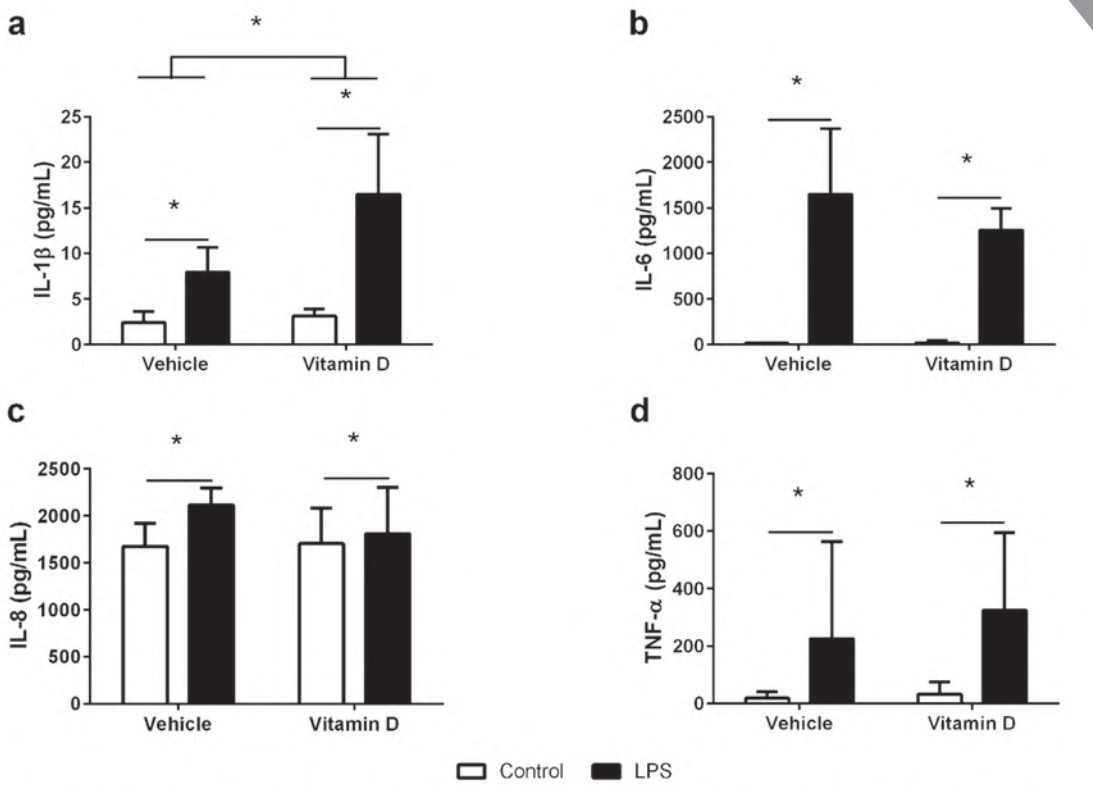

Figure 3. Production of IL-1β (**a**), IL-6 (**b**), IL-8 (**c**) and TNF-α (**d**) in the supernatant of macrophages with (black bars) and without (white bars) LPS in the presence of vehicle or vitamin D ($1,25(OH)_2D_3$). Data are represented as mean (SD), *indicates $p < 0.05$, n = 7/group for IL-1β testing, n = 5/group for IL-6 testing, n = 7/group for IL-8 testing, and n = 8/group for TNF-α testing.

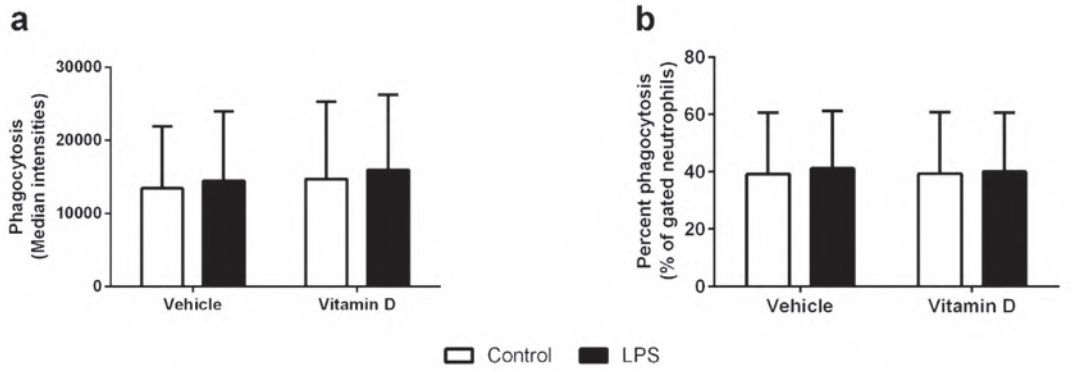

Figure 4. Phagocytic capacity of neutrophils measured as the total phagocytosis of *E. coli* (**a**) or the percentage of cells that phagocytosed *E. coli* (**b**) in neutrophils treated with (black bars) or without (white bars) LPS in the presence of vehicle or vitamin D ($1,25(OH)_2D_3$). Data are represented as mean (SD), n = 6/group.

to controls. Interestingly, while $1,25(OH)_2D_3$ had no effect on the production of IL-6 ($p = 0.297$) or TNF-α ($p = 0.728$) by the neutrophils, it increased the IL-8 response ($p = 0.007$) in both the LPS treated and untreated neutrophils. Neither LPS ($p = 0.153$) nor $1,25(OH)_2D_3$ had any effect on IL-1β production (Fig. 2a, $p = 0.600$).

Cytokine production by macrophages. 24 hours after exposure to LPS, the production of IL-1β (Fig. 3a, $p < 0.001$), IL-6 (Fig. 3b, $p < 0.001$), IL-8 (Fig. 3c, $p = 0.049$) and TNF-α (Fig. 3d, $p < 0.001$) was increased in macrophages compared to controls. In contrast to the neutrophils, while $1,25(OH)_2D_3$ had no effect on the production of IL-6 ($p = 0.531$), IL-8 ($p = 0.297$) or TNF-α ($p = 0.095$), it increased the production of IL-1β ($p = 0.001$) in both the LPS treated and untreated cells.

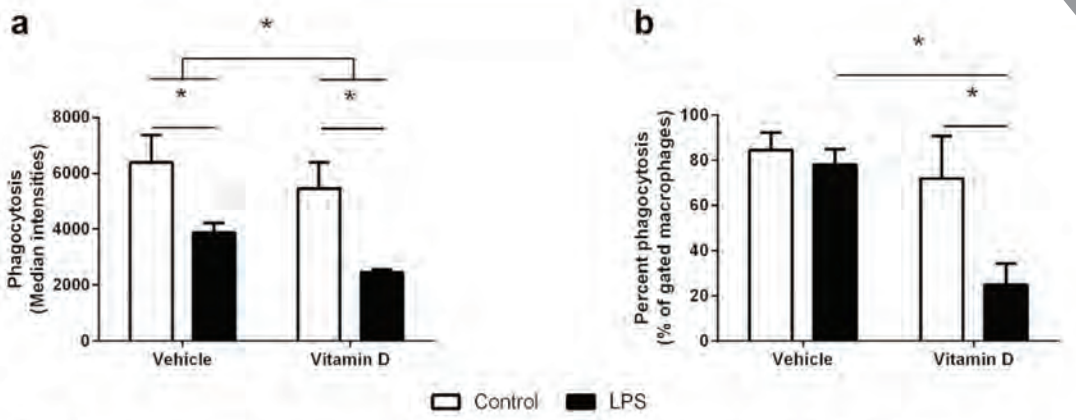

Figure 5. Phagocytic capacity of macrophages measured as the total phagocytosis of *E. coli* (**a**) or the percentage of cells that phagocytosed *E. coli* (**b**) in macrophages treated with (black bars) or without (white bars) LPS in the presence of vehicle or vitamin D ($1,25(OH)_2D_3$). Data are represented as mean (SD), *indicates $p < 0.05$, $n = 3$/group.

Phagocytic capacity. There was no effect of LPS treatment or $1,25(OH)_2D_3$ on total phagocytic capacity (Fig. 4a) (LPS, $p = 0.732$; $1,25(OH)_2D_3$, $p = 0.802$) or the percentage of cells that engulfed the *E. coli* (Fig. 4b) (LPS, $p = 0.788$; $1,25(OH)_2D_3$, $p = 0.936$) in neutrophils. In contrast, both LPS ($p < 0.001$) and $1,25(OH)_2D_3$ ($p = 0.019$) reduced the phagocytic capacity of macrophages (Fig. 5a). In the case of LPS and $1,25(OH)_2D_3$, in isolation, the effect seemed to be due to the reduced capacity of individual cells because the percentage of cells that phagocytosed *E. coli* was not changed in response to LPS ($p = 0.516$) or $1,25(OH)_2D_3$ ($p = 0.219$) alone (Fig. 5b). However, in combination, LPS and $1,25(OH)_2D_3$ significantly reduced the number of macrophages with phagocytic capacity (Fig. 5b, $p < 0.001$).

Discussion

Data on the effect of vitamin D deficiency on responses to respiratory tract infections are conflicting. We aimed to examine the effect of supplemental vitamin D on cytokine production and phagocytic capacity in three cell types that are important in the innate response to respiratory infections; airway epithelial cells, neutrophils and macrophages. We found that $1,25(OH)_2D_3$, the active form of vitamin D, enhanced IL-8 and IL-1β production by neutrophils and macrophages respectively. $1,25(OH)_2D_3$ also decreased the total phagocytic potential of macrophages and, in combination with LPS, reduced the ability of individual macrophages to engulf *E. coli*. These data suggest that vitamin D has differing effects on the response to individual cell types to a bacterial stimulus. On the one hand the addition of vitamin D enhanced the production of cytokines but reduced the capacity of the macrophages to phagocytose pathogens. This may explain the conflicting associations between vitamin D and respiratory tract infections.

The airway epithelium is the first point of contact for invading pathogens in the respiratory tract. In response to bacterial stimuli, airway epithelial cells produce a range of pro-inflammatory mediators such as IL-6 and IL-8[22] which act to recruit mononuclear phagocytes[23] and neutrophils[24]. These phagocytes play important roles in responding to, and clearing respiratory infections by phagocytosing and producing reactive oxygen species (ROS) to eliminate pathogens[25]. As expected, we saw increased production of IL-6 and IL-8 by airway epithelial, BEAS-2B, cells in response to LPS stimulation. These responses were not altered by vitamin D suggesting that vitamin D does not modulate important innate responses by epithelial cells to bacterial infections in the airway.

Macrophages and neutrophils are key cells in generating the innate immune response to invading pathogens. When a pathogen crosses the epithelial barrier and begins to replicate in the tissues of the host, it is immediately recognized by macrophages, that reside in the tissues, which, along with epithelial cells, produce chemokines to recruit large numbers of neutrophils to sites of infection[10]. Macrophages and neutrophils respond to pathogens, in part, by releasing a plethora of cytokines and chemokines in order to orchestrate the immune response[26]. Neutrophils have a high phagocytic capacity and, when a pathogen is engulfed, release IL-8, a neutrophil chemoattractant[27], which recruits additional neutrophils to site of infection[24]. Neutrophils also act to eliminate the pathogen by producing an oxidative burst[28]. As expected, we found that LPS increased the production of IL-8 by neutrophils. Interestingly, $25(OH)_2D_3$ in our study facilitated the production of IL-8 suggesting that vitamin D may boost the capacity of neutrophils to respond to invading pathogens by recruiting additional neutrophils to the site of infection. This is in contrast to other studies on respiratory pathogens including tuberculosis where vitamin D had no effect on neutrophilia[29]. Similarly, vitamin D has been shown down-regulate IL-8 release in hyper-inflammatory macrophages[21]. The disparities in these observations suggest that the impact of vitamin D on the production of IL-8 by innate immune cells is dependent on the cell that is producing the cytokine and the infectious stimulus.

Activated macrophages produce a range of inflammatory mediators in response to bacterial infections. Consistent with previous studies[30], we found that macrophages increased production of all the cytokines measured, IL-1β, IL-6, IL-8 and TNF-α, in response to LPS stimulation. However, pre-treatment with $1,25(OH)_2D_3$ during macrophage differentiation period facilitated LPS induced IL-1β production but had no effects on the

production of IL-6, IL-8 and TNF-α which is in contrast to a recent study which showed that $1,25(OH)_2D_3$ significantly reduced the levels of IL-6 and TNF-α in alveolar macrophages in response to a combination of LPS and IFN-γ[31]. It is important to note that our study used differentiated PBMCs that were primed with vitamin D during differentiation rather than primary alveolar macrophage, so it is possible that the inconsistencies in these studies reflects different macrophage phenotypes. Again, this suggest that vitamin D can both potentiate and inhibit the inflammatory response under different conditions.

Our study is also in contrast to an early study which suggested that $1,25(OH)_2D_3$ inhibits the proliferation of blood lymphocytes and IL-1 production[32], however, recent studies, in line with our data, have found that vitamin D generally boosts infection-stimulated cytokine and chemokine responses; including enhanced IL-1β expression in macrophages[18], enhanced macrophage survival and reduced mycobacterial burden by stimulating the anti-mycobacterial capacity of co-cultured lung epithelial cells. IL-1β exerts its protective action against infections by rapidly recruiting neutrophils to inflammatory sites. For example, IL-1 receptor knock-out mice recruit fewer neutrophils in response to influenza infection[33]. In addition, *in vitro* studies have shown that IL-1β restricts intracellular *Mycobacterium tuberculosis* (*M. tuberculosis*) growth in murine and human macrophages by regulating TNF signalling and caspase-3 activation[34]. Similar to our observation in neutrophils, these data suggested that vitamin D enhances the capacity of macrophages to respond to bacterial stimuli.

Phagocytosis of pathogens by neutrophils and macrophages is one of their key functions[35]. While localization of neutrophils to the site of inflammation is crucial for clearance of the infection, which vitamin D appears to enhance through increased IL-8 production, neither LPS nor vitamin D had any impact on the phagocytic capacity of neutrophils when challenged with *E. coli*. Interestingly, a previous study has clearly demonstrated that vitamin D enhances the phagocytic potential of macrophages that have low phagocytic capacity to live *M. tuberculosis*[36]. In contrast, our results have shown that both LPS and $1,25(OH)_2D_3$ independently decreased the phagocytic capacity of individual macrophages to engulf *E. coli* with a decrease in overall phagocytosis (overall fluorescence) but approximately the same number of cells involved in phagocytosis. Interestingly, LPS and $1,25(OH)_2D_3$ together decreased the number of macrophages that were able to phagocytose *E. coli*. While there are several differences between these studies that may explain this difference, including the length of prior treatment with vitamin D (2 vs 6 days), the pathogen used, and the length of pathogen challenge (45 minutes vs 3 hours), these observation suggest that vitamin D is a potent modulator of the phagocytic capacity of macrophages. In line with our data, *in vivo* studies have shown that mice injected with LPS had reduced phagocytic capacity in macrophages that may be due to the fact that the LPS drives macrophages to a more inflammatory, antigen presentation state[37]. There are numerous receptors involved in bacterial recognition by macrophages[38], such that, if the macrophage receptors involved in phagocytosis are reduced, occupied, or internalized by stimuli, the phagocytic capacity can be attenuated[39].

Other studies have shown that administration of vitamin D (10^{-8} M of $1,25(OH)_2D_3$) had no effects on the phagocytic capacity of human alveolar macrophages, but increased the levels of the antimicrobial peptide cathelicidin (LL-37)[31]. We assessed LL-37 levels in the supernatants from our BEAS-2B cell and macrophage experiments and found that LL-37 levels were not increased in macrophages by pre-stimulation with vitamin D (10^{-7} M of $1,25(OH)_2D_3$) during differentiation and were undetectable in BEAS-2B cells (Supplementary material; Figure S1). This again highlights the importance of cell phenotype, and protocol, in influencing the cellular response to stimulation with vitamin D. Studies have shown that vitamin D has an effect on the morphology and function of monocyte-derived macrophages[40] by inhibiting M1 macrophage activation and promoting M1-M2 phenotype switching[41,42]. However, our data appear to contradict this and it possible that by priming with vitamin D during differentiation we facilitated M1 phenotype switching with increased the production of IL-1β. These data have important implications for the clearance of pathogens whereby the effect of vitamin D on macrophage phagocytosis and antimicrobial activity seems to be dependent on the type of pathogen, the period of exposure to vitamin D, the presence of pre-existing inflammation, and macrophage phenotype.

In conclusion, vitamin D exerted different effects on different cell types. While there was no effect of vitamin D on LPS induced inflammation in epithelial cells, vitamin D enhanced the production of IL-8 in neutrophils and the production of IL-1β macrophages. Vitamin D had no effect on neutrophils phagocytic capacity but exaggerated the LPS induced decrease in macrophage phagocytic capacity. While acknowledging that we have studied these responses in isolation *in vitro*, and that these cells have the capacity to modulate $1,25(OH)_2D$ levels *in vivo* through the upregulation of 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1)[43], our data demonstrate the complexity in understanding the effect of vitamin D on responses to infection. In some instances, the net effect of vitamin D on the response to infection may be neutral due to the competing effect of vitamin D on phagocytosis and the production of cytokines that are important in the resolution of infection. This area requires further investigation if we are to understand the complex role of vitamin D in the innate immune response.

Methods

Study subjects. Bronchial epithelial cells (BEAS-2B) were purchased from American Type Culture Collection (ATCC; Manassas, VA). Whole blood samples were collected from normal healthy (no acute or chronic illnesses) volunteers (aged 20–35 years; 5 males and 5 females) to isolate peripheral blood neutrophils and monocytes. The University of Tasmania Research Ethics Committee approved all the studies, and subjects gave written, informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

Neutrophil and monocyte isolation. Neutrophils and human peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Lympholyte-poly cell separation media (Cedarlane Labs, Burlington, Canada) composed of sodium diatrizoate and dextran 500[44]. 30 mL of freshly drawn blood was collected in Vacutainer collection tubes (BD, San Jose, CA) containing 15% K_3EDTA solution and then

mixed at a ratio of 1:1 (vol/vol) with PBS at room temperature and 15 mL of the mixture was layered with 15 mL of Lympholyte-poly in a 50 mL tube. After centrifugation at 450 g for 35 min at 20 °C, neutrophils and PBMCs buffy coats were collected separately. Neutrophils buffy coats were washed with ice-cold HBSS (without Ca^{2+}/Mg^{2+}) and centrifuged at 450 g for 5 min. The remaining red blood cells were lysed by hypotonic treatment with Red Blood Cell Lysis Buffer (Roche, Basel, Switzerland). This method has been show to yield samples of >95% neutrophils with >95% viability[45]. PBMCs buffy coats were washed with ice-cold PBS containing 0.1% BSA and 2 mM EDTA and centrifuged at 450 g for 5 min. Monocytes were purified from freshly isolated PBMCs using MACS CD14 microbeads according to the manufacturer's introductions (Miltenyi Biotec, Bergisch Gladbach, Germany), which are used for positive selection of human monocytes and macrophages from PBMCs[46]. Briefly, monocytes suspension was passed through the Pre-Separation filter then incubated with CD14 microbeads for 15 min at 4 °C, to allow separation of $CD14^+$ monocytes.

Cytokines. The concentrations of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α) in the supernatants from the cell culture medium were measured using DuoSet ELISA kits (R&D Systems, Minneapolis, MN). The absorbance was read at 450 nm/570nm using a spectrophotometer (Spectramax M2, Molecular Devices, Sunnyvale, CA).

BEAS-2B inflammatory response. BEAS-2B cells were cultured in bronchial epithelial cell growth medium (BEGM) (Lonza, Walkersville, MD) at 37 °C in a 5% CO_2 humidified incubator. BEAS-2B cells were seeded in 12-well plates at a density of 5×10^5 cells/mL with either 0.1% ethanol (vehicle control) or 10^{-7} M of $1,25(OH)_2D_3$ (Enzo Life Sciences, Farmingdale, NY) for 24 hours. After 24 hours, the culture media was changed, and cells were treated with 10 ng/mL of LPS (Sigma-Aldrich, St.Louis, MO) in the presence of vehicle control or 10^{-7} M of $1,25(OH)_2D_3$ for 24 hours at which point cell supernatants were collected for enzyme-linked immune sorbent assay (ELISA) assessment of IL-1β, IL-6, IL-8 and TNF-α.

Neutrophil inflammatory response. Isolated neutrophils were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA) supplied with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St.Louis, MO) and 1% Penicillin-Streptomycin (Pen-Strep) (Sigma-Aldrich, St.Louis, MO) at 37 °C in a 5% CO_2 humidified incubator. Neutrophils were seeded in 12-well plates at a density of 1×10^6 cells/mL with either 0.1% ethanol (vehicle control) or 10^{-7} M of $1,25(OH)_2D_3$ for 24 hours prior to treatment with 10 ng/mL of LPS in the presence of vehicle control or 10^{-7} M of $1,25(OH)_2D_3$ for another 24 hours. Cells suspensions were centrifuged and the supernatant collected for ELISA assessment of IL-1β, IL-6, IL-8 and TNF-α.

Monocyte inflammatory response. The enriched $CD14^+$ monocytes from PBMCs were cultured in RPMI-1640 medium supplied with 10% FBS and 1% Pen-Strep at 37 °C in a 5% CO_2 humidified incubator. Monocytes were seeded in 12-well plates at a density of 5×10^5 cells/mL, and differentiated using 100 ng/mL of macrophage colony stimulating factor (M-CSF) (Sigma-Aldrich, St.Louis, MO) in the presence of vehicle control or 10^{-7} M of $1,25(OH)_2D_3$ for 6 days with fresh $1,25(OH)_2D_3$ added every day[30]. At day 7, M-CSF containing media was changed, and PBMCs differentiated macrophages were treated with 10 ng/mL of LPS in the presence of vehicle control or 10^{-7} M of $1,25(OH)_2D_3$ for 24 hours. The supernatants were centrifuged and the supernatant collected for ELISA assessment of IL-1β, IL-6, IL-8 and TNF-α.

Phagocytic capacity of neutrophils and macrophages. Fluorescein conjugated Escherichia coli (K-12 strain) (Thermo Fisher Scientific, Waltham, MA) was used to assess phagocytic capacity. Briefly, after 24 hours of stimulation with LPS, neutrophils or macrophages were incubated with Fluorescein conjugated *E. coli* at multiplicity of infection (MOI) of 5 for 45 minutes at 37 °C in a 5% CO_2 humidified incubator. The reaction was stopped with ice. Cells were collected and washed 3 times with cold PBS, then phagocytes were fixed in 1% paraformaldehyde before been measured by flow cytometer BD FACSCANTO II (BD Biosciences, San Jose, CA). Optimal removal of macrophages required vigorous pipetting. Flow cytometry was performed to estimate the percentage of phagocytes which had engulfed bacteria and quantify the fluorescence intensities of *E. coli* in engulfed phagocytes. Flow cytometry data were analysed using FCS Express 6 (De Novo Software, Glendale, CA).

Statistical analysis. Group comparisons were made using two-way analysis of variance (Two-way ANOVA) with Holm-Sidak posthoc tests. Data were log-transformed where necessary to satisfy the assumptions of the test (normality and homoscedasticity). Data were analysed in SigmaPlot (Systat, Erkrath, Germany) and reported as mean (SD). P values of <0.05 were considered statistically significant.

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Author Contributions

L.C. and M.E. carried out the experiments in the study. L.C. and G.R.Z. analysed the data and interpreted the results. L.C. and G.R.Z. conceived the study, and participated in its design. L.C. coordinated and drafted the manuscript. All authors have read and approved the final manuscript.

