

## Serum 25-Hydroxyvitamin D and Incidence of Fatal and Nonfatal Cardiovascular Events: A Prospective Study With Repeated Measurements

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**Context:** Several studies suggested that low serum concentrations of 25-hydroxyvitamin D (25(OH)D) are associated with an increased risk of cardiovascular disease (CVD). However, the evidence is still inconclusive, mostly based on CVD mortality and studies with single 25(OH)D measurements.

**Objective:** We aimed to assess the association of 25(OH)D with fatal and nonfatal CVD in the same study population, using repeated 25(OH)D measurements and competing risks analysis.

**Design:** This was a population-based cohort study (ESTHER study, baseline 2000–2002). Follow-up data, including survival status, were collected after 2, 5, and 8 years. The response rate for survival was 99.9%.

**Setting:** Participants were recruited during a health screening examination by their general practitioners. 25(OH)D was measured in blood samples collected at baseline and the 5-year follow-up visit.

**Patients or Other Participants:** A total of 9949 men and women, aged 50 to 74 years at baseline, with sufficient knowledge of the German language and resident in the German state of Saarland were included in the study.

**Main Outcome Measures:** Outcomes included CVD, coronary heart disease (CHD), and stroke, in total and differentiated into fatal and nonfatal events.

**Results:** Overall, 854 study participants had a nonfatal and 176 a fatal CVD event during 8 years of follow-up. Comparing subjects with 25(OH)D levels below 30 nmol/L and above 50 nmol/L resulted in a hazard ratio of 1.27 (95% confidence interval = 1.05–1.54) for total CVD and 1.62 (95% confidence interval = 1.07–2.48) for fatal CVD in a model adjusted for important potential confounders. No significant association for nonfatal CVD was observed. In dose-response analysis, we observed an increased cardiovascular risk at 25(OH)D levels below 75 nmol/L. Results for CHD and stroke were comparable to the results obtained for the composite outcome CVD.

**Conclusions:** Our results support evidence that low 25(OH)D levels are associated with moderately increased risk of CVD and indicate that the observed association is much stronger for fatal than for nonfatal events. (*J Clin Endocrinol Metab* 98: 4908–4915, 2013)

Several studies, including meta-analyses of prospective studies (1–5), seem to indicate an increased risk of cardiovascular disease (CVD) at low levels of 25-hydroxyvitamin D (25(OH)D), and a number of biological

mechanisms for the observed association have been suggested (6). In particular, the highest-powered population-based study to date found a 40% increased risk for ischemic heart disease and a 64% increased risk for

myocardial infarction (MI) among individuals with lower 25(OH)D concentrations (4). These findings were endorsed by a recent meta-analysis on ischemic heart disease and 25(OH)D levels, which included 18 studies and 82 982 participants and reported a 39% increased risk of ischemic heart disease by comparing the lowest with the highest quartile of plasma 25(OH)D (4). In another recent meta-analysis including 10 studies and 58 384 participants, an increasing risk of ischemic stroke with decreasing concentrations of 25(OH)D was observed (5). Similar results were reported by a meta-analysis including 65 994 participants and focusing on the association between 25(OH)D concentrations and CVD risk (1).

Low concentrations of 25(OH)D have also been associated with risk factors for CVD. It has been reported that vitamin D deficiency reduces insulin secretion, thus contributing to the pathogenesis of type 2 diabetes mellitus (DM), and its replenishment improves pancreatic  $\beta$ -cell function and glucose tolerance (7). Furthermore, there is evidence that vitamin D deficiency is associated with blood levels of inflammatory factors (8), and treatment with vitamin D<sub>3</sub> might reduce cardiovascular risk of death among patients with end-stage renal disease (9).

Vitamin D can be supplied by food, but, especially in summer months, it is mainly produced in the skin under UV-B radiation exposure and metabolized to 25(OH)D in the liver and to the active compound 1,25-dihydroxyvitamin D in the kidney. The last step is under feedback control of PTH (10), which is inversely correlated with 25(OH)D (11) and whose excess has been associated with the development of CVD (8), including a 30% increased risk for heart failure (11). PTH has also been associated with the regulation of blood pressure among individuals with 25(OH)D concentrations  $\leq 75$  nmol/L (12). It is speculated that, *inter alia*, the inverse relationship of 25(OH)D with PTH, coupled with the effects of 25(OH)D on the renin-angiotensin system and on cardiac tissues, might contribute to the development of CVD (6).

Although several observational studies have analyzed the association between serum concentrations of 25(OH)D and CVD risk, the evidence is still insufficient because several prospective studies associating circulating concentrations of 25(OH)D with CVD have been judged not to meet the criteria for study quality, as graded by the Agency for Healthcare and Quality (2). Furthermore, most observational studies that found a strong association between low serum levels of 25(OH)D and CVD used CVD death as the main outcome measure. Although this is in line with the bulk of the literature showing a strong inverse association between 25(OH)D concentrations and mortality (13–15), evidence for the association between 25(OH)D and nonfatal CVD is still very limited, with only a population-

based study distinguishing between fatal and nonfatal ischemic heart disease in the same study population (4).

