

Circulating Vitamin D, Supplement Use, and Cardiovascular Disease Risk: The MrOS Sleep Study

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Context: Evidence suggests an inverse association between circulating 25(OH) vitamin D and cardiovascular disease (CVD).

Objective: To determine the association between serum 25(OH) vitamin D and risk for CVD events.

Setting and Design: From March 2000 to April 2002, participants were recruited for the Osteoporotic Fractures in Men (MrOS) study. Between December 2003 and March 2005, members of the MrOS cohort were invited to participate in the MrOS Sleep Study. Participants were recruited from 6 clinical centers across the United States and followed for a mean of 5.9 years. Three-thousand-one-hundred-thirty-five men ages 65 and older were included from the MrOS cohort, of whom 116 were excluded for missing vitamin D or CVD data. Participants were divided into two groups based on serum 25(OH) vitamin D levels, <20 ng/mL and \geq 20 ng/mL. Participants were followed for CVD endpoints including coronary heart disease (CHD) and cerebrovascular events. Age- and multi-variable-adjusted hazard ratios were calculated and stratified by use of vitamin D containing supplements.

Results: We observed no significant association between circulating 25(OH) vitamin D and risk of CVD event (HR, 0.91; 95% confidence interval (CI), 0.73–1.13) and CHD event (HR, 0.81; 95% CI, 0.61–1.07). For cerebrovascular events, men with vitamin D deficiency exhibited a higher risk (HR, 1.44; 95% CI, 1.00–2.08) using the minimally adjusted model and after excluding supplement users (HR, 1.70; 95% CI, 1.02–2.83).

Conclusions: 25(OH) vitamin D was not associated with risk of CVD and CHD events. However, vitamin D deficiency may be associated with an increased risk of cerebrovascular events. (*J Clin Endocrinol Metab* 99: 3256–3262, 2014)

More than 80 million adults in the United States have cardiovascular disease (CVD) (1). Heart disease in particular is the leading cause of death in the United States (2). Likewise, stroke/cerebrovascular disease is the fourth

most common cause of death and a leading cause of disability (1, 2). Interest in identifying common, potentially modifiable risk factors for both diseases continues to grow. As early as the 1970s, (3) scientists have been trying

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Abbreviations: BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; MrOS, Osteoporotic Fractures in Men Study; PASE, Physical Activity Scale for the Elderly; RR, relative risk; TIA, transient ischemic attack; VDR, vitamin D receptor.

to ascertain the relationship between vitamin D and CVD risk. Epidemiologic studies have shown mixed results but many observational studies suggest an inverse association between vitamin D levels and CVD risk. A recent meta-analysis (4) of 19 published prospective studies found that pooled data of the lowest levels of 25-hydroxy (OH) vitamin D were significantly associated with an increased risk of total CVD (incident CVD and CVD mortality) (relative risk (RR), 1.52; 95% CI (confidence interval), 1.30–1.77), coronary heart disease (CHD) (RR, 1.38; 95% CI, 1.21–1.57), and stroke (RR, 1.64; 95% CI, 1.27–2.10) when compared with the highest categories of 25(OH) vitamin D. In another meta-analysis of pooled data from nine prospective cohort studies, (5) vitamin D concentration was inversely associated with risk of cerebrovascular disease events, specifically, when divided by circulating vitamin D levels, individuals in the top third had an RR of 0.60 (95% CI, 0.48–0.72) when compared with those in the bottom third.

It is conceivable that vitamin D could play a role in development of CVD. Experimental studies have explored the role of vitamin D in various physiological pathways on a cellular level. There is a vitamin D receptor (VDR) that binds the active metabolite of 25(OH) vitamin D and is located on several cell types involved in cardiovascular functioning (6, 7). On endothelial cells, VDR-mediated action is thought to decrease inflammation and increase flow-mediated dilation, both actions that inhibit atherosclerosis. On smooth muscle cells, VDR activation affects gene expression of factors involved in plaque formation and thrombosis. Signaling through the VDR on cardiac myocytes has been shown to have direct antihypertrophic activity in the heart (8). The active metabolite of vitamin D is also known to be a negative regulator of the renin-angiotensin system via a VDR-regulated mechanism altering renin gene transcription (9).