Additional Information

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Association of Vitamin D Status with SARS-CoV-2 Infection or COVID-19 Severity: A Systematic Review and Meta-analysis

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ABSTRACT

This systematic review was conducted to summarize and clarify the evidence on the association between 25-hydroxyvitamin-D [25(OH)D] concentrations and coronavirus disease 2019 (COVID-19) risk and outcomes. PubMed, Scopus, and Web of Science databases and Google Scholar were searched up to 26 November 2020. All retrospective and prospective cohort, cross-sectional, case-control, and randomized controlled trial studies that investigated the relation between 25(OH)D and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and COVID-19 severity were included. Thirty-nine studies were included in the current systematic review. In studies that were adjusted (OR: 1.77; 95% CI: 1.24, 2.53; I^2: 44.2%) and nonadjusted for confounders (OR: 1.75; 95% CI: 1.44, 2.13; I^2: 33.0%) there was a higher risk of SARS-CoV-2 infection in the vitamin D deficiency (VDD) group. Fifteen studies evaluated associations between VDD and composite severity. In the studies that were adjusted (OR: 2.57; 95% CI: 1.65, 4.01; $I^2 = 0.0\%$) and nonadjusted for confounders (OR: 10.61; 95% CI: 2.07, 54.23; $I^2 = 90.8\%$) there was a higher severity in the VDD group. Analysis of studies with crude OR (OR: 2.62; 95% CI: 1.13, 6.05; I^2: 47.9%), and adjusted studies that used the Cox survival method (HR: 2.35; 95% CI: 1.22, 4.52; I^2: 84%) indicated a significant association of VDD with mortality, while in adjusted studies that used logistic regression, no relation was observed (OR: 1.05; 95% CI: 0.63, 1.75; I^2: 76.6%). The results of studies that examined relations between VDD and intensive care unit (ICU) admission, pulmonary complications, hospitalization, and inflammation were inconsistent. In conclusion, although studies were heterogeneous in methodological and statistical approach, most of them indicated a significant relation between 25(OH)D and SARS-CoV-2 infection, COVID-19 composite severity, and mortality. With regard to infection, caution should be taken in interpreting the results, due to inherent study limitations. For ICU admission, inflammation, hospitalization, and pulmonary involvement, the evidence is currently inconsistent and insufficient. *Adv Nutr* 2021;00:1–23.

Keywords: COVID-19, vitamin D, severity, infection, SARS-CoV-2

Introduction

Vitamin D deficiency (VDD) and insufficiency in adults and children, as a global problem, is associated with several disorders, including metabolic disorders, autoimmune diseases, cardiovascular disease, diabetes, and infections, and has been widely considered by researchers and clinicians (1). In particular, several studies have investigated the link between the risk of respiratory tract infections and VDD (2). For instance, Mamani et al. (3) reported an association between incidence of community-acquired pneumonia and low serum concentrations of 25-hydroxyvitamin D [25(OH)D], and adverse outcomes were observed in acute respiratory distress syndrome (ARDS) patients with VDD (4).

Vitamin D is a fat-soluble vitamin that plays an important role in several physiological processes, such as bone metabolism, calcium and phosphorus absorption, and immune system function (5). It may reduce the risk of microbial infections through stimulating innate cellular immunity, inhibiting the cytokine storm, decreasing proinflammatory cytokine production, and modulating the adaptive immune response (6). Vitamin D3 and vitamin D2 are 2 primary metabolites of vitamin D (7). Unstable 7-dehydrocholesterol in the skin is transformed to pre-vitamin D3 and stable vitamin D3, respectively, when exposed to UV-B radiation (8). Vitamin D3, or cholecalciferol, can also be found in foods, such as dairy products, eggs, and fish (9). Vitamin D3 is subsequently converted to 25-hydroxyvitamin D3

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($25(OH)D_3$) through 25-hydroxylase enzyme activity during the hydroxylation process in the liver. The $25(OH)D_3$ form then transfers to the kidney and converts to 1α,25-dihydroxyvitamin D3 via 1α-hydroxylase, otherwise known as calcitriol, the active form of vitamin D (8, 10).

Currently, the global community is involved in a novel pandemic named coronavirus disease 19 (COVID-19), a respiratory tract infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (11). The WHO reported the total global cases of SARS-CoV-2 infection and death as >61.8 and 1.4 million, respectively (weekly epidemiological update, 1 December 2020) (12). This novel coronavirus (SARS-CoV-2), like the other viruses of the β-coronavirus family, is extremely contagious, and COVID-19 symptoms vary from initially mild symptoms such as dry cough, fever, fatigue, and gastrointestinal symptoms, to severe situations requiring admission to an intensive care unit (ICU) or death in severe cases (13, 14). In some cases, inflammation can increase following both local and systemic immune responses generated by this virus and an increased number of leukocyte and concentrations of plasma proinflammatory cytokines have been reported in patients infected with SARS-CoV-2 (15).

Several studies have investigated the association of $25(OH)D_3$ concentrations and supplementation with the risk and severity of respiratory virus infections (16, 17). Indeed, Martineau et al. (18) conducted a meta-analysis that included 25 placebo-controlled clinical trials (total of 10,933 people) and concluded that vitamin D supplementation reduces the risk of acute respiratory infections, especially in people with the lowest 25(OH)D concentrations.

Recently, a growing body of evidence has emerged regarding potential factors affecting the incidence and severity of COVID-19 (19–21). Recent reports highlight that certain factors may be effective in controlling this pandemic or reducing the damage caused by it. Indeed, based on the global prevalence of VDD (22), it has attracted considerable attention as a potential factor associated with the risk or severity of COVID-19, and several studies have reported on this possible association (6, 23–25). However, results currently preclude a clear consensus. Thus, we conducted this systematic review to summarize and clarify the evidence on the association between 25(OH)D concentrations and COVID-19 risk and outcomes.

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Supplemental Tables 1–15 and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.
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Abbreviations used: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ICU, intensive care unit; RCT, randomized controlled trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGF-β, transforming growth factor β; Th, T-helper; VDD, vitamin D deficiency; VDR, vitamin D receptor; WMD, weighted mean difference; 25(OH)D, 25-hydroxyvitamin-D.

Methods

The protocol of this study has been registered in PROSPERO International Prospective Register of Systematic Reviews (www.crd.york.ac.uk/prospero/index.asp, identifier CRD42020203903). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was used in developing and conducting this systematic review (26).

Search strategy and study selection

PubMed, Scopus, and Web of Science databases and the first 500 Google Scholar search results were searched up to 26 November 2020, with no restriction in language. Reference lists of included studies and relevant review articles were also scanned for additional relevant studies. The following search strategy was used for our search: (Coronavirus or COVID-19 or SARS-CoV-2) AND (vitamin D or 25-OH-D or cholecalciferol or 25-hydroxycholecalciferol or calcitriol or 25-hydroxyvitamin D or hydroxycholecalciferols or 25-hydroxyvitamin D3).

Two reviewers independently assessed the eligibility of studies. Studies that met the following criteria were included: *1*) study design as retrospective, prospective, or cross-sectional, or case-control studies reporting serum/plasma concentrations of 25(OH)D; *2*) participants as patients diagnosed with COVID-19 with no restriction on age; *3*) exposure/intervention as serum/plasma concentrations of vitamin D either reported as a continuous or categorical variable (deficiency vs. sufficiency); and *4*) outcome as SARS-CoV-2 infection or COVID-19 severity, with severity defined as at least 1 of the following outcomes—ARDS and/or mechanical ventilation, ICU admission, length of hospitalization, and death. The exclusion criteria were as follows: *1*) case reports, abstracts, and summaries of discussion; *2*) insufficient data on vitamin D measurement or COVID-19 outcomes; *3*) preprint studies without peer review; and *4*) studies that were not individual based (compared countries or regions).

Data extraction and quality assessment

The following data were extracted independently by 2 reviewers: first author, study design, start and completion date, geographical location, age and gender composition of patients, objective of the study [if the aim of the study was to assess association of 25(OH)D status with risk of SARS-CoV-2 infection or to assess the association with severity of disease], definition of VDD, time of serum 25(OH)D measurement, prevalence of VDD and insufficiency, definition of disease severity, the number of events and nonevents in the case and control groups, relative risk and 95% CIs for SARS-CoV-2 infection and disease severity, and adjustment factors.

Quality assessment of observational studies was assessed using the Newcastle–Ottawa Scale, which included 3 items: selection, comparability, and outcome (27). Studies with a score of $\geq$7 were defined as high quality. The Cochrane risk-of-bias tool was used to evaluate quality assessment

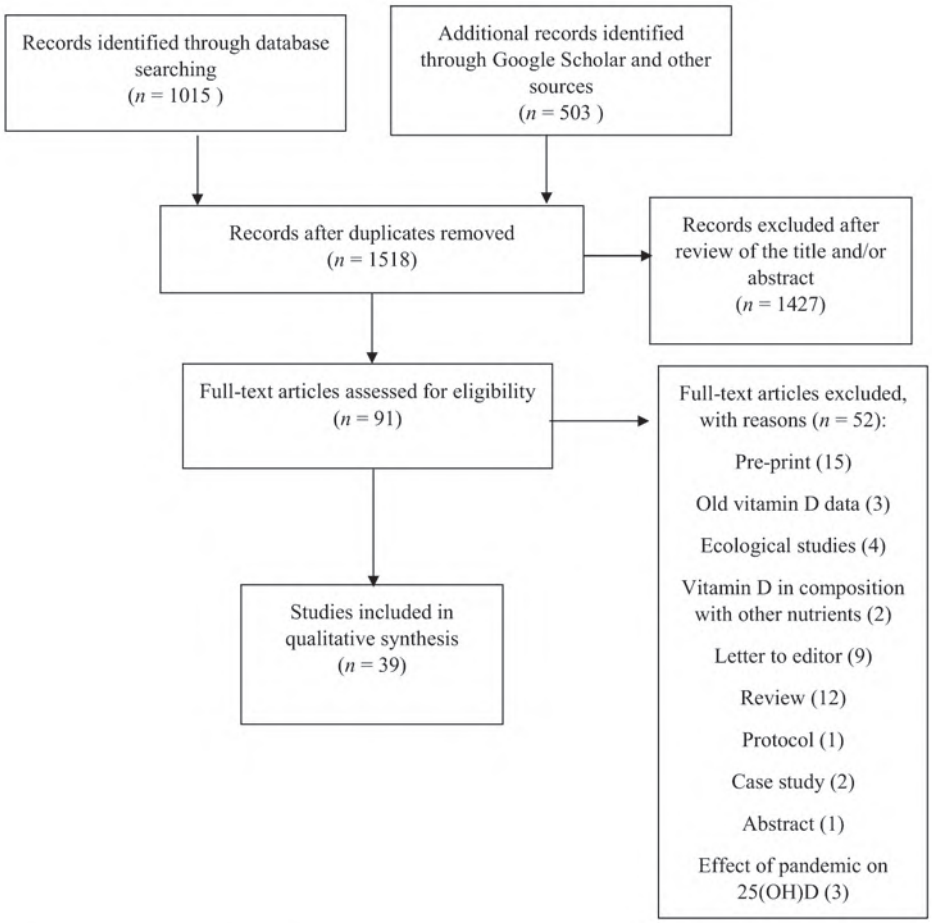

FIGURE 1 Summary of the process for selecting studies that investigated the association of vitamin D status with SARS-CoV-2 infection and COVID-19 severity. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; 25(OH)D, 25-hydroxyvitamin-D.

of randomized trials. This tool included selection bias, performance and detection bias, attrition bias, reporting bias, and the other biases (28).

Statistical analysis

Wherever it was probable, we pooled data and conducted meta-analysis (SARS-CoV-2 infection, disease severity, ICU admission, and mortality). We used ORs to estimate the association between VDD and SARS-CoV-2 infection and COVID-19 severity. ORs with 95% CIs were obtained using a random-effects model. In studies that did not report relative risk, the OR was calculated by the number of events and nonevents in the case and control groups; these studies together with studies with crude ORs were analyzed separately from the studies that reported adjusted relative risk. To compare concentrations of 25(OH)D_3 between groups, we used the weighted mean difference (WMD) and its 95% CI. Heterogeneity was evaluated using Cochran's Q test, deriving its magnitude from the I^2. If at least 10 studies were available, we explored potential small-study effects, such as publication bias, using visual examination of the funnel plot and Egger's test (29). All analyses were conducted using Stata version 13 software (StataCorp).

Results

Characteristics of the study population

As described in **Figure 1**, 1518 records were obtained by the literature search. Of these, 57 articles met the inclusion criteria; however, 3 studies were excluded because they used old 25(OH)D data, and 15 papers were preprints (**Supplemental Table 1**). Finally, 39 studies were included, with different geographical locations and ethnic backgrounds, including Europe (n = 17 studies), North America (United States) (n = 2), South America (n = 2), West Asia (n = 9), South Asia (n = 4), East Asia (n = 4), and Africa (n = 1). Ten studies were of a case-control design, 19 cross-sectional, 2 retrospective cohorts, 2 randomized controlled trials (RCTs), 2 quasi-experimental design, and 4 studies were only descriptive. All studies were conducted in adults, except for 1 study in children and 1 study in pregnant women. All studies,

except for 2, included both male and female participants; in 1 study, participants were only male (30), and in another, only females were included (31). Nine studies were not included in the analysis because 4 of them were only descriptive [only reported concentration of 25(OH)D in patients; **Supplemental Table 2**] (31–34), 1 study was in children (35), and 4 were different in design from other studies [they assessed the effect of 25(OH)D_3 supplementation instead of 25(OH)D measurement] (14, 36–38).

Twenty-one studies examined the association of 25(OH)D concentrations with the severity, 14 studies with SARS-CoV-2 infection, whereas 10 of them assessed severity as a secondary outcome. Characteristics of studies that examined the association of vitamin D with SARS-CoV-2 infection are summarized in **Table 1**, and those examining COVID-19 severity are summarized in **Table 2**.

Association of 25(OH)D status with SARS-CoV-2 infection

Nine studies evaluated the relation between VDD and SARS-CoV-2 infection. Studies that were adjusted ($n = 3$) (39–41) (OR: 1.77; 95% CI: 1.24, 2.53; I^2: 44.2%; **Figure 2**A) and nonadjusted for confounders ($n = 5$) (42–45, 46) (OR: 1.75; 95% CI: 1.44, 2.13; I^2: 33%; Figure 2B) indicated higher risk of infection in the VDD group (Figure 2). The Blanch-Rubió et al. (37) study was not included in analysis, because of a different design. This study was a cross-sectional study including 2102 patients with noninflammatory rheumatic conditions and found that no association between intake of vitamin D supplement and COVID-19 (risk ratio: 0.91; 95% CI: 0.62, 1.34).

Twelve studies compared 25(OH)D concentration between COVID-19 patients and healthy subjects. The pooled analysis of 10 studies (41–49) revealed a lower concentration of 25(OH)D in cases compared with controls (WMD = −7.0 ng/mL; 95% CI: −9.49, −4.50; $I^2 = 92.4\%$; cases, $n = 1899$; controls, $n = 11{,}122$; **Supplemental Figure 1**). Subgroup analysis indicated a greater difference in the studies that measured 25(OH)D after a SARS-CoV-2 test (WMD = −10.28 ng/mL; 95% CI: −14.41, −6.16; $I^2 = 90.1\%$; $n = 6$ studies) compared with studies that used 25(OH)D data collected before a SARS-CoV-2 test (WMD = −3.0 ng/mL; 95% CI: −5.15, −0.86, $I^2 = 80.3\%$; $n = 4$ studies). Two studies were not included in the analysis (35, 50); both studies indicated that 25(OH)D concentrations were significantly lower in cases compared with controls. In 1 study, the participants were children (35); the other study only reported that COVID-19 patients had a significantly lower 25(OH)D concentration compared with healthy counterparts; however, the mean ± SD values of 25(OH)D were not provided (50). Results of studies are summarized in **Supplemental Table 3**.

Association of vitamin D status with COVID-19 severity

Twenty-one studies assessed the association of VDD with severity (composite severity or 1 feature of severity) as a primary outcome, and 10 studies as a secondary outcome.

Composite severity

Fifteen studies evaluated the association between VDD and composite severity. Studies that were adjusted (38, 41, 44, 46, 51, 52) (OR: 2.57; 95% CI: 1.65, 4.01; $I^2 = 0.0\%$; **Figure 3**A) and nonadjusted for confounders (42, 45, 53–55) (OR: 10.61; 95% CI: 2.07, 54.23, $I^2 = 90.8\%$; Figure 3B) revealed a higher severity in the VDD group. Four studies were not included in the analysis; one of these studies was conducted in children and found a negative correlation between fever symptom and 25(OH)D concentration ($P = 0.02$), while no significant correlations were found between other clinical parameters and 25(OH)D concentration (35). The other study had a quasi-experimental design and indicated that vitamin D3 supplementation was inversely associated with Ordinal Scale for Clinical Improvement (OSCI) score for COVID-19 ($\beta = -3.84$; 95% CI: −6.07, −1.62; $P = 0.001$) (56). The third study, which assessed vitamin D supplementation in patients with a past history of COVID-19, found that it reduces the risk of exacerbation and worsening of the disease (OR: 0.29; 95% CI: 0.10, 0.083; $P = 0.02$) (57). The last study did not provide sufficient data, and only reported that VDD was significantly associated with severity; however, no data were available to indicate this (58). Results of studies have been summarized in **Supplemental Table 4**.

ICU admission or stay

Four studies examined the relation between VDD and ICU admission and 1 study between VDD and ICU stay duration. Pooled analysis of 3 studies (38, 44, 59) with unadjusted ORs indicated no significant relation between VDD and ICU admission (OR: 1.17; 95% CI: 0.67, 2.03; $I^2 = 69.3\%$), while an RCT that was not pooled with these studies revealed a lower risk of ICU admission in the intervention group compared with the control group (OR: 0.03; 95% CI: 0.003, 0.25; $P = < 0.001$) (36). Carpagnano et al. (59) verified the association of VDD with ICU stay, highlighting that 10 patients with severe VDD had a median ICU stay of 8 d with the interquartile range (IQR) of 6 to 11.25., while 32 patients without VDD had a median stay of 12.5 d (IQ25 8, IQ75 20.5) (**Supplemental Table 5**).

Pulmonary complications

Eight studies investigated the association of VDD with one of the pulmonary complication indicators. In Abrishami et al. (60), an increase in 25(OH)D concentrations yielded a reduction in the development of severe lung involvement (OR: 0.96; 95% CI: 0.93, 0.98; $P = 0.04$). Pizzini et al. (61) found no significant difference between 25(OH)D concentrations in patients with or without computed tomographic (CT) abnormalities (22 vs. 21.6 ng/mL; $P = 0.83$). Three studies assessed the relation between 25(OH)D concentration and progression to ARDS. In a prospective study in 33 hospitalized patients, the patients who progressed to ARDS had a lower serum 25(OH)D concentration on presentation to the hospital compared with non-ARDS patients [mean (SD): 10.8 (4.8) ng/mL in ARDS and 16.4 (7.6) ng/mL in non-ARDS patients; $P = 0.03$] (30), while there was no difference

TABLE 1 Characteristics of studies investigated association of vitamin D status with SARS-CoV-2 infection[1]

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Definition of VitD deficiency	Time of VitD ascertainment	Objective/study question	Adjusting factors
Bahat (31)	April and June, 2020	A tertiary referral hospital, Turkey	Descriptive	44 SARS-CoV-2-positive (+) pregnant women who were hospitalized, >8 wk of gestation	Mean age: 28.57; female: 100%	Serum 25(OH)D <20 ng/mL	On the day of admission	To measure serum 25(OH)D concentration in SARS-CoV-2+ pregnant women	—
Baktash (47)	March 1 and April, 2020	General hospital in the UK	Prospective cohort	105 elderly (>65 y) participants, 70 SARS-CoV-2+, 35 SARS-CoV-2 negative (–)	Mean age: 81.28; patients: 60% male; healthy: 40%	Serum 25(OH)D ≤12 ng/mL	Concurrent with SARS-CoV-2 test	Relation between VDD and SARS-CoV-2 infection	No adjustment for confounders; another limitation is vitamin D intake after the acute phase of illness
Blanch-Rubió (37)	March 1 to May 3, 2020	Rheumatology service of hospital, Spain	Cross-sectional	2102 patients with noninflammatory rheumatic conditions	Mean age: 66.4; 80.5% female	—	—	Effect of vitamin D intake on COVID-19 incidence	Sex, age, comorbidities, treatment, and drugs
D'Avolio (48)	March 1 to April 14, 2020	Switzerland	Retrospective cohort	27 SARS-CoV-2+, 80 SARS-CoV-2–	Median age: 73, IQR (63 to 81); male: 54.2%	—	The vitamin D analysis was required to be conducted within 7 wk of the SARS-CoV-2 PCR result	Describing the 25(OH)D plasma concentrations in a cohort of patients from Switzerland	—
De Smet (42)	March 16 to April 16, 2020	General hospital in Belgium	Retrospective observational study	186 SARS-CoV-2+ hospitalized patients and 2717 diseased controls	Patients: median age, (IQR): 69 (52–80); male: 58.6%; controls: 68 (49–82); male: 36.8%	Serum 25(OH)D <20 ng/mL	Measured after SARS-CoV-2 test	Are lower 25(OH)D concentrations correlated with COVID-19?	—
Ferrari (43)	February to April, 2020	The San Raffaele Hospital, Milan, Italy	Retrospective cohort	128 SARS-CoV-2+, 219 SARS-CoV-2–	Patients: 64.8% males; male age: 62.7; female age: 69.3; healthy: 48.85% males; male age: 62.8, female age: 54.3	Serum 25(OH)D ≤30 ng/mL	The average time interval between SARS-CoV-2 test and their corresponding 25(OH)D measurements for the positive group was 33.9 and for the negative group was 33.33 d	—	—

(*Continued*)

TABLE 1 (*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Definition of VitD deficiency	Time of VitD ascertainment	Objective/study question	Adjusting factors
Hernández (44)	March 10 to March 31, 2020	University Hospital, Spain	Retrospective case-control study	216 SARS-CoV-2+ and 197 population-based controls; in COVID-19 patients: number of VDD: 35; number of non-VDD: 162	Cases: age, median (IQR): 61.0 (47.5–70.0); controls: 61.0 (56.0–66.0); male: 62.4% in both groups	Serum 25(OH)D <20 ng/mL	At admission	To assess serum 25(OH)D concentrations in hospitalized patients with COVID-19 and to analyze the possible influence of vitamin D status on disease severity	—
Im (45)	February to June, 2020	Inha University Hospital, South Korea	Case-control	50 patients with SARS-CoV-2+ and 150 controls	Mean age: 57.5 in case and 52.2 in control groups; male: 58%	Serum 25(OH)D_3 <20 ng/mL	Within 7 d of admis?sion	Prevalence of VDD among COVID-19 patients, comparing vitamin D status between COVID-19 patients and healthy individuals	Control group was matched for age and sex with the COVID-19 group
Kerget (50)	March 24, to May 15, 2020	University Hospital in Turkey	Case-control	88 SARS-CoV-2+, 20 SARS-CoV-2–	Mean age: cases: 49.1; male: 60%; controls: 35.2; male: 40%	—	Fifth day of admission to hospital	To determine the relation of serum vitamin D concentration between patients and healthy controls	—
Luo (46)	February 27 to March 21, 2020	Hospital in China	Cross-sectional	335 COVID-19 patients, age- and sex-matched population of 560 individuals	Patients: median (IQR) age: 56 (43–64); male: 44.2%; controls: age: 55 (49.0–60.0); male: 45.9%	Serum 25(OH)D <30 ng/mL	In control, serum 25(OH)D concentrations were measured during the same period from 2018–2019; in patients, serum 25(OH)D concentrations were measured on admission	To investigate whether VDD is associated with COVID-19 incidence	Age, sex, comorbidities, smoking status, and BMI
Mardani (49)	March, 2020	A general clinic, Iran	Case-control	63 SARS-CoV-2+, 60 SARS-CoV-2–	Median age of 39; male: 52%	Deficient [25(OH)D <10 ng/mL], insufficient [25(OH)D: 10–30 ng/mL]	At baseline of the study	Relation between VDD and SARS-CoV-2 infection	Not adjusted

TABLE 1 (*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Definition of VitD deficiency	Time of VitD ascertainment	Objective/study question	Adjusting factors
Meltzer (39)	March 3 to April 10, 2020	Academic hospital in USA	Retrospective cohort study	63 SARS-CoV-2+, 365 SARS-CoV-2–	Mean age: 45.7; male: 25.2%	VDD was defined by the most recent 25(OH)D <20 ng/mL or 1,25(OH)D <18 pg/mL	Within 1 y before SARS-CoV-2 test (subjects received treatment in this duration were excluded)	Is VDD associated with positive test for SARS-CoV-2?	Demographic and comorbidity
Merzon (40)	February 1 to March 30, 2020	Health Services in Israel	Retrospective cohort study	782 SARS-CoV-2+, 7025 SARS-CoV-2–	SARS-CoV-2+: mean age: 35.6; male: 49.23%; SARS-CoV-2–: mean age: 47.4; male: 40.6%	"Suboptimal" or "low": plasma 25(OH)D <30 ng/mL	At least 1 previous blood test for plasma 25(OH)D concentration	Is VDD risk factor for SARS-CoV-2 infection?	Demographic variables, psychiatric and somatic disorders
Sun (34)	February to February, 2020	Hospital University in Wuhan, China	Descriptive	241 patients with confirmed COVID-19	Median age: 65 (IQR: 55–72); male: 46.4%	—	Within 24 h of admission	25(OH)D concentration in SARS-CoV-2+ adults	—
Ye (41)	February to March, 2020	A Hospital in China	Case-control	62 SARS-CoV-2+, 80 healthy controls	Controls: median age (IQR): 42 (31–52); male: 40%; patients: age: 43 (32–59); male: 37%	25(OH)D <20 ng/mL	At admission	To examine the relation between serum 25(OH)D concentration and SARS-CoV-2 infection	Demographics and comorbidities
Yılmaz (35)	March to May, 2020	University Hospital in Turkey	Case-control	85 children (40 SARS-CoV-2+ and hospitalized, 45 healthy children in control group)	COVID-19 patients: 101.76 mo; male: 47.5%; controls: 75.68 mo; male: 60%	25(OH)D <12 ng/mL	From retrospective file records	Is VDD a risk factor for COVID-19 in children?	None

[1]COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction; ref, reference; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VDD, vitamin D deficiency; VitD, vitamin D; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D3, 25-hydroxyvitamin D3; 1,25(OH)D, 1,25-hydroxyvitamin D.

TABLE 2 Characteristics of studies investigated association of vitamin D status with COVID-19 severity[1]

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Objective/study question	Severity definition/vitamin deficiency definition	Time of VitD ascertainment	Adjusting factors
Abrishami (60)	February to April, 2020	Academic hospital in Iran	Retrospective study	73 SARS-CoV-2–positive (+) patients	Mean age: 55.18; male: 46.4%	To evaluate the prognostic role of serum 25(OH)D_3 on the extent of lung involvement and final outcome in patients with COVID-19	Lung involvement and mortality; serum 25(OH)D <25 ng/mL	At admission	For mortality, multivariate linear regression analysis adjusted for potential confounders including sex, age, and comorbidity
Anjum (62)	March to June, 2020	A hospital in Pakistan	Prospective	140 SARS-CoV-2+ patients	Mean age: 42.46; age range: 15–75; male: 58.57%	To determine the association between severe VDD and mortality in patients with COVID-19	Severity was defined as mortality; severe VDD was defined as 25(OH)D <10 ng/ml	At admission	—
Annweiler (56)	March to April, 2020	Nursing home in France	Quasi-experimental study with mean follow-up of 36 d	66 frail elderly nursing-home residents: intervention, *n* = 57; comparator, *n* = 9	Experiment: mean age: 87.7; male: 21% Comparator: mean age: 87.4; male: 33%	To evaluate COVID-19 severity and the use of COVID-19 drugs; the primary and secondary outcomes were COVID-19 mortality and OSCI score in acute phase	OSCI score	The intervention group received VitD3 (single dose of 80,000 IU every 2–3 mo) during COVID-19 or in the preceding month; the comparator group corresponded to all other participants	Age, gender, drugs, functional abilities, albuminuria
Annweiler (51)	March to May, 2020	One geriatric acute care unit dedicated to COVID-19 patients in France	Quasi-experimental study	Group 1 (*n* = 29), group 2 (*n* = 16), group 3 (*n* = 32)	Mean age: 88; male: 51%	14-day mortality and highest (worst) score on the OSCI measured during COVID-19 acute phase	To determine whether vitamin D3 supplementation taken either regularly over the preceding year or after the diagnosis of COVID-19 was	Group 1 (*n* = 29): supplemented regularly with VitD over the preceding year Group 2 (*n* = 16): supplemented with VitD after	Potential confounders were age, gender, functional abilities, undernutrition, chronic

(*Continued*)

TABLE 2 (*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Objective/study question	Severity definition/vitamin deficiency definition	Time of VitD ascertainment	Adjusting factors
							effective in improving survival among hospitalized frail elderly COVID-19 patients; severe COVID-19 defined as an OSCI score ≥5	COVID-19 diagnosis Group 3 ($n = 32$): comparator received no VitD	disease, drugs; the 3 groups were similar in the treatments used for COVID-19
Arvinte (63)	May, 2020	ICU of medical center in Colorado, USA	Cross-sectional, descriptive	21 critically ill COVID-19 patients hospitalized; 11 survived, 10 died	Median age 61; age range: 20–94; male: 71.4%	To measure serum $25(OH)D_{2,3}$ in patients with critical COVID-19 illness and to assess if VDD correlated with other illness risk factors	Severity was defined as mortality	At ICU admission	—
Baktash (47)	March to April, 2020	General hospital in the UK	Prospective cohort study	70 elderly SARS-CoV-2+ individuals (aged ≥65 y); VDD patients: ($n = 39$); non-VDD patients: ($n = 31$)	Mean age: 81.28; Male: 60% in COVID-19 patients and 40% in non–COVID-19 patients	Vitamin D status and outcomes for hospitalized older patients with COVID-19	Noninvasive ventilation and high-dependency unit; clinical markers of disease severity; 25(OH)D ≤12 ng/mL	Concurrent with SARS-CoV-2 test	Not adjusted for confounders; another limitation is the supplementation of VitD after the acute phase of illness
Bagheri (57)	March to May 2020	University hospital in Iran	Cross-sectional	103 outpatients and 28 hospitalized patients	Mean age: 43.74 in outpatients and 58.77 in inpatients	The vitamin D supplementation pattern in past history of patients with COVID-19 in a cross-sectional inquiry	Severity was considered as hospitalization	Supplemented or not supplemented with vitamin D	Adjusted for the factors affecting the severity of this disease
Carpagnano (64)	March 11 to April 30, 2020	Italy, hospital policlinic	Retrospective, observational study	42 patients with ARF due to COVID-19, treated in respiratory intermediate care unit, and no need of intubation or invasive ventilation	Mean age: 65; male: 71%	Assessing any correlations with disease severity and prognosis	Transfer to ICU, death; vitamin D insufficiency, moderate deficiency, and severe deficiency were defined as 25(OH)D concentrations of 20–29, 10–19, and <10 ng/mL, respectively	Measured after SARS-CoV-2 test	—

TABLE 2 (*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Objective/study question	Severity definition/vitamin deficiency definition	Time of VitD ascertainment	Adjusting factors
Entrenas Castillo (36)	May, 2020	University hospital, Spain	RCT	76 patients hospitalized with SARS-CoV-2 infection (50 in the intervention and 26 in the control)	Mean age: 53; male: 59%	Effect of calcifediol treatment on ICU admission and mortality rate among patients hospitalized for COVID-19	Admission to ICU, (0.53 mg VitD at admission, 0.26 mg at day 3 and 7, and then weekly until discharge or ICU admission)	Not measured	Adjusted for variables that were different between groups at baseline (HTN, DM); MLR analysis for probability of the ICU admission
Cereda (65)	March to April, 2020	Italian tertiary referral hospital	Single-center cohort study	129 COVID-19 patients: VDD group, $n = 99$; non-VDD, $n = 30$	Median age: 77 (IQR, 65.0, 85.0); male: 54.3%	To determine the prevalence of VDD in COVID-19 patients and explore its association with clinical outcomes of disease severity	Clinical outcomes (severe pneumonia, admission to ICU and in-hospital mortality) and biochemical markers of disease severity 25(OH)D <20 ng/mL	Within 48 h since hospital admission	Age, sex, CRP, IHD, and severe pneumonia
De Smet (42)	March 1 to April 7, 2020	Belgium, general hospital	Retrospective observational study	186 hospitalized SARS-CoV-2–infected patients	Age 68.5; male: 58.6%	Are lower 25(OH)D concentrations correlated with COVID-19 severity?	Patients were classified based on the radiological lesion as early stage 1 (ground-glass opacities), progressive stage 2 (crazy paving pattern), or peak stage 3 (consolidation), 25(OH)D <20 ng/mL	Measured after SARS-CoV-2 test	None
Haraj (33)	April 17 to May 26, 2020	Endocrinology service in Morocco	Descriptive observational study	41 patients admitted to the endocrinology service for additional care after a stay in ICU	Mean age: 55 <45 years (26.8%), 45–70 years (48.8%), >70 (24.4%); 51.2% male	To assess the vitamin D status of patients with COVID-19 after a stay in intensive care	—	At the beginning of the study	—

(*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Objective/study question	definition/vitamin deficiency definition	Time of VitD ascertainment	Adjusting factors
Pérez (66)	—	Hospital Central Military Mexico		172 patients with COVID-19; cases: those who died ($n = 35$); controls: those who survived	Mean age: 51.44; male: 77.3%	Determine the association between 25(OH)D concentrations and mortality in hospitalized patients with COVID-19	Mortality was considered as severe; 25(OH)D <20 ng/dL	—	—
Radujkovic (13)	March to June, 2020	Medical university hospital, Heidelberg, Germany	Cohort	185 patients; patients with VDD ($n = 41$); non-VDD ($n = 144$); outpatients: 92; inpatients: 93	Median age: 60, IQR (49–70); male: 51%	To explore possible associations of vitamin D status with disease severity and survival	Decision for inpatient vs. outpatient admission was based on spontaneous oxygen saturation, comorbidities, and the overall performance status; based on COVID-19 severity classifications, all inpatients had severe disease (defined as tachypnea, oxygen saturation <93% at rest, or ICU requirement); 25(OH)D <12 ng/mL	At the time of admission	Adjusted for age, gender, and comorbidities
Rastogi (14)	—	Tertiary care hospital in north India	RCT	40 Asymptomatic or mildly symptomatic SARS-CoV-2+ with VDD [$25(OH)D_3$ <20 ng/mL]	Median age in the intervention group: 50.0, IQR (36–51); male: 37.5% Control: 47.5 (39.3 to 49.2); male: 58.3%	Effect of high-dose oral cholecalciferol supplementation on SARS-CoV-2 viral clearance	—	At the beginning of study	—
Ye (41)	February to March, 2020	Guangxi People's Hospital, China	Case-control	80 healthy controls and 62 patients diagnosed with COVID-19	Median age in controls: 42, IQR (31–52); male: 40% Age in cases: 43(32–59); male: 37%	To examine the relation between serum $25(OH)D_3$ concentration and COVID-19 severity, and its clinical case characteristics	Severe COVID-19 case was defined according to the guidelines of the National Health Commission of China[5]; 25(OH)D <20 ng/dL	At admission	Demographics and comorbidities

(*Continued*)

TABLE 2 (*Continued*)

First author (ref)	Study date	Country, setting	Design	Sample size, *n*	Age (y); sex	Objective/study question	Severity definition/vitamin deficiency definition	Time of VitD ascertainment	Adjusting factors
Yılmaz (35)	March to May 2020	Turkey, Dicle University Faculty of Medicine	Case-control	85 children (40 patients who were diagnosed with COVID-19 and hospitalized, 45 healthy children in the control group)	COVID-19 patients: 101.76 ± 27.91 mo; male: 47.5% Controls: 75.68 ± 27.34 mo; male: 60%	To determine the prevalence and clinical importance of VDD in children and adolescent patients who were hospitalized with the diagnosis of COVID-19	Mild: cases with upper respiratory tract infection with normal respiratory system examination Moderate: pneumonia with fever and cough but without symptoms of dyspnea and hypoxemia or cases with findings of COVID-19 on CT scan without any symptoms Severe: fever and cough in the early period who develop dyspnea and central cyanosis Critical: develop ARDS or RF rapidly 25(OH)D < 20 ng/mL	From retrospective file records	None

[1]ARF, acute respiratory failure; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CT, computed tomography; DM, diabetes mellitus; ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; GFR, glomerular filtration rate; HTN, hypertension; ICU, intensive care unit; IHD, ischemic heart disease; MAS, macrophage activation syndrome; MLR, multivariate logistic regression; OSCI, Ordinal Scale for Clinical Improvement; PaO_2, partial oxygen pressure; RCT, randomized controlled trial; ref, reference; RF, renal failure; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SpO_2, oxygen saturation; VDD, vitamin D deficiency; VitD, vitamin D; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D3, 25-hydroxyvitamin D3; 1,25(OH)D, 1,25-hydroxyvitamin D.

[2]Chinese Clinical Guideline for classification of COVID-19 severity. Moderate: fever and pulmonary symptoms along with pneumonia on radiologic imaging. Severe: the presence of any of the following criteria: *1*) respiratory distress (≥30 breaths/min), *2*) oxygen saturation ≤93% at rest, *3*) PaO_2/FiO_2 ≤300 mmHg or chest imaging shows obvious lesion progression >50% within 24–48 h.

[3]Commission and State Administration of Traditional Chinese Medicine: *1*) mild: mild symptoms with no signs of pneumonia on imaging; *2*) moderate: fever, respiratory symptoms with radiological evidence of pneumonia; *3*) severe [i.e., meeting any of the following: respiratory distress, respiratory rate ≥30 breaths/min, hypoxemia, SpO_2 ≤93% (at rest), or lung infiltrates of >50% within 24–48 h]; and *4*) critical (i.e., meeting any of the following criteria: respiratory failure requiring mechanical ventilation, shock, or multiple organ dysfunction requiring ICU monitoring and treatment).

[4]CDC criteria were used for the disease severity and prognosis, which includes mild–moderate (mild respiratory symptoms and fever on an average of 5–6 d after infection), severe disease (dyspnea, respiratory frequency ≥30 breaths/min, blood oxygen saturation ≤93%, and/or lung infiltrates >50% of the lung field within 24–48 h) and critical (respiratory failure, septic shock, and/or multiple-organ dysfunction/failure).

[5]Per Guidelines of the National Health Commission of China severe cases met at least 1 of the following criteria: *1*) respiratory rate >30 breaths/min, *2*) pulse oximeter SpO_2 ≤93% when breathing ambient air, *3*) ratio of PaO_2 to FiO_2 ≤300 mmHg (1 mmHg = 0.133 kilopascal), and *4*) lung imaging showing significant progression of >50% within 24 to 48 h. Critical cases were defined as having at least 1 of the following: *1*) respiratory failure (PaO_2 <60 mmHg when breathing ambient air), *2*) hemodynamic shock (persisting hypotension requiring vasopressors to maintain mean arterial pressure >65 mmHg and serum lactate concentration >2 mmol/L despite volume resuscitation, and *3*) organ failure or admittance to ICU.

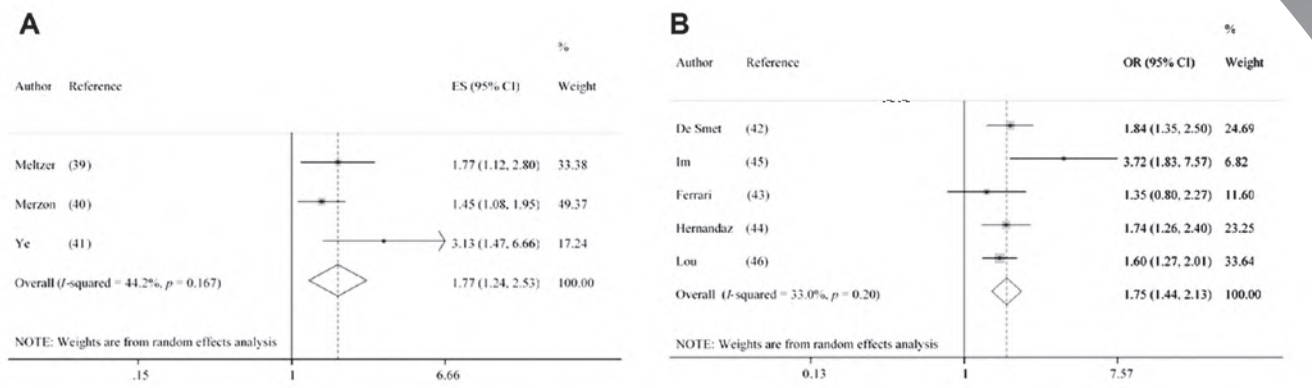

FIGURE 2 Relation between vitamin D deficiency and risk of SARS-CoV-2 infection in studies that adjusted for confounders (adjusted OR) (A) and studies that did not adjust for confounders (crude OR) (B). ES, effect size; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

between concentrations of 25(OH)D in ARDS [mean (SD): 16.8 (10.5) ng/mL) and non-ARDS [21.8 (15.8)] patients in Kerget et al. (50) ($P = 0.10$). Similarly, no significant association between VDD and ARDS was observed in the Maghbooli et al. (38) study (17.1% in VDD vs. 11.7% in non-VDD that progressed to ARDS; $P = 0.33$); moreover, bilateral lung involvement was observed in 33.3% in VDD versus 31.7% in non-VDD ($P = 0.86$) in this study. Three remaining studies evaluated the relation between VDD and risk of ventilation requirement. In a prospective study, VDD increased the risk of invasive mechanical ventilation and/or death (HR: 6.12; 95% CI: 2.79, 13.42; $P < 0.001$) (13). Consistently, another study indicated a significant relation between VDD and ventilation requirement (OR: 4.15; 95% CI: 1.05, 16.34; $P = 0.042$) (47), while one reported no relation (22.8% in VDD vs. 17.14% in non-VDD; $P = 0.58$) (44). Confounders were adjusted in the Radujkovic et al. (13) and Abrishami et al. (60) studies, while not adjusted in the Baktash et al. (47), Hernández et al. (44), Kerget et al. (50), Faul et al. (30), Maghbooli et al. (38), Pizzini et al. (61), and Im et al. (45) studies, respectively. Results of studies are summarized in **Supplemental Table 6**.

Hospitalization

Three studies investigated the relation between 25(OH)D and hospital admission and 2 with hospital stay. A significant association between VDD and risk of hospitalization was observed in Radujkovic et al. (13) (31% hospitalization in VDD vs. 69% in non-VDD, $P = 0.004$) and a marginally significant relation in Merzon et al. (40) (adjusted OR: 1.95; 95% CI: 0.98, 4.845; $P = 0.06$). The third study was a cross-sectional study that compared history of vitamin D3 supplement intake between inpatients and outpatients (57), where vitamin D3 intake was reported in 30% of outpatients versus 16.5% of hospitalized patients ($P = 0.001$).

Hernández et al. (44) found a significant relation between VDD and hospital stay [median (IQR) of 12.0 d (8.0–16.0) in patients with VDD vs. 8.0 d (6.0–14.0) in non-VDD patients; $P = 0.01$], while Luo et al. (46) failed to find a significant relation between serum 25(OH)D concentrations and length

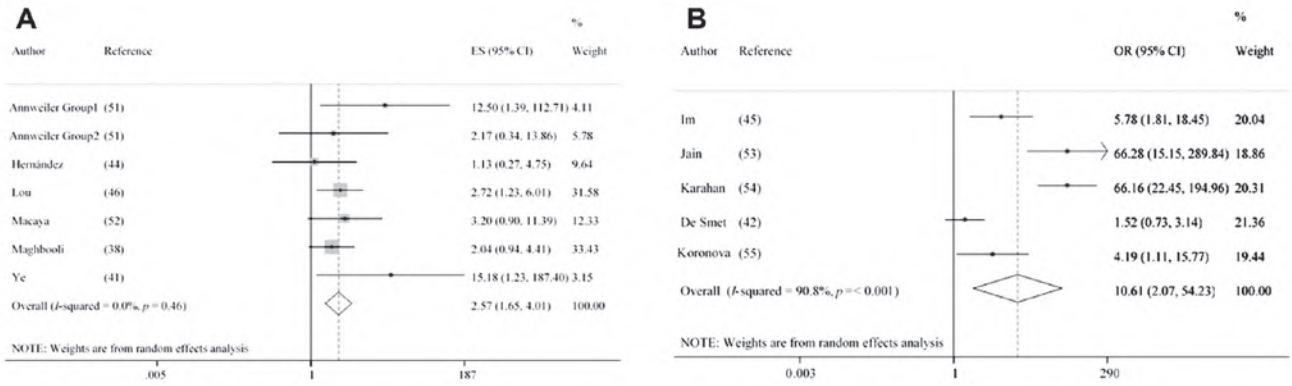

FIGURE 3 Relation between vitamin D deficiency and COVID-19 severity in studies that adjusted for confounders (adjusted OR) (A) and studies that did not adjust for confounders (crude OR) (B). COVID-19, coronavirus disease 2019; ES, effect size.

f hospital stay (B = −0.03, P = 0.64) (**Supplemental Table 7**).

Concentration of 25(OH)D between severe and less severe status of disease

Thirteen studies compared the serum concentration of 25(OH)D between patients with severe and nonsevere status of COVID-19 (either composite or 1 feature of severity). Analysis of 12 studies (13, 30, 41, 42, 43, 46, 50, 53–55, 59, 61), with 806 cases and 1024 controls, indicated that serum concentrations of 25(OH)D in patients with severe status of disease was lower (WMD = −7.17 ng/mL; 95% CI: −9.99, −4.34; I^2 = 87.6%) compared with less-severe counterparts (**Supplemental Figure 2**). In all of the studies except for one (43), 25(OH)D was measured after SARS-CoV-2 testing. One study was not included in the analysis, since the sample size according to hospitalization was not reported. Indeed, in this retrospective study, mean concentrations of 25(OH)D were 18.38 ng/mL (95% CI: 16.79, 19.96) in hospitalized and 20.45 ng/mL (95% CI: 20.22, 20.68) in nonhospitalized individuals (P < 0.001) (40) (**Supplemental Table 8**).

Inflammatory markers

We assessed the association of VDD with C-reactive protein (CRP), IL-6, D-dimer, and ferritin in COVID-19 patients. Nine studies examined the association of at least 1 of these markers with VDD. In an RCT in 40 COVID-19 patients, cholecalciferol supplementation did not significantly reduce CRP and D-dimer (14). A retrospective study in 42 patients with acute respiratory failure due to COVID-19 (64) revealed no statistically significant differences in inflammation indices among the 4 vitamin D groups (normal, insufficiency, deficiency, severe deficiency). Another retrospective study in 97 COVID-19 patients revealed that only ferritin, but not CRP, IL -6, and D-dimer, was significantly higher in VDD compared with non-VDD (44). In a prospective multicenter observational study in 109 patients, the correlation between 25(OH)D concentrations at follow-up and CRP, IL-6, ferritin, and D-dimer was not significant. The same was true for 25(OH)D concentrations measured at disease onset and CRP (r = 0.152, P = 0.45), IL-6 (r = 0.050, P = 0.80), and ferritin (r = 0.070, P = 0.73). In contrast, D-dimer concentrations were moderately associated with 25(OH)D concentrations (r = 0.437, P < 0.05) (61). Karahan and Katkat (54) in their retrospective study in 149 COVID-19 patients found a significant negative relation between serum 25(OH)D concentration and CRP (r = −0.253, P = 0.002). Kerget et al. (50) found a significant negative correlation only with CRP (r = −0.297, P = 0.01), but not IL-6, ferritin, and D-dimer. In a prospective study in 70 elderly individuals, it was reported that the VDD group demonstrated higher peak CRP, lactate dehydrogenase (LDH), and ferritin concentrations (47). Maghbooli et al. (38) in a cross-sectional study in 235 patients indicated that a relative risk of CRP >40 mg/L (inpatient mortality serum concentrations) was significantly higher in VDD. In Radujkovic et al. (13), IL-6 concentration was significantly higher in VDD versus non-VDD [median (IQR): 70.5 pg/mL (32.0–326.3) vs. 29.7 pg/mL (14.3–59.9); P = 0.01]. Only Maghbooli et al. and Radujkovic et al. adjusted for confounders, whereas the other studies did not report any adjustment. Results of studies are listed in **Supplemental Table 9**.

Mortality

Among 15 studies that assessed the relation between mortality and VDD, 13 studies were included in the analysis. Pooled analysis of 4 adjusted studies that used the Cox survival method (13, 51, 56, 60) (HR: 2.35; 95% CI: 1.22, 4.52; I^2: 84%; **Figure 4**A) and 5 studies (44, 47, 53, 55, 62) with crude OR (OR: 2.62; 95% CI: 1.13, 6.05; I^2: 47.8%; Figure 4B) indicated a significant association of VDD with mortality, while in adjusted studies that used logistic regression (54, 59, 65), no relation was observed (OR: 1.05; 95% CI: 0.63, 1.75; I^2: 76.6%). Two studies were not included in the analysis since 1 study had an RCT design (36) and another one used different statistical methods (64). In the RCT, 2 deaths in the control group versus no deaths in the intervention group were observed (36). In the other study, which had a retrospective design, patients with serum 25(OH)D <10 ng/mL had a 50% probability of mortality, while those with 25(OH)D ≥10 ng/mL had a 5% mortality risk after 10 d of hospitalization (P = 0.02) (64).

Moreover, 6 studies compared serum concentrations of 25(OH)D between deceased patients and those who survived (50, 54, 55, 60, 63, 66); pooled analysis of studies indicated lower concentrations of 25(OH)D in patients who died compared with those who survived (WMD: −9.05 ng/mL; 95% CI: −13.86, −4.23; I^2: 87.8%; **Supplemental Figure 3**). Results of studies are summarized in **Supplemental Table 10**.

Publication bias and quality assessment

Assessment of publication bias was conducted for 25(OH)D concentration between SARS-CoV-2–positive and –negative subjects as well as between severe and less-severe COVID-19 groups. Based on Egger's test, publication bias was evident in comparison of SARS-CoV-2–positive with –negative subjects (P = 0.002) and the funnel plot was asymmetric (**Supplemental Figure 4**A). The probable reason for publication bias may be that the studies with 25(OH)D data collected before SARS-CoV-2 testing had larger sample sizes and detected smaller differences compared with the studies that measured 25(OH)D after SARS-CoV-2 testing. There was no publication bias in the comparison of severe and less-severe COVID-19 patients (P = 0.60); however, a small deviation towards an WMD ∼ −5 and an SE ≈2 was observed in a funnel plot (Supplemental Figure 4B); this implies that studies with a smaller SE (more precision) indicate less difference in 25(OH)D concentration compared with the pooled WMD. Therefore, it should be considered that a small overestimation is probable. The quality of most of the studies was classified as poor (**Supplemental Tables 11–14**). Moreover, the strength and limitations of studies are summarized in **Supplemental Table 15**.

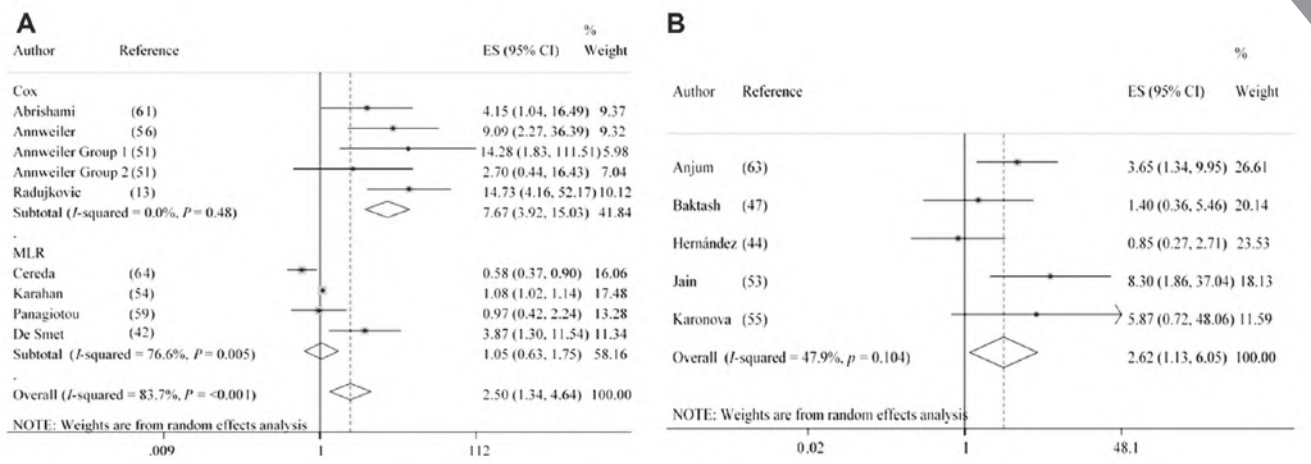

FIGURE 4 Relation between vitamin D deficiency and risk of mortality from COVID-19 in studies that adjusted for confounders (adjusted HR) (A) and studies that did not adjust for confounders (crude OR) (B). COVID-19, coronavirus disease 2019; ES, effect size; MLR, multiple logistic regression.

Discussion

In this systematic review, we investigated the relation between 25(OH)D concentrations and risk of SARS-CoV-2 infection and COVID-19 severity. For this purpose, we systematically reviewed and, where appropriate, meta-analyzed the related retrospective, cohort, cross-sectional, and clinical trial studies that assessed the association of 25(OH)D concentrations and the risk of SARS-CoV-2 infection, composite severity, or 1 feature of severity.

Higher risk of SARS-CoV-2 infection was observed in VDD and serum concentrations of 25(OH)D were lower in COVID-19 patients compared with healthy counterparts, as indicated by pooled results of both adjusted and nonadjusted studies. Among the 3 adjusted studies, 2 measured 25(OH)D in the preceding year before SARS-CoV-2 infection (39, 40); the sample sizes in one of these studies were sufficiently powered (case/control: 782/7025) (39). The nonadjusted studies measured 25(OH)D at admission and the sample sizes were sufficient in 4 studies (186/2700, 197/197, 128/219, 335/560) (39, 43, 39, 39). Moreover, concentrations of 25(OH)D were lower in COVID-19 patients compared with healthy subjects. Based on the findings, VDD is associated with increased risk of SARS-CoV-2 infection; however, caution should be made in interpreting these results, since the studies have inherent limitations.

All of the studies indicated a lower concentration of 25(OH)D with more severe status (composite severity) of disease. Furthermore, VDD was associated with composite severity in studies that were both adjusted and not adjusted for confounders. The significant relation between VDD and composite severity was evident in all of the primary studies, except for the Hernández et al. (44) and De Smet et al. (42) studies, where De Smet et al. revealed such a relation only in males but not in females. Zero heterogeneity was estimated for adjusted studies based on the I^2 statistic. It should be noted that the heterogeneity I^2 statistic can be biased in small meta-analyses and so an I^2 of 0.0% does not necessarily reflect perfect homogeneity (67).

Pooled results from the studies that were unadjusted and adjusted studies using Cox survival analysis indicated a higher risk of mortality in VDD; however, the adjusted studies that used logistic regression failed to find a significant relation. The Cox model estimates the instantaneous probability of death at a particular time, while logistic regression estimates the cumulative probability; instantaneous risk could be important as the cumulative probability can be conditioned by a complex clinical outcome. Moreover, it is noteworthy to mention that the Cox model tends to have greater statistical power to detect a significant exposure effect than logistic regression (68). Among the 4 adjusted studies that used logistic regression, 1 study indicated higher risk of mortality in VDD, 2 revealed no significant relation, and 1 study unexpectedly found a lower risk of mortality in VDD. In this study, the prevalence of $\geq$2 comorbidities was higher in the non-VDD (46.7%) versus the VDD group (30.3%). Although this difference between groups was not statistically significant, it could be important because of the small sample size ($n = 30$ in non-VDD and $n = 99$ in VDD). The authors adjusted for some confounders (age, sex, CRP, ischemic heart disease, and severe pneumonia), but the effects of other chronic diseases that were more prevalent in the non-VDD versus VDD groups (albeit nonsignificant) were not adjusted. Moreover, the population in this study was old (mean age of 77 y) and so at high risk for other nutrient deficiencies. The 2 studies that were not included in the analysis also indicated a significant relation, in which 1 study was an RCT (36, 64). Consistently, pooled results indicated a higher concentration of 25(OH)D in patients who survived versus those who died. Overall, evidence indicates that VDD greatly increases the risk of mortality.

Pooled analysis of unadjusted studies failed to detect any significant relation between 25(OH)D concentration and

ICU admission, although an RCT indicated a significant association (36).

For pulmonary complications, results of studies were inconsistent; 4 studies found a significant relation between 25(OH)D concentration and an increased risk of pulmonary involvement, while 4 studies failed to find any relation. Among them, only Radujkovic et al. (13) and Abrishimi et al. (60) were adjusted for confounders, and both found a significant association between VDD and risk of pulmonary involvement. Radujkovic et al. had some other strengths, such as a cohort design and larger sample size, as compared with the other studies. Although this study indicated a very large risk in VDD, the HR in this study was for the combination of both ventilator requirement and death. In Abrishami et al., increases in 25(OH)D concentration led to only a 4% reduction in severe lung involvement. Therefore, it seems pragmatic to suggest that no conclusion can be drawn regarding the relation between 25(OH)D and pulmonary complications.

All 3 studies that examined the association between VDD and hospitalization indicated a significant relation (13, 40, 57). One study adjusted for confounders and had a good quality design (13); another study adjusted for confounders and had a large sample size but the authors used vitamin D data that were measured in the past (40), while the third study did not adjust for confounders and had a poor design (57). With regard to the relation between 25(OH)D concentration and hospital length of stay, 1 study found a significant relation (44), while the other failed to find any relation (46). In total, the evidence is not adequate to draw a conclusion with regard to the association of vitamin D with hospitalization admission and length of stay.

We assessed CRP, D-dimer, ferritin, and IL-6 as the inflammatory markers. Five studies indicated a positive association between 25(OH)D concentration and inflammation. In 2 studies peak CRP and CRP >40 mg/L were evaluated in related to VDD (38, 47). In 1 study, only IL-6 was measured, and in the other 2 studies, the relation was examined using Pearson correlation coefficients (50, 54). Four studies failed to detect a significant relation (14, 44, 64, 61); among them, the highest-quality study was a clinical trial that failed to discern the effect of cholecalciferol supplementation on CRP and D-dimer (14), although it does not appear that 25(OH)D concentration is correlated with inflammation in nonacute phases, given that the evidence is currently not sufficient.

Several mechanisms are involved in elucidating the relation between VDD and SARS-CoV-2 infection risk and outcomes. Vitamin D improves cellular immunity and can decrease the plasma concentrations of proinflammatory cytokines, such as TNF-α and IFN-γ, that have been produced as part of the cytokine storm by the innate immune system in viral infections such as COVID-19, in addition to increasing concentrations of anti-inflammatory markers (69). Furthermore, vitamin D can regulate adaptive immunity response by stopping the T-helper (Th) cell type 1 (Th1) reaction, elevating production of cytokine by Th2, and increasing the induction of T-regulatory cells (70–72).

In addition, due to the highly expressed concentrations of vitamin D receptors (VDRs) in B- and T-lymphocytes (73), vitamin D can affect immune system function. VDR is a member of the nuclear hormone receptor (NHR) family, which is a known transcription factor (74); indeed, VDR is present in both T and B immune cells and regulates a variety of metabolic pathways, such as those involved in the immune response and cancer (75). High concentrations of transforming growth factor β (TGF-β) have been reported in the acute phase of COVID-19, where TGF-β signaling is closely related to SARS-CoV-2 and is suppressed by VDR via genomic competition with Mothers against decapentaplegic homolog 3 (Smad3) occupancy on proinflammatory (e.g., IL-6) genes and therefore creating a stable physiologic situation (76).

Another probable mechanism is that vitamin D can induce cathelicidin, IL-37, and defensins as antimicrobial peptides, and promote cellular innate immunity and reduce virus replication (77–79).

It has been posited that vitamin D can enhance the expression of some genes related to antioxidant systems, such as the glutathione reductase gene (80); accordingly, some studies have reported that vitamin D metabolites have vascular-related functions including anticoagulant effects through modifying the expression of thrombomodulin and tissue factor in monocyte and aortic cells (81, 82).

Because of the worldwide increasing prevalence of COVID-19 as a novel pandemic, it is important to research potential antiviral treatments or preventions. Therefore, we conducted this systematic review to investigate the association of vitamin D concentration with SARS-CoV-2 infection and various clinical outcomes.

Some systematic reviews have investigated the association between vitamin D3 and COVID-19 risk and severity (83, 84), in addition to a meta-analysis by Pereira et al. (85), which included 27 studies. The priority of the present study was to include a higher number of studies and exclude preprint articles that had not been peer reviewed and studies with high risk of bias. Moreover, problematically, studies that did and did not adjust for confounding variables were pooled together in the Pereira et al. study, while we analyzed these studies separately.

The main limitation of the present systematic review is the inclusion of studies that were heterogeneous in design, methodology, and statistical approach, and since most of the studies were observational, causality cannot be inferred. Sex and age are important factors that have been shown to be related to both COVID-19 and 25(OH)D concentrations independently. Thus, it is of high importance that the relation between COVID-19 and vitamin D be verified in different subgroups of age and sex. Indeed, we were unable to do so due to the results not being reported separately in the included studies.

In conclusion, although studies were heterogeneous in methodological and statistical approach, and some inherent

limitations were present, the findings of the present study indicated a significant relation between 25(OH)D concentration and SARS-CoV-2 infection, COVID-19 composite severity, and mortality. For infection, caution should be taken in interpreting the results due to inherent limitations of studies. For ICU admission, inflammation, hospitalization, and pulmonary involvement, the evidence is currently inconsistent and insufficient. Moreover, future studies should investigate the association of COVID-19 with vitamin D in subgroups of age and sex.

Acknowledgments

The authors' responsibilities were as follows—AK: designed the study; AK, VM, and SKA: performed the literature search and screening and wrote the initial draft, which was modified after feedback from all coauthors; AK, VM, and MG: performed data extraction; MG and AK: performed quality assessment; CCTC and SB: critical revising of the manuscript; AK: had primary responsibility for content; and all authors: read and approved the final manuscript.

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[8]

by Nancy Munoz, DCN, MHA, RDN, FAND

Vitamin D is a fat-soluble vitamin [9] that is not commonly found in foods. Good sources of vitamin D include fortified cereal, fortified milk, and fish with high fat content such as salmon. This nutrient is also produced endogenously through skin exposure to the ultraviolet rays from the sun. Vitamin D made available to the body through sun exposure, food, and supplements is organically inactive and must go through a chemical process that introduces a hydroxyl group (-OH) into an organic compound (hydroxylation). This chemical process converts vitamin D to calcidiol and calcitriol, which the body can absorb.[1]

Vitamin D in the Body

Vitamin D is necessary in the body to promote calcium absorption, bone growth, and bone remodeling. Low levels of vitamin D have been associated with many chronic illnesses [10], inflammatory conditions, and increased risk for mortality. Vitamin D deficiency and insufficiency have been associated with common cancers, autoimmune ailments, infectious conditions, and cardiovascular disease.[2] Deficiency causes rickets in children and

osteomalacia in adults. In conjunction with calcium, vitamin D helps to prevent osteoporosis in older adults. Vitamin D has receptors in almost every cell of the body.[3]

Aside from promoting bone health, vitamin D is involved in other body processes such as cell growth modulation, immune function, inflammation reduction, and neuromuscular activity. Over 200 genes, including those involved in cell proliferation, cell differentiation, angiogenesis, and apoptosis, need vitamin D.[3] Vitamin D is needed to regulate cells in various tissues, including epidermal keratinocytes. This is done by modifying growth factors and cytokines. Vitamin D can affect wound healing by increasing the production of epidermal and platelet growth factors.[4]

Dietary Reference Intakes

The Food and Nutrition Board of the National Academy of Medicine defined the Dietary Reference Intakes (DRI) for vitamin D based on the daily intake required to promote bone health and adequate calcium metabolism in healthy adults. The DRI of vitamin D is 400 international units (IU) for children up to 12 months of age, 600 IU for ages 1 to 70, and 800 IU for people over 70.[3]

Vitamin D and Wound Healing

Insufficient sun exposure and chronic illness continues to vitamin D deficiency in people of all ages. Vitamin D deficiency, defined as less than 20 ng/mL, and insufficiency with levels between 20-30 ng/mL affect approximately 1 billion individuals globally.[5]

Vitamin D has recently been shown to display beneficial effects in various vascular diseases [11] by promoting angiogenesis and inhibiting inflammatory responses. A study examining the role of vitamin D in cutaneous wound healing in streptozotocin-induced diabetic mice concluded that supplementation with vitamin D can significantly accelerate wound healing rate.[6]

Razzaghi and colleagues examined the effect of vitamin D supplementation on wound healing and metabolic status in individuals with diabetic foot ulcers.[7] Their study concluded that vitamin D supplementation had a positive effect on stabilizing blood glucose and cholesterol levels. The study also reported that vitamin D could have a secondary effect on wound healing as a result of the improved glycemic control status seen in the study subjects.[7] After assessing the relationship between vitamin D and pressure injuries in community-dwelling older adults, Kalava and associates

concluded that vitamin D deficiency was not an independent risk factor for pressure injury development.[8]

Implications for Practice

Although the relationship between vitamin D supplementation and wound healing has not been clearly established, we know that this nutrient is essential to maintain bone health. Vitamin D deficiency and insufficiency are very common and can have a negative impact on health and well-being. Reversing vitamin D deficiency is simple and inexpensive and has been linked to many health benefits.

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Review Article

Links between Vitamin D Deficiency and Cardiovascular Diseases

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The aim of the present paper was to review the most important mechanisms explaining the possible association of vitamin D deficiency and cardiovascular diseases, focusing on recent experimental and clinical data. Low vitamin D levels favor atherosclerosis enabling vascular inflammation, endothelial dysfunction, formation of foam cells, and proliferation of smooth muscle cells. The antihypertensive properties of vitamin D include suppression of the renin-angiotensin-aldosterone system, renoprotective effects, direct effects on endothelial cells and calcium metabolism, inhibition of growth of vascular smooth muscle cells, prevention of secondary hyperparathyroidism, and beneficial effects on cardiovascular risk factors. Vitamin D is also involved in glycemic control, lipid metabolism, insulin secretion, and sensitivity, explaining the association between vitamin D deficiency and metabolic syndrome. Vitamin D deficit was associated in some studies with the number of affected coronary arteries, postinfarction complications, inflammatory cytokines and cardiac remodeling in patients with myocardial infarction, direct electromechanical effects and inflammation in atrial fibrillation, and neuroprotective effects in stroke. In peripheral arterial disease, vitamin D status was related to the decline of the functional performance, severity, atherosclerosis and inflammatory markers, arterial stiffness, vascular calcifications, and arterial aging. Vitamin D supplementation should further consider additional factors, such as phosphates, parathormone, renin, and fibroblast growth factor 23 levels.

1. Introduction

Vitamin D exists in two forms: D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D3, "the sunshine vitamin," is synthetized in the human epidermis via ultraviolet irradiation, or it may be consumed in the form of oily fish or supplements. Vitamin D2 is found in plants, as a product of irradiation of ergosterol [1]. The vitamin is converted in the liver and kidney to calcidiol and calcitriol, respectively, and acts on specific target tissues via vitamin D receptors. Calcitriol, the active form of vitamin D, binds to vitamin D receptors in the intestines, bones, and kidneys to increase calcium absorption from the intestines, promote calcium deposition in bones, and decrease parathyroid hormone concentrations (PTH). Its extraosseous effects are less known. Vitamin D receptors were found in other tissues, as well, including the brain, cardiomyocytes, vascular smooth muscle cells, endothelial cells, pancreatic beta-cells, skeletal muscle, breast, prostate, colon, macrophages, and skin, exerting several pleiotropic effects, and their expression decreases with age. The vitamin D receptor is closely related to the thyroid, retinoid, and peroxisome proliferator-activator receptors [2]. Recent studies have found active 1 alpha hydroxylase in several extra renal tissues, such as the heart and vascular smooth muscle cells [3–5]. Activated vitamin D may influence cellular growth, proliferation and apoptosis, oxidative stress, membrane transport, matrix homeostasis, cell adhesion, and immune system functions and may regulate a large number of genes and healthy aging [6, 7].

Vitamin D insufficiency is a common public health problem, very often unrecognized and untreated, associated with rickets, dental caries, and growth retardation in children and osteomalacia, osteopenia, osteoporosis, decreased muscle strength, falls, and increased risk of fracture in adults.

Vitamin D insufficiency is associated with indoor lifestyle, sun avoidance strategies, obesity, diabetes mellitus, low HDL cholesterol, older age, distance from the equator, darker skin, winter season, air pollution, smoking, malabsorption, renal and liver disease, and medication (anticonvulsants, glucocorticoids, antirejection, and human immunodeficiency virus therapy) [1–11]. The biologically active form of vitamin D is 1,25 dihydroxyvitamin D, but the best indicator of vitamin D status in individuals free of kidney disease is 25-hydroxyvitamin D, the substrate for the renal and nonrenal production of calcitriol, with a longer biological half-life and a higher concentration than 1,25 dihydroxyvitamin D, reflecting the total endogenous and exogenous production of vitamin D [12, 13].

Recent research has linked inadequate vitamin D status to nonskeletal major chronic diseases, especially cardiovascular diseases [8]. Existing data from laboratory studies, epidemiologic and experimental research and prevention trials, suggest that vitamin D reduces the risk of cardiovascular disease, and a large, randomized, primary prevention trial, with adequate dosing, combining cholecalciferol and omega-3 fatty acids, is ongoing: the VITAL study. Poor vitamin D status was associated with cardiovascular and overall mortality, despite unconvincing results of vitamin D supplementation on mortality [13]. Food-based strategies for enhancement of vitamin D status in the population could lower cardiovascular risk if a causal link between low vitamin status and cardiovascular pathology would be demonstrated [14].

The aim of the present paper was to review the most important mechanisms explaining the possible association of vitamin D deficiency and cardiovascular diseases, focusing on recent experimental and clinical data.

2. Definition of Vitamin D Deficiency

Optimal serum concentration of 25-hydroxyvitamin D considers only bone health and was defined as the concentration that maximally suppresses serum parathyroid hormone [15]. Most experts define vitamin D deficiency as a calcidiol level of <20 ng/mL and insufficiency as 21–29 ng/mL [1, 16]. Vitamin D is sufficient if >30 ng/mL, and vitamin D intoxication is considered if >150 ng/mL [16]. There are variations among professional bodies regarding the cut-off values for insufficient or deficient vitamin D level [17].

According to a report of the Institute of Medicine (IOM), vitamin D at doses of 600 IU/day is beneficial for the bones, but it is not certain if higher doses could reduce the risk of chronic diseases, including cancer and cardiovascular pathology [17]. A threshold effect between vitamin D status and cardiovascular risk was suggested [11]. Zittermann et al. found a vitamin D level of 30–35 ng/L as the best choice for risk reduction in cardiovascular mortality [18].

3. Vitamin D and Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAA) maintains vascular resistance, due to angiotensin II synthesis, and extracellular fluid volume homeostasis, considering the release of aldosterone [19]. Studies by different groups, in both animals and humans, demonstrated that vitamin D *decreases RAA activity* [20], suppressing *renin gene expression* [12]. Vitamin D regulates the genes involved in renin production, through a *cis*-DNA element in the renin gene promoter [1, 21], downregulating the RAA system.

Vitamin D receptor-null mice had a sustained elevation of renin expression, maintaining a normal level of blood electrolytes [19]. Increased renin synthesis leads to an elevated angiotensin II production, which is a strong vasoconstrictor, enabling the development of hypertension and left ventricular hypertrophy [19]. Similarly, 1-alpha-hydroxylase deficient mice, unable to synthetize the active metabolite 1,25-dihydroxyvitamin D, develop also high blood pressure and left ventricular hypertrophy [4]. Studies using renal arteries from hypertensive patients reported that calcitriol *reduces the expression of the angiotensin-1 receptor in endothelial cells,* improving endothelial function and preventing reactive oxygen species overproduction [22]. Secondary *hyperparathyroidism* in vitamin D receptor-null mice may also contribute to renin upregulation [19], considering that intravenous infusion of PTH increases plasma renin activity and renin release [23, 24].

Vitamin D regulation of renin expression is independent of calcium metabolism, and calcitriol markedly suppresses renin transcription by a vitamin D receptor—mediated mechanism in cell cultures [19]. Ferder et al. suggested a possible feedback link between vitamin D and the renin-angiotensin system (RAS), considering that vitamin D and angiotensin II receptors are distributed in the same tissues, changes in RAA activity and activation of the vitamin D receptors seem inversely related, and vitamin D deficiency could be explained by the cellular *inflammatory response activity* induced by the RAA system [25]. Therapy should combine RAS blockade and VDR stimulation [25].

D hypervitaminosis induces vascular and soft-tissue calcifications. *Calcium deposition in the vascular smooth muscle cells* may also lead to RAA activation [26–28].

Suppression of renin production and downregulation of the RAA may explain the direct myocardial and vascular effects through *modulation of hypertrophic stimuli* [10].

4. Vitamin D and Atherosclerosis

Vitamin D suppresses inflammation via several pathways, such as inhibition of prostaglandin and cyclooxygenase pathways, upregulation of anti-inflammatory cytokines, decrease of cytokine induced expression of adhesion molecules, reduction of matrix metalloproteinase 9, and downregulation of the RAA [11, 25]. Vitamin D deficiency stimulates *systemic and vascular inflammation,* enabling atherogenesis [1]. On the other hand, as already mentioned, hypertension is also associated with lack of vitamin D, due to activation of the RAA system, enabling *endothelial dysfunction*, the first step in plaque formation. The proinflammatory nuclear factor kB mediates partly the association between endothelial dysfunction and low vitamin D status [11].

Large epidemiological studies have highlighted vitamin D deficiency as a marker of cardiovascular risk [29], promoting

accelerated atherosclerosis [12, 30] and subsequent cardiovascular events [11]. Chronic vitamin D deficiency causes *secondary hyperparathyroidism, increasing insulin resistance*, impairing beta-pancreatic cell function, and enabling the development of metabolic syndrome and diabetes mellitus [1]. Calcitriol regulates the genes involved in insulin production in the pancreas [1].

Vitamin D has also some *antiatherogenic functions*, inhibiting the formation of foam cells, cholesterol uptake by the macrophages, and enabling HDL transport [31]. Lower serum 25-hydroxyvitamin D was associated with the metabolic syndrome and its components, especially HDL cholesterol concentration [32].

Vascular smooth muscle cells and endothelial cells express receptors for vitamin D, enabling conversion of calcidiol to calcitriol [12], and vitamin D is involved in *regulation of growth and proliferation of smooth muscle cells and cardiomyocytes* [12, 33, 34]. Vitamin D inhibits proliferation of vascular smooth muscle cells by acute influx of calcium into the cell and increases calcification of smooth muscle cells [34].

The cardiovascular protective effects of vitamin D include also the anti-inflammatory effects, inhibition of vascular smooth muscle cells proliferation, suppression of proatherogenic T lymphocytes, preservation of endothelial function [22, 35–40], and protection against advanced glycation products [2].

Vitamin D deficiency was associated with *vascular stiffness*, which is a known predictor of cardiovascular morbidity and mortality [11] and a marker of subclinical atherosclerosis.

5. Vitamin D Insufficiency and Hypertension

Low vitamin D levels have been associated with increased prevalence of hypertension [41–43] or elevated diastolic blood pressure [44–46]. Clinical studies demonstrated an *inverse, dose-response relationship* between plasma 1,25(OH)2D3 concentration and blood pressure or *renin activity* in both normotensive and hypertensive patients [43, 47–49]. Wang et al. reported associations of hypertension risk and plasma 25-hydroxyvitamin D and vitamin D receptor polymorphism, respectively [8]. Rats with experimentally induced vitamin D deficiency developed hypertension and cardiomegaly [43, 50]. Mice lacking vitamin D receptor had an increased renin expression and angiotensin II production and developed also hypertension and cardiac hypertrophy [19, 43, 51]. Lower end-diastolic pressures were noted in Dahl salt-sensitive rats treated with paricalcitol, a vitamin D receptor activator, compared to untreated animals [52].

Ultraviolet light exposure enables vitamin D synthesis and has blood pressure lowering effects [53, 54]. Hypertensive patients exposed to a tanning bed significantly raised their concentration of 25-hydroxyvitamin D after 3 months and became normotensive [54]. Vitamin D3 supplementation reduces blood pressure in patients with essential hypertension [55, 56] and reduces also plasma renin activity and angiotensin II levels in hyperparathyroidism patients [57, 58]. Erythemal and preerythemal doses of UV irradiation decrease vascular resistance, with diffuse skin vasodilatation, related to nitric oxide release [2, 59].

Calcitriol exerts a protective effect on human renovascular function, restoring the impaired *endothelium-dependent relaxation* in renal arteries, accompanied by the normalization of oxidative-stress related proteins [22]. The augmented production of *reactive oxygen species* is induced by angiotensin II in human renal arteries and endothelial cells and impairs vascular function enabling the development of hypertension [22]. Vitamin D metabolites reduced endothelium-dependent vascular smooth muscle contractions and vascular tone in hypertensive rats by affecting calcium influx across endothelial cells [60]. *In vitro*, vitamin D receptor activation induces a concentration-dependent increase of nitric oxide production in endothelial cells and improves the angiogenic properties of endothelial progenitor cells [61, 62].

Hypertensive patients with vitamin D deficiency were associated with a twofold risk of cardiovascular events, including myocardial infarction, angina, prolonged chest pain with documented ECG changes, stroke, transient ischemic attack, peripheral claudication, and heart failure [12].

Not all studies demonstrated blood pressure lowering effects of vitamin D. High-dose intermittent vitamin D therapy was given every 2 months in patients with resistant hypertension, on, at least, 3 antihypertensive agents, for 6 months, with no reduction of office blood pressure, 24-hour ambulatory blood pressure, or left ventricular mass [63].

The relation of vitamin D deficiency and *preeclampsia* is controversial. Vitamin D deficiency in pregnancy has been associated with an increased risk of preeclampsia [64, 65], suggesting that vitamin D supplementation in early pregnancy could prevent preeclampsia [64] and decreased calcidiol was found at diagnosis of early onset severe preeclampsia [66]. On the other hand, several authors reported no adverse pregnancy outcomes, including preeclampsia, with low 25-hydroxyvitamin D [67, 68]. An inverse association was reported between calcium intake and maternal blood pressure, as well, as the incidence of preeclampsia syndrome, explained, probably, by the influence on parathyroid hormone release and intracellular calcium availability, but the relationship between calcium and risk of hypertension in pregnancy seems to be inconsistent and inconclusive [69]. Preeclampsia is associated with reduced placental perfusion and maternal endothelial dysfunction [62]. Vitamin D increases capillary formation in endothelial colony forming cells, probably mediated by increased expression of *vascular endothelial growth factor and promatrix metalloproteinase activity* [62].

The mode of *vitamin D prophylaxis during infancy* (continuous daily supplementation, bolus doses of vitamin D forte every three months during the first year of life, or bolus doses during winter combined with continuous daily drops during summer) did not influence the blood pressure level in early adolescence, and no adverse effects were reported despite exceeding daily doses [70].

Vitamin D levels were significantly lower in patients with *thostatic hypotension*, but lower vitamin D status was not sociated with impaired orthostatic hemodynamics [46]. tamin D deficiency may also be involved in orthostatic potension development in elderly patients [71]. Serum vels of vitamin D should be checked during the evaluation those patients, considering that orthostatic hypotension associated with falls, fractures, cardiovascular events, and gnificant mortality in the elderly [46, 71]. Vitamin D ceptors are found in vascular smooth muscle, endothelial d cardiac cells, enabling involvement in the cardiac and scular response during orthostasis [72], and vitamin D ficiency downregulates the RAA system and was also sociated with endothelial dysfunction [46]. On the other nd, both orthostatic hypotension and vitamin D deficiency e more prevalent in the elderly.

Women's Health Initiative study of *postmenopausal omen* found no reduction in blood pressure with supementation of 1,000 mg/day of calcium and 400 IU/day vitamin D3, probably because the vitamin dose was sufficient for beneficial blood pressure effects [73].

The parathormone (PTH) is a crucial regulator of calcium d phosphate balance. Higher PTH concentrations were sociated with several cardiovascular risk factors, including pertension and arterial stiffness [74]. The mechanisms aking PTH and blood pressure include upregulation of RAA ie to increase of serum calcium and sympathetic activity 4]. PTH correlated with blood pressure and hypertension cidence in a cross-sectional study, including 1,205 elderly bjects; serum vitamin D was not associated with blood essure, probably due to the relatively high levels in the study opulation [75].

The antihypertensive properties of vitamin D include suppression of the RAA system, renoprotective effects including tiproteinuric and anti-inflammatory effects, direct effects endothelial cells and calcium metabolism, inhibition growth of vascular smooth muscle cells, prevention of condary hyperparathyroidism, and beneficial effects on mmon cardiovascular risk factors and are important in tamin D deficient, hypertensive patients [42, 44, 76]. bservational studies suggest that vitamin D deficiency is sociated with high blood pressure, but randomized clinical als did not yield conclusive results [20].

Vitamin D and Metabolic Syndrome

rdiovascular risk factors such as hyperlipidemia, abdomal obesity, hypertension, and diabetes often cluster in the me individual [42]. A small cross-sectional study, including iddle-aged men, found that vitamin D metabolites were lated to lipid and glucose metabolism and serum urate 7]. Serum calcitriol was inversely correlated to the blood essure and triglycerides and calcidiol to fasting insulin d lipoprotein lipase activity both in adiposal tissue and in eletal muscle [77].

Statins exert beneficial effects, not only on lowering olesterol but also on diabetes, bone metabolism, and flammatory states, probably related to vitamin D [78]. orvastatin used in patients with acute coronary syndromes decreased cholesterol levels and significantly increased vitamin D levels, due to the shared metabolic pathway of cholesterol and vitamin D [42, 79].

Obese individuals are at higher risk of hypertension, hypercholesterolemia, diabetes, cardiovascular mortality, and vitamin D deficiency. Besides vitamin D sequestration in subcutaneous fat and making stores less available to become biologically activated, obese persons may have a sedentary lifestyle and be less active outdoors, and skin production of vitamin D may be impaired due to clothing habits [42, 80].

Many cellular, experimental, and observational studies support the role of vitamin D in the pathogenesis of type 1 and type 2 diabetes [81]. Type 1 and type 2 diabetic patients have a higher incidence of hypovitaminosis D [81]. A lower incidence of type 2 diabetes was found after vitamin D supplementation in high risk individuals, supporting the hypothesis that high vitamin D status protects against type 2 diabetes [82]. Insulitis and type 1 diabetes mellitus were prevented by pharmacologic doses of vitamin D in nonobese mice, possibly by immune modulation and direct effect on beta-cell function [81]. An inverse association between circulating 25-hydroxyvitamin D and incident type 2 diabetes was demonstrated by a systematic review and meta-analysis, including only prospective studies [83]. The plausible mechanisms explaining the mentioned associations involve vitamin D receptors in pancreatic beta-cells influencing insulin secretion and vitamin D effects on insulin sensitivity, or through the effects of vitamin D on calcium metabolism, but there is no demonstrable evidence of causality yet [27, 83, 84]. Sun exposure implies greater outdoor physical activity, improving insulin sensitivity [84]. Further mechanism connecting vitamin D and diabetes mellitus involves pancreatic tissue and cells of the immune system expressing not only vitamin D receptors but also vitamin D binding protein, some allelic gene variations involving vitamin D metabolism and vitamin D receptors, associated with glucose intolerance, insulin secretion and sensitivity, and inflammation [81]. Thus, vitamin D has an important role in glycemic control, which may influence cardiovascular outcomes [27].

Vitamin D may influence several components of the metabolic syndrome, especially hypertension, hyperglycemia, insulin resistance, and hyperlipidemia.

7. Vitamin D, Coronary Heart Disease, and Heart Failure

A strong association was found between vitamin D deficiency and *slow coronary flow, endothelial dysfunction and subclinical atherosclerosis,* in patients with normal or near-normal coronary arteries at coronary angiography [85]. Decreased levels of vitamin D binding protein were found in the plasma of survivors of a myocardial infarction at young age, statistically correlated with the number of affected coronary arteries [86]. Low vitamin D levels have been linked to inflammation, *higher coronary artery calcium scores, increased mean platelet volume,* and *increased vascular stiffness* [11, 28]. Abnormally high mean platelet volume has been associated with cardiovascular diseases, considering the higher risk to block arteries, due to the ability to aggregate more rapidly with

collagen, the higher thromboxane A2 level, and expression of more glycoprotein Ib and IIb/IIIa receptors than smaller platelets [28]. The increased release of proinflammatory cytokines in patients with vitamin D deficiency increases oxidative stress and enables release of immature and activated platelets from the bone marrow, with an increased mean platelet volume [28].

Vitamin D deficiency was associated with coronary heart disease and myocardial infarction [87] and was found in a high proportion of patients with myocardial infarction [9, 88]. Vitamin D status is prognostic for major *postinfarction adverse events*, such as heart failure hospitalizations, recurrent acute myocardial infarction, death [89, 90], or restenosis after percutaneous coronary intervention [11]. A significant, moderate association was found between circulating vitamin D concentration and the risk of all-cause mortality, especially deaths due to coronary disease [6].

Vitamin D level was not associated with the severity of coronary lesions in patients with ST-segment elevation myocardial infarction [88]. On the other hand, the severity of coronary artery stenosis, assessed according to the Gensini score, a validated measure of the angiographic severity of coronary heart disease, was associated with vitamin D deficiency [91].

Arnson et al. examined the effects of short-term vitamin D supplementation on *inflammatory cytokines* after an acute myocardial infarction, reporting a reduction of vascular cell adhesion molecules, C-reactive protein, and interleukin-6, supporting the cardioprotective anti-inflammatory effects of vitamin D on the vascular system [92].

Racial differences have been reported regarding the associations between serum 25-hydroxyvitamin D and the risk of coronary heart disease in a multiethnic community-based cohort of adults without clinical cardiovascular disease. The association of low calcidiol and increased risk of coronary heart disease was demonstrated in white and Chinese study participants, but not in black or Hispanic [93].

Vitamin D exerts biological effects on cardiac myocytes, stimulating calcium-ATPase activity and calcium uptake in cardiac myocytes [11]. Lack of vitamin D could cause diastolic dysfunction, and the Hoorn study found a trend towards increased risk of diastolic dysfunction in persons with vitamin D deficiency, considering 614 persons from a population-based cohort of older men and women [94]. No significant association was found between vitamin D levels and left ventricular diastolic performance, including left atrial volume index in a retrospective observational study including 1,011 unselected patients (involving patients with hypertension and diabetes) [95]. Several explanations were found for the lack of association between vitamin D level and diastolic dysfunction, such as the cross-sectional study design and insufficient information about the duration of vitamin D deficiency [95].

The majority of congestive heart failure patients have insufficient vitamin D, due to reduced sunlight exposure, difficult mobilization and outdoor activity, nutritional factors, and malabsorption of vitamin D due to intestinal edema in severe right heart failure and comorbidities, such as obesity and renal and hepatic failure [10]. Lack of vitamin D causes hypocalcemia and *secondary hyperparathyroidism*. Indeed, serum parathormone (PTH) was elevated in patients with congestive heart failure due to ischemic or dilated cardiomyopathy and hypovitaminosis D was present [96]. Osteomalacia and osteoporosis and fracture rates should be, probably, evaluated in individuals with congestive heart failure [10]. Osteoporosis and cardiovascular pathology share common backgrounds, including osteoprotegerin and receptor activator of nuclear factor kappa-B ligand, involved in osteoclast activation and vascular calcification and atherosclerosis; bone morphogenetic protein, involved in osteoblastic differentiation and atherosclerotic lesions, and age-related estrogen deficiency [97]. An inverse association between PTH and vitamin D level persists until the vitamin D value exceeds 30 ng/mL [72]. The presence of hypocalcemia, osteopenia, or osteomalacia could justify vitamin D supplementation in heart failure patients, despite controversial causative link between vitamin D deficiency and heart failure. An *autocrine/paracrine vitamin D system* also exists, independent of PTH level, besides the endocrine vitamin D system [74].

It is important to determine the vitamin D level in patients with myocardial infarction and correct deficient levels. Vitamin D repletion to prevent cardiac remodeling after a myocardial infarction deserves future study [87]. Vitamin D signaling plays an important cardioprotective role after myocardial infarction through anti-inflammatory, *antifibrotic, and antiapoptotic* mechanisms [98]. Despite several studies revealing vitamin deficiency in congestive heart failure patients, no clear data on improvement of outcome with vitamin D supplementation exist, despite reduction of inflammatory markers and PTH level [10]. The RECORD randomized controlled trial demonstrated that vitamin D supplementation might *protect against cardiac failure* in the elderly, but not against myocardial infarction or stroke [99].

Vitamin D receptor knockout mice had upregulated matrix metalloproteinases, involved in cardiac remodeling, impaired cardiac relaxation and contractility, and developed left ventricular hypertrophy [27, 100]. It seems that both matrix metalloproteinases and inhibitors of metalloproteinases expressions are modulated by vitamin D [100].

Vitamin D decreases fibrosis in mesenchymal multipotent cells through the increased expression and nuclear translocation of vitamin D receptors, decreasing profibrotic factors (transforming growth factor B1 and plasminogen activator inhibitor) and several collagen isoforms and increasing expression of antifibrotic factors [101].

Vitamin D deficiency is associated with an increased prevalence of coronary heart disease with adverse outcomes, considering the proatherogenic and profibrotic effects, impaired coronary perfusion, and cardiac remodeling.

8. Vitamin D and Left Ventricular Hypertrophy

Murine models, lacking vitamin D receptor, exhibit increased ventricular mass, higher atrial natriuretic peptides, and impaired homeostasis of metalloproteinases and fibroblasts, leading to ventricular dilatation and impaired electromechanical coupling [2]. Considering *hypertension* associated

with low vitamin D levels, left ventricular hypertrophy could also be a consequence. O'Connell et al. demonstrated that calcitriol increases *myocyte protein levels and cell size,* suggesting that it induces cardiac myocyte hypertrophy [33]. Blocking the S phase of the cell cycle is the mechanism by which 1,25(OH)2D3 regulates *myocyte proliferation* [33].

Vitamin D reduces cardiac hypertrophy in spontaneously hypertensive rats [102] and in salt-sensitive rats via modulation of several protein kinase pathways [52, 103]. Among the proposed cardioprotective effects of vitamin D, reduced expression of mediators of myocardial hypertrophy, including atrial natriuretic peptides, and growth factors promoting cell proliferation were mentioned [10].

Intravenous calcitriol treatment, used to control secondary hyperparathyroidism in hemodialysis patients, caused regression of myocardial hypertrophy and reduction of QT interval dispersion, suggesting a cardioprotective effect of vitamin D [104]. The addition of calcitriol to cardiomyocytes inhibits cell proliferation without apoptosis, promoting cardiac differentiation [87]. A significant relationship between vitamin D level and interventricular septum and left ventricular mass index was found after adjusting for age, hypertension, and vitamin D therapy status, in a large retrospective study, suggesting the role of vitamin D in ventricular remodeling [95]. *Calcium* is also involved in cellular proliferation and activates AKT, a protein kinase involved in the development of cardiac hypertrophy [105]. Calcium increases after vitamin D supplementation and could also enable cardiac hypertrophy, and calcium overload causes also myocyte apoptosis and cardiac arrhythmias [105].

The results reporting the effect of vitamin D on left ventricular hypertrophy are not convincing, ranging from favorable influences to negative results [106].

9. Vitamin D and Atrial Fibrillation

Conflicting results were also found regarding the association of low vitamin status and atrial fibrillation. A relationship between vitamin D deficiency and nonvalvular atrial fibrillation was reported by several studies [107, 108]. Serum 25-hydroxyvitamin D level correlated with the *left atrial diameter, high-sensitive C reactive protein, and pulmonary systolic pressure* and was significantly associated with atrial fibrillation in Chinese patients with nonvalvular persistent atrial fibrillation [108]. Direct *electromechanical effects* on the left atrium were revealed by Hanafy et al. for vitamin D, enabling prevention or termination of atrial fibrillation [109].

Rienstra et al. evaluated 2,930 participants of the Framingham Heart Study during a follow-up period of 9.9 years and found no relation between vitamin D status and incident atrial fibrillation, concluding that vitamin D deficiency does not promote the development of atrial fibrillation [110].

10. Vitamin D and Stroke

Epidemiological studies have shown that vitamin D deficiency is an independent risk factor for arterial hypertension and stroke [111]. A recent "umbrella" review stated that the association between high vitamin D level and low stroke risk is possible, but not convincing [112].

Additional *neuroprotective* actions of vitamin D have also been reported [111], which may reduce cognitive impairment in poststroke patients [113], and the neuromuscular and osteoprotective effects may improve mobility. It is premature to recommend vitamin D supplementation for the prevention and treatment of stroke, considering that randomized controlled trials did not confirm that vitamin D reduces stroke incidence [111]. The high prevalence of vitamin D deficiency in patients with hypertension and stroke, associated with musculoskeletal pathology, could justify the evaluation, prevention, and treatment of vitamin deficiency in these patients [111].

11. Vitamin D and Peripheral Arterial Disease

Vitamin D receptors may be also found in the vascular wall, suggesting that vitamin D status might play a role in the pathogenesis of arterial disease [114]. Among individuals with peripheral artery disease, low vitamin D status was associated with a *faster decline of functional performance* but not with mortality [115]. Vitamin D deficiency was highly prevalent in patients with occlusive and aneurysmatic arterial disease, independent of traditional cardiovascular risk factors, and showed a strong association with the severity of the arterial disease and atherosclerotic markers: *carotid artery intima-media thickness and ankle-brachial index and high sensitive C reactive protein* [114]. It was suggested that the relationship between low vitamin D status and arterial disease is mediated by an independent arterial wall effect [114]. Severe vitamin D deficiency results in a disrupted adaptive *immune response and an inflammatory milieu,* promoting vascular dysfunction and insulin resistance [2, 116].