Even though two very high-powered population-based studies (4, 5) not included among the studies graded by the Agency for Healthcare and Quality have recently contributed to the literature, they might be limited, similarly to all previous prospective observational studies on CVD and 25(OH)D levels, by the use of single 25(OH)D measurements, thus possibly underestimating the effects of 25(OH)D levels due to changes in 25(OH)D during follow-up (2). Also, a general limitation of previous observational studies is the lack of standardization of vitamin D measurements with liquid chromatography tandem-mass spectrometry (LC-MS/MS), which might limit their accuracy and comparability (16).

Randomized controlled trials on the efficacy of vitamin D supplementation in CVD prevention have yielded conflicting results, and so far, available results cannot provide final evidence whether vitamin D deficiency contributes to the development of CVDs (17). Because new randomized controlled trials, adequately designed and powered for that study question, will not be finalized within the next 5 years (18), evidence based on large and methodologically robust prospective observational studies is still needed.

Our analysis, conducted with a large sample size, aimed to assess the relationship between 25(OH)D levels and incident CVDs by including repeated 25(OH)D measurements, standardized with LC-MS/MS, and by differentiating the cardiovascular endpoints total CVD, coronary heart disease (CHD), and stroke into fatal and nonfatal categories in the same study population. We also aimed to contribute to the literature by using a competing risk of death approach (19).

## Subjects and Methods

### Study population

Our analyses are based on data from the ESTHER study, a population-based cohort study including 9949 older adults resident in the state of Saarland, Germany, described in detail elsewhere (20). In brief, participants were recruited between July 2000 and December 2002 by their general practitioner (GP) during a regular health check-up. Inclusion criteria were residence in the state of Saarland, age between 50 and 74 years, and sufficient knowledge of the German language. Socio-demographic, lifestyle, and medical characteristics were obtained by a standardized self-administered questionnaire from the study participants and from the medical records of the GP.

Follow-up questionnaires, including information on incident CVDs, were sent to study participants and their GPs after 2, 5, and 8 years with response rates among survivors of 96%, 87%, and 79%, respectively. Blood samples were collected at baseline during the health check-up and at the 5- and 8-year follow-up

visits. The samples were centrifuged, sent to the study center, and stored at  $-80^{\circ}\text{C}$  until biomarker measurement.

For the present analysis, we excluded subjects with a history of physician-diagnosed CVD ( $n = 1949$ ) at baseline, subjects with unknown history of CVD ( $n = 4$ ), and subjects with missing baseline measurements of 25(OH)D ( $n = 287$ ), resulting in a study population of 7709.

## 25(OH)D measurements

Baseline serum levels of 25(OH)D were measured for women and men in the framework of two different projects with the DiaSorin-Liaison (Diasorin, Inc) and the IDS-iSYS (Immunodiagnostic Systems GmbH) immunoassays in 2006 and 2010, respectively. The latter method was also used for 25(OH)D measurements at the 5-year follow-up for both sexes.

All obtained 25(OH)D values were retrospectively standardized with LC-MS/MS in the Department of Clinical Chemistry, Canisius Wilhelma Hospital, Nijmegen, The Netherlands, as described previously (16). In brief, for each of the two assays employed, random baseline serum samples of 97 study participants were drawn and remeasured with isotope-dilution LC-MS/MS in the Department of Clinical Chemistry, Canisius Wilhelma Hospital, Nijmegen, The Netherlands. Spearman rank correlation between measurements with Diasorin-Liaison and LC-MS/MS and between IDS-iSYS and LC-MS/MS was high ( $r = 0.83$  and  $r = 0.86$ , respectively). Therefore, the following ordinary least-squares linear regression equations were fitted, and results were employed for standardization of 25(OH)D levels for all study participants: 25(OH)D LC-MS/MS (nanomoles per liter) =  $0.7989 \times 25(\text{OH})\text{D Diasorin-Liaison (nanomoles per liter)} + 17.58 \text{ nmol/L}$ ; 25(OH)D LC-MS/MS (nanomoles per liter) =  $0.9526 \times 25(\text{OH})\text{D IDS-iSYS (nanomoles per liter)} - 0.3222 \text{ nmol/L}$ . Standardization on average increased the 25(OH)D levels measured with Diasorin-Liaison by 10.3 nmol/L and decreased 25(OH)D levels measured with IDS-iSYS by 2.9 nmol/L.

## Covariate assessment

Information on age, sex, height, weight, smoking, physical activity, fish consumption, intake of multivitamin supplements, including vitamin D<sub>3</sub>, and family history of CVD were taken from the study participant's questionnaire. Diabetes mellitus and hypertension diagnoses were recorded by the GP during the health check-up. From stored serum samples, C-reactive protein (CRP) (turbidimetric) and total cholesterol were determined on the Beckman Synchron LX. Cystatin C was measured by immunonephelometry on a Behring Nephelometer II (Siemens Dade-Behring). Chronic kidney disease (CKD) was defined by an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m<sup>2</sup> according to the equation:  $\text{eGFR} = 74.835/(\text{cystatin C}^{1.333})$  (21).

