Epidemiology

Vitamin D Deficiency and Risk of Cardiovascular Disease

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Background—Vitamin D receptors have a broad tissue distribution that includes vascular smooth muscle, endothelium, and cardiomyocytes. A growing body of evidence suggests that vitamin D deficiency may adversely affect the cardiovascular system, but data from longitudinal studies are lacking.

Methods and Results—We studied 1739 Framingham Offspring Study participants (mean age 59 years; 55% women; all white) without prior cardiovascular disease. Vitamin D status was assessed by measuring 25-dihydroxyvitamin D (25-OH D) levels. Prespecified thresholds were used to characterize varying degrees of 25-OH D deficiency (<15 ng/mL, <10 ng/mL). Multivariable Cox regression models were adjusted for conventional risk factors. Overall, 28% of individuals had levels <15 ng/mL, and 9% had levels <10 ng/mL. During a mean follow-up of 5.4 years, 120 individuals developed a first cardiovascular event. Individuals with 25-OH D <15 ng/mL had a multivariable-adjusted hazard ratio of 1.62 (95% confidence interval 1.11 to 2.36, $P=0.01$) for incident cardiovascular events compared with those with 25-OH D ≥15 ng/mL. This effect was evident in participants with hypertension (hazard ratio 2.13, 95% confidence interval 1.30 to 3.48) but not in those without hypertension (hazard ratio 1.04, 95% confidence interval 0.55 to 1.96). There was a graded increase in cardiovascular risk across categories of 25-OH D, with multivariable-adjusted hazard ratios of 1.53 (95% confidence interval 1.00 to 2.36) for levels 10 to <15 ng/mL and 1.80 (95% confidence interval 1.05 to 3.08) for levels <10 ng/mL (*P* for linear trend=0.01). Further adjustment for C-reactive protein, physical activity, or vitamin use did not affect the findings.

Conclusions—Vitamin D deficiency is associated with incident cardiovascular disease. Further clinical and experimental studies may be warranted to determine whether correction of vitamin D deficiency could contribute to the prevention of cardiovascular disease. (***Circulation***. 2008;117:503-511.)

Key Words: cardiovascular diseases ■ risk factors ■ vitamin D

Vitamin D deficiency is highly prevalent in the United States and worldwide.[1] Low levels of 25-hydroxyvitamin D (25-OH D), the principal circulating storage form of vitamin D, are present in as many as one third to one half of otherwise healthy middle-aged to elderly adults.[1–4] Limited cutaneous synthesis due to inadequate sun exposure or pigmented skin and inadequate dietary intake are the principal causes of low 25-OH D levels.

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Although the best-characterized sequelae of vitamin D deficiency involve the musculoskeletal system, a growing body of evidence suggests that low levels of vitamin D may adversely affect the cardiovascular system.[5] Vitamin D receptors have a broad tissue distribution that includes vascular smooth muscle,[6,7] endothelium,[8] and cardiomyocytes.[1] In vitro, activated 1,25-dihydroxyvitamin D (1,25-OH D) directly suppresses renin gene expression,[9,10] regulates the growth and proliferation of vascular smooth muscle cells and cardiomyocytes,[11] and inhibits cytokine release from lymphocytes.[12] Studies in knockout mice confirm that the absence of vitamin D receptor activation leads to tonic upregulation of the renin-angiotensin system, with the development of hypertension and left ventricular hypertrophy.[10,13,14]

Clinical studies have reported cross-sectional associations between lower vitamin D levels and plasma renin activity,[15] blood pressure,[16,17] coronary artery calcification,[18,19] and prevalent cardiovascular disease.[20–22] Additionally, ecological studies have reported higher rates of coronary heart disease and hypertension with increasing distance from the

equator, a phenomenon that has been attributed to the higher prevalence of vitamin D deficiency in regions with less exposure to sunlight.[23–26]

Despite these clinical observations, prospective data are needed because vitamin D deficiency could be a consequence of cardiovascular disease rather than a cause. Moreover, results of hospital- or clinic-based studies may be influenced by selection bias or confounding bias, because chronic illnesses may affect access to sunlight or diet. Thus, we prospectively investigated the relation of vitamin D status to the incidence of cardiovascular events in a large, ambulatory, community-based sample of individuals free of cardiovascular disease at baseline.