Only few studies examined the effects of vitamin D on vascular function and the results are contradictory [2]. Vitamin D also affects *aortic stiffness and vascular aging* [117, 118]. Activation of the RAA system and subsequent synthesis of angiotensin II increase vascular tone and arterial stiffness, preceding the development of hypertension [2]. A study, including 62 diabetic participants, identified no beneficial effects on cardiovascular risk, insulin resistance, and arterial stiffness after 24 weeks of vitamin D supplementation [119]. The lack of reduction in arterial stiffness might be due to the negative effects of vitamin D supplementation on arterial-stiffness related cardiovascular risk factors and the insufficient duration of the therapy [119].

Vascular calcifications, the result of calcium-phosphate deposition, major determinants of mortality and morbidity in affected patients, are associated with excessive vitamin D and hyperphosphatemia. Arterial calcifications occur in the vascular intima, associated with atherosclerosis and lipid accumulation, or in the media, associated with arteriosclerosis due to age, diabetes, and end-stage renal failure; both forms increase vascular stiffness [34, 120].

Physiologic vascular vitamin D actions include inhibitions of proatherogenic processes and intimal and medial artery calcification, including release of proinflammatory

cytokines and adhesion molecules, migration, and proliferation of vascular smooth muscle cells [121].

12. Renal Implications of Vitamin D Deficiency Related to Cardiovascular Pathology

The kidneys are involved in the synthesis of the metabolically active form of vitamin D, since the second hydroxylation, stimulated by the parathyroid hormone, occurs in the kidneys. Serum phosphate levels influence the renal hydroxylation of vitamin D through a negative feedback mechanism [122]. Individuals with renal disease have a deficiency of 1,25-dihydroxyvitamin D, impairing calcium and phosphate balance [122].

Cardiovascular diseases are more prevalent in patients with chronic kidney disease compared to patients with normal kidney function, and several links between vitamin D deficiency and poor cardiovascular outcomes were described in patients with renal disease [43].

Mortality, due mainly to cardiovascular causes, was associated with low vitamin D levels and high parathyroid hormone in patients with chronic renal disease [43]. The randomized Japan Dialysis Active Vitamin D Trial (J-DAVID), with the following primary outcomes: fatal or nonfatal cardiovascular events, coronary interventions, and lower limb artery intervention in hemodialysis patients, will, probably, provide valuable data regarding cardiovascular events in patients with chronic kidney disease stage 5, considering active vitamin D [43].

Vascular calcifications were found also in experimental uremic models with low levels of vitamin D [123]. They are associated with an increased cardiovascular mortality in stage 5 chronic kidney disease [120], and renal osteodystrophy and its therapy, the use of warfarin, and, probably, other elements of the uremic milieu may contribute to its etiology [34].

Vitamin D deficient patients with chronic renal failure had *enhanced atherosclerotic lesions* with arterial stiffening and reduced flow-mediated dilatation [97]. Animal studies evaluating the effects of vitamin D compounds on uremic vascular calcifications and pulse wave velocity revealed a dose-response relationship on vascular calcifications and a differential effect of different compounds, suggesting different mechanisms of action [43, 120]. Low doses of calcitriol and paricalcitol, sufficient to correct secondary hyperparathyroidism, were protective against aortic calcification in a mouse model of chronic kidney disease, but higher doses stimulate further calcification [124]. Calciphylaxis, a highly morbid and severe type of vascular calcification, was reported to be more prevalent in patients treated with calcitriol, but not with selective vitamin D analogues [43].

Patients on *chronic dialysis* are at increased risk of vitamin D deficiency, and six months of cholecalciferol therapy did not improve *blood pressure, arterial stiffness, and cardiac function* [125].

Elevated PTH contributes probably to the development of *uremic cardiomyopathy*, considering the correlations between PTH and left ventricular hypertrophy in chronic renal failure [126]. Vitamin D given in hemodialysis patients enabled regression of myocardial hypertrophy and reduction of QT interval dispersion, a marker of ventricular arrhythmia risk [104].

Vitamin D receptor polymorphisms, such as B alleles of BsmI, with altered vitamin D signaling, are genetic risk factors for the development of left ventricular hypertrophy in kidney disease [127–129]. Left ventricular hypertrophy is a strong cardiovascular risk marker in patients with end-stage renal disease [127–129]. The possible mechanisms responsible for the increased mortality associated with BsmI polymorphism in hemodialysis patients are as follows: modification of vitamin D receptor sensitivity and expression in cardiac and vascular tissues, modification of the circulating levels of vitamin D due to the influence of vitamin D receptors on the feedback mechanism for the regulation of alpha-1-hydroxylase, hyperparathyroidism with calcium-phosphate imbalance, which predisposes to cardiac and vascular calcifications, and hampered calcitriol effects [130].

13. Why Conflicting Results?

At present, the data regarding the causal link between low vitamin D status and cardiovascular disease are mixed, conflicting, and ambiguous. Several reasons for conflicting results were found, including significant heterogeneity of vitamin D doses, baseline concentration, therapy duration and compounds, differences of absorption and metabolism among individuals, genetic differences in the vitamin D receptor, private use of vitamin D, biases due to different diseases, study-design related factors, variations in definitions [43], several potentially confounding factors, including age, body mass index, medication, diet, sunlight exposure, physical activity, and concomitant intake of calcium [114, 131, 132], latency of the effect of vitamin D, inappropriate follow-up time or lack of a control group with normal vitamin D level [9], lack of standardization of 25-hydroxyvitamin D assay, different ethnic populations [83], autocrine and paracrine vitamin D systems, local tissue vitamin D intoxication, concomitant hyperphosphatemia, PTH level, and counterregulatory hormones, such as fibroblast growth factor 23 [74, 133]. Assessment of vitamin D status only from dietary questionnaires has, probably, a high degree of subjectivity. Future investigations should focus also on bioavailability rather than total 25-hydroxyvitamin D [43]. A high cardiovascular disease incidence and prevalence were found at high latitudes and geographical areas with low exposure to ultraviolet B radiation [20]. Winter and spring months would probably show higher proportions of patients with vitamin D deficiency. The risk of mortality was significantly higher in studies with lower baseline use of vitamin D [6]. Calcium intake may affect the results, because oral calcium may increase the risk of cardiovascular disease [134]. Most of the studies are observational and they should be replicated in randomized controlled trials [112]. The studies are very different, including observational studies of plasma vitamin D concentrations, cross-sectional, longitudinal, systematic literature reviews, and randomized controlled trials of vitamin D supplementation and experimental studies exploring the mechanisms of the associations. Even meta-analyses of

randomized studies may not be convincing, especially due to limited sample and low level of significance. Bias due to selection of participants, comparability of study groups, and selection of outcomes of interest [6] could also contribute to different results.

It is still not clear if vitamin D supplementation is needed only if vitamin D is deficient in order to exert its cardioprotective effects. Which type of vitamin D or vitamin D analogue is effective is another question still requiring an answer. Lower doses in vitamin D2 supplementation and shorter intervention periods were associated with a higher mortality [6].

The question about the benefit of vitamin D supplementation for cardiovascular outcomes cannot be answered certainly for the moment [76], but perhaps the outcomes of the VITAL prevention trial and J-DAVID trial will provide more answers.

Another concern is related to the vitamin D level with benefic effects for cardiovascular disease, considering that doses recommended for osteoporosis treatment are neither beneficial nor harmful in cardiovascular disease [29]. Consumption of high amounts of vitamin D may interfere with the regulation of phosphate metabolism by fibroblast growth factor 23 and the Klotho gene product [133]. It is therefore important to identify and use new markers for phosphate homeostasis, such as salivary phosphate secretion [105], during vitamin D therapy.

It still remains uncertain whether the association between low vitamin D status and cardiovascular diseases is causal or just a bystander. It is likely that unidentified factors and relationships with other endocrine networks are also involved in vitamin D biology, emphasizing the need of further research in this area [74].

14. Conclusions

Maintaining an optimal vitamin D serum level seems important not only for calcium homeostasis but also for cardiovascular risk, blood pressure control, prevalence of stroke, metabolic syndrome, and peripheral artery disease. Observational data support the link between vitamin D status and cardiovascular diseases, and vitamin D deficiency can be considered a cardiovascular risk marker. Vitamin D exerts its cardiovascular effects by reducing the activity of the renin-angiotensin-aldosterone system, lowering blood pressure values, and having an anti-inflammatory, antiproliferative, antihypertrophic, antifibrotic, antidiabetic, and antithrombotic effect and beneficial modulation of classical cardiovascular risk factors. The mentioned effects might be very important for public health, considering the high prevalence of vitamin D deficiency, the aging population, and the indoor oriented lifestyle.

Vitamin D deficiency is treatable and supplementation is inexpensive. Vitamin D could be combined with antihypertensive agents in order to control blood pressure, as a simple, inexpensive, and important prophylactic method in order to prevent cardiovascular morbidity, especially in the elderly. Even small gains in prevention are important from a public health perspective. Further proteomics and basic research studies are needed in order to identify the missing pieces in the vitamin D-cardiovascular disease puzzle. Large randomized controlled trials could confirm the promising findings of observational studies, considering endothelial function, arterial stiffness, and patients undergoing percutaneous coronary interventions. Guidelines are needed in order to establish optimal vitamin D level and intake, to maintain a healthy vitamin D status in patients with cardiovascular diseases, and to include vitamin D blood tests, genotyping for vitamin D receptor variants, and serum calcium and phosphates level and bone mineral density as mandatory in evaluating patients with cardiovascular disease. The benefits of screening and treating vitamin D deficiency would be, probably, reflected by reduced cardiovascular morbidity and mortality. Vitamin D supplementation should further consider additional factors, such as phosphates, PTH, RAA, and fibroblast growth factor 23.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Special Article

Effect of vitamin D supplementation on serum lipid profiles: a systematic review and meta-analysis

Daniel T. Dibaba

Context: *Vitamin D deficiency is highly prevalent across the world. The existing evidence suggests vitamin D may have beneficial effects on serum lipid profiles and thus cardiovascular health.* ***Objective:*** *The objective of this systematic review and meta-analysis was to examine the effect of vitamin D supplementation on serum lipid profiles.* ***Data Source:*** *Original randomized controlled trials (RCTs) examining the effect of vitamin D supplementation on serum lipid profiles and published before July 2018 were identified by searching online databases, including PubMed, Google Scholar, and ScienceDirect, using a combination of relevant keywords.* ***Data Extraction:*** *Data on study characteristics, effect size, measure of variation, type of vitamin D supplementation, and duration of follow-up were extracted by the author.* ***Data Analysis:*** *PRISMA guidelines for systematic reviews were followed. Random effects (DerSimonian and Laird [D-V)] models were used to pool standardized mean differences in total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides between the active and the placebo arms of RCT studies. Between-study heterogeneities were assessed using Cochrane Q and I^2, and publication bias was assessed using Begg's test, Egger's test, and funnel plot.* ***Results:*** *A total of 41 RCTs comprising 3434 participants ($n = 1699$ in the vitamin D supplementation arm and $n = 1735$ in the placebo arm) were identified and included in the meta-analysis. Approximately 63.4% of study participants were women, with 14 studies conducted entirely among women. Approximately 24% of the trials had follow-up duration >6 months, whereas the remaining 76% had follow-up duration of <6 months. The standardized mean differences (SMDs) and 95% confidence intervals (CIs) for comparing the change from baseline to follow-up between the vitamin D supplementation arm and the placebo (control) arm were as follows: total cholesterol = −0.17 (−0.28 to −0.06); LDL cholesterol = −0.12 (−0.23 to −0.01); triglycerides = −0.12 (−0.25 to 0.01); and HDL cholesterol = −0.19 (−0.44 to 0.06). After removing a trial that was an outlier based on the magnitude of the effect size, the SMD for triglycerides was −0.15 (−0.24 to −0.06) and that for HDL cholesterol was −0.10 (−0.28 to 0.09). The improvements in total cholesterol and triglycerides were more pronounced in participants with baseline vitamin D deficiency.* ***Conclusions:*** *Vitamin D supplementation appeared to have a beneficial effect on reducing serum total cholesterol, LDL cholesterol, and triglyceride levels but not HDL cholesterol levels. Vitamin D supplementation may be useful in hypercholesterolemia patients with vitamin D insufficiency who are at high risk of cardiovascular diseases.*

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INTRODUCTION

The prevalence of vitamin D deficiency (serum vitamin D $\leq$ 20 ng/mL) in the United States was estimated to be around 40% in general and higher among blacks, Hispanics, Asians, children, and elderly populations.[1,2] Globally, however, vitamin D deficiency is highly prevalent even in those living in low altitudes assumed to have enough ultraviolet (UV) radiation and in developed countries where vitamin D fortification has been implemented.[3–6] Low serum vitamin D is associated with several chronic diseases, including cardiovascular diseases, stroke, and diabetes.[7–11] The results of intervention studies examining the effect of vitamin D on serum lipid profiles are inconsistent. Some studies have shown favorable lipid profiles in those supplemented with vitamin D[12–14] and in those supplemented with calcium and vitamin D[14,15] compared with the control (placebo) arms. However, some of these studies also found unfavorable outcomes for high-density lipoprotein (HDL) cholesterol after vitamin D[13,16] or calcium and vitamin D[15] supplementation. Many other intervention studies have documented favorable but statistically nonsignificant effects of vitamin D[16–19] on serum lipid profiles. A 2012 meta-analysis documented statistically nonsignificant effects of vitamin D supplementation on total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, and triglycerides.[5] Since then, several more RCTs have been conducted to further evaluate the association between vitamin D supplementation and serum lipid profiles. Thus, the current study aimed to examine the effect of vitamin D supplementation on serum lipid profiles among participants in published RCTs using a systematic review and a large meta-analysis. The study was conducted according to the PRISMA guidelines, and the PRISMA checklist is included in the Supporting Information online.

METHODS

Data source and study selection

The author searched for original RCT studies relating vitamin D to serum lipid profiles published before July 2018 in PubMed, ScienceDirect, Google Scholar, and ClinicalTrials.gov using the medical subject header (MeSH) terms "vitamin D" or "VitD" or "25-hydroxy vitamin D" or "25(OH)D3" or "OH25D" or "(OH)25D2" or "(OH)25D3" or "cholecalciferol" and "serum lipid profiles" or "HDL" or "LDL" or "cholesterol" or "total cholesterol" or "TG" or "TAG" or "triglyceride" or "triacylglycerol". Additional RCTs were identified from reference lists of relevant full-text articles retrieved.

To be included studies had to 1) examine the effect of vitamin D on serum lipid profiles, 2) be conducted in a human population aged $\geq$18 years, 3) be an original study, 4) report mean and standard deviations of the lipid profiles, and 5) be a randomized placebo-controlled clinical trial or compare the supplementation arm to a control arm.

The exclusion criteria included 1) nonhuman study, 2) non-RCT study, 3) studies without clear control arm or placebo arm, 4) study duration <1 month, and 5) studies without baseline and end-of-trial serum lipid profiles or without changes in lipid profiles with a related measure of variation. The participant, intervention, comparisons, outcome, and study design (PICOS) criteria are presented in Table 1. The database search resulted in the identification of 8269 studies, and after exclusion of the studies that did not meet the inclusion criteria and duplicate results, a total of 41 RCTs were included in the meta-analysis (Figure 1). Study quality was evaluated using the Jadad scales.[20] An emphasis was put particularly on items directly related to bias, including randomization, double-blinding, and reporting of dropout rates/loss to follow-up (*see Table S1 in the Supporting Information online*). Each of the 3 items was given a score from 0 to 5 points with the maximum total adding to 15 if all 3 items were mentioned and the method was described and appropriate. A score of 0 was given if an item was not mentioned, and points were subtracted from the maximum 5 for each item if the item was mentioned but the method was not appropriate.

Data extraction

The author extracted the data. The extracted data included first author's last name, year of publication, sample sizes in the active arm and control arm, changes in mean from baseline to the end of the study of HDL cholesterol, LDL cholesterol, total cholesterol, and triglycerides, and related pooled standard deviations. The name of supplemented vitamin D, treatment dose, treatment duration (months), general health status of participants, percentage female, and vitamin D status (Table 2 and *see Table S1 in the Supporting Information online*) were also extracted.

Data synthesis

For studies for which the lipid profiles were reported as mean changes and associated 95% confidence intervals (CIs), the CIs were converted into SD using $\sqrt{n}$ *(UCI − LCI)/3.92, where n is the sample size and UCI and LCI are upper and lower confidence intervals, respectively. Data reported in millimoles per liter were converted to milligrams per deciliter by multiplying with

Table 1 **PICOS criteria for inclusion and exclusion of studies**

Parameter	Inclusion criteria	Exclusion criteria
Participants	Studies with adult human population aged >18 y	Nonhuman studies (animal studies), studies among children
Intervention	Studies with vitamin D supplementation	Studies with follow-up duration <1 month
Comparison	Studies with placebo or control arm	Studies without a clear comparison group
Outcomes	Studies with mean and standard deviation in total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides at baseline and end of trials or mean changes and standard deviations in the serum lipid profiles in both the vitamin D supplementation and placebo or control arms	Studies not reporting mean and standard deviation in the serum lipid profiles or not reporting mean changes and standard deviations in serum lipid profiles
Study Design	Randomized controlled trials with parallel design	Observational studies, pre/post and cross-over randomized control trials (excluded from the meta-analysis but reviewed), studies without a placebo or control arm, editorials and opinion pieces

38.67 for HDL, LDL, and total cholesterol and by 88.57 for triglyceride.[21] For studies for which the medians and the first and the third quartiles were reported, the medians were converted to mean using, $\bar{x} \approx \frac{X_{Q3} + X_{Q2} + X_{Q1}}{3}$, where X_{Q3} is the 3rd quartile, X_{Q2} is the median, and X_{Q1} is the 1st quartile, and the interquartile range was converted to standard deviation (SD) using $S \approx \frac{X_{Q3} - X_{Q1}}{2 * \Phi^{-1}\left(\frac{0.75 * n - 0.125}{n + 0.25}\right)}$, where n is the sample size and Φ^{-1} is the inverse of the cumulative standard normal distribution.[22] When not given in the original study, change from the baseline was computed for each lipid profile by subtracting the baseline from the end-of-trial values. When the SD in changes in the lipid profile from baseline to the end of trial was not reported, the SD was computed using $\sqrt{S_b^2 + S_e^2 - 2 * 0.5 * S_b * S_e}$, where S_b is baseline SD, S_e is SD at the end of trial, and the correlation between the baseline and the end of trial was assumed to be 0.5 for the given lipid profile.[23]

Statistical analysis

Random effects (DerSimonian and Laird [D-V])[24] meta-analysis models were conducted to pool standardized mean differences (SMDs) between the active arm and the placebo arm of the trials. The SMD was calculated using $\frac{\mu_t - \mu_p}{SD_p}$, where μ_t is the mean of the active arm, μ_p is the mean of the placebo arm, and SD_p is the pooled standard deviation. The risk of publication bias was assessed using Begg's test.[25] Cochran's χ^2 test was used to examine heterogeneity among the included studies, and computed I^2, which is the proportion of the total variation due to heterogeneity between studies, was used to determine the degree of inconsistency across studies. Meta-regression analysis was conducted to explore covariates that might explain the heterogeneity among trials. For the meta-regression analyses, the effect sizes based on the random effect meta-analysis as a dependent variable were regressed on each or on a combination of study-level summary characteristics such as study duration; baseline and end-of-study levels of LDL, HDL, and total cholesterol and triglycerides; publication year; country; baseline health status of the participants/disease; treatment dose; sample size; percentage female; and mean age. The restricted maximum likelihood estimation method was used to estimate the between-study variances, and adjusted R^2 for the proportion of between-study variance explained by a covariate or covariates was reported. The statistical hypothesis of 0 SMD was tested using χ^2 and associated P value. A study with extreme effect size having 95%CIs not covered by the 95%CI of at least 2 other study was considered as an outlier. This was also confirmed if the effect size (SMD) was above the third quartile plus 1.5 times the interquartile range or below the first quartile minus 1.5 times the interquartile range, and such studies were excluded in the sensitivity analysis. All analyses were conducted using STATA statistical software (version 13, STATA Corp, College Station, TX, USA). All statistical tests were 2-sided, and a P value ≤ 0.05 was considered statistically significant.

RESULTS

Overall, 41 RCTs[12,14,16–19,26–58] consisting of 3434 participants with 1699 participants in the active arm and 1735 in the placebo arm were included in the meta-analysis. Fourteen of the trials for which sex information was reported were conducted among women only, with women constituting 63.4% of participants in trials for which sex was reported. The mean age of the participants was 55 years (SD = 11.6); the age range of the participants began at 19 years, but most of the participants were aged > 45 years. The study duration ranged from

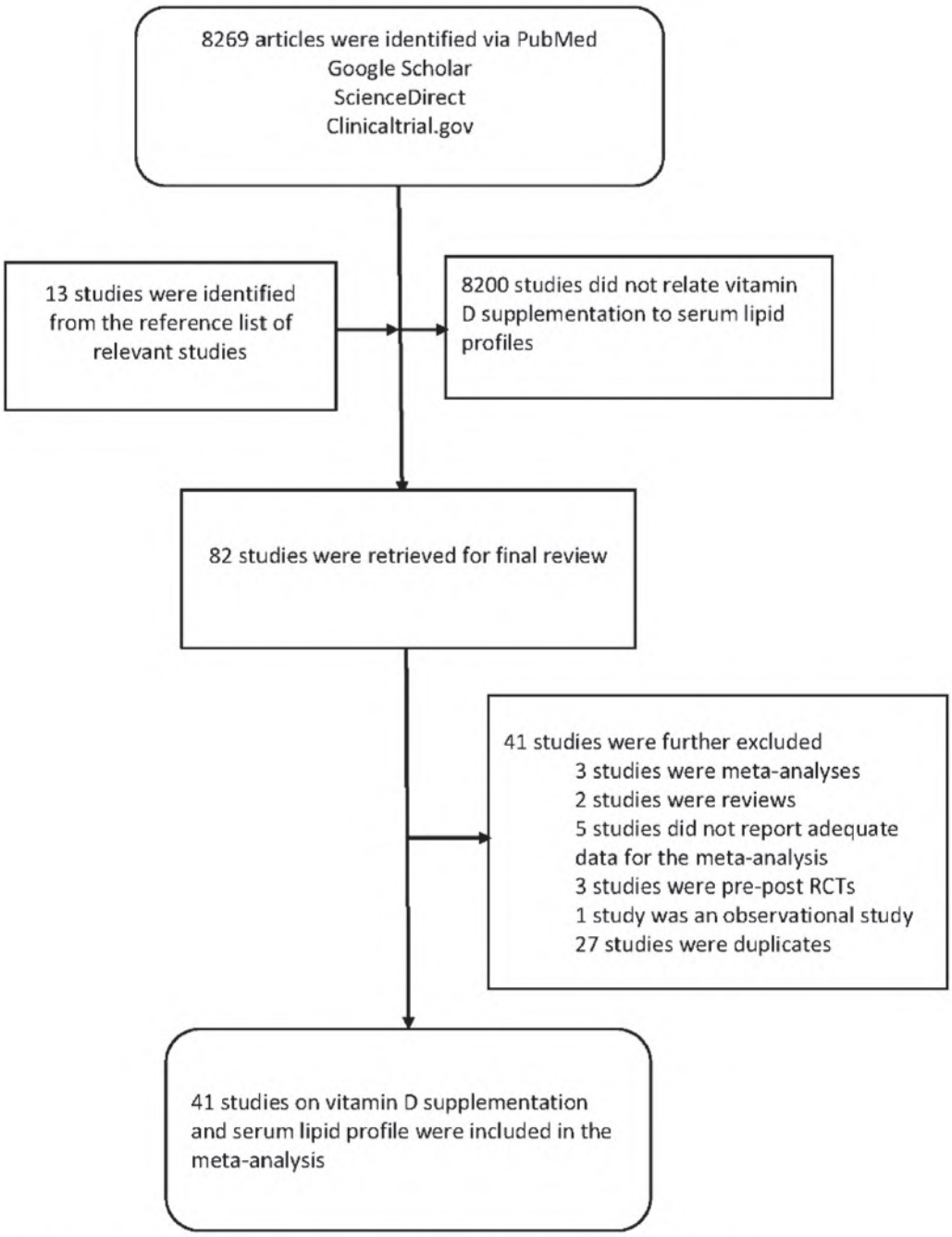

Figure 1 **Flow diagram of the literature search process.** *Abbreviation:* RCT, randomized controlled trial.

6 weeks to 3 years. The mean duration of the study was 6.9 (SD = 7.5) months (Table 2). Mean vitamin D supplement per day was 2795IU (range, 20–8570 IU). Twenty-one trials were conducted on participants with diabetes, 13 trials were conducted on apparently healthy participants, and 3 trials were conducted on those who were obese or overweight. In 24 (68.6%) trials for which vitamin D deficiency (≤20 ng/mL) at baseline was reported, participants were vitamin D sufficient at the end of the trial (see *Table S1 in the Supporting Information online*). In 4 (11.4%) trials, no improvement was seen in serum vitamin D after the trial. In 7 (20%) trials, participants had sufficient vitamin D (>20 ng/mL) both at baseline and at the end of the study. In 6 trials either both baseline and end-of-trial serum vitamin D or end-of-trial serum vitamin D was not reported (*see Table S1 in the Supporting Information online*).

Total cholesterol

The random effect model pooled SMD in changes from baseline to the end of the trials between the supplementation arm and the placebo arm for total cholesterol was −0.17 (95%CI, −0.28 to −0.06; I^2=54.6%) (Figure 2). The nonstandardized mean total cholesterol difference was −3.69 (95%CI, −5.78 to −1.59). In meta-regression analysis, end-of-trial total cholesterol in the vitamin D arm explained 100% of the between-study heterogeneity; the adjusted R^2 was 100%. There was no evidence of publication bias (Begg's $P = 0.425$).

Low-density lipoprotein cholesterol

For LDL cholesterol, the SMD was −0.12 (95%CI, −0.23 to −0.01; I^2=52%) (Figure 3). The nonstandardized LDL cholesterol mean difference was −2.92 (95%CI, −5.27 to −0.58). There was no statistically significant publication bias for LDL cholesterol (Begg's $P = 0.06$). Removing the Kampmann[40] trial that appeared to be an outlier, the effect remained (SMD, −0.10; 95%CI, − 0.20 to −0.003) (*see Figure S1 in the Supporting Information online*). In meta-regression analysis, the mean changes in LDL cholesterol in each arm explained 100% of the between-study heterogeneity; the adjusted R^2 was 100%.

High-density lipoprotein cholesterol

For HDL cholesterol, the SMD was −0.19 (95%CI, −0.44 to 0.06; I^2 =91.1%) (Figure 4). The nonstandardized HDL cholesterol mean difference was −1.09 (95%CI, −2.46 to 0.28). In meta-regression analysis, baseline and end-of-trial HDL cholesterol in the placebo arm explained 100% of the between-study heterogeneity; the adjusted R^2 was 100%. There was no evidence of publication bias on the association of vitamin D with HDL cholesterol (Begg's $P = 0.478$). Removing the Sai[50] trial that appeared to be an outlier, there was no material change (SMD, −0.10; 95%CI, −0.28 to 0.09) (*see Figure S2 in the Supporting Information online*).

Triglycerides

The SMD for triglycerides was −0.12 (95%CI, −0.25 to 0.01; I^2=69.1%) (Figure 5). The nonstandardized triglyceride mean difference was −6.92 (95%CI, −11.97 to −1.86). Baseline and end-of-trial triglycerides in the treatment arm and mean change in triglycerides in the placebo arm explained 100% of the between-studies heterogeneity; the adjusted R^2 was 100%. Furthermore, removing the Sai[50] trial that appeared to be an outlier, vitamin D supplementation reduced serum triglycerides (SMD, −0.15; 95%CI, − 0.24 to −0.06; I^2 = 32.9%) (*see Figure S3 in the Supporting Information online*). Because there was an evidence of publication bias (Begg's $P = 0.003$) on the association of vitamin D supplementation with triglycerides, trim-and-fill analysis was conducted with the assumption that trials with direct association may have been suppressed. In the trim-and-fill analysis 12 data points in the positive direction were filled, and vitamin D no longer had a beneficial effect on serum triglycerides (SMD, 0.04; 95%CI, −0.09 to 0.17).

Stratified analysis

In a stratified analysis by trial duration (≤6 months vs >6 months), the effects for total cholesterol, LDL cholesterol, and triglycerides remained for the trials with duration ≤6 months and overall pooled results but disappeared for trials with a duration >6 months (data not shown). The difference might be attributed to the small number of trials (<10) with duration >6 months. There was no marked difference for HDL cholesterol by trial duration. In a stratified analysis by baseline serum vitamin D (≤20 ng/mL and >20 ng/mL) (*see Table S1 in the Supporting Information online*), the observed associations remained for total cholesterol and triglycerides in studies among participants with serum vitamin D deficiency at baseline, whereas in studies among participants with sufficient baseline serum vitamin D there was no beneficial effect; for LDL cholesterol the reverse was observed. Baseline serum vitamin D levels were not reported for 4 studies[14,16,41,50] and were not included in the baseline serum vitamin D stratified analysis. The pooled SMD for total cholesterol was −0.15 (95%CI, −0.24 to −0.05) for studies among participants with baseline serum vitamin D deficiency and –0.13 (95%CI, −0.28 to 0.03) for the studies among participants with sufficient baseline vitamin D; the overall SMD for total cholesterol was –0.14 (95%CI, −0.22 to −0.06). The SMD for triglycerides for studies among participants with serum vitamin D deficiency at baseline was −0.16 (95%CI, −0.29 to −0.02) and was −0.08 (95%CI, −0.24 to 0.08) for studies among participants with sufficient baseline serum vitamin D; the overall SMD was −0.13 (95%CI, −0.23 to −0.03). The SMD for LDL cholesterol was −0.13 (95%CI, −0.29 to 0.02) for studies among participants with baseline vitamin D deficiency, and −0.17 (95%CI, −0.33 to −0.02) for studies among participants with sufficient baseline vitamin D; the overall SMD for LDL cholesterol was −0.15 (95%CI, −0.24 to −0.05). The SMD for HDL cholesterol was not appreciably different based on baseline vitamin D levels. The SMD for HDL cholesterol for the

studies among participants with baseline serum vitamin D deficiency was −0.18 (95%CI, −0.41 to 0.05) and was −0.11 (95%CI, −0.51 to 0.29) for studies among participants with baseline serum vitamin D sufficiency; the overall SMD was −0.16 (95%CI, −0.36 to 0.03).

DISCUSSION

A meta-analysis of 41 RCTs evaluating the effect of vitamin D supplementation on lipids revealed that vitamin D supplementation has a beneficial effect on serum total cholesterol, LDL cholesterol, and triglycerides but not on HDL cholesterol. This is the largest meta-analysis to date evaluating this association; previous meta-analyses were based on ≤20 studies and focused on those with specific underlying health conditions, including type 2 diabetes[59] or gestational diabetes.[60] The present meta-analysis is the most comprehensive meta-analysis, including 41 vitamin D supplementation RCTs.

These results are similar to the findings of previous meta-analyses. For instance, a previous meta-analysis in 2012 reported that vitamin D has a beneficial effect on LDL cholesterol but not HDL cholesterol, triglyceride, or total cholesterol.[5] Another recent (2016) meta-analysis of 17 articles in participants with type 2 diabetes observed that vitamin supplementation lowered total cholesterol and LDL cholesterol but had no beneficial effect on triglycerides and HDL cholesterol.[59] Another meta-analysis in 2017 among women with gestational diabetes observed that vitamin D supplementation had a beneficial effect on serum LDL cholesterol, but in that meta-analysis, vitamin D was not beneficial on total cholesterol, HDL cholesterol, or triglycerides.[60] One study found vitamin D has a synergetic effect with cholesterol medications. In that study, vitamin D reduced LDL and total cholesterol compared with the arm that took only cholesterol medication.[61] One trial that was not included in this meta-analysis because the reported results are outliers found that vitamin D improved total cholesterol, LDL cholesterol, and HDL cholesterol but not triglycerides.[62] Three trials compared post–vitamin D supplementation serum lipid profiles to baseline serum lipid profiles and did not have a control arm. Among them, Al-Daghri et al reported vitamin D supplementation reduced total cholesterol, LDL cholesterol, and triglycerides,[63] whereas Manoy et al reported LDL cholesterol and HDL cholesterol improved after supplementation but total cholesterol and triglycerides were not different.[28] The Amarasekera et al pre/post trial did not find a beneficial effect of vitamin D supplementation among healthy adults in a trial that lasted 3 months.[64]

The magnitude of the baseline or end-of-trial lipid profiles or changes from baseline to the end of the trials in both arms explained almost all of the heterogeneity among the included studies. Among other trial-level covariates, including trial duration, publication year, country, the health status of the participants/disease, treatment dose, sample size, percentage female, and mean age, that were included in meta-regression analyses, only treatment duration appeared to explain some of the between-trial heterogeneity. In an analysis stratified by duration, the result remained consistent for trials of a short duration (≤6 months) but was no longer for trials of a long duration (>6 months). This difference by trial duration was no longer apparent when RCTs that appeared to be outliers, including the trials conducted by Sai et al[50] and Kampmann et al,[40] were removed. The Kampmann et al[40] and the Sai et al[50] trials appeared to be outliers in some of the analyses, and removal of the Kampmann et al trial from the analysis on LDL cholesterol and the Sai et al trial from the analysis on triglycerides resulted in stronger associations. The Sai et al study was designed to study the effect of estrogen and vitamin D supplementation in apparently healthy postmenopausal elderly women, and it was relatively large and had a long follow-up duration (3 y), but there was no mention of double blinding. However, at baseline, there was no evidence of differences in total cholesterol, HDL cholesterol, and triglycerides, but there was a difference in mean baseline LDL cholesterol. The Kampmann et al study was a double-blinded RCT among patients with type 2 diabetes with vitamin D insufficiency at baseline. It had a 3 month follow-up time but had a relatively small sample size (n =8 in each trial arm). Because none of the other covariates explained the heterogeneity among the trials, stratified analysis by those covariates was not conducted.

Because the meta-analysis indicated an evidence of publication bias for triglycerides, trim-and-fill analyses were conducted.[65] Trim-and-fill analysis tries to compensate for a publication bias by generating hypothetical missing studies with effects opposite to those likely favored and reported and pools those generated studies with studies included in a meta-analysis. The trim-and-fill analysis suggested 12 missing studies for triglycerides, and the augmented analysis suggested no beneficial effect for triglycerides. However, because there was no evidence of publication bias for total cholesterol in which 40 available trials were included, the augmented analysis that filled in 12 data points in the positive direction for potentially missing trials may have over-augmented. In meta-analyses of triglycerides, 35 trials were included, only 5 trials less than the analysis on total cholesterol and 1 trial less than the analysis on HDL cholesterol, both of which had no publication bias present.

The mechanism through which vitamin D affects circulating cholesterol levels may be through the action of vitamin D on the transcription activity of vitamin D receptor and insulin-induced gene-2 (Insig-2) expression. Insig-2 downregulates sterol regulatory-element binding protein-2 (SREBP-2) activation and inhibits 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (HMGR) expression, an enzyme critical to cholesterol synthesis, thus reducing cholesterol synthesis.[66] Animal studies also support the role of vitamin D in cholesterol synthesis through inhibition of SREBP-2.[67] In a skeletal muscle cell, calcitriol altered lipid partitioning and lipid droplet packaging in a way that favored lipid turnover.[68] An animal study also indicated vitamin D regulates the level of lipogenic genes and controls lipid synthesis via the deactivation of SREBP.[69] In an experimental study, active vitamin D also resulted in the reduction of triglycerides in differentiated adipocytes, increased fatty acid ß-oxidation, and reduced de novo fatty acid synthesis.[70]

A strength of this review is that the inclusion of data from 41 RCTs provided enough power to detect the effect of vitamin D on serum lipid profiles. Generally, there was no evidence of major publication bias, especially for trials on total cholesterol. Most of the included studies were high-quality trials with randomization and double blinding minimizing the risks of residual confounding and bias. There were low dropout rates in the original trials, and per protocol analyses were used in most trials. One of the limitations of this review is that the follow-up period was short in most trials. Another limitation is that data on season was available in only 1 trial,[48] thus precluding examination of the role of vitamin D on serum lipid profile by season. Furthermore, none of the included studies evaluated potential differences in the effect of vitamin D supplementation by race.

CONCLUSION

In conclusion, this meta-analysis of RCTs indicates that vitamin D supplementation improved serum total cholesterol, LDL cholesterol, and triglycerides but not HDL cholesterol levels. It may be beneficial for patients at risk of cardiovascular diseases to be evaluated clinically for hypercholesterolemia and vitamin D deficiency, and clinicians may consider supplementing regular cholesterol treatments with vitamin D in vitamin D–deficient patients.

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Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Figure S1 **Forest plot of the standardized mean difference (SMD) in changes from baseline of the low-density lipoprotein (LDL) cholesterol in vitamin D intervention studies. The horizontal bar indicates the 95% confidence interval. The size of the rectangle at the center of the horizontal bar is proportional to the weight of the given study. The diamond at the bottom indicates the pooled SMD. The Kampmann et al[40] trial was omitted**

Figure S2 **Forest plot of the standardized mean difference (SMD) in changes from baseline of the high-density lipoprotein (HDL) cholesterol in vitamin D intervention studies. The horizontal bar indicates the 95% confidence interval. The size of the rectangle at the center of the horizontal bar is proportional to the weight of the given study. The diamond at the bottom indicates the pooled SMD. The Sai et al[50] trial was omitted**

Figure S3 **Forest plot of the standardized mean difference (SMD) in changes from baseline of the triglyceride in vitamin D intervention studies. The horizontal bar indicates the 95% confidence interval. The size of the rectangle at the center of the horizontal bar is proportional to the weight of the given study. The diamond at the bottom indicates the pooled SMD. The Sai et al[50] trial was omitted**

Table S1 **Characteristics of trials included in the meta-analysis**

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REVIEW

Role of vitamin D in diabetes mellitus and chronic kidney disease

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Abstract

Approximately 30%-50% of people are recognized to have low levels of vitamin D, and insufficiency and deficiency of vitamin D are recognized as global health problems worldwide. Although the presence of hypovitamin D increases the risk of rickets and fractures, low vitamin D levels are also associated with hypertension, cancer, and cardiovascular disease. In addition, diabetes mellitus (DM) and chronic kidney disease (CKD) are also related to vitamin D levels. Vitamin D deficiency has been linked to onset and progression of DM. Although in patients with DM the relationship between vitamin D and insulin secretion, insulin resistance, and β-cell dysfunction are pointed out, evidence regarding vitamin D levels and DM is contradictory, and well controlled studies are needed. In addition, vitamin D influences the renin-angiotensin system, inflammation, and mineral bone disease, which may be associated with the cause and progression CKD. There is increasing evidence that vitamin D deficiency may be a risk factor for DM and CKD; however, it remains uncertain whether vitamin D deficiency also predisposes to death from DM and CKD. Although at this time, supplementation with vitamin D has not been shown to improve glycemic control or prevent incident DM, clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed. This review focuses on the mechanism of vitamin D insufficiency and deficiency in DM or CKD, and discusses the current evidence regarding supplementation with vitamin D in patients with these diseases.

Key words: Vitamin D; Vitamin D deficiency; Diabetes mellitus; Chronic kidney disease; Cardiovascular disease

Core tip: Vitamin D plays an essential role in diabetes

mellitus (DM) and chronic kidney disease (CKD). The relationship between vitamin D and insulin secretion, insulin resistance, and β-cell dysfunction are pointed out. Vitamin D deficiency has been linked with the renin-angiotensin system and inflammation, which may be associated with the cause and progression CKD. There is increasing evidence that vitamin D deficiency may be a risk factor for DM and CKD. Clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed.

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INTRODUCTION

Diabetes mellitus (DM) and chronic kidney disease (CKD) are common diseases worldwide, and their prevalence continues to increase[1,2]. Vitamin D deficiency is also recognized as a worldwide health problem[3], and is associated with rickets and fracture. In addition, hypovitamin D has recently been considered a responsible factor in the onset and progression of DM and CKD. There has been increasing evidence suggesting that an inverse vitamin D status is prevalent in patients with DM or CKD[4]. Furthermore, supplementation of vitamin D in patients with DM or CKD has been reported in several trials and a meta-analysis[5]. In this review, we provide current clinical data on the mechanism of vitamin D deficiency and the effects of vitamin D on patients with DM or CKD.

VITAMIN D PHYSIOLOGY

Vitamin D is a fat-soluble steroid hormone derived from dietary intake as well as synthesis through the skin *via* exposure to sunlight (Figure 1). Vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) are produced through solar ultraviolet B radiation (UVB; wavelength 290 to 315 nm). Vitamin D_3 is manufactured from previtamin D_3, which is changed through UVB irradiation from provitamin D_3[6]. Most 25-hydroxyvitamin (25[OH]D) is derived from skin conversion. An alternative source is from dietary intake, mainly from foods of plant or animal origin. In general, animals and fish contain vitamin D_3, and mushrooms contain vitamin D_2[7]. Vitamin D from the skin and diet is either stored in adipose tissue or converted to 25(OH)D in the liver. Vitamin D metabolism requires two hydroxylations to form its active metabolite. The first hydroxylation of vitamin D takes place in the liver where vitamin D is metabolized to 25(OH)D by cytochrome P 2R1 (CYP2R1). 25(OH)D binds to vitamin D-binding protein (DBP) and can flow into the blood in a stable form. 25(OH)D-DBP complex is excreted into the urine and reabsorbed through megalin, a multiligand scavenger receptor in the proximal tubules[8,9], where the complex is converted by 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) and changed to its active form 1,25-dihydroxyvitamin $(OH)_2D$, although other tissues have 1α-hydroxylase enzymatic activity[10]. CYP27B1 gene expression in the kidney is mediated by various factors. Parathyroid hormone (PTH), hypocalcemia, hypophosphatemia, and calcitonin affect the activation of CYP27B1 and can increase 1,25-$(OH)_2D$ levels. On the other hand, 1,25-$(OH)_2D$ and fibroblast growth factor-23 (FGF-23) inhibit CYP27B1 and can decrease 1,25-$(OH)_2D$ levels[11].

The binding of 1,25$(OH)_2D$ to the vitamin D receptor (VDR) in the nuclear receptor affects gene transcription. In general, 1,25$(OH)_2D$ promotes dietary calcium and phosphorus absorption in the intestine and regulates reabsorption of calcium in the renal tubules. Because VDR is expressed in a variety of organs, such as the heart, liver, blood vessels, and the central nervous system, 25-hydroxyvitamin D-1α-hydroxylase is also expressed in these tissues[12].

It is widely believed that 25(OH)D is the only precursor of 1,25$(OH)_2D$ and does not influence individual tissues. However, recent reports revealed that 25(OH)D has a weak binding capacity for VDR and affects several tissues in the autocrine or paracrine system[13,14]. In addition, extrarenal 1α-hydroxylase enzymatic activity is controlled in different ways that that in renal tubular cells[15].

EPIDEMIOLOGY OF VITAMIN D DEFICIENCY

Because 1,25$(OH)_2D$ has a short half-life (approximately 15 h), 1,25$(OH)_2D$ levels are not considered a good indicator of vitamin D levels. As 25(OH)D is more stable in the blood than 1,25$(OH)_2D$, blood concentrations of 25(OH)D are 500 to 1000 times higher than 1,25$(OH)_2D$ concentrations. Therefore, to evaluate vitamin D deficiency and insufficiency, serum 25(OH)D concentrations are considered an adequate biomarker. The United States Institute of Medicine defines vitamin D deficiency as 25(OH)D levels less than 20 ng/mL and greater than 20 ng/mL is sufficient upon evidence related to bone health[16]. Several studies reported that people with 25(OH)D levels less than 20 ng/mL is the risk factor of fracture[17] and have greater subsequent rates of bone loss[18]. On the other hand, the Endocrine Society's guidelines, which are based on patients with endocrine disorders, define vitamin D insufficiency as 25(OH)D levels of 21-29 ng/mL[19,20]. Despite these different definitions, both guidelines agree that vitamin D insufficiency and deficiency are common problems in certain populations.

About 1 billion people worldwide lack vitamin D[21,22]. Vitamin D deficiency and insufficiency are prevalent conditions not only in elderly people but also

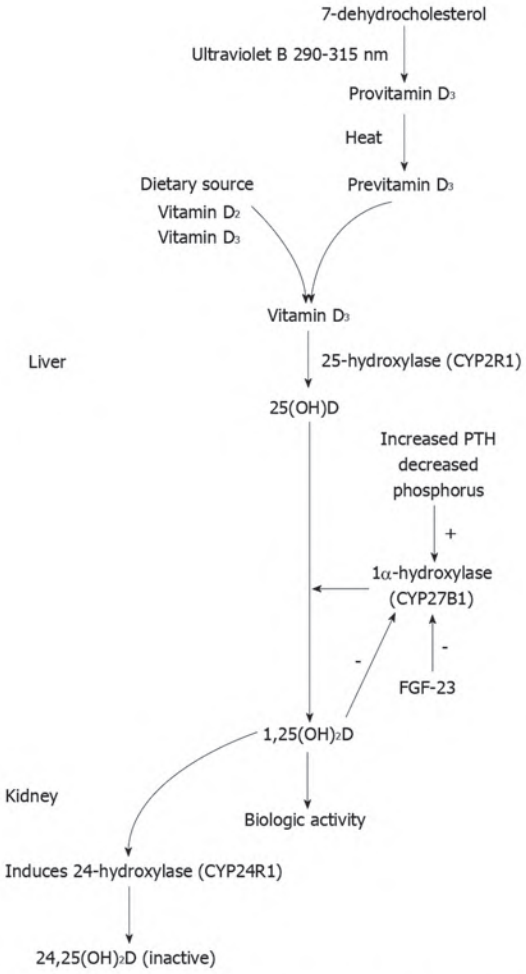

Figure 1 Mechanism of vitamin D synthesis. FGF-23: Fibroblast growth factor-23.

in adolescents[23] and children[24]. One study reported that almost one half of participants had 25(OH)D levels less than 40 nmol/L during the winter and spring[25]. In this study, 7437 people from a British birth cohort study who were 45 years old had 25(OH)D levels measured. Although the prevalence of hypovitamin D, defined as levels below 40 nmol/L, was 15.4% during the spring and summer, the proportion was 46.6% during the winter and autumn. Other studies showed that vitamin D deficiency was especially common in older persons (67-95 years)[26,27], and more than 50% of postmenopausal women taking medication for osteoporosis had 25(OH)D levels below 30 ng/mL[28]. Various factors, including age, sex, location, nutrition status, and physical fitness, affect vitamin D status[29]. In addition, diabetes, renal function, hypoalbuminemia, and albuminuria are also risk factors for vitamin D deficiency[30,31].

Recently, the relationship between 25(OH)D levels and genetic polymorphisms of DBP were reported[32]. It was previously known that 25(OH)D concentrations differed between black Americans and whites[33]. Although it was generally thought that nutritional, environmental, and hormonal factors affected racial differences[34], the detailed mechanisms behind these differences are unknown. Powe *et al*[32] reported that although total 25(OH)D and DBP were lower in black subjects than in white subjects, concentrations of estimated bioavailable 25(OH)D were similar between black and white subjects. In addition, because the affinity of DBP to 25(OH)D differs in the DBP gene polymorphism, genetic polymorphisms of DBP genes (rs7041 and rs4588) provide a likely explanation for racial variations in levels of DBP and 25(OH)D[35]. The combination of rs7041 and rs4588 produces amino acid changes resulting in variant DBPs (Gc1F, Gc1S, and Gc2). The phenotype of Gc1F, which is common in black homozygotes, was associated with the lowest levels of DBP (Gc1F/Gc1F homozygotes). On the other hand, Gc1S, which is common in white subjects, was associated with the highest DBP levels (Gc1S/Gc1S homozygotes). The Gc2/Gc2 homozygotes and Gc1F/Gc1S heterozygotes were associated with intermediate DBP levels. These findings suggest that racial differences in the distribution of DBP and total 25(OH)D are caused by DBP polymorphisms, and low total 25(OH)D levels do not indicate vitamin D deficiency. For purposes of cross-racial evaluations of vitamin D deficiency, it might be appropriate to estimate serum total 25(OH)D concentrations using DBP polymorphisms and DBP.