## Cardiovascular outcomes ascertainment

Analyzed cardiovascular outcomes were CVD, CHD, and stroke, both in total as well as differentiated into fatal and nonfatal events. Subjects with prevalent CVD at baseline were excluded from all analyses. We defined nonfatal incident CVD as MI, stroke, bypass surgery, balloon catheterization of the coronary arteries, lung embolism, or a CHD diagnosis reported by the study participants in mailed standardized questionnaires at the 2-, 5-, or 8-year follow-up for the first time, covering a follow-up period until the end of 2010. Nonfatal CHD diagnoses (includ-

ing CHD and self-reported MI) and strokes were validated by medical records. Only a minority of self-reported cases could not be validated due to nonresponse of the GP, and the final overall proportions of validated cases included in the analyses were 91.1% for CHD and 87.9% for stroke.

To ascertain fatal cardiovascular outcomes, deaths between 2000 and 2010 were identified by inquiry at the residents' registration offices in Saarland, and information on the vital status could be obtained for 99.9% of the ESTHER participants. Death certificates were provided for 97.7% of deceased participants by public health departments. Fatal CVD, CHD, and stroke were identified by deaths coded with ICD-10 codes I00 to I99, I20 to I25, and I60 to I69, respectively.

Total incident CVD, CHD, and stroke were recorded by combining the ascertained respective nonfatal and fatal events.

## Statistical analyses

Cox proportional hazards models were employed to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the association of vitamin D deficiency (25(OH)D <30 nmol/L) and vitamin D insufficiency (25(OH)D 30 to <50 nmol/L) compared with sufficient vitamin D status (25(OH)D  $\geq 50$  nmol/L) with respect to incident CVD, CHD, and stroke (22). Analyses were additionally carried out separately for nonfatal and fatal events. All Cox proportional hazards models used repeated measurements from the 5-year follow-up (25(OH)D and covariates) by fitting time-dependent variables. We employed 3 different models. In model 1, analyses were adjusted for age (continuous variable), sex (male/female), and season of blood draw (2-month intervals starting with January/February). In model 2, analyses were additionally adjusted for the following potential confounders: BMI (body mass index) (<25/ $\geq 25$ -<30/ $\geq 30$  kg/m<sup>2</sup>), smoking (never/former/current), physical activity (low/medium or high), total cholesterol (<200/ $\geq 200$  mg/dL), CRP ( $\leq 3$ / $> 3$  mg/L), family history of CVD (yes/no), fish consumption (<1/ $\geq 1$  time/wk), and regular multivitamin supplement intake (yes/no). In model 3, analyses were further adjusted for factors possibly mediating the association between 25(OH)D and CVD: hypertension (yes/no), DM (yes/no), and CKD (yes/no). Potential interactions of vitamin D deficiency and insufficiency with the covariates with respect to the outcomes CVD, CHD, and stroke were tested for statistical significance by adding pertinent product terms to model 3. Analyses were additionally stratified for covariates that showed a significant interaction with vitamin D deficiency and/or insufficiency with any of the cardiovascular outcomes.

Dose-response relationships were assessed with restricted cubic splines (23), adjusted for variables of model 2, without time-dependent modeling, and employing predefined knots at 30, 50, and 100 nmol/L 25(OH)D with 75 nmol/L 25(OH)D as the reference. Disease-free survival curves were derived using the Kaplan-Meier method to compare subjects with levels of 25(OH)D <30 nmol/L,  $\geq 30$  nmol/L to <50 nmol/L, and  $\geq 50$  nmol/L. The log-rank test for statistical comparison of Kaplan-Meier curves was also employed.

In sensitivity analyses, the competing risk of death was considered by fitting a proportional subdistribution hazards regression model with an extension of the Fine and Gray method (24, 25) as implemented in the SAS macro of Heinze, Medical University of Vienna, Austria (26). All analyses were also repeated with exclusion of events not validated by general practitioners.

To deal with missing values, multiple imputation was employed, and details are given in the Supplemental Data (published on The Endocrine Society's Journals Online website at <http://jcem.endojournals.org>). All statistical tests were two-sided using an  $\alpha$ -level of 0.05 and conducted with the software package SAS version 9.2 (SAS Institute Inc).

## Results

Characteristics of the overall study population are shown in Table 1. The study population included more women than men (59.3% vs 40.7%), and the majority (59%) of the participants had inadequate 25(OH)D levels (<50 nmol/L). The prevalence of vitamin D deficiency was comparable at baseline and 5-year follow-up in both men and women (data not shown).

During a mean follow-up duration of 9.2 years for mortality and 6.5 years for the other cardiovascular endpoints (ranging from 6.2 years for CVD to 6.8 years for stroke), 854 study participants had a nonfatal and 176 a fatal CVD event, 460 had a nonfatal and 79 a fatal CHD event, and 313 had a nonfatal and 41 a fatal stroke. The sum of the fatal and nonfatal individual events exceeds the respective total sum of events because of multiple events of single individuals. Of the 1011 CVD events in total, 493 (48.8%) occurred ahