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Original Contribution

Vitamin D Deficiency and Cardiovascular Events in Patients With Coronary Heart Disease: Data From the Heart and Soul Study

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A growing body of evidence supports an association between vitamin D and cardiovascular disease. However, the mechanisms underlying this association are unknown. From 2000 to 2002, we identified 946 participants with stable cardiovascular disease in San Francisco, California, and followed them prospectively for cardiovascular events (heart failure, myocardial infarction, stroke, or cardiovascular death). We then examined the extent to which the association was attenuated by adjustment for poor health behaviors, comorbid health conditions, and potential biological mediators. During a median follow-up period of 8.0 years (through August 24, 2012), 323 subjects (34.1%) experienced a cardiovascular event. Following adjustment for sociodemographic factors, season of blood measurement, health behaviors, and comorbid conditions, 25-hydroxyvitamin D levels under 20 ng/mL remained independently associated with cardiovascular events (hazard ratio = 1.30, 95% confidence interval: 1.01, 1.67). However, after further adjustment for potential biological mediators, the independent association was no longer present (hazard ratio = 1.11, 95% confidence interval: 0.85, 1.44). Parathyroid hormone, a potentially modifiable biological factor downstream from 25-hydroxyvitamin D, was responsible for the majority of this attenuation. These findings highlight the need for randomized controlled trials to determine whether vitamin D supplementation in persons with deficiency could be beneficial for the primary or secondary prevention of cardiovascular events.

cardiovascular events; coronary heart disease; nutrition; vitamin D; vitamin D deficiency

Abbreviations: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; 25(OH)D, 25-hydroxyvitamin D.

Editor's note: *An invited commentary on this article appears on page 1288, and the authors' response appears on page 1291.*

Vitamin D deficiency is highly prevalent; approximately 25%–57% of the US population (1) and more than 1 billion persons worldwide (2) are either insufficient (defined as serum 25-hydroxyvitamin D (25(OH)D) levels of 20–29.9 ng/mL) or deficient (defined as serum 25(OH)D levels under 20 ng/mL) in vitamin D. Several lines of evidence support the hypothesis that vitamin D deficiency may contribute to cardiovascular disease (CVD) (3). Ecological studies have demonstrated a higher burden of hypertension and CVD at greater distances from the equator (4, 5). Observational studies have shown inverse associations between 25(OH)D levels and hypertension (6, 7), body mass index (8), congestive heart failure (9, 10), myocardial infarction (11–13), stroke (12, 14), peripheral arterial disease (15, 16), mortality (13, 17) and combined cardiovascular events (18, 19). A recent meta-analysis suggested that vitamin D supplementation at moderate-to-high doses may reduce CVD risk (20).

However, it is unclear whether vitamin D deficiency is simply a marker for poor health or whether this association is mediated by biological changes that increase risk of cardiovascular events. For example, persons with vitamin D deficiency may have comorbid health conditions or poor health behaviors which cause them to stay indoors. Therefore,

vitamin D deficiency could simply be a surrogate for poor health. Alternatively, however, a variety of plausible biological mechanisms (blood pressure elevation (21), insulin resistance (22), inflammation (23), obesity (8), endothelial dysfunction (24), or vascular remodeling due to hyperparathyroidism (25)) have been proposed by which vitamin D deficiency may cause cardiovascular events.

A better understanding of the mechanisms underlying the association between vitamin D deficiency and CVD would be informative for determining the potential benefit of vitamin D supplementation. If low 25(OH)D levels simply identify persons with poor overall health, vitamin D supplementation is unlikely to be effective in the primary or secondary prevention of CVD. Alternatively, if low vitamin D levels produce biological changes which mediate CVD risk, repletion of vitamin D could have substantial public health benefit. Such relationships may be particularly beneficial in secondary prevention, as the risks of recurrent CVD and death are much higher in populations with established CVD. Therefore, in a population of outpatients with stable coronary heart disease (CHD), we evaluated the association between 25(OH)D levels and cardiovascular events and sought to elucidate the potential mediators underlying this association.

METHODS

The Heart and Soul Study is a prospective cohort study originally designed to investigate psychosocial factors and health outcomes in patients with stable CHD. Details regarding recruitment methods and study design have been published previously (26, 27). In brief, 1,024 outpatients with stable CHD were recruited from 12 outpatient clinics in the San Francisco Bay Area. Eligible participants met 1 or more of the following criteria: 1) history of myocardial infarction; 2) evidence of at least 50% stenosis in one or more coronary vessels upon cardiac catheterization; 3) evidence of exercise-induced ischemia by treadmill electrocardiogram or nuclear perfusion stress imaging; or 4) a history of coronary revascularization. Subjects were excluded if they had a history of myocardial infarction in the previous 6 months, were unable to walk 1 block, or were planning to move out of the local area within 3 years. The study was approved by the institutional review boards of the University of California, San Francisco, and the San Francisco VA Medical Center, and all participants provided written informed consent.

Between September 2000 and December 2002, all participants attended a baseline study appointment which included completion of a medical history, a physical examination, and a comprehensive health status questionnaire. Of the 1,024 original study participants, we excluded 78 with missing covariate data. The remaining 946 patients were followed for cardiovascular events through August 24, 2012.

25(OH)D levels

The night prior to the baseline examination, participants completed an overnight (12-hour) fast, except for taking their regularly prescribed medications. Blood samples were drawn into chilled tubes containing ethylenediaminetetraacetic acid; plasma was aliquoted and stored at −70°C until April 2011. We used an API 5000 LC/MS/MS mass spectrometer (Ab Sciex, Framingham, Massachusetts) to measure 25-hydroxyvitamin D_3 and D_2 levels. The analytical measuring range for the $25(OH)D_3$ and $25(OH)D_2$ assays was 5–100 ng/mL. The intraassay coefficient of variation of both assays at a concentration of 40 ng/mL was 3.6%; the interassay coefficient of variation for both assays was 8.9%. Total 25(OH)D levels (referred to throughout this article as 25(OH)D) were calculated by the addition of $25(OH)D_2$ and $25(OH)D_3$ levels.

Cardiovascular events

The primary outcome was any cardiovascular event, which was defined as a composite of hospitalization for myocardial infarction, stroke, heart failure, or cardiovascular mortality. We also evaluated the individual outcomes of myocardial infarction, stroke, heart failure, cardiovascular mortality, and all-cause mortality as secondary outcomes. We conducted annual follow-up interviews with participants or their proxies to inquire about interval death or hospitalization for "heart trouble." For any reported event, we retrieved medical records, which 2 independent and blinded physician adjudicators reviewed. If the adjudicators agreed on the outcome classification, their classification was binding. In the event of a disagreement, a third blinded adjudicator was consulted.

Myocardial infarction was defined using the American Heart Association diagnostic criteria (28). Stroke was defined as a new neurological deficit not known to be secondary to brain trauma, tumor, infection, or other cause. Heart failure was defined as hospitalization for a clinical syndrome based on the Framingham heart failure criteria (28). Cardiovascular mortality was defined as a death which occurred as a result of a cardiovascular cause. Cardiovascular mortality and all-cause mortality were determined by review of death certificates.

Other patient characteristics

Age, sex, race/ethnicity, and medical history were self-reported. Season of the year in which the blood draw occurred was recorded. Weight and height were measured, and body mass index (weight (kg)/height $(m)^2$) was calculated. Estimated glomerular filtration rate was calculated using the combined creatinine-cystatin C equation (29). Medication use was recorded by study personnel. Depression was assessed by means of Patient Health Questionnaire 2. Tobacco use, multivitamin use, educational level, and physical activity (defined as exercise performed at least 1–2 times per week) were self-reported. Medication nonadherence was defined as a self-report of taking prescribed medications 75% of the time or less (30).

Potential biological mediators

Blood pressure was measured in the supine position after 5 minutes of rest. Lipids (mg/dL) and levels of hemoglobin A_{1c} (%), C-reactive protein (mg/dL), parathyroid hormone (mg/dL), calcium (mg/dL), albumin (g/dL), and phosphorus

(mg/dL) were measured from the fasting blood samples. Fibroblast growth factor 23 (relative units/mL) was also measured from fasting blood samples, as previously described in detail (31, 32). Urinary albumin:creatinine ratio (mg/g) was measured from 24-hour urine specimens as previously described (33).

Statistical analyses

We first performed exploratory analyses using an age-adjusted cubic spline, which revealed that the association was nonlinear, with a sharp increase in CVD risk at 25(OH)D levels less than 20 ng/mL. Therefore, we chose a cutpoint of 20 ng/mL for our analysis. This value also corresponds to a clinically relevant cutpoint (deficiency is currently defined by most experts as a 25(OH)D level less than 20 ng/mL) (2).

We compared baseline differences in participant characteristics by 25(OH)D level across categories using χ^2 tests for dichotomous variables and *t* tests for continuous variables. Data on triglycerides, C-reactive protein, parathyroid hormone, urinary albumin:creatinine ratio, and fibroblast growth factor 23 were log-transformed for the analysis because they were not normally distributed. We used Cox models to evaluate the association of 25(OH)D with time to cardiovascular events, adjusting for potential confounding and mediating factors which met our a priori criteria of either face validity (age, sex, race/ethnicity, season) or an association across categories of vitamin D at $P < 0.1$. The reference category was a 25(OH)D level greater than or equal to 20 ng/mL. We developed sequential models: Model 1 adjusted for sociodemographic factors; model 2 adjusted for model 1 variables plus health behaviors; model 3 adjusted for model 2 variables plus comorbid health conditions; and model 4 adjusted for all model 3 variables plus potential biological mediators. We assessed proportional hazards assumptions by visual inspection of Schoenfeld residuals and log-minus-log plots, and found no evidence of violation.

To better define the individual contributions of each of the potential mediators, we also evaluated how much each potential mediator changed the strength of the association between 25(OH)D and cardiovascular events. For each covariate in models 3 and 4, we calculated the percent change in the magnitude of the association (log hazard ratio, adjusted for all model 2 covariates) before and after adjustment for the potential mediator of interest. We then performed stratified analyses to determine whether the association differed by age (≥65 years), sex, race/ethnicity, hypertension, diabetes, obesity (body mass index ≥30), chronic kidney disease (defined as an estimated glomerular filtration rate of ≥60 mL/minute/1.73 m^2), albuminuria (urinary albumin:creatinine ratio ≥30 mg/g), or hyperparathyroidism (parathyroid hormone concentration ≥65 mg/dL). For all analyses, including interactions, *P* values less than 0.05 were considered significant. All analyses were conducted using STATA, version 11.0 (StataCorp LP, College Station, Texas).

RESULTS

Baseline characteristics of the 946 study participants are displayed in Table 1. The mean 25(OH)D concentration in this sample was 25.8 ng/mL. The prevalence of vitamin D deficiency (25(OH)D level <20 ng/mL) was 32%. Compared with participants with 25(OH)D levels greater than or equal to 20 ng/mL, those with levels under 20 ng/mL were younger, less likely to be male, and less likely to have graduated from college. Participants with vitamin D deficiency were more likely to use tobacco, less likely to take multivitamins, and less likely to engage in physical activity. Consistent with prior studies, participants with vitamin D deficiency were more likely to have hypertension, diabetes, and depression. They had higher systolic and diastolic blood pressures, higher levels of hemoglobin A_{1c}, C-reactive protein, parathyroid hormone, fibroblast growth factor 23, and serum phosphorus, and higher urinary albumin:creatinine ratios. Serum calcium levels did not differ across categories (Table 1).

During a median follow-up period of 8.0 years, 323 subjects (34.1%) experienced a cardiovascular event. We observed a nonlinear association, with a sharp increase in cardiovascular events at 25(OH)D levels less than 20 ng/mL (Figure 1). The question of whether 25(OH)D levels of 20–29.9 ng/mL (often referred to as vitamin D insufficiency) also confer adverse health consequences is currently controversial. Therefore, we performed additional exploratory analyses to compare annual cardiovascular event rates at 3 different 25-OH levels: <20 ng/mL, 20–29.9 ng/mL, and ≥30 ng/mL. These analyses confirmed that the cardiovascular event rates observed in participants with 25(OH)D levels of 20–29.9 ng/mL were similar to the rates observed in participants with 25(OH)D levels greater than or equal to 30 ng/mL (Figure 2).

After adjustment for age, sex, race/ethnicity, and season of blood draw, participants with 25(OH)D levels less than 20 ng/mL had a 50% greater rate of cardiovascular events (hazard ratio (HR) = 1.50, 95% confidence interval (CI): 1.19, 1.90) than participants with 25(OH)D levels of 20 ng/mL or higher. Further adjustment for poor health behaviors (tobacco use, no multivitamin use, low physical activity) modestly attenuated the association (HR = 1.31, 95% CI: 1.02, 1.69). Adjustment for comorbid health conditions (diabetes, hypertension, depression, higher body mass index) did not materially alter findings (HR = 1.30, 95% CI: 1.01, 1.67). Following further adjustment for potential biological mediators (systolic and diastolic blood pressure, high-density lipoprotein cholesterol, triglycerides, hemoglobin A_{1c}, C-reactive protein, parathyroid hormone, phosphorus, and fibroblast growth factor 23), the association was no longer significant (HR = 1.11, 95% CI: 0.85, 1.44) (Table 2). These associations were similar across individual outcomes (Table 3). Results revealed that parathyroid hormone most strongly attenuated the association, resulting in a 10.5% change in the size of the association (Figure 3).

Stratified analyses revealed that the association between 25(OH)D levels and cardiovascular events was stronger in participants with diabetes ($n = 249$; HR = 1.62, 95% CI: 1.06, 2.48) than in participants without diabetes ($n = 695$; HR = 1.09, 95% CI: 0.79, 1.51 (*P* for interaction < 0.001)). The association was also stronger in participants with albuminuria ($n = 207$; HR = 1.66, 95% CI: 1.07, 2.57) than in those without albuminuria ($n = 731$; HR = 1.07, 95% CI: 0.77, 1.47 (*P* for interaction < 0.001)). The association was modestly greater in participants with chronic kidney disease ($n = 290$;

Table 1. Baseline Characteristics of 946 Participants in the Heart and Soul Study, by 25-Hydroxyvitamin D Status, 2000–2002

Characteristic	25-Hydroxyvitamin D Level, ng/mL						*P* Value
	<20 (*n* = 304)			≥20 (*n* = 642)			
	%	Mean (SD)	Median (IQR)[a]	%	Mean (SD)	Median (IQR)	
Demographic factors							
Age, years		65 (11)			67 (11)		0.001
Male sex	77			83			0.02
Race/ethnicity							<0.001
White	49			66			
Black	33			8			
Hispanic	9			9			
Asian	7			13			
Other	2			4			
Season of year							
Spring	24			24			0.14
Summer	18			24			
Fall	33			28			
Winter	25			24			
College graduation	19			42			<0.001
Health behaviors							
Tobacco use	28			16			<0.001
Alcohol use	27			30			0.27
Medication nonadherence	10			7			0.17
Multivitamin use	16			45			<0.001
Physical activity	35			54			<0.001
Comorbid conditions							
Hypertension	76			68			0.007
Diabetes	23			33			0.001
Myocardial infarction	53			55			0.41
Left ventricular ejection fraction	62			61			0.11
eGFR, mL/minute/1.73 m^2		71.3 (1.4)			70.4 (0.8)		0.56
Body mass index[b]		29.2 (0.4)			28.0 (0.2)		0.001
Depression	24			17			0.01

Table continues

HR = 1.44, 95% CI: 0.99, 2.08) than in those without chronic kidney disease ($n = 656$; HR = 1.27, 95% CI: 0.88, 1.81 (P for interaction < 0.001)). The association did not differ by age, sex, race/ethnicity, hypertension, obesity, or hyperparathyroidism.

DISCUSSION

In this ambulatory population of persons with stable CHD, we found that participants with vitamin D deficiency (25(OH)D level <20 ng/mL) had a 50% greater rate of cardiovascular events than participants who were sufficient in vitamin D (25(OH)D level ≥20 ng/mL). Even following adjustment for sociodemographic factors, poor health behaviors, and comorbid health conditions, 25(OH)D levels less than 20 ng/mL remained associated with a 30% greater rate of cardiovascular events. However, after further adjustment for parathyroid hormone level and other potentially mediating biological factors, the association was no longer significant. These findings raise the possibility that correction of vitamin D deficiency could reduce CVD risk by preventing elevations in parathyroid hormone levels and other biological changes.

Two prior prospective studies have examined the association between vitamin D and cardiovascular events in participants with established CVD, both with differing conclusions. In a large study of over 3,000 patients who were referred for cardiac catheterization, Dobnig et al. (34) found inverse associations between 25(OH)D levels and cardiovascular mortality. However, in a German study of 1,000 patients who were referred for cardiac rehabilitation after an acute cardiovascular

Table 1. Continued

Characteristic	25-Hydroxyvitamin D Level, ng/mL						*P* Value
	<20 (*n* = 304)			≥20 (*n* = 642)			
	%	Mean (SD)	Median (IQR)[a]	%	Mean (SD)	Median (IQR)	
Medication use							
Aspirin	73			73			0.99
Statins	63			66			0.34
Beta blockers	61			57			0.22
Angiotensin inhibitors	52			52			0.88
Potential biological mediators							
Systolic blood pressure, mm Hg		136 (23)			132 (20)		0.01
Diastolic blood pressure, mm Hg		76 (12)			74 (11)		0.02
Hemoglobin A_{1c}, %		6.1 (1.3)			5.9 (1.1)		0.004
LDL cholesterol, mg/dL		103 (32)			105 (34)		0.42
HDL cholesterol, mg/dL		44 (13)			46 (14)		0.08
Triglycerides, mg/dL			109 (78–174)			110 (72–159)	0.07
C-reactive protein, mg/dL			2.7 (1.2–6.6)			2.0 (0.8–4.1)	<0.001
Parathyroid hormone, mg/dL			61 (45–82)			50 (39–65)	<0.001
Calcium, mg/dL		9.5 (0.5)			9.5 (0.5)		0.21
Corrected calcium,[c] mg/dL		9.6 (0.5)			9.6 (0.5)		0.17
Phosphorus, mg/dL		3.8 (0.7)			3.7 (0.6)		0.01
Urinary albumin:creatinine ratio, mg/g			9.4 (5.3–23.5)			8.3 (5.0–15.8)	0.01
Fibroblast growth factor 23, RU/mL			50 (31–103)			41(28–62)	0.004

Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; RU, relative units; SD, standard deviation.

[a] 25th–75th percentiles.

[b] Weight (kg)/height $(m)^2$.

[c] Corrected calcium = total calcium + (4.0 − serum albumin) × 0.8.

event, Grandi et al. (35) found no association between 25(OH)D levels and secondary cardiovascular events or mortality. The authors noted that the inconsistencies in the two studies could be attributable to the differences in the patient populations. In the Grandi study, all participants had suffered a recent acute event (35), whereas the Dobnig study was comprised of patients both with and without CHD, and a stratified analysis revealed a greater association in nondiseased participants (34).

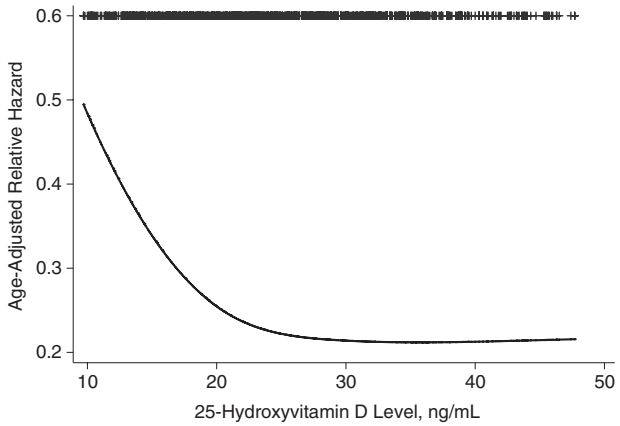

Figure 1. Nonlinearity of the association between 25-hydroxyvitamin D levels and subsequent cardiovascular events among 946 participants in the Heart and Soul Study, 2000–2012.