We have previously reported the relationship of serum 25(OH) vitamin D levels with the 4-year risk of both CVD event and cardiovascular mortality in a subset of 813 men enrolled in the Osteoporotic Fractures in Men (MrOS) Study (10). During a median follow-up of 4.4 years, we found no significant association between levels of circulating 25(OH) vitamin D and risk of CVD incidence when comparing the lowest quartile (4.9–20.1 ng/mL) vs. the highest quartile (30.2–55.4 ng/mL) of serum 25(OH) vitamin D: HR, 1.18; 95% CI, 0.69–2.03. Here, we report a larger sample of data with longer follow-up from the Outcomes of Sleep Disorders in Older Men (MrOS Sleep Study) cohort, an ancillary to the MrOS Study in which 3019 men were enrolled from six United States study sites to prospectively evaluate the association between serum

25(OH) vitamin D and risk of any CVD, CHD, and cerebrovascular events.

Materials and Methods

Study population

Details of the MrOS Study have been described previously (11, 12). Briefly, the MrOS Study is a prospective cohort study designed to determine risk factors for osteoporosis and fractures in older men. From March 2000 to April 2002, MrOS recruited 5994 community-dwelling men ages 65 and older at six recruitment sites: Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Pittsburgh, PA; Portland, OR; and, San Diego, CA. Exclusion criteria were: 1) inability to walk without assistance from another person, 2) bilateral hip replacements, and 3) inability to provide self-reported data. At baseline of the parent cohort, a food frequency questionnaire was administered, blood serum samples were taken in the morning while fasting, and physical activity was assessed using the Physical Activity Scale for the Elderly (PASE) (13). Participants were also asked to bring all prescription and nonprescription medications used within the 30 days preceding the clinic visit (14). All medications recorded by the clinics were stored in an electronic medications inventory database (San Francisco Coordinating Center, San Francisco, CA). Each medication was matched to its ingredient(s) based on the Iowa Drug Information Service Drug Vocabulary (College of Pharmacy, University of Iowa, Iowa City, IA).

Between December 2003 and March 2005, participants from the MrOS cohort were invited to participate in the MrOS Sleep Study. Participants were excluded from the sleep study if they reported any of the following: 1) sleeping with a continuous positive airway pressure or bilevel positive airway pressure mask in the last 3 months, 2) sleeping with a mouthpiece in the last 3 months, 3) having an open tracheostomy, or 4) using oxygen therapy during sleep in the last 3 months. Upon enrollment in the MrOS Sleep Study, 3135 participants completed a self-administered questionnaire, including medical history, lifestyle summary, a clinical interview questionnaire (which included information about medication use), and clinical measurements, including anthropometric measures and blood pressure (BP).

This study was approved by the Institutional Review Board at each participating institution. All subjects gave written informed consent.

Assessment of vitamin D

Serum for vitamin D analysis was collected at baseline of the MrOS Sleep Study and immediately frozen at -20°C . Concentrations of serum 25(OH) vitamin D₂ and 25(OH) vitamin D₃ in fasting plasma samples collected at the Sleep Study visit, were measured at the Mayo Clinic Reference Laboratories (R.J. Singh, PhD, Mayo Clinic Laboratory, Rochester, MN) by liquid chromatography-tandem mass spectrometry (ThermoFisher Scientific and Applied Biosystems-MDS Sciex), an accurate and precise method free of the artifacts that have affected other radio immunoassay-based methods (15). Using three different target markers as quality controls for each assay, the interassay coefficient of variability for 25(OH) vitamin D₃ was 9.7% at 9.0 IUs, 7.5% at 29 IUs, and 5.8% at 76 IUs. For 25(OH) vitamin D₂, coefficients of variability were 11.2% at 11 IUs, 8.5% at 28 IUs,

and 7.7% at 74 IUs. We used total serum 25(OH) vitamin D for our primary analyses, combining 25(OH) vitamin D2 and 25(OH) vitamin D3.