Methods

Study Sample
The Framingham Offspring cohort was initiated in 1971 with the enrollment of 5124 offspring (and their spouses) of the original Framingham Heart Study participants.[27] Between 1996 and 2001, 1972 consecutive participants attending the sixth or seventh quadrennial Offspring examinations had measurement of 25-OH D levels. From this group, 233 participants were excluded from the present investigation for prevalent cardiovascular disease (n=225) or kidney disease (serum creatinine >2.0 mg/dL or missing; n=8). A total of 1739 participants (947 women) remained eligible. Because of the historical design of the Framingham Heart Study, all participants in the Framingham Offspring Cohort were white. All protocols were approved by the Boston University Medical Center and Tufts-New England Medical Center institutional review boards, and participants provided written informed consent.

Clinical Evaluation and End-Point Review
A physician-administered medical history, examination, and laboratory assessment of vascular risk factors were performed at the sixth and seventh Offspring examinations.[27] Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive therapy.[28] Criteria for diabetes mellitus were a fasting glucose ≥126 mg/dL or use of insulin or hypoglycemic medications.[29] Current smoking denoted regular use of cigarettes in the preceding year. Physical activity was assessed with a physical activity index, calculated from the number of hours spent each day at various activity levels, weighted according to the estimated oxygen consumption required for each activity.[30,31] This index previously has been related to the incidence of coronary heart disease and cerebrovascular events.[30,32] Information regarding vitamin D intake from supplements and total vitamin D dietary intake was obtained with a detailed food-frequency questionaire.[33]

Medical records were obtained for hospitalizations and physician visits related to cardiovascular disease during follow-up. In some cases, records were obtained after the participant self-reported a cardiovascular event. In other cases, medical records were forwarded by hospitals or doctor's offices based on knowledge of the individual's participation in the Framingham Heart Study. Records were reviewed prospectively by a committee of 3 experienced investigators. Cardiovascular events included myocardial infarction, coronary insufficiency (prolonged chest pain with documented ECG changes), angina, stroke, transient ischemic attack, peripheral claudication, or heart failure. Criteria for each of these diagnoses have been described previously.[34]

Laboratory Assays
Serum samples were obtained in the morning after an overnight fast and frozen at −70°C for no more than 3 years. Serum 25-OH D was determined by radioimmunoassay (DiaSorin, Stillwater, Minn). The limit of detection for 25-OH D was 1.5 ng/mL, and no samples had concentrations below the limit of detection. All samples were run in duplicate and the values averaged. Total (intra-assay and interassay) coefficients of variation for control values of 14.4 and 54.7 ng/mL were 8.5% and 13.2%, respectively. High-sensitivity C-reactive protein was measured with a Dade Behring BN100 nephelometer (Deerfield, Ill). The mean intra-assay coefficient of variation was 3.2%.

Statistical Analyses
We examined the association between 25-OH D status and the risk of first cardiovascular events using Cox proportional hazards regressions. We verified that the assumption of proportionality of hazards was not violated using plots of the standardized score processes and a Kolmogorov-type supremum test.[35]

On the basis of the experimental evidence,[1] we postulated that 25-OH D deficiency was associated with higher cardiovascular risk and that this association observed a threshold. Previous clinical studies suggest that the association of vitamin D deficiency with other sequelae, such as hyperparathyroidism and hyperglycemia, is nonlinear.[36,37] Thus, in our primary analysis, we modeled vitamin D status using a categorical variable. All cut points were chosen a priori, based on previous studies. We used the 25-OH D cut point of 15 ng/mL in our primary analyses, in keeping with cross-sectional studies in Framingham[38] and hospital-based[36] samples. For additional analyses, we defined "severe" vitamin D deficiency as 25-OH D <10 ng/mL, which approximates the lowest thresholds used in prior studies.[36,39] The referent group for the above analyses was prespecified as those having 25-OH D ≥15 ng/mL. Although levels >30 ng/mL are considered optimal for bone metabolism, only 10% of the study sample had levels in this range. In secondary analyses, we examined 25-OH D as a linear continuous variable. We also examined the potential nonlinearity of the relation between 25-OH D and cardiovascular risk using generalized additive Cox models with penalized splines.[40]

We estimated age- and sex-adjusted models, as well as multivariable models. Additional covariates used for adjustment in multivariable models included systolic blood pressure, use of antihypertensive therapy, diabetes mellitus, cigarette smoking, total-to–high-density lipoprotein cholesterol ratio, body mass index, and serum creatinine. In additional models, we adjusted for C-reactive protein levels, given the postulated relations between vitamin D deficiency and vascular inflammation.[12] We also estimated a model with adjustments for the use of vitamin D–containing supplements, physical activity, and educational attainment (<12 years). For the present analysis, 25-OH D levels were standardized within each of the 4 seasons, to account for seasonal variation in 25-OH D; vitamin D deficiency was defined as the bottom quartile of seasonally standardized 25-OH D, with the top 3 quartiles serving as the referent group.