In this large sample of outpatients, all of whom had established CHD, we observed an inverse association between 25(OH)D levels and subsequent cardiovascular events. In contrast to the Grandi population of patients who had recently suffered an acute event (35), our population was comprised entirely of patients with stable CHD (those who had had a myocardial infarction in the prior 6 months were excluded). Thus, our results suggest that in patients who have established, stable CHD, vitamin D deficiency is associated with subsequent cardiovascular events.

Poor health behaviors explained a portion of the association between 25(OH)D and cardiovascular events, but they did not explain the entire association. The association was mostly explained by biological mediators; in particular, parathyroid hormone, which is downstream of 25(OH)D. Hyperparathyroidism has been proposed as one mechanism by which vitamin D deficiency could mediate cardiovascular events, since it may promote cardiac hypertrophy (25),

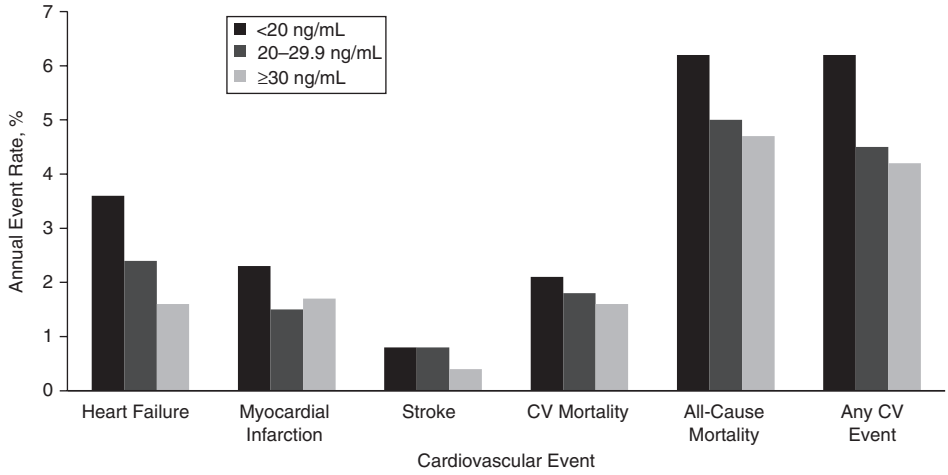

Figure 2. Incidence of cardiovascular (CV) events during a median follow-up period of 8.0 years, by 25-hydroxyvitamin D status, among 946 participants in the Heart and Soul Study, 2000–2012.

vascular remodeling (36, 37), and inflammation (38). In some studies, parathyroid hormone has also been identified as an independent risk factor for cardiovascular events both in the general population (39) and in patients with existing CVD (40). Our findings suggest that the elevations in parathyroid hormone which accompany vitamin D deficiency could mediate cardiovascular events. Moreover, the association between 25(OH)D and cardiovascular outcomes was similar in persons with and without overt hyperparathyroidism, indicating that even small elevations in parathyroid hormone may mediate CVD risk.

We also observed stronger associations between 25(OH)D and cardiovascular events in participants with diabetes, chronic kidney disease, and albuminuria. The high degree of overlap in these risk factors and the very strong *P* values for interaction (all *P*'s < 0.001) strengthen the likelihood that these post hoc analyses may be correctly identifying a high-risk subgroup. However, further analyses are warranted for validation of these subgroup results, as well as to parse out which of these factors is the primary contributor.

For each of these 3 subgroups, fairly compelling reasons exist which could explain why the association between 25(OH)D and cardiovascular events may be particularly strong. If vitamin D deficiency causes insulin resistance, these associations may be even more pronounced in persons who already have overt diabetes. Alternatively, it may be that persons with diabetes are at greater risk simply because of their higher prevalence of albuminuria and chronic kidney disease. Patients with chronic kidney disease may be at greater risk because chronic kidney disease is associated with lower 1-α-hydroxylase activity, which results in even lower levels of 1,25-hydroxyvitamin D. Therefore, if vitamin D deficiency leads to cardiovascular events, vitamin D deficiency may be particularly harmful when superimposed on chronic kidney disease. Finally, participants with albuminuria may be at higher risk because albuminuria is associated with endothelial dysfunction and is an independent risk factor for cardiovascular events (41). Randomized controlled trials have demonstrated reductions in albuminuria with vitamin D administration (42, 43). Thus, these data raise the possibility that correction of vitamin D deficiency could modify CVD risk by preventing or reducing albuminuria.