Associations between vitamin D levels and mortality have been shown by several observational studies[36,37]. Low vitamin D levels have also been shown to be associated with obesity, fractures, and infections[38]. Several observational studies have revealed potential links between low vitamin D levels and cardiovascular disease[39]. It is well known that people who live at high altitudes are at higher risk for hypertension and cardiovascular disease[40,41]. In a study of patients with hypertension who were exposed to UVB radiation three times a week for 3 mo, 25(OH)D concentrations increased by about 180%, and blood pressure became normal[42]. A prospective, nested, case-control study of 1484 women without hypertension and with low 25(OH)D levels showed that women with lower 25(OH)D levels had a higher rate of incident hypertension than controls. Low 25(OH)D concentrations have been shown to be inversely related to developing hypertension[43]. A recent Mendelian randomization study of vitamin D status and blood pressure concluded that increased plasma concentrations of 25(OH)D might reduce the risk for hypertension[44]. Cardiovascular disease such as coronary arterial disease[45], myocardial infarction[46], heart failure[47], and stroke[48] are also associated with vitamin D deficiency. However, a recent study showed that high levels of 25(OH)D were also associated with cardiovascular disease mortality[49]. This prospective, observational, cohort study analyzed 247574 citizens from Denmark and showed that a 25(OH)D level below 12.5 nmol/L was associated with a higher risk for mortality [hazard ratio (HR) = 1.59] compared with the reference range (50-75 nmol/L); however, those with

levels higher than 125 nmol/L had the highest mortality risk (HR = 1.95). There is a possibility that maintaining adequate vitamin D levels is essential for human health.

As mentioned above, vitamin D status and cardiovascular disease are strongly associated. Animal models offer several mechanisms to explain this association. Activation of the renin-angiotensin-aldosterone system (RAAS) has been seen in VDR knockout mice[50], and vitamin D has been shown to regulate the nuclear factor kappa beta pathway in renal failure model mice[51]. In vascular endothelial cells, transcription of nitric oxide synthase has been shown to be inhibited by vitamin D in mice[52]. In addition, vitamin D has been shown to activate the Keap1-Nrf pathway, which opposes oxidative stress, in renal failure model mice[53].

VITAMIN D AND DM

Type 1 DM

Type 1 DM is caused by a complex autoimmune destruction of pancreatic islet β-cells, leading to absolute insulin deficiency. The autoimmune nature of type 1 DM has been clarified with the detection of autoantibodies against islet β-cells and their infiltration by T cells, B cells, and macrophages[54]. Vitamin D has been shown to have immunomodulatory properties as well. Many immunomodulatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease have been reported to be associated with vitamin D deficiency[55,56]. Type 1 DM is also said to be related to vitamin D deficiency[57]. As VDR are expressed in human T and B lymphocytes, vitamin D is thought to modify the Th1/Th2 cytokine profile[58]. In addition, vitamin D is also thought to be associated with the immune system *via* its inhibition of lymphocyte proliferation[59]. Non-obese diabetic (NOD) mice with vitamin D deficiency showed an increased incidence and severity of diabetes[60]. Using $1,25(OH)_2D$ reduced the manifestation of diabetes in NOD mice by decreasing the number of effector T cells[61,62]. Another study reported that $1,25(OH)_2D$ also counteracted cytokine-induced expression of Fas, which regulates cell death in human islet cells[63].

The relationship between sunlight exposure and the incidence of type 1 DM has been reported[64]. One study showed that providing vitamin D supplements to infants in North Europe, where daylight hours are shorter than in other countries, decreased the risk for new-onset type 1 DM[65]. Although children suspected of having rickets during the study period had a relative risk (RR) of 3.0 (1.0-9.0) for type 1 DM, children who had taken 2000 IU vitamin D daily had a RR = 0.22 (0.05-0.89). Some studies were designed to clarify the effect of vitamin D on the preservation of β-cell function after the onset of type 1 DM[66]. Two studies found no significant effects of administration on vitamin D in protecting β-cell function[67,68]. However, another study reported significant effects of vitamin D administration on maintaining β-cell function after the development of type 1 DM. Thirty-eight patients with new-onset type 1 DM were randomly assigned to receive daily oral therapy with cholecalciferol, 2000 IU, or placebo[69]. The cumulative incidence of progression to undetectable ($\leqslant$ 0.1 ng/mL) fasting C-peptide and stimulated C-peptide levels was lower in the cholecalciferol group than in the placebo group. In another study, alfacalcidol (0.25 μg/d) preserved β-cell function in children with newly diagnosed type 1 DM[70]. Further studies are needed to clarify whether the administration of 25(OH)D or $1,25(OH)_2D$ can inhibit the onset of type 1 DM.

Type 2 DM

As VDRs in pancreatic β-cells play an important role in the progression of type 2 DM[71], vitamin D deficiency is related to insulin secretion, insulin resistance, and β-cell dysfunction in the pancreas[72] (Figure 2). The secretion of pancreatic insulin is inhibited by vitamin D deficiency in the diabetic animal model[73,74]. Administration of vitamin D restores glucose-stimulated insulin secretion and promotes β-cell survival by modulating the generation and effects of cytokines[75,76]. Insulin secretion is also influenced by calcium concentration and flux through the β-cells[77]. Vitamin D regulates the function of calbindin, a systolic calcium-binding protein found in pancreatic β-cells, and acts as a modulator of depolarization-stimulated insulin secretion *via* regulation of intracellular calcium[78]. PTH, which has its concentration regulated by vitamin D, is associated with insulin synthesis and secretion in the pancreas[79].

Insulin sensitivity is also associated with vitamin D. By stimulating the expression of insulin receptors, vitamin D regulates insulin sensitivity[80,81]. In addition, vitamin D enhances insulin sensitivity by promoting the expression of peroxisome proliferator-activated receptor (PPAR) delta, which is a widely expressed member of the PPAR family of nuclear receptor fatty acid sensors and regulates fatty acids in skeletal muscle and adipose tissue[82]. Intracellular calcium is a key factor of peripheral insulin resistance *via* an impaired signal transduction pathway leading to decreased glucose transporter activity[83,84].

The indirect effect of vitamin D is exerted by regulating calcium flux through the cell membrane and intracellular calcium. While low vitamin D induces secondary hyperparathyroidism, increased PTH levels are also associated with diabetes. A recent observational study of 494 women undergoing serial metabolic characterization revealed that hypovitamin D levels with increased PTH levels were an independent predictor of β-cell dysfunction, insulin resistance, and glycemia[85]. Vitamin D affects insulin resistance through the RAAS. One animal study demonstrated that vitamin D negatively regulated expression of renin genes in a mice model[86]. Furthermore, low levels of $1,25(OH)_2D$ increased renal renin production and activated the RAAS system in an animal model[87]. Finally, angiotensin Ⅱ inhibited the action of insulin in vascular and skeletal muscle tissues, leading to impaired glucose uptake[88].

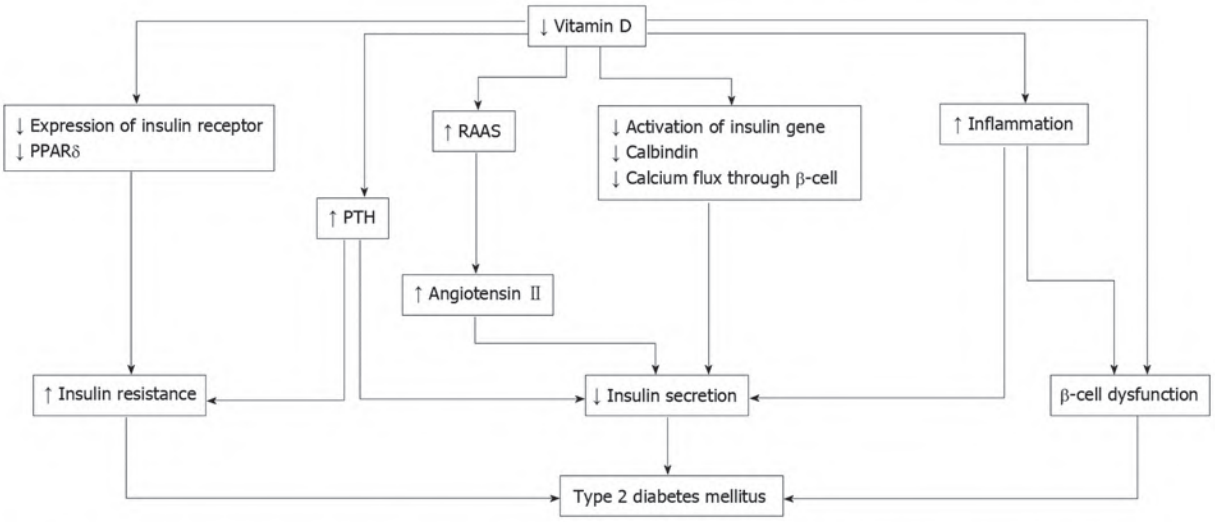

Figure 2 Putative scheme of effect of vitamin D on type 2 diabetes mellitus. PPAR: Peroxisome proliferator-activated receptor; PTH: Parathyroid hormone; RAAS: Renin-angiotensin-aldosterone system.

Systemic inflammation has an important role in insulin resistance and cardiovascular events in patients with type 2 DM[89]. As β-cells in the pancreas are affected *via* cytokine-induced apoptosis, high levels of inflammation cause worsening glycemic control. Vitamin D could decrease the effects of systemic inflammation and protect against β-cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines, as has been shown in animal models[90]. In patients with type 2 DM, incubation of isolated monocytes with $1,25(OH)_2D$ decreased the expression of inflammatory cytokines affecting insulin resistance, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α[91].

A prospective cohort study designed in the United Kingdom showed that baseline 25(OH)D concentrations in patients without diabetes were inversely related with the risk for hyperglycemia and insulin resistance at 10 years of follow-up visits[92]. Moreover, a similar study reported that low 25(OH)D levels were a risk factor for type 2 DM[93]. This prospective, cohort study was conducted over 29 years among 9841 subjects without diabetes. Lower vitamin D levels were a risk factor for incident type 2 DM. However, a recent Mendelian randomization approach study found that low 25(OH)D levels were not genetically associated with the risk for type 2 DM[94]. This result suggests that the association between 25(OH)D concentrations and type 2 DM is not causal. A meta-analysis of 16 studies reported that the odds ratio for type 2 DM was 1.5 (1.33-1.70) for the bottom *vs* top quartile of 25(OH)D levels[95]. Numerous randomized controlled studies have investigated whether vitamin D supplementation influences glycemic homeostasis[96,97]. As described above, vitamin D is thought to improve insulin resistance and promote insulin secretion. Therefore, clinical trials often use outcomes such as homeostasis model assessment of insulin resistance, fasting plasma glucose levels, and hemoglobin A1c levels. Some clinical trials have assessed the combined effects of vitamin D and calcium supplementation on glucose homeostasis of patients with diabetes[98,99] and without diabetes[100]. These studies suggest that vitamin D plus adequate calcium levels might be needed for an improvement in glycemic status. However, a recent meta-analysis concluded that vitamin D supplementation given to address concerns with glycemic control and insulin resistance in patients with diabetes is not recommended, although the doses of vitamin D supplementation may not have been optimal; almost all of the included trials used vitamin D doses of at least 2000 IU/d[101]. Because most trials focused on glycemic status and insulin resistance over short durations (12 mo or less), we should await the results of ongoing trials with longer follow-up periods to provide new evidence regarding the potential role of vitamin D supplementation in type 2 DM[102].

One study was designed to examine the protective effect of vitamin D against the development of type 2 DM[103]. A total of 2447 older people (mean age, 77 years) were allocated to 800 IU daily vitamin D_3 and 1000 mg calcium both, or placebo for 24-62 mo. Vitamin D in combination with calcium was not able to prevent the development of diabetes or an increase in the need for medication in patients with diabetes. The Women's Health Initiative Calcium/Vitamin D Study, a randomized, placebo-controlled trial of 33951 postmenopausal women, followed participants receiving 1000 mg elemental calcium plus 400 IU of vitamin D_3 daily, or placebo for 7 years. Calcium plus vitamin D_3 supplementation did not reduce the risk for developing diabetes over 7 years[104]. These results suggest that vitamin D supplementation at doses of 400 to 800 IU/d, with or without calcium, does not prevent new-onset type 2 DM.

Although at this time, supplementation with vitamin D has not been shown to improve glycemic control or

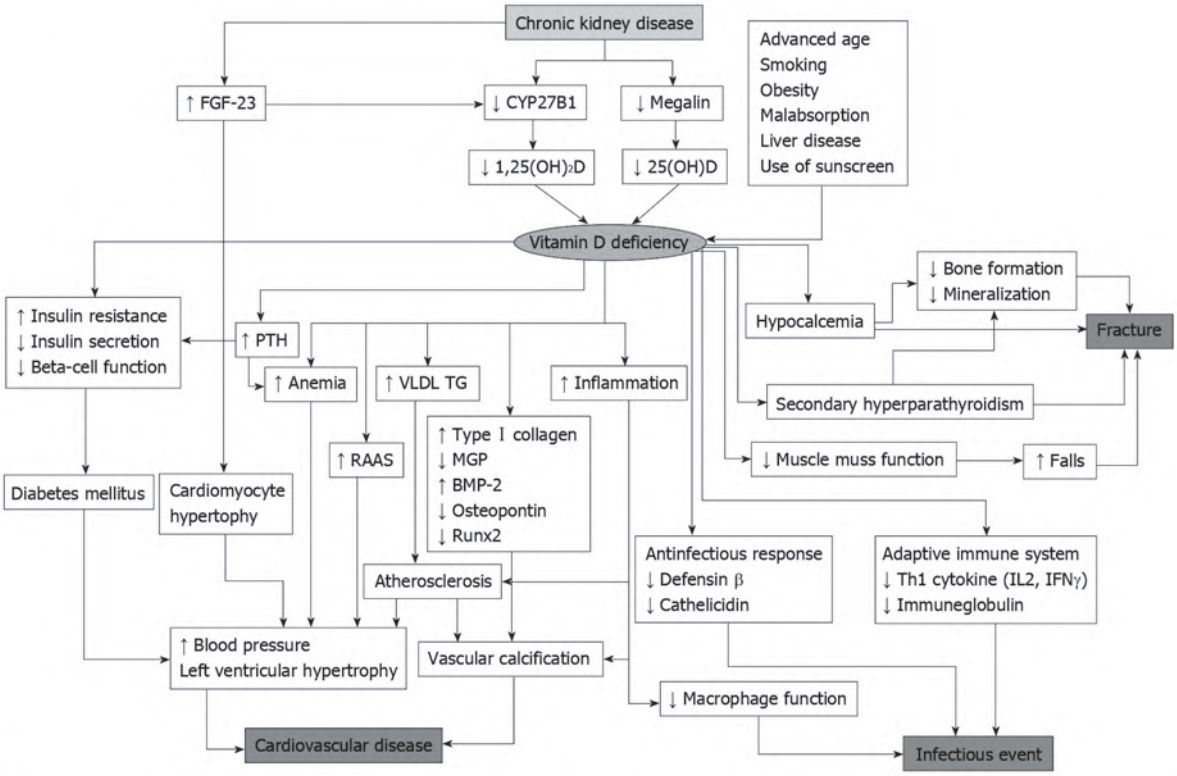

Figure 3 Vitamin D deficiency and cardiovascular disease. FGF-23: Fibroblast growth factor-23; PPAR: Peroxisome proliferator-activated receptor; PTH: Parathyroid hormone; RAAS: Renin-angiotensin-aldosterone system; VLDL: Very low density lipoprotein; TG: Triglycerides; IL: Interleukin; IFN: Interferon; MGP: Matrix gla protein; BMP: Bone morphogenetic protein.

prevent incident type 2 DM, clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed. In addition, it is important that future studies of vitamin D use primary outcomes, such as all-cause mortality and cardiovascular disease, as endpoints.

VITAMIN D AND CKD

One study revealed that paricalcitol diminished residual albuminuria in patients with diabetic nephropathy[105]. In this study, patients were randomly assigned (1:1:1) to receive placebo, 1 μg/d paricalcitol, or 2 μg/d paricalcitol for 24 wk to investigate the effect on mean urinary albumin-to-creatinine ratio (UACR). Patients receiving 2 μg paricalcitol showed a nearly sustained reduction in UACR, ranging from -18% to -28% (P = 0.014 *vs* placebo). However, few trials have used a vitamin D receptor antagonist (VDRA) for patients with diabetes, and none has a sufficient number of patients or follow-up period. The effect of vitamin D_3 and VDRA on hard outcomes, such as progression of diabetes, cardiovascular disease, and all-cause mortality, requires larger and longer-term trials.

Some studies indicate that $1,25(OH)_2D$ levels decrease in patients with CKD[106]. There are the several theories about the pathogenesis of vitamin D deficiency in CKD. Megalin, which is present in endocytic receptors in proximal tubule cells, is involved in the reabsorption of DBP from glomerular ultrafiltrates[107]. In addition, megalin also mediates the subsequent intracellular conversion of 25(OH)D to its active form. As kidney function declines, megalin expression in the proximal tubule decreases[108]. Megalin function is also attenuated with reduced kidney function, because of damages from low molecular weight proteinuria. The activity of CYP27B1 is also associated with decreasing kidney function[109]. As FGF-23 reduces expression of cotransporters NaPi-Ⅱa and NaPi-Ⅱc, of the brush border in the proximal tubules, these mechanisms inhibit phosphorus absorption and CYP27B1 activity.

In addition to the decline of $1,25(OH)_2D$ levels, 25(OH)D levels also decrease in patients with CKD. There are the several plausible mechanisms that explain the decreases in 25(OH)D. The complex of 25(OH)D and DBP leaks with proteinuria. Uptake of 25(OH)D decreases due to down-regulation of megalin levels. One study showed that 25(OH)D concentrations in patients with CKD were low[110]. The prevalence of vitamin D deficiency is 35% among about 4000 patients with CKD in the United States[111].

There is some evidence that vitamin D status is associated with poor clinical outcomes in patients with CKD[112] (Figure 3). Low 25(OH)D levels are associated with all-cause mortality and cardiovascular disease in patients with CKD[113]. The risk for end stage renal disease is higher in patients with low vitamin D status. Among patients undergoing hemodialysis and peritoneal

dialysis, low 25(OH)D levels are also associated with cardiovascular disease[114].

There is some evidence regarding restitution of vitamin D in patients with CKD[115,116] and as well as in patients undergoing dialysis [117,118]. As previously described, patients with kidney failure usually have insufficient 1,25(OH)$_2$D levels, and a VDRA is used for these patients. One study revealed that paricalcitol diminished albuminuria in patients with diabetic nephropathy[105]. In the VITAL study, which was designed to compare the effectiveness between paricalcitol and placebo, the paricalcitol group showed a decreased UACR of -16% compared with placebo[105]. However, as of yet, no other studies have investigated the effectiveness of vitamin D supplementation for protection of kidney function; thus, future studies are needed. Another study showed that paricalcitol led to decreases in levels of brain natriuretic peptide (BNP) in patients with CKD[119]. On the other hand, a recent study reported that treatment with paricalcitol did not improve left ventricular mass and function in patients with CKD[120]. There is controversial evidence regarding the role of VDRA to cardiovascular disease and surrogate makers. It is thought that as 1,25(OH)$_2$D inhibits activation of the RAAS, it leads to organ protection[121]. In addition, there is some evidence regarding VDRAs in patients undergoing hemodialysis. A retrospective cohort study showed that VDRA users had a lower mortality rate than non-VDRA users[122]. However, the Dialysis Outcomes and Practice Patterns Study revealed that taking vitamin D agents did not improve clinical outcome in patients undergoing dialysis. In addition, a recent study reported that pharmacological doses of alfacalcidol were associated with accelerated progression of aortic stiffness in patients undergoing hemodialysis[123]. To date, various discussions have taken place regarding the use of VDRA in patients undergoing dialysis, but adequate clinical studies are needed before any recommendations can be made.

According to the Kidney Disease Improving Global Outcomes guidelines, 25(OH)D levels should be determined in patients with CKD stage 3-5, and if levels are low, physicians should consider vitamin D supplementation[124]. Low 25(OH)D levels are associated with all-cause mortality and cardiovascular disease in patients with CKD as well as in patients undergoing dialysis[125]. Another study showed that among these patient groups, those with low levels of 25(OH)D and high levels of FGF-23 have worse outcomes[38]. However, there is not sufficient evidence regarding vitamin D supplementation for patients with CKD and those undergoing dialysis[126]. Although studies have reported that cholecalciferol decreases albuminuria[127,128] and improves PTH levels[129] in patients with CKD, there is no study with set clinical outcomes such as all-cause mortality or cardiovascular disease. In patients undergoing dialysis, cholecalciferol decreases BNP levels and reduces left ventricular hypertrophy[130]. As VDRAs increase calcium and phosphorus levels in patients undergoing dialysis, it is usually recommended that physicians only need to monitor calcium and phosphorus levels when using a VDRA[131]. On the other hand, vitamin D$_3$, such as cholecalciferol, does not increase calcium and phosphorus levels[132,133]. As with patients with CKD, there is no evidence with hard endpoints regarding the use of vitamin D$_3$ supplementation in patients undergoing hemodialysis.

CONCLUSION

Emerging evidence is accumulating on the important role of vitamin D in the pathogenesis of diabetes and CKD. Many prospective studies have shown associations between vitamin D status and chronic disease, including diabetes and CKD. However, there are contradictory findings regarding whether restitution of normal vitamin D levels modifies the occurrence or clinical course of these diseases. Although there is a concern that vitamin D may be a surrogate marker for poor health status, further well-designed clinical trials are needed in this area.

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D-Revive

Cholecalciferol

20000 IU
40000 IU

BEST QUALITY API FROM DENMARK

Vegetable Capsule Shell

The finest Vitamin D for healthy life

Vitamin D Status[2]

≥30 ng/ ml	Sufficiency
21-29 ng/ ml	Insufficiency
11-20 ng/ ml	Deficiency
≤10 ng/ ml	Severe deficiency

*ng=Nanogram

Dosage & Administration[3]

Treatment of Vitamin D deficiency

Adults: 40000 IU once a week for 7 weeks, followed by maintenance therapy (equivalent to 1400-2000 IU a day, such as two or three 20000 IU capsules per month), based on physician advice.

Children (12-18 years):
20000 IU once every 2 weeks for 6 weeks.

Prevention of Vitamin D deficiency

Adults:
20000 IU once a month.

Children (12-18 years):
20000 IU every 6 weeks.

D-Revive

Cholecalciferol

20000 IU
40000 IU

BEST QUALITY API FROM DENMARK

Vegetable Capsule Shell

The finest Vitamin D for healthy life

Regulates various body functions (4-14)

- Boost up immune system
- Ensures healthy bone & teeth
- Ensures healthy skin
- Ensures rapid wound healing after surgery
- Decreases the chances of hypertension
- Decreases the chances of diabetes
- Reduces the chances of muscle pain
- Reduces the chances of depression
- Reduces the risks of stroke
- Decreases LDL, TG & Total Cholesterol to control lipid profile
- Prevents pre-eclampsia & preterm birth

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