Assessment of CVD

This analysis includes participants in the MrOS Sleep Study who were actively followed for both fatal and nonfatal CVD endpoints for a mean of 5.9 years (median, 7.2 years). Participants were surveyed for potential incident cardiovascular events by postcard and/or phone contact every 4 months with a 99% response rate. Relevant medical records and supporting documentation from any potential incident clinical events identified by phone or postcard contact were obtained by the clinical center and forwarded to the coordinating center for centralized adjudication. For all fatal events, death certificates were forwarded for centralized adjudication. When no medical records were available for out-of-hospital deaths, proxy interviews with next of kin were obtained.

For both nonfatal and fatal cardiovascular events, all documents were adjudicated by a board-certified cardiologist using a prespecified adjudication protocol developed using methods that had been successfully employed at the coordinating center for both prior randomized trials and epidemiological studies of CVD. Interrater agreement was periodically evaluated by one or more expert adjudicator(s) in a random subset of events to ensure quality control in the outcomes adjudication process.

Confirmed events were grouped as follows: 1) CHD event (acute myocardial infarction (ST or non-ST elevation), CHD sudden death, coronary artery bypass surgery, coronary revascularization, hospitalization for unstable angina, ischemic congestive heart failure, or other CHD event not listed above); 2) cerebrovascular event (stroke or transient ischemic attack (TIA)); and 3) all-cause CVD event (combines CHD event, cerebrovascular event, and peripheral vascular disease).

Statistical analysis

Of the 3135 men in the MrOS Sleep Study, 90 did not have samples archived for vitamin D measurement and an additional 26 had missing CVD data, leaving 3019 for these analyses. During a mean follow-up of 5.9 years, 749 men had at least one CVD event, 503 men had at least one CHD event, and 214 men had at least one cerebrovascular event. We evaluated differences in baseline characteristics of men with and without a follow-up CVD event and across categories of total 25(OH) vitamin D using a χ^2 test for categorical variables and an ANOVA for continuous normally distributed variables or a Kruskal-Wallis test for continuous non-normal variables.

We examined the association of low serum levels of 25(OH) vitamin D on risk of subsequent CVD, CHD, and cerebrovascular disease event outcomes using Cox proportional hazards models. We estimated HR and 95% CI for 1) a minimally adjusted model adjusted for age (continuous), nonwhite race (yes/no), study site (Birmingham/Minneapolis/Palo Alto/Pittsburgh/Portland/San Diego), season of blood draw (Winter/Spring/Summer/Fall), and laboratory batch for vitamin D assay (batch 1/batch 2); and 2) a fully adjusted multivariable model additionally adjusted for education (high school education or greater), body mass index (BMI) (BMI, continuous), PASE score (continuous), systolic BP (continuous), diastolic BP (continuous), history of smoking (yes/no), alcohol use (drinks per week, continuous), statin use (yes/no), history of hypertension (yes/no),

history of diabetes (yes/no), and history of cardiovascular event (yes/no). The full cohort (N = 3019) was used for the analysis using the minimally adjusted model; analysis using the fully adjusted model was performed on 2990 men from the cohort and reflects missing values for some covariates. Level of serum 25(OH) vitamin D was divided into two categories (<20 ng/mL and \geq 20 ng/mL) based on the generally accepted definition for vitamin D deficiency as being levels less than 20 ng/mL (16). The higher category (sufficient levels) was used as the reference. In secondary analyses, we used serum 25(OH) vitamin D as a continuous variable.

We examined the effect of vitamin D supplement use by performing secondary analyses on the subset of men (N = 1123) remaining after excluding those men (N = 1867) who reported they took supplements (in the form of a pill, specifically a vitamin D supplement or any other pill that was likely to have vitamin D as an ingredient, such as a multivitamin) at the time of blood collection. Similarly, to eliminate potential bias due to prevalent CVD, we performed analyses by removing men who reported a prior CVD event at baseline, leaving 306 (17.6%) men with incident CVD, 181 (10.4%) with incident CHD, and 109 (6.3%) men with incident cerebrovascular disease among a subset of 1742 men without CVD at baseline. Further, in specifically exploring risk of cerebrovascular events, as stroke events imply a higher risk of mortality than TIAs, in other secondary analysis, we defined cerebrovascular events as stroke only rather than stroke or TIA.

Lastly, we performed sensitivity analysis on the cutoff points used to stratify the serum 25(OH) vitamin D levels. We repeated our analyses using vitamin D categories of <20 ng/mL (deficient; N = 408), 20–30 ng/mL (insufficient; N = 1399), and >30 ng/mL (sufficient; N = 1183), to try to determine whether a stricter cutoff for vitamin D sufficiency would reveal an association. We also conducted analyses with a stricter cutoff for severe vitamin D deficiency by defining vitamin D categories of \leq 12 ng/mL (severely deficient; N = 68), 12.1–19.9 ng/mL (mildly to moderately deficient; N = 340), and \geq 20 ng/mL (sufficient; N = 2582).

Data collected from the study was compiled and analyzed using the SAS software (version 9.2). A *P* value <0.05 was considered statistically significant.

Results

Table 1 shows baseline characteristics of all 3019 participants by categories of circulating 25(OH) vitamin D. Variables that differed significantly across serum 25(OH) vitamin D levels were age (*P* = .002), BMI (*P* < .001), PASE score (*P* < .001), Caucasian race (*P* < .001), alcohol intake (*P* = .007), history of diabetes (*P* < .001), education (*P* = .01), and season of blood draw (*P* < .001).

Table 2 shows associations between circulating 25(OH) vitamin D level and risks of CVD outcomes using minimally adjusted and fully adjusted models. Overall, there was no significant association between serum 25(OH) vitamin D level and risks of CVD or CHD events in any of the models. In the full cohort using the multivariable model, compared with those with sufficient levels of vi-

Table 1. Baseline Characteristics for Entire Cohort (n = 3019), Overall and Per Serum 25(OH) Vitamin D Categories

Characteristic	Serum 25(OH) Vitamin D by Level		
	<20 ng/mL	≥20 ng/mL	Overall
N	416	2603	3019
Age, mean ± SD, y	77.2 ± 5.7	76.3 ± 5.5	76.4 ± 5.5
Caucasian, n (%)	334 (80.3)	2385 (92)	2719 (90.1)
College education or greater, n (%)	319 (76.7)	2062 (79.2)	2381 (78.9)
BMI, mean ± SD, kg/m ²	28.2 ± 4.4	27.0 ± 3.7	27.2 ± 3.8
PASE score, mean ± SD	131.0 ± 72.4	149.0 ± 71.8	146.5 ± 72.1
Systolic blood pressure, mean ± SD, mm Hg	128.0 ± 18.5	126.7 ± 16.0	126.9 ± 16.3
Diastolic blood pressure, mean ± SD, mm Hg	67.6 ± 10.4	67.7 ± 9.3	67.7 ± 9.5
Past or current smoker, n (%)	249 (59.9)	1579 (60.7)	1828 (60.6)
Alcohol intake in past 12 mo, mean ± SD, (drinks/wk)	1.7 ± 1.7	1.9 ± 1.7	1.9 ± 1.7
Caffeine intake, mean ± SD, mg/d	255.5 ± 263.9	232.9 ± 243.3	236.0 ± 246.3
History of diabetes, n (%)	79 (19.1)	318 (12.2)	397 (13.2)
History of CVD, n (%)	191 (46.3)	1064 (40.9)	1255 (41.7)
History of congestive heart failure, n (%)	35 (8.5)	145 (5.6)	180 (6.0)
History of stroke, n (%)	13 (3.1)	99 (3.8)	112 (3.7)
History of hypertension, n (%)	220 (53.1)	1283 (49.3)	1503 (49.8)
Statin use, n (%)	170 (40.9)	1082 (41.6)	1252 (41.5)
Supplemental vitamin use, n (%)	119 (28.6)	1764 (67.8)	1883 (62.4)
Season of blood draw			
Winter, n (%)	31 (44.9)	793 (30.5)	988 (32.7)
Spring, n (%)	16 (23.2)	656 (25.2)	756 (25.0)
Summer, n (%)	7 (10.1)	651 (25.0)	705 (23.4)
Fall, n (%)	15 (21.7)	503 (19.3)	570 (18.9)

tamin D, the group that was deficient in vitamin D had an HR of 0.91 (95% CI, 0.73–1.13) for incident CVD events and an HR of 0.81 (95% CI, 0.61–1.07) for incident CHD events. There was no evidence of an interaction between serum 25(OH) vitamin D levels and self-reported use of vitamin D supplements for prediction of risks of any CVD

or CHD outcomes ($P = .82$ and $P = .88$, respectively), and results overall were similar among older men who reported no current use of vitamin D supplements (Table 3).

In the full cohort, we observed a borderline statistically significant positive association between circulating 25(OH) vitamin D and risk of cerebrovascular events in men with

Table 2. Association Between Serum 25(OH) Vitamin D Level and Risk of CVD Event^a

Serum 25(OH) Vitamin D by Level	Full Cohort Minimally Adjusted ^b				Full Cohort Multivariate Model ^c			
	N	Events	Person Years	HR (95% CI)	N	Events	Person Years	HR (95% CI)
CVD ^d								
<20 ng/mL	416	104	2390	1.03 (0.83–1.27)	408	100	2348	0.91 (0.73–1.13)
≥20 ng/mL	2603	645	15 691	1.00 reference	2582	640	15 571	1.00 reference
CHD ^e								
<20 ng/mL	416	65	2517	0.95 (0.73–1.25)	408	62	2474	0.81 (0.61–1.07)
≥20 ng/mL	2601	438	16 357	1.00 reference	2580	433	16 237	1.00 reference
Cerebrovascular events ^f								
<20 ng/mL	414	38	2571	1.44 (1.00–2.08)	406	37	2529	1.34 (0.92–1.95)
≥20 ng/mL	2596	176	17 360	1.00 reference	2575	176	17 230	1.00 reference

^a $P < .05$ considered significant.

^b Minimally adjusted model adjusted for age, race, study site, season of blood draw, and laboratory batch for vitamin D assay.

^c Multivariate model adjusted for age, race, study site, season of blood draw, laboratory batch for vitamin D assay, education, BMI, PASE score, systolic blood pressure, diastolic blood pressure, history of smoking, alcohol use, statin use, history of hypertension, history of diabetes, and history of cardiovascular event.

^d Includes all CHD events, cerebrovascular events, as well as peripheral vascular disease (acute arterial dissection, acute arterial occlusion, acute arterial rupture, and vascular surgery).

^e Includes acute myocardial infarction (MI), coronary artery bypass surgery, ischemic congestive heart failure, coronary revascularization, non-ST elevation MI, ST elevation MI, hospitalization for unstable angina, sudden CHD death, and other CHD events.

^f Includes stroke and transient ischemic attacks.

Table 3. Association Between Serum 25(OH) Vitamin D Level and Risk of CVD Event Excluding Vitamin D Supplement Use^a

Serum 25(OH) Vitamin D by Category	No Vitamin D Supplement Use, Multivariate Model ^b	
	Events/N	HR (95% CI)
CVD ^c		
<20 ng/mL	74/291	0.97 (0.72–1.3)
≥20 ng/mL	210/832	1.00 reference
CHD ^d		
<20 ng/mL	45/291	0.85 (0.59–1.24)
≥20 ng/mL	139/830	1.00 reference
Cerebrovascular events ^e		
<20 ng/mL	28/289	1.70 (1.02–2.83)
≥20 ng/mL	53/829	1.00 reference

^a $P < .05$ considered significant.

^b Multivariate model adjusted for age, race, study site, season of blood draw, laboratory batch for vitamin D assay, education, BMI, PASE score, systolic blood pressure, diastolic blood pressure, history of smoking, alcohol use, statin use, history of hypertension, history of diabetes, and history of cardiovascular event.

^c Includes all CHD events, cerebrovascular events, as well as peripheral vascular disease (acute arterial dissection, acute arterial occlusion, acute arterial rupture, and vascular surgery).

^d Includes acute myocardial infarction (MI), coronary artery bypass surgery, ischemic congestive heart failure, coronary revascularization, non-ST elevation MI, ST elevation MI, hospitalization for unstable angina, sudden CHD death, and other CHD events.

^e Includes stroke and transient ischemic attacks.

vitamin D deficiency using the minimally adjusted model (HR, 1.44; 95% CI, 1.00–2.08). This risk was slightly attenuated after adjusting for additional confounders (multivariate HR, 1.34; 95% CI, 0.92–1.95), but remained statistically significant after excluding men who used vitamin D–containing supplements (multivariate HR, 1.70; 95% CI, 1.02–2.83). When specifically examining strokes ($n = 151$), there was no association between serum 25(OH) vitamin D level and risk for stroke (HR, 1.29; 95% CI, 0.83–2.00) in the full cohort using the multivariate model, nor after we excluded men who used vitamin D–containing supplements (HR, 1.45; 95% CI, 0.77–2.71).

Results remained essentially unchanged after excluding 1,265 men (41.9%) with prevalent CVD or cerebrovascular disease at baseline (data not shown).

When using serum 25(OH) vitamin D as a continuous variable per SD decrease, none of the associations with our main disease outcomes, including the positive association we observed for cerebrovascular events among men without vitamin D supplement use, were significant (for CVD: HR per SD decrease, 0.91; 95% CI, 0.80–1.03; $P = .14$; for CHD: HR per SD decrease, 0.86; 95% CI, 0.73–1.01; $P = .06$; and for cerebrovascular events: HR per SD decrease, 1.00; 95% CI, 0.81–1.23; $P = .98$).

Sensitivity analyses using different vitamin D cut-points were similar. For example, when we used a stricter cutoff for vitamin D sufficiency, in multivariate analyses, neither deficient levels (<20 ng/mL serum 25(OH) vitamin D; HR, 0.88; 95% CI, 0.69–1.13; $P = .33$) nor relative insufficient levels of vitamin D (20–30 ng/mL serum 25(OH) vitamin D; HR, 0.96; 95% CI, 0.82–1.14; $P = .66$) were associated with CVD risk. Further, there was no significant association between circulating vitamin D levels and risk for CHD events or cerebrovascular events using these cut-off values for serum vitamin D levels. When we repeated our analyses with a stricter cutoff for severe vitamin D deficiency, in the multivariate model, groups severely deficient in vitamin D (≤ 12 ng/mL) when compared with those with a sufficient vitamin D level (≥ 20 ng/mL), had an HR of 0.73 (95% CI, 0.43–1.24) for CVD risk, and those with mild to moderate deficiency had an HR of 0.94 (95% CI, 0.75–1.19) for CVD risk. Again, there was no significant association between circulating 25(OH) vitamin D levels and CHD events nor cerebrovascular events using these cutoff values for serum 25(OH) vitamin D levels.

Discussion

In this prospective cohort study of 3019 older men from six sites across the United States, there was no association between low levels of serum 25(OH) vitamin D and overall risk of CVD events.

There was some evidence of an association among men with lower vitamin D levels (ie, <20 ng/mL) not taking vitamin D supplements with a higher risk of any cerebrovascular event. Although this could be due to chance, an alternative explanation could be that men who use vitamin D–containing supplements were taking it as part of a multivitamin or were taking other vitamin supplements that may alleviate the risk for cerebrovascular events in these men, even at low levels of serum 25(OH) vitamin D. Vitamin D–containing supplement use could also be a marker of other health behaviors that we did not measure, or measure adequately, that might alter the risk for cerebrovascular events.