We found no significant mediation by several other commonly proposed biological processes. Inflammation (10, 23) and blood pressure elevation (44) have also been proposed as plausible biological pathways by which vitamin D deficiency

Table 2. Association Between Baseline 25-Hydroxyvitamin D Level (<20 ng/mL vs. ≥20 ng/mL) and Subsequent Cardiovascular Events[a] (*n* = 323) After Multivariate Adjustment Among 946 Participants in the Heart and Soul Study, 2000–2012

Model	Hazard Ratio	95% Confidence Interval	*P* Value
Model 1[b]	1.50	1.19, 1.90	0.001
Model 2[c]	1.31	1.02, 1.69	0.03
Model 3[d]	1.30	1.01, 1.67	0.04
Model 4[e]	1.11	0.85, 1.44	0.43

[a] Subsequent cardiovascular events were defined as heart failure, myocardial infarction, stroke, or cardiovascular mortality.

[b] Results were adjusted for sociodemographic factors (age, sex, white race/ethnicity, season of blood draw, and college graduation).

[c] Results were adjusted for all model 1 covariates plus likely confounders, including health behaviors (tobacco use, multivitamin use, and physical activity).

[d] Results were adjusted for all model 2 covariates plus comorbid health conditions (diabetes, hypertension, depression, and body mass index).

[e] Results were adjusted for all model 3 covariates plus potential biological mediators (systolic blood pressure, diastolic blood pressure, hemoglobin A_{1c}, triglycerides, high-density lipoprotein cholesterol, C-reactive protein, phosphorus, parathyroid hormone, and fibroblast growth factor 23).

Table 3. Association Between Baseline 25-Hydroxyvitamin D Level (<20 ng/mL vs. ≥20 ng/mL) and Subsequent Cardiovascular Events,[a] With Multivariate Adjustment, Among 946 Participants in the Heart and Soul Study, 2000–2012

Model	Heart Failure ($n = 172$)		Myocardial Infarction ($n = 127$)		Stroke ($n = 49$)		Cardiovascular Mortality ($n = 141$)		All-Cause Mortality ($n = 369$)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Model 1[b]	1.75	1.27, 2.41	1.43	0.99, 2.09	1.27	0.69, 2.34	1.45	1.01, 2.08	1.39	1.11, 1.74
Model 2[c]	1.56	1.11, 2.19	1.34	0.90, 1.99	1.42	0.74, 2.72	1.21	0.83, 1.77	1.22	0.97, 1.55
Model 3[d]	1.44	1.02, 2.02	1.27	0.85, 1.90	1.39	0.72, 2.68	1.23	0.84, 1.80	1.25	0.98, 1.58
Model 4[e]	1.25	0.87, 1.79	1.19	0.78, 1.82	1.08	0.54, 2.18	1.13	0.76, 1.70	1.18	0.92, 1.52

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Subsequent cardiovascular events were defined as heart failure, myocardial infarction, stroke, or cardiovascular mortality.

[b] Results were adjusted for sociodemographic factors (age, sex, white race/ethnicity, season of blood draw, and college graduation).

[c] Results were adjusted for all model 1 covariates plus likely confounders, including health behaviors (tobacco use, multivitamin use, and physical activity).

[d] Results were adjusted for all model 2 covariates plus comorbid health conditions (diabetes, hypertension, depression, and body mass index).

[e] Results were adjusted for all model 3 covariates plus potential biological mediators (systolic blood pressure, diastolic blood pressure, hemoglobin A_{1c}, triglycerides, high-density lipoprotein cholesterol, C-reactive protein, phosphorus, parathyroid hormone, and fibroblast growth factor 23).

could lead to cardiovascular events. However, we found that adjustment for C-reactive protein, hypertension, and blood pressure resulted in no substantial change. Depression has also been proposed as a potential mediator of the association, because vitamin D deficiency is associated with incident depression (45, 46) and depression is known to be an independent risk factor for CVD (26). However, we found no evidence of mediation by depression.

Our study had several important limitations. First, our study was observational in nature. Although we attempted to control for potentially confounding factors prior to performing our mediation analysis, we cannot rule out the

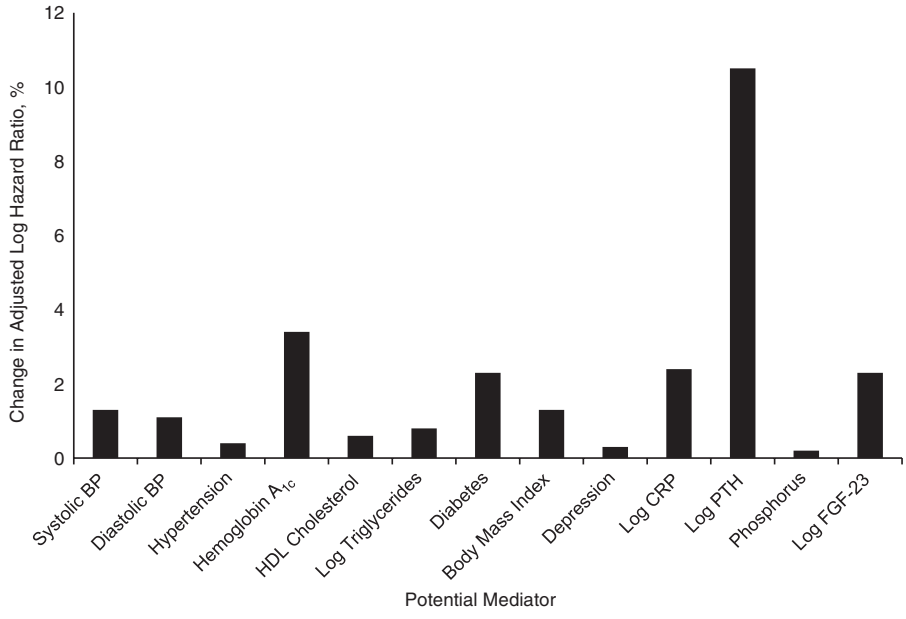

Figure 3. Change in the strength of the association between 25-hydroxyvitamin D level (<20 ng/mL vs. ≥20 ng/mL) and cardiovascular events after adjustment for potential mediators (expressed as percent change in the age-adjusted log hazard ratio) among 946 participants in the Heart and Soul Study, 2000–2012. All models adjusted for sociodemographic factors (age, sex, white race/ethnicity, season of blood draw, and college graduation) and health behaviors (tobacco use, multivitamin use, and physical activity). BP, blood pressure; CRP, C-reactive protein; FGF-23, fibroblast growth factor 23; HDL, high-density lipoprotein; PTH, parathyroid hormone.

possibility that residual confounding may have remained. Second, the study participants were mostly white men, and all had stable CHD. Generalizability to other settings is unknown. Third, the potential mediators we evaluated in this study were measured at the same time as the 25(OH)D levels; therefore, we cannot determine the direction of the association. Finally, over the course of the follow-up period, subjects may have developed interval elevations in blood pressure, insulin resistance, or comorbid conditions which were not accounted for in this study.

In summary, we found that vitamin D deficiency was associated with cardiovascular events in participants with stable CHD, independent of sociodemographic factors, comorbid health conditions, and poor health behaviors. We observed a substantial change in the magnitude of the association after adjustment for parathyroid hormone concentration, indicating that parathyroid hormone could mediate the association. We also discovered that the association was strongest in participants with diabetes, chronic kidney disease, and albuminuria. Elevations in parathyroid hormone and albuminuria are both potentially modifiable by administration of vitamin D; therefore, these findings raise the possibility that correction of vitamin D deficiency could reduce CVD risk. These observations highlight the importance of randomized controlled trials, such as the ongoing Vitamins and Lifestyle (VITAL) Study, in determining whether vitamin D supplementation could play a role in reducing the public health burden of CVD.

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