All models were stratified by baseline examination (sixth or seventh examination of the Offspring cohort). Because vitamin D has been linked to blood pressure regulation, we also prespecified subgroup analyses according to hypertension status. These models incorporated the same covariates (including systolic blood pressure) as the original models. Additionally, we performed tests for effect modification by age, gender, hypertension status, diabetes mellitus, and body mass index by including multiplicative interaction terms with these variables and 25-OH D status. We also performed an analysis replacing serum creatinine with estimated glomerular filtration rate using the Modification of Diet in Renal Disease formula.[41]

Analyses were performed with SAS software, version 9.1 (SAS Institute, Cary, NC). A 2-sided probability value <0.05 was considered statistically significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Baseline characteristics of the 1739 participants are shown in Table 1. The mean 25-OH D concentration was 19.7 ng/mL. The overall prevalence of 25-OH D <15 ng/mL was 28%, with 9% having 25-OH D <10 ng/mL.

Table 1.   Baseline Characteristics According to Vitamin D Status

Characteristic	Whole Sample (n=1739)	Serum 25-OH Vitamin D Levels		P*
		≥15 ng/mL (n=1258)	<15 ng/mL (n=481)	
Age, y	59±9	59±9	59±9	0.53
Women, %	55	54	55	0.91
Body mass index, kg/m^2	28.1±5.3	27.5±4.9	29.6±6.0	<0.001
Cigarette smoking, %	14	12	17	0.01
Systolic BP, mm Hg	127±18	127±18	130±19	<0.001
Antihypertensive therapy, %	27	26	30	0.13
ACE inhibitor, %	12	11	14	0.16
ARB, %	2	2	1	0.12
Thiazide diuretic, %	8	8	10	0.16
Loop diuretic, %	1	1	1	0.71
Hypertension, %	40	39	42	0.20
Diabetes mellitus, %	8	7	11	0.002
Total/HDL cholesterol ratio	4.2±1.4	4.1±1.3	4.5±1.5	<0.001
Serum creatinine, mg/dL	1.2±0.2	1.2±0.2	1.1±0.2	0.10
Total vitamin D intake, IU	427±312	483±328	278±203	<0.001
Vitamin D supplement, %	4	5	1	<0.001
Multivitamin, %	41	48	21	<0.001
Physical activity index†	36.0±6.5	36.1±6.6	35.6±6.3	0.14

BP indicates blood pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; and HDL, high-density lipoprotein.

Values are percentages or mean±SD for continuous variables.

*P for comparison of 25-OH vitamin D groups.

†Physical activity index calculated as described in the Methods.

During a maximum of 7.6 years of follow up (mean 5.4 years), 120 participants (57 women) developed a first cardiovascular event. Events included 65 fatal or nonfatal coronary heart disease events (myocardial infarction, angina, or coronary insufficiency), 28 fatal or nonfatal cerebrovascular events (stroke or transient ischemic attack), 8 occurrences of claudication, and 19 occurrences of heart failure. None of the strokes were hemorrhagic.

As shown in Table 2, the age- and sex-adjusted 5-year rate of cardiovascular disease was approximately twice as high in those with 25-OH D <15 ng/mL as in those with 25-OH D ≥15 ng/mL. The highest rate of cardiovascular disease was observed in those with hypertension and vitamin D defi-

Table 2.   Five-Year Rates of Cardiovascular Disease, Adjusted for Age and Sex

	Events/ Person-Years	25-OH Vitamin D Levels, 5-Year Rate (95% CI)	
		≥15 ng/mL	<15 ng/mL
Whole sample (n=1739)	120/8069	4.4 (2.8–5.9)	8.9 (5.5–12.2)
With hypertension (n=688)	70/3145	5.8 (3.0–8.6)	14.2 (7.3–20.5)
Without hypertension (n=1051)	50/4924	3.5 (1.6–5.3)	5.1 (1.7–8.3)

No. of events by 25-OH D category: in whole sample, 70 (25-OH D ≥15 ng/mL) and 50 (25-OH D <15 ng/mL); in hypertensive participants, 37 and 33; and in nonhypertensive participants, 33 and 17, respectively.

ciency. Figure 1A and 1B display Kaplan-Meier curves showing the cumulative probability of cardiovascular events according to 25-OH D status in individuals with and without hypertension.

Results of multivariable Cox proportional hazards regressions are shown in Table 3. Levels of 25-OH D <15 ng/mL were associated with an age- and sex-adjusted hazard ratio of 2.04 (95% confidence interval [CI] 1.42 to 2.94, P<0.001) for cardiovascular events. After adjustment for conventional cardiovascular risk factors and renal function, the association between 25-OH D deficiency and risk of cardiovascular events remained significant (multivariable-adjusted hazard ratio 1.62, 95% CI 1.11 to 2.36, P=0.01). Adjustment for C-reactive protein levels did not attenuate this association. Results were also similar in a model that accounted for physical activity, vitamin D supplementation, educational attainment, and season (multivariable-adjusted hazard ratio 1.70, 95% CI 1.08 to 2.67, P=0.02). In models dividing 25-OH D status into 3 categories (≥15, 10 to <15, and <10 ng/mL), there was a stepwise increase in cardiovascular risk across categories (multivariable-adjusted hazard ratios 1.00, 1.53, and 1.80; P for linear trend=0.01).

Table 4 displays the results of prespecified subgroup analyses with the sample divided according to hypertension status. Among hypertensive participants, vitamin D deficiency (25-OH D <15 ng/mL) was associated with a multivariable-adjusted hazard ratio of 2.13 (95% CI 1.30 to 3.48, P=0.003) for cardiovascular events. A stepwise in-
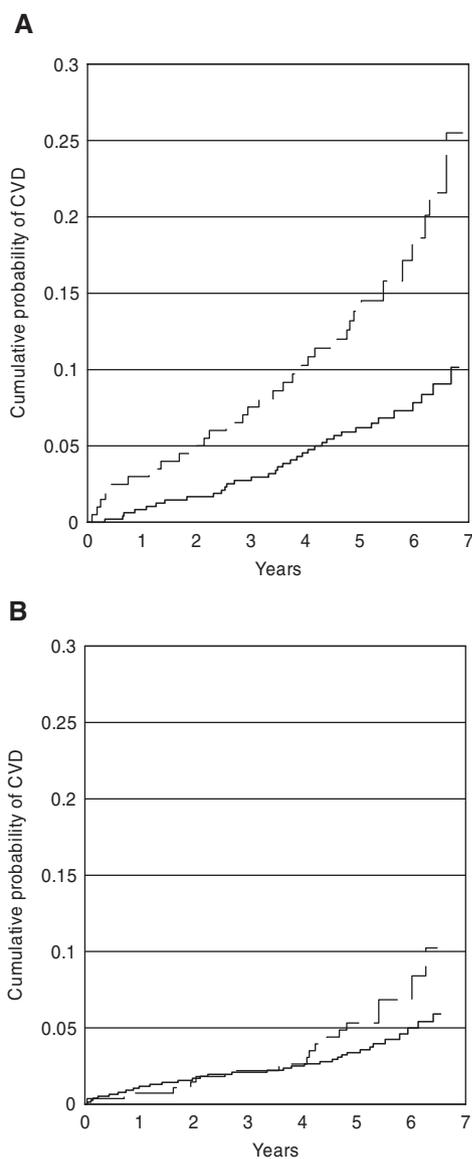
A

B

Figure 1. Kaplan-Meier curves showing the crude cumulative probability of first cardiovascular events in participants with (A) and without (B) hypertension. The group with a 25-OH D level ≥15 ng/mL is represented by solid line, whereas the group with a 25-OH D level <15 ng/mL is represented by dashed line. CVD indicates cardiovascular disease.

crease in hazard ratios was observed across categories of 25-OH D status in the hypertensive sample (1.00, 1.93, and 2.51; *P* for trend=0.002). In contrast, in nonhypertensive individuals, vitamin D deficiency was not associated with the risk of cardiovascular events in either the 2- or 3-category models. The multiplicative interaction term between vitamin D deficiency and hypertension had a borderline statistical significance (*P*=0.08 for both 2- and 3-category 25-OH D models).

In secondary analyses, we performed multivariable Cox regression models with continuous 25-OH D to assess for a linear relation between 25-OH D and cardiovascular events. Compared with threshold models, we observed an attenuated association between continuous 25-OH D and events (*P*=0.10), but there was a significant interaction between

25-OH D and hypertension (*P*=0.015). Analyses stratified by hypertension status demonstrated a strong association between continuous 25-OH D and cardiovascular risk among individuals with hypertension (multivariable-adjusted hazard ratio per 1-ng/mL increase in 25-OH D, 0.95; 95% CI 0.91 to 0.98, *P*=0.003). Exploratory analyses with regression splines suggested a nonlinear relation between 25-OH D levels and cardiovascular risk, with increased hazard for cardiovascular events at 25-OH D levels below 15 to 20 ng/mL (Figure 2).

In additional analyses that incorporated multiplicative interaction terms, neither age, gender, diabetes mellitus, nor body mass index modified the association between 25-OH D deficiency and cardiovascular risk. Multivariable-adjusted hazard ratios associated with 25-OH D deficiency were similar in models with and without variables potentially in the causal pathway, including diabetes mellitus, systolic blood pressure, and C-reactive protein. Replacement of serum creatinine with estimated glomerular filtration rate did not alter the results.

Discussion

These prospective, community-based data suggest that vitamin D deficiency is associated with increased cardiovascular risk, above and beyond established cardiovascular risk factors. The higher risk associated with vitamin D deficiency was particularly evident among individuals with hypertension, in whom 25-OH D levels <15 ng/mL were associated with a 2-fold risk of cardiovascular events. Strengths of the study include the use of a large, ambulatory cohort; the longitudinal study design and long-term follow-up; the standardized adjudication of cardiovascular events; and the use of multivariable analyses to account for comorbid conditions.

1,25-OH D is the biologically active, hormonal form of vitamin D; however, serum 25-OH D is regarded as the best indicator of vitamin D status in individuals without kidney disease, because it is the substrate for the renal and nonrenal production of 1,25-OHD, has a longer biological half-life than 1,25-OHD, and circulates in much higher concentrations. Serum 25-OH D reflects the total production of vitamin D from both endogenous and exogenous sources, including exposure to ultraviolet-B radiation and intake of various dietary forms. Our data indicate that increased cardiovascular risk is present at 25-OH D levels (<15 ng/mL) compatible with at least moderate vitamin D deficiency. With this cut point, the prevalence of vitamin D deficiency in the present cohort (28%) was substantial but consistent with that reported in other epidemiological studies.[42]

The present findings extend the results of smaller, cross-sectional studies that have examined the association between vitamin D status and cardiovascular risk.[20–22,43] In these studies, lower 25-OH D levels have been observed in individuals with acute myocardial infarction,[20] stroke,[21] heart failure,[22] and cardiovascular disease.[43] In 2 studies, 25-OH D was assayed on presentation to the hospital with the cardiovascular event,[20,21] which suggests that the low 25-OH D levels predated the cardiovascular event, because 25-OH D has a half-life of several weeks. Furthermore, a recent prospective study showed an association between vitamin D

Table 3.   Results of Multivariable Analyses (n=1739)

	Adjusted for Age and Sex	Adjusted for Clinical Covariates*	Adjusted for Clinical Covariates and CRP
Two-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D <15 ng/mL	2.04 (1.42–2.94)	1.62 (1.11–2.36)	1.66 (1.13–2.43)
P	<0.001	0.01	0.01
Three-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D 10 to <15 ng/mL	1.80 (1.18–2.75)	1.53 (1.00–2.36)	1.59 (1.03–2.45)
25-OH D <10 ng/mL	2.63 (1.57–4.38)	1.80 (1.05–3.08)	1.81 (1.03–3.18)
P (linear trend)	<0.001	0.01	0.01

CRP indicates C-reactive protein.

Values are multivariable-adjusted hazard ratios, with 95% CIs in parentheses.

*Clinical covariates are age, sex, systolic blood pressure, antihypertensive treatment, diabetes mellitus, serum creatinine, total-to–high-density lipoprotein cholesterol ratio, cigarette smoking, and body mass index.

deficiency and increased cardiovascular mortality in hemodialysis patients.[44]

Potential Mechanisms

Several mechanisms may explain the link between vitamin D deficiency and cardiovascular disease. First, experimental studies indicate that 1,25-OH D participates in the regulation of renin-angiotensin axis by directly suppressing renin gene expression.[10,14] Renin overexpression can be produced in wild-type mice by pharmacological inhibition of vitamin D synthesis.[10] Second, vascular smooth muscle cells and endothelial cells express receptors for vitamin D and have the ability to convert circulating 25-OH D to 1,25-OH D.[7,45] Putative vascular effects of vitamin D are wide-ranging and include modulation of smooth muscle cell proliferation,[46] inflammation,[12] and thrombosis.[47] Interestingly, transgenic rats constitutively expressing vitamin D-24-hydroxlyase, the enzyme that catalyzes the breakdown of 1 to 25-OH D, develop substantial atherosclerosis.[48] Third, vitamin D deficiency triggers secondary hyperparathyroidism. Parathyroid hormone (PTH) promotes myocyte hyper-

Table 4.   Results of Multivariable Analyses by Hypertension Status

	Adjusted for Age and Sex	Adjusted for Clinical Covariates	Adjusted for Clinical Covariates and CRP
Participants with hypertension (n=688)			
Two-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D <15 ng/mL	2.42 (1.51–3.89)	2.13 (1.30–3.48)	2.19 (1.33–3.63)
P	<0.001	0.003	0.002
Three-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D 10 to <15 ng/mL	2.07 (1.19–3.61)	1.93 (1.09–3.42)	2.07 (1.16–3.69)
25-OH D <10 ng/mL	3.19 (1.70–5.99)	2.51 (1.30–4.82)	2.43 (1.23–4.80)
P (linear trend)	<0.001	0.002	0.003
Participants without hypertension (n=1051)			
Two-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D <15 ng/mL	1.50 (0.83–2.71)	1.04 (0.55–1.96)	1.06 (0.56–2.00)
P	0.18	0.90	0.86
Three-category models			
25-OH D ≥15 ng/mL	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
25-OH D 10 to <15 ng/mL	1.45 (0.74–2.82)	1.06 (0.53–2.13)	1.05 (0.52–2.13)
25-OH D <10 ng/mL	1.66 (0.64–4.28)	1.00 (0.35–2.85)	1.08 (0.37–3.16)
P (linear trend)	0.17	0.94	0.86

CRP indicates C-reactive protein.

Values are multivariable-adjusted hazard ratios, with 95% CIs in parentheses. Clinical covariates are age, sex, systolic blood pressure, antihypertensive treatment (if applicable), diabetes mellitus, serum creatinine, total-to–high-density lipoprotein cholesterol ratio, cigarette smoking, and body mass index.
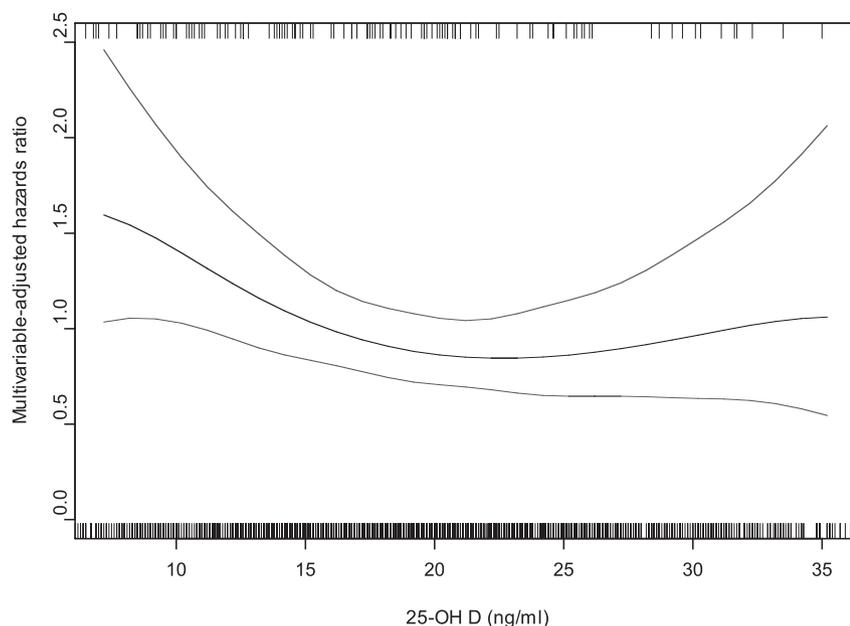
Figure 2. Nonlinearity of multivariable-adjusted relation between baseline vitamin D status and incident cardiovascular events. Solid lines show estimated relation of adjusted hazard ratios (with 95% confidence limits) and 25-OH D levels when time to cardiovascular event is modeled as a function of penalized regression splines of 25-OH D levels with adjustment for all other covariates. Hatched lines on the horizontal axis represent cardiovascular events (top axis) and censored individuals (bottom axis).

trophy[49] and vascular remodeling.[50,51] Other studies suggest that PTH has a proinflammatory effect, stimulating the release of cytokines by vascular smooth muscle cells.[7,52]

The present data also highlight a potential interaction between vitamin D deficiency and hypertension. Hypertension plays a key role in the development of left ventricular hypertrophy and vascular remodeling. Because vitamin D deficiency may also influence cardiac and vascular remodeling, hypertension could magnify the adverse effects of vitamin D deficiency on the cardiovascular system. Also, experimental and clinical data suggest that vitamin D deficiency directly promotes the development of hypertension,[10,53] which provides another potential mechanism linking vitamin D deficiency, hypertension, and cardiovascular risk. Because a formal test for interaction was of borderline significance ($P=0.08$), the results of analyses in hypertension subgroups require further validation.

The present findings in categorical versus linear models are consistent with the hypothesis that the relation between 25-OH D and cardiovascular risk is nonlinear; however, the exact threshold at which risk for cardiovascular disease may increase is unclear. Relatively high levels of 25-OH D (>30 ng/mL) are required to maintain normal PTH levels, but optimal levels for cardiovascular protection may differ from those for bone metabolism or normal PTH physiology.[42,54] We found increased cardiovascular risk at 25-OH D levels well below 30 ng/mL. We had inadequate statistical power to evaluate the effect of milder degrees of vitamin D deficiency (15 to 30 ng/mL), given the low proportion of individuals in the cohort with levels >30 ng/mL (10%). Indeed, it is noteworthy that we observed the increased cardiovascular risk associated with decreased 25-OH D levels in analyses in which the reference group included individuals with "mild" deficiency.

An alternate explanation for the present findings is that vitamin D deficiency is a marker of chronic nonspecific illness rather than a direct contributor to disease pathogenesis.

Individuals may get less exposure to sunlight when they are sick and cannot go outdoors; however, the present investigation was restricted to ambulatory participants attending a voluntary Framingham Heart Study examination, which makes confounding by immobility or chronic illness unlikely. Additionally, we adjusted for multiple characteristics at baseline, including physical activity.

Clinical Implications

The possibility of a causal link between vitamin D deficiency and cardiovascular disease is supported by biological plausibility, the demonstration of a temporal association, and the finding of a dose response between 25-OH D deficiency and risk. These data raise the possibility that treatment of vitamin D deficiency, via supplementation or lifestyle measures, could reduce cardiovascular risk. However, treatment strategies suggested by observational data are not always borne out by randomized trials, as evidenced by studies of hormone replacement therapy[55] and B vitamins for homocysteine lowering.[56] Problems related to the use of observational data include indication bias, confounding, and reverse causation. Several features of the study design suggest that the present data may be less susceptible to these problems. First, we used a direct, objective measure of vitamin D status (25-OH D levels) rather than relying on self-reported vitamin D intake or sunlight exposure. Second, 25-OH D levels were assessed on blood samples drawn at baseline, years before the acute cardiovascular events. Third, cardiovascular risk factors and comorbid diagnoses are well characterized in the Framingham Offspring cohort, which has been followed for >3 decades.

In small clinical trials, vitamin D supplementation has promoted reductions in blood pressure,[57,58] left ventricular hypertrophy,[59] and inflammatory cytokines.[60] On the other hand, Hsia and colleagues[61] recently reported that use of calcium and vitamin D supplements was not associated with a reduction in cardiovascular events in the Women's Health

Initiative. These apparently discrepant findings could be attributable to several factors.[62] First, the Women's Health Initiative was a fracture-prevention trial and was not designed to evaluate cardiovascular risk.[63] Second, the dose of vitamin D in the treatment arm (400 IU/d) was far below the amount necessary to correct vitamin D deficiency (≥800 IU/d) or doses used in previous cardiovascular studies.[60,62] Third, patients in the placebo arm were allowed to take vitamin D supplements, which resulted in a mean consumption of vitamin D in the placebo arm of nearly 400 IU/d. Lastly, baseline 25-OH D levels were not measured in the Women's Health Initiative; thus, the trial did not address whether vitamin D supplementation benefited individuals with vitamin D deficiency, because enrollment was performed irrespective of vitamin D status. It is noteworthy that vitamin D did appear to reduce cardiovascular risk in obese individuals, who are prone to endogenous vitamin D deficiency, and also in those with multiple coronary risk factors.[61] The mean body mass index among individuals with vitamin D deficiency in the present study was ≈30 kg/m^2, in the overweight-to-obese range.

The present data, although observational, permit an assessment of cardiovascular risk across a broad range of baseline 25-OH D levels and could motivate additional prospective or interventional studies. Still, barriers to future trials of vitamin D for cardiovascular prevention exist. A primary prevention trial would require a very large sample and may be hampered by the perception that it would be unethical to randomize individuals with moderate vitamin D deficiency to placebo therapy. In this regard, additional observational studies could be used to identify surrogate end points or subgroups most likely to benefit from vitamin D supplementation, which may facilitate smaller trials.

Study Limitations

Several limitations of the study deserve comment. Because we did not assess PTH levels, we cannot determine whether the association between vitamin D status and cardiovascular risk was mediated in part by secondary hyperparathyroidism. Nonetheless, even if secondary hyperparathyroidism were present, its treatment would require repletion of vitamin D, which justifies the focus on assessment of vitamin D stores. Notably, in vitamin D–receptor knockout mice, correction of calcium and PTH toward wild-type levels by use of a rescue diet does not ameliorate the hyperreninemia,[10] which supports a direct effect of vitamin D deficiency.

Although we attempted to adjust for all relevant covariates in the multivariable models, it is important to acknowledge the possibility of residual confounding. Unmeasured characteristics associated with vitamin D deficiency, rather than vitamin D deficiency itself, could account for the increased cardiovascular risk.

Given the interval between study visits, we had follow-up information on blood pressure for only a limited proportion of participants subsequent to the baseline examination. It is conceivable that the development of hypertension in individuals with vitamin D deficiency accounted for some of the increase in cardiovascular risk.[53] We had limited statistical power to assess the relation between 25-OH D levels and specific components of the cardiovascular end point. Experimental studies suggest that vitamin D deficiency influences both vascular and cardiac remodeling, but clinical data are limited. Lastly, because the present cohort was white, our results may not be generalizable to nonwhite individuals, including blacks, who have a higher prevalence of vitamin D deficiency.[4]

Conclusions

In summary, the results of the present study suggest that moderate to severe vitamin D deficiency is a risk factor for developing cardiovascular disease. These findings may have potentially broad public health implications, given the high prevalence of vitamin D deficiency in developed countries, the contribution of lifestyle and geography to vitamin D status, and the ease, safety, and low cost of treating vitamin D deficiency. Further clinical and experimental studies may be warranted to validate our findings, to investigate the mechanisms underlying increased cardiovascular risk, and to determine whether correction of vitamin D deficiency could contribute to the prevention of cardiovascular disease.

Disclosures

Dr Wolf has received honoraria from Abbott Laboratories and Genzyme. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Vitamin D deficiency is highly prevalent in the United States and worldwide. Low levels of 25-hydroxyvitamin D (25-OH D), the principal circulating storage form of vitamin D, are present in as many as one third to one half of otherwise healthy middle-aged to elderly adults. Although the best-characterized sequelae of vitamin D deficiency involve the musculoskeletal system, a growing body of experimental evidence suggests that low levels of vitamin D may adversely affect the cardiovascular system. We studied 1739 Framingham Offspring Study participants without prior cardiovascular disease; 28% had 25-OH D levels <15 ng/mL, and 9% had levels <10 ng/mL. During a mean follow-up of 5.4 years, 120 individuals developed a first cardiovascular event. Individuals with 25-OH D <15 ng/mL had a multivariable-adjusted hazard ratio of 1.62 (95% confidence interval 1.11 to 2.36; $P=0.01$) for incident cardiovascular events compared with those with 25-OH D ≥15 ng/mL. This effect was evident in participants with hypertension (hazard ratio 2.13, 95% confidence interval 1.30 to 3.48) but not in those without hypertension (hazard ratio 1.04, 95% confidence interval 0.55 to 1.96). There was a graded increase in cardiovascular risk across categories of 25-OH D, with multivariable-adjusted hazard ratios of 1.53 (95% confidence interval 1.00 to 2.36) for levels 10 to <15 ng/mL and 1.80 (95% confidence interval 1.05 to 3.08) for levels <10 ng/mL (P for linear trend$=0.01$). In summary, moderate to severe vitamin D deficiency is associated with increased risk for developing cardiovascular disease. Further prospective studies may be warranted to determine whether correction of vitamin D deficiency could contribute to the prevention of cardiovascular disease.