Our findings differ from those shown in a recent meta-analysis by Wang et al (4). In this meta-analysis, study authors searched MEDLINE and EMBASE from 1966 through February 2012 for prospective studies of 25(OH) vitamin D and CVD risk. Nineteen studies were included for a total of 65,994 participants and 6,123 CVD cases in the primary analyses. In defining vitamin D “deficiency” vs. “sufficiency,” cutoffs of vitamin D levels varied among studies and final analyses was performed comparing in

general the lowest levels as defined by the individual study compared with the highest level as defined by the individual study. Results showed significant positive associations between the lowest levels of vitamin D compared with the highest levels for total CVD (RR, 1.52; 95% CI, 1.30–1.77), CVD mortality (RR, 1.42; 95% CI, 1.19–1.71), CHD event (RR, 1.38; 95% CI, 1.21–1.57), and stroke (RR, 1.64; 95% CI, 1.27–2.10). These associations remained significant even when baseline cases of CVD were excluded. As is expressed by the study authors, the meta-analysis is limited by publication bias: positive results are more likely to be published than papers such as ours, which show null findings.

The present study is an expansion of our previous publication (10) on the association between serum 25(OH) vitamin D level and risk of CVD events using a smaller sample ($n = 813$ men) from within the MrOS Sleep Study cohort. In this previous analysis, we measured 25(OH) vitamin D at the baseline of the parent MrOS study, which is approximately 2 years earlier than our current evaluation, which used serum levels taken at the baseline of the MrOS Sleep Study. In this earlier analysis, during a median follow-up of 4.4 years (with a median 25(OH) vitamin D level of 23.5 ng/mL), there were 140 prevalent CVD cases, including 115 with CHD and 25 with cerebrovascular events. In multivariate analyses, the lowest quartile of serum 25(OH) vitamin D (4.9–20.1 ng/mL) was not significantly associated with CVD incidence (HR, 1.18; 95% CI, 0.69–2.03) when compared with the highest quartile (30.2–55.4 ng/mL). Excluding prevalent cases did not alter these results, and overall, the findings from these previous analyses are in line with our current findings, using the full MrOS Sleep Study cohort.

Our study has several important strengths, including its relatively large size, with 3,019 subjects and 749 CVD events. Further, there was a wide geographic distribution of subjects: the six study sites were each at different latitudes, and presumably different levels of sun, and consequently, vitamin D exposure. Our multivariate model accounts for nutritional factors (BMI) and comorbidities (hyperlipidemia as represented by statin use, diabetes, and hypertension.) Additional adjustment for biomarkers potentially relevant to disease outcomes (e.g., serum albumin, serum creatinine as a marker of renal function, and mineral metabolism parameters including serum calcium and serum phosphate) did not change our results and were therefore not included in our final models. It is possible that the cutoffs commonly used for 25(OH) vitamin D deficiency and sufficiency in many other studies only describe serum 25(OH) vitamin D levels that are optimal for bone health but not representative of vitamin D as a marker of cardiovascular health. We performed sensitivity

analyses in which we expanded our cutoffs for 25(OH) vitamin D deficiency and sufficiency and found that altering the cutoff points did not alter our findings significantly. There is an aspect of reverse causality that must be considered in that presence of CVD generally decreases exercise and functional tolerance implying less time spent outdoors and consequently less vitamin D exposure. We addressed this by also performing extensive secondary analyses to evaluate potential biases related to prevalent CVD cases, and adjusting for a range of potential confounders that were not considered in our previous study.

The MrOS Study comprises only men ages 65 and older who are 90% Caucasian, which limits its generalizability to other members of the population. Our study results may also be limited by the relatively small size of the vitamin D deficient group compared with the sufficient group ($N = 416$ compared with $N = 2603$). A larger sample size in the deficient group would have increased power, which is potentially important in particular for certain less common outcomes such as stroke. Another limitation in our study relates to the secondary analyses on the effect of vitamin D supplementation and CVD risk. The data we collected on supplementation was not specific enough to only exclude vitamin D-containing supplements; instead we broadly excluded any supplement use, which would have included those men taking vitamin pills that were not composed of any vitamin D. Whether vitamin D intake alters risk for CVD is an important question; hence, future studies are needed, which better define vitamin D supplement use.

In sum, although our study does not support an association between serum 25(OH) vitamin D levels and risk for CVD events, the significantly higher risk of cerebrovascular disease among men with mild-to-moderate vitamin D deficiency not using vitamin D supplements warrants additional exploration. Future observational studies of vitamin D should consider interactions with other ingredients in vitamin D-containing multivitamins.

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