25-Hydroxyvitamin D and Risk of Myocardial Infarction in Men

A Prospective Study

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Background: Vitamin D deficiency may be involved in the development of atherosclerosis and coronary heart disease in humans.

Methods: We assessed prospectively whether plasma 25-hydroxyvitamin D (25[OH]D) concentrations are associated with risk of coronary heart disease. A nested case-control study was conducted in 18 225 men in the Health Professionals Follow-up Study; the men were aged 40 to 75 years and were free of diagnosed cardiovascular disease at blood collection. The blood samples were returned between April 1, 1993, and November 30, 1999; 99% were received between April 1, 1993, and November 30, 1995. During 10 years of follow-up, 454 men developed nonfatal myocardial infarction or fatal coronary heart disease. Using risk set sampling, controls (n=900) were selected in a 2:1 ratio and matched for age, date of blood collection, and smoking status.

Results: After adjustment for matched variables, men deficient in 25(OH)D (≤15 ng/mL [to convert to nanomoles per liter, multiply by 2.496]) were at increased risk for MI compared with those considered to be sufficient in 25(OH)D (≥30 ng/mL) (relative risk [RR], 2.42; 95% confidence interval [CI], 1.53-3.84; $P<.001$ for trend). After additional adjustment for family history of myocardial infarction, body mass index, alcohol consumption, physical activity, history of diabetes mellitus and hypertension, ethnicity, region, marine ω-3 intake, low- and high-density lipoprotein cholesterol levels, and triglyceride levels, this relationship remained significant (RR, 2.09; 95% CI, 1.24-3.54; $P=.02$ for trend). Even men with intermediate 25(OH)D levels were at elevated risk relative to those with sufficient 25(OH)D levels (22.6-29.9 ng/mL: RR, 1.60 [95% CI, 1.10-2.32]; and 15.0-22.5 ng/mL: RR, 1.43 [95% CI, 0.96-2.13], respectively).

Conclusion: Low levels of 25(OH)D are associated with higher risk of myocardial infarction in a graded manner, even after controlling for factors known to be associated with coronary artery disease.

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A VARIETY OF OBSERVATIONS are not easily explained by known cardiovascular disease (CVD) risk factors. In most populations studied, the rate of CVD-related death is elevated at higher latitudes, increases during the winter months, and is lower at high altitudes. As noted elsewhere,[1] this pattern is consistent with an adverse effect of hypovitaminosis D, which is more prevalent at higher latitudes, during the winter, and at lower altitudes. Alternative explanations for these observations are possible, but a variety of plausible biological mechanisms support a role for vitamin D. The vitamin D axis affects vascular smooth muscle cell proliferation, inflammation, vascular calcification, the renin-angiotensin system (RAS), and blood pressure,[1] all of which affect risk of CVD and myocardial infarction (MI).

Despite these suggestive ecologic data and plausible mechanisms, data directly linking vitamin D levels to risk of MI are sparse. A Danish study[2] examined 25-hydroxyvitamin D (25[OH]D) levels measured in 128 patients admitted to the hospital with ischemic heart disease (75 with angina pectoris and 53 with acute MI) and 409 control subjects and found that 25(OH)D levels were significantly lower in those with angina (23.5 ng/mL [to convert to nanomoles per liter, multiply by 2.496]) or MI (24.0 ng/mL) than in controls (28.8 ng/mL). In a New Zealand case-control study[3] of 179 patients with MI, cases had a lower mean 25(OH)D level ($P=.02$), which was more pronounced in the winter-spring ($P=.03$) than in the summer-autumn ($P=.21$). The relative risk (RR) of MI decreased across increasing quartiles of 25(OH)D (<10 ng/mL: RR, 1 [reference]; 10-13 ng/mL: RR, 0.56 [95% confidence in-

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terval [CI], 0.32-1.03]; 13.1-16.8 ng/mL: RR, 0.33 [95% CI, 0.17-0.64]; and >16.8 ng/mL: RR, 0.30 [95% CI, 0.15-0.61]). Multivariate analyses of major CVD risk factors did not appreciably alter the results. A small, nested, case-control study[4] of MI based in the Tromso Heart Study (northern Norway) with only 30 cases and 60 matched controls found a slightly nonsignificant lower 25(OH)D level in cases (23.6 ng/mL) compared with controls (25.4 ng/mL).

Because hypovitaminosis D is prevalent and easily correctable,[5] establishing the relationship between vitamin D and risk of MI is important. Thus, we prospectively examined 25(OH)D concentrations relation to risk of MI in a large cohort of US men, which is called the Health Professionals Follow-up Study (HPFS).

METHODS

STUDY POPULATION

The HPFS is a prospective cohort investigation in 51 529 US male health care professionals aged 40 to 75 years at baseline in 1986. This cohort was designed primarily to evaluate associations between diet and chronic disease incidence.[6] We assessed information about health and disease every 2 years by using a self-administered questionnaire and about diet every 4 years by using a self-administered food frequency questionnaire.[7] Between January 1, 1993, and December 31, 1995, a blood sample was requested from all surviving cohort participants; 18 225 provided samples. Respondents were somewhat younger but were otherwise similar to nonparticipants. Based on the cohort that provided samples, and after the exclusion of participants with a history of CVD before 1994, we identified 454 participants with incident nonfatal MI or fatal coronary heart disease (CHD) between the date of blood collection and January 31, 2004. We assessed disease status through January 31, 2004, for 97.3% of the men. Controls were randomly selected from participants with a blood sample who were alive and who did not have a history of CVD at case ascertainment. For controls, we used a 2:1 ratio and matched for age, month and year of blood collection, and smoking status (risk set sampling).[8] Eight participants were originally selected as controls and subsequently had an event during follow-up, leaving 900 controls available for analysis. No control was selected twice during the random selection process. All the participants gave written informed consent, and the Harvard School of Public Health human subjects committee review board approved the study protocol.

ASSESSMENT OF NONFATAL MI AND FATAL CHD

Study physicians reviewed the medical records of all the participants for whom nonfatal MI or fatal CHD was reported during follow-up. The reviewers were masked to participants' exposure status. Each questionnaire, mailed biennially to HPFS participants, contains a question on whether the man has had "professionally diagnosed . . . myocardial infarction (heart attack)" in the preceding 2 years. Myocardial infarction was confirmed if it met the World Health Organization's criteria, which include symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes.[9] For approximately 70% of the men who self-reported MI, we confirmed the diagnosis using these methods. Reasons for nonconfirmation of MI were that either no further information was available, typically because the participant did not consent or the hospital did not send the hospital records, or a reported case was rejected based on the medical record information received. We excluded nonconfirmed participants from the control selection process. Deaths were identified from state vital statistics records and the National Death Index or were reported by next of kin or by the postal system. Fatal CHD was considered to have occurred if there was fatal MI confirmed by hospital records or autopsy findings or if CHD was listed as the cause of death on the death certificate, if it was the underlying and most plausible cause, and if evidence of previous CHD was available. In this analysis, 352 participants had nonfatal MI and 102 had fatal CHD as the qualifying event.

ASSESSMENT OF MEDICAL HISTORY, ANTHROPOMETRIC DATA, AND DIET AND LIFESTYLE FACTORS

For this analysis, anthropometric data, lifestyle factors, and diet were based on the 1994 questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Nutrient intake was computed based on a validated semiquantitative food frequency questionnaire, which inquires about average food intake during the past year using composition values from the US Department of Agriculture sources,[10] supplemented with other data. Physical activity was expressed as metabolic equivalent task–hours based on self-reported types and durations of activities over the previous year.[11] One metabolic equivalent task–hour is equivalent to energy expenditure while sitting quietly for 1 hour. We derived medical history information from questionnaires completed between January 1, 1986, and December 31, 1994. There have been previous reports[7,12-16] on the validity and reproducibility of the collected data and measurements.

MEASUREMENT OF BIOCHEMICAL VARIABLES

Blood samples were collected in three 10-mL liquid EDTA blood tubes, placed on ice packs, stored in polystyrene foam containers, and returned to the HPFS blood storage and processing facility at the Harvard School of Public Health via overnight courier. More than 95% of the samples arrived within 24 hours of collection. The blood samples were then centrifuged and aliquoted for storage in the vapor phase of liquid nitrogen freezers (–130°C or colder). Fewer than 15% of the samples were slightly hemolyzed, and few were moderately hemolyzed (<3%), lipemic (<1%), or not cooled on arrival (<0.5%).

Plasma 25(OH)D levels were determined by means of radioimmunoassay as previously described.[17] The coefficient of variation for 25(OH)D was 11.5%. In a subcohort of the 144 men who provided baseline blood samples in 1993-1994 and again in 1997 (mean [SD] of 3.03 [0.46] years apart), the Pearson product moment correlation coefficient for 25(OH)D was 0.70 ($P < .001$).[18] Low-density lipoprotein cholesterol levels were measured using a homogeneous direct method from Genzyme Corp, Cambridge, Massachusetts,[19] high-density lipoprotein cholesterol levels using a direct enzymatic colorimetric assay,[20] and triglyceride levels enzymatically with correction for endogenous glycerol.[21] The assays used for lipoprotein and lipid analysis are approved by the US Food and Drug Administration for clinical use, and coefficients of variation were less than 6%.

STATISTICAL ANALYSES

Plasma 25(OH)D levels were divided into 4 categories based on common definitions of "deficient" (≤15 ng/mL), "insufficient" (15.1-29.9 ng/mL), and "sufficient" (≥30 ng/mL) 25(OH)D levels.[22] We further dichotomized the insufficient

Table 1. Baseline Characteristics of Men With Incident MI and Matched Control Subjects During 10 Years of Follow-up[a]

Characteristic	Cases (n=454)	Controls (n=900)	*P* Value
Age, mean (SD), y	63.8 (8.6)	63.8 (8.6)	NA
Current smoker, No. (%)	42 (9.3)	80 (8.9)	NA
Body mass index, mean (SD)[b]	26.1 (3.3)	25.6 (3.4)	.01
Race/ethnicity, No. (%)			
White	427 (94.1)	851 (94.6)	.70
African American	0	2 (0.2)	.55
Asian	3 (0.7)	5 (0.6)	>.99
Other	24 (5.3)	42 (4.7)	.62
Region of residence, No. (%)			
South	197 (43.4)	426 (47.3)	.17
Northeast	146 (32.2)	313 (34.8)	.34
Midwest	111 (24.4)	161 (17.9)	.004
Family history of MI before age 60 y, No. (%)	72 (15.9)	100 (11.1)	.01
Current aspirin use, ≥2/wk, No. (%)	177 (39.0)	310 (34.4)	.10
History of diabetes mellitus, No. (%)	42 (9.3)	33 (3.7)	<.001
History of hypertension, No. (%)	169 (37.2)	265 (29.4)	.004
Fat intake, mean (SD), % energy			
Total	30.6 (6.7)	30.2 (6.9)	.40
Saturated fat	10.2 (2.7)	10.0 (2.9)	.22
Marine ω-3	0.12 (0.16)	0.13 (0.20)	.85
Alcohol consumption, median (IQR), g/d	4.2 (0-14.6)	6.9 (0.9-17.8)	.002
Multivitamin use, No. (%)	211 (46.5)	436 (48.4)	.49
Physical activity, median (IQR), MET-h/wk	22.8 (9.5-46.5)	26.1 (11.9-48.8)	.05
Cholesterol, mean (SD), mg/dL			
Total	210.7 (39.0)	202.4 (36.3)	<.001
HDL	42.2 (11.1)	46.0 (12.6)	<.001
LDL	133.9 (34.5)	126.0 (31.2)	<.001
Triglycerides, mean (SD), mg/dL	164.4 (100.4)	142.6 (101.4)	<.001
25(OH)D, mean (SD), ng/mL	23.0 (7.6)	24.5 (8.3)	.002

Abbreviations: HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MET-h, metabolic equivalent task–hours; MI, myocardial infarction; NA, not applicable; 25(OH)D, 25-hydroxyvitamin D.

SI conversion factors: To convert total, HDL, and LDL cholesterol to millimoles per liter, multiply by 0.0259; 25(OH)D to nanomoles per liter, multiply by 2.496; and triglycerides to millimoles per liter, multiply by 0.0113.

[a] Age, smoking status, and month of blood collection were matched variables.

[b] Calculated as weight in kilograms divided by height in meters squared.

range at its midpoint into 15.1 to 22.5 ng/mL and 22.6 to 29.9 ng/mL. For the main analysis, to investigate the association between 25(OH)D concentrations and the incidence of nonfatal MI or fatal CHD (events), we used conditional logistic regression. For tests for trend, we modeled the median value of the 4 categories as continuous variables in the regression model. In the multivariate model, we further adjusted for family history of MI before the age of 60 years (yes or no), alcohol intake (nondrinker; 0.1-4.9, 5.0-14.9, 15.0-29.9, or ≥30.0 g/d; or missing), BMI (continuous), physical activity (quintiles), history of diabetes mellitus (yes or no) and hypertension (yes or no), ethnicity (white or other), region (Northeast, mid-Atlantic, Midwest, or South), marine ω-3 intake (quintiles), and low- and high-density lipoprotein cholesterol and triglyceride levels (quintiles) at baseline. Because of the design of this study, the odds ratio derived from logistic regression directly estimates the incidence rate (hazard) ratio and, therefore, the RR.[8,23] We assessed the goodness of fit of the models using the method described by Hosmer and Lemeshow,[24] which did not show any significant lack of fit.

For the stratified analysis, we used unconditional logistic regression adjusted for matched variables (age [<50, 50-54, 55-59, 60-64, or ≥65 years], smoking status [never, past, or current], and month of blood collection [5 categories]). For the main analysis, unconditional logistic regression with adjustment for matched variables yielded similar results as conditional logistic regression. Tests for interaction were based on the Wald test for the interaction term (variable × 25[OH]D level), with the variable and vitamin D in the model as continuous variables. All *P* values are 2-tailed; $P<.05$ was considered statistically significant. All analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Selected characteristics and biomarker levels of cases and controls are given in **Table 1**. As expected, cases had a higher BMI, a lower level of physical activity, and less alcohol intake and were more likely to have a family history of MI before the age of 60 years and a history of diabetes mellitus and hypertension. In addition, cases had higher levels of total and low-density lipoprotein cholesterol and triglycerides but lower levels of high-density lipoprotein cholesterol. Plasma 25(OH)D levels were significantly lower in cases (23.0 ng/mL) than in controls (24.5 ng/mL) ($P=.002$).

We next measured levels or percentages of selected risk factors across 25(OH)D concentrations in controls (**Table 2**). Men with lower 25(OH)D levels were more likely to be current smokers, less physically active, and heavier and less likely to be white and to have a parental history of MI. They were also less likely to take a multivitamin supplement, they drank less alcohol, and they were more likely to live in northern states. Men with lower 25(OH)D levels had a suggestive but not statistically significant higher prevalence of diabetes mellitus and hypertension and significantly higher levels of triglycerides and lower levels of high-density lipoprotein cholesterol.

Table 3 provides RRs of MI (incident nonfatal MI plus fatal CHD) during 10 years of follow-up across the categories of plasma 25(OH)D. After adjustment for matching factors, men with deficient levels of 25(OH)D had a significantly elevated risk of MI (RR, 2.42; 95% CI, 1.53-3.84; $P<.001$ for trend). In the multivariate model without plasma lipids, the RR was somewhat attenuated but remained significant (RR, 2.01; 95% CI, 1.22-3.30; $P=.02$ for trend). After further adjustment for plasma lipids, the RR was 2.09 (95% CI, 1.24-3.54; $P=.02$ for trend). The results did not change appreciably after excluding the first 2 years of follow-up (RR, 1.97; 95% CI, 1.10-3.51; $P=.05$ for trend across levels of 25[OH]D).

In alternative analyses, we examined season-specific quintiles of 25(OH)D in relation to MI risk. For those in the bottom vs top quintiles, the multivariate RR was 1.94 (95% CI, 1.23-3.06). Compared with men with ad-

equate 25(OH)D levels, those with insufficient values of 25(OH)D were associated with approximately a 50% increase in risk. In the full multivariate model, the β coefficient for a 1-ng/mL increment in 25(OH)D was −0.0214 (SE, 0.0099), indicating that the risk of MI decreased by 2.1% per 1-ng/mL increment in 25(OH)D. We did not have adequate power to test for differences between nonfatal (n=352) and fatal (n=102) cases, but the β coefficient for MI risk per 1-ng/mL increase in 25(OH)D suggested a stronger association for fatal MI (4.3% decrease in risk) than for nonfatal MI (1.8% decrease in risk). Furthermore, the multivariate RR for deficient vs sufficient 25(OH)D values did not change appreciably when we included C-reactive protein (RR, 2.04; 95% CI, 1.20-3.46) or estimated glomerular filtration rate based on measured plasma creatinine level, age, sex, and race (RR, 2.09; 95% CI, 1.23-3.54) in the full multivariate model.

We tested for interaction across various factors to examine whether the association between 25(OH)D level and MI risk varied. We did not find statistical evidence of interaction by age ($P=.85$ for interaction), high blood pressure ($P=.84$), BMI ($P=.87$), aspirin use ($P=.92$), physical activity ($P=.21$), alcohol use ($P=.53$), low-density lipoprotein cholesterol level ($P=.33$), or triglyceride level ($P=.73$). Suggestive evidence was observed for an interaction by high-density lipoprotein cholesterol level, with a suggestive stronger inverse association with 25(OH)D level in men with a higher high-density lipoprotein cholesterol level ($P=.06$). Excluding men taking cholesterol-lowering drugs at baseline, the multivariate RR for deficient vs sufficient 25(OH)D concentrations was 2.30 (95% CI, 1.33-3.97). Because this subset included a limited number of participants with diabetes mellitus at baseline, we could not calculate effect estimates in this subgroup; however, exclusion of these participants did not substantively alter the results. Similarly, the exclusion of participants with a parental history of MI before the age of 60 years and current smokers did not affect the results.

Table 2. Baseline Characteristics of Selected Covariates by Plasma 25(OH)D Level in Control Subjects[a]

Characteristic	25(OH)D, ng/mL ≤15 (n=87)	15.1-22.5 (n=307)	22.6-29.9 (n=299)	≥30 (n=207)	*P* Value (Trend)
25(OH)D, mean, ng/mL	12.0	19.2	25.9	35.5	NA
Age, mean, y	63.8	63.0	64.9	63.4	NA
Current smoker, %	20.7	7.0	8.0	8.1	.01
Body mass index[b]	26.4	25.9	25.5	24.9	<.001
White race, %	93.1	95.2	92.9	96.7	.31
Region of residence, %					
South	43.7	42.8	50.3	51.3	.15
Northeast	36.8	40.0	31.4	31.3	.10
Midwest	19.5	16.9	18.3	17.4	.97
Family history of MI before age 60 y, %	6.9	8.7	14.2	12.0	.02
Current aspirin use, ≥2/wk, %	36.8	34.6	31.3	37.9	.80
History of diabetes mellitus, %	5.7	3.5	4.4	2.1	.12
History of hypertension, %	31.0	31.6	28.2	27.4	.19
Fat intake, % energy					
Total	30.7	30.2	30.2	30.0	.23
Saturated fat	10.1	10.0	10.0	9.8	.21
Marine ω-3	0.09	0.13	0.13	0.14	.59
Alcohol consumption, g/d	11.5	12.0	13.1	15.5	.009
Multivitamin use, %	39.1	48.3	49.0	51.8	.01
Physical activity, MET-h/wk	27.1	35.6	39.7	42.3	.002
Cholesterol level, mg/dL					
Total	200.1	202.9	200.7	205.3	.20
HDL	42.0	43.9	47.0	49.4	<.001
LDL	122.8	126.0	125.0	128.6	.21
Triglycerides, mean (SD), mg/dL	155.3	156.8	133.9	128.8	.001

Abbreviations: See Table 1.

SI conversion factors: To convert total, HDL, and LDL cholesterol to millimoles per liter, multiply by 0.0259; 25(OH)D to nanomoles per liter, multiply by 2.496; and triglycerides to millimoles per liter, multiply by 0.0113.

[a] All variables other than age and 25(OH)D level are age standardized.

[b] Calculated as weight in kilograms divided by height in meters squared.

COMMENT

In this cohort study, men with circulating 25(OH)D levels of at least 30 ng/mL had approximately half the risk of MI, independent of other CVD factors. The association was suggestively stronger for fatal CHD, but the number of cases was too small to make definitive conclusions. Although traditional CVD risk factors (eg, lipids, hypertension, diabetes mellitus, and smoking) remained strong risk factors in this population, vitamin D deficiency seemed to be an independent risk factor. Only 23% of the men in the HPFS had levels of 25(OH)D of at least 30 ng/mL. This percentage is typical of many populations, and the prevalence of deficiency is even higher in subpopulations such as dark-skinned individuals and elderly persons. In individuals in sun-rich environments, where clothing or cultural practices do not appreciably limit vitamin D production, 25(OH)D levels of 54 to 90 ng/mL are attained,[25] but from the present study we cannot evaluate whether levels greater than 35 ng/mL would be associated with an even greater MI risk reduction.

The prospective design, the high follow-up rate, and the use of a plasma marker largely precluded major sources of bias, such as recall bias and selection bias. We excluded men diagnosed as having CVD at baseline and, furthermore, results were similar after excluding the first 2 years of follow-up, arguing against reverse causation bias, which might occur if men predisposed to an MI stayed indoors and, thereby, avoided sun exposure. We controlled for and stratified by major covariates that could affect MI risk and 25(OH)D concentrations, including exercise (which increases sun exposure), BMI, region, race, multivitamin use, and marine ω-3 intake, as fatty fish is the only significant natural source of dietary vitamin D. We controlled for the major lipid risk factors of MI. In addition, we considered the estimated glomer-

Table 3. Estimated RRs of MI by Level of 25(OH)D at Baseline During 10 Years of Follow-up

Variable	Plasma 25(OH)D, ng/mL				*P* Value (Trend)
	≤15.0	15.1-22.5	22.6-29.9	≥30.0	
Cases/controls, No.	63/87	156/307	165/299	70/207	NA
RR (95% CI)					
Matching variables	2.42 (1.53-3.84)	1.65 (1.15-2.37)	1.72 (1.22-2.42)	1 [Reference]	<.001
MV1[a]	2.01 (1.22-3.30)	1.45 (0.99-2.12)	1.56 (1.09-2.22)	1 [Reference]	.02
MV2[b]	2.09 (1.24-3.54)	1.43 (0.96-2.13)	1.60 (1.10-2.32)	1 [Reference]	.02

Abbreviations: CI, confidence interval; MI, myocardial infarction; MV, multivariate; NA, not applicable; 25(OH)D, 25-hydroxyvitamin D; RR, relative risk.
SI conversion factor: To convert 25(OH)D to nanomoles per liter, multiply by 2.496.
[a]MV1: matching variables (age, month and year of blood collection, and smoking status) and family history of MI before the age of 60 years, history of diabetes mellitus, history of hypertension, alcohol intake, body mass index, physical activity, region, race, multivitamin use, marine ω-3 intake, and fasting status.
[b]MV2: all the variables in MV1 and high- and low-density lipoprotein cholesterol and triglyceride levels.

ular filtration rate because circulating 25(OH)D levels could be lower in individuals with chronic kidney disease, which is a risk factor for CVD.[26] Controlling for the estimated glomerular filtration rate did not change the results. Given the strength of the association observed between 25(OH)D level and MI risk, and the fact that controlling for these factors did not appreciably affect the magnitude of the association, substantial residual confounding by these factors is not likely but cannot be ruled out.

Because 25(OH)D levels are largely affected by sun exposure, it is plausible that some other consequence of sun exposure other than vitamin D production is responsible for the observed association with MI. Nevertheless, much evidence supports mechanisms whereby vitamin D could affect CVD risk. Of the potentially relevant mechanisms, vitamin D affects vascular smooth muscle cell proliferation, inflammation, vascular calcification, and blood pressure through the RAS.[1]

The RAS helps regulate blood pressure, electrolyte levels, and volume homeostasis, and excessive RAS stimulation is associated with hypertension. Animal studies[27] show that vitamin D is an important regulator of the RAS system and that 1,25-dihydroxyvitamin D, the activated form of vitamin D, suppresses renin gene expression. Disruption of the vitamin D receptor gene leads to elevated renin production, cardiac hypertrophy, and elevated blood pressure in mice.[28] In a randomized controlled trial (RCT)[29] of either UV-B or UV-A administered through tanning booths, UV-B, which increased 25(OH)D levels by 162%, was effective in reducing 24-hour ambulatory blood pressure (by –6/–6 mm Hg, $P<.001$), whereas UV-A did not affect 25(OH)D levels or blood pressure. In another RCT[30] of individuals with low vitamin D status (<20 ng/mL; mean, approximately 10 ng/mL), supplementation with 800 IU of vitamin D resulted in an increase in serum 25(OH)D of 72% ($P<.01$), a decrease in systolic blood pressure of 9.3% ($P=.02$), and a suggestive decrease in diastolic blood pressure of 8.5% ($P=.10$). In the HPFS and the Nurses' Health Study,[31] during 4 years of follow-up, men and women who had plasma 25(OH)D levels less than 15 ng/mL were 3 times as likely to have a new diagnosis of hypertension in the next 4 years compared with those with 25(OH)D levels greater than 30 ng/mL. An inverse association between 25(OH)D levels and blood pressure was also found using Third National Health and Nutrition Examination Survey data.[32]

Calcification is a common feature of atherosclerosis, and nearly all angiographically significant lesions are calcified.[33] Calcification of coronary arteries has been associated with increased risk of MI[34] and poorer 5-year survival.[35] Atherosclerotic calcification is a process regulated in ways similar to skeletal osteogenesis.[36] A significant association exists between osteoporosis and vascular calcification, suggesting that osteoregulatory mechanisms related to bone development may affect calcification in the vasculature. Levels of 1,25-dihydroxyvitamin D have been shown to be inversely associated with vascular calcification,[36] suggesting that vitamin D may affect MI risk through its effects on vascular calcification.

Other mechanisms could account for or contribute to the association between 25(OH)D and MI risk. Vitamin D deficiency, possibly combined with low calcium intake, has been associated with impaired fasting glucose and possibly risk of type 2 diabetes mellitus,[37-40] risk factors for CVD. Vitamin D deficiency has also been associated with a cytokine profile that favors greater inflammation (eg, higher C-reactive protein and interleukin 6 levels and lower interleukin 10 levels),[41-46] which could predispose to heightened MI risk. Finally, seasonal respiratory tract infections, particularly influenza, have been proposed to account for the winter increase in mortality due to CVD,[47] and hypovitaminosis D could contribute to these infections.[48,49]

Two case-control studies[2,3] and a small prospective study[4] found that individuals with low 25(OH)D levels were at higher risk for ischemic heart disease. The strongest test of the hypothesis that vitamin D lowers MI risk would be from an RCT. Two RCTs reported on CVD. In a UK study of 2686 men and women, the participants were randomized to receive 830 IU of vitamin D daily (administered as 100 000 IU of oral vitamin D3 every 4 months) or placebo for 5 years. The in-study 25(OH)D levels were 29.7 ng/mL in the vitamin D group and 21.4 ng/mL in the placebo group. There was a nonsignificant decrease in CVD incidence (RR, 0.90; 95% CI, 0.77-1.06) and CVD mortality (RR, 0.84; 95% CI, 0.65-1.10) in the intervention group. Based on the present study, a difference of 8.3 ng/mL in 25(OH)D concentration would be associated with an RR of 0.92, which is compatible with the previous results. A recent meta-

analysis[50] of total mortality as a secondary end point of RCTs with varying levels of vitamin D vs placebo controls found a statistically significant 8% reduction in risk of total mortality in individuals who had received vitamin D. Although the authors could not evaluate cause-specific mortality, the relatively immediate effect of a large enough magnitude to affect total mortality would suggest a benefit on CVD risk.

The largest RCT of vitamin D (and calcium) supplementation and CVD risk was from the Women's Health Initiative,[51] in which 36 282 postmenopausal women received either calcium (1000 mg/d) and vitamin D3 (cholecalciferol) (400 IU/d) or placebo. No reduction was observed in MI- or CHD-related deaths (hazard ratio, 1.04; 95% CI, 0.92-1.18). These results seem to be in contrast to the present findings, suggesting 2 possible explanations. First, despite our efforts to exclude confounding, it is possible that uncontrolled or residual confounding explained these results. Alternatively, the range of vitamin D studied was much wider in the HPFS, which allowed us to detect an association. The difference between the medians of the top and bottom categories, for which we observed an RR of approximately 2, was 23.5 ng/mL (35.5–12.0 ng/mL), and the calculated reduction in MI risk per increment of 1 ng/mL of 25(OH)D was 2%. In the Women's Health Initiative study, the effect of the treatment on 25(OH)D levels was not reported, but based on the dose and compliance, Lappe et al estimated it to be only 2 ng/mL.[52] Based on the present data, such an increment would be expected to have only a 4% reduction in risk. To increase 25(OH)D levels from 12 to 35.5 ng/mL would require approximately 3000 IU of vitamin D daily.[53] Although such intakes may seem high by current standards, increasing evidence demonstrates no toxic effects at intakes below 10 000 IU/d.[54] Because current sources of vitamin D provide much less (eg, a glass of milk has approximately 100 IU), those who achieve high levels such as 35 ng/mL naturally do so largely through sun exposure.

Vitamin D deficiency has been related to an increasing number of conditions[5] and to total mortality.[50] These results further support an important role for vitamin D in MI risk. If this association is causal, which remains to be established, the amount of vitamin D required for optimal benefit may be much higher than would be provided by current recommendations (200-600 IU/d), especially in those with minimal sun exposure. Thus, the present findings add further support that the current dietary requirements of vitamin D need to be increased to have an effect on circulating 25(OH)D levels substantially large enough for potential health benefits.[25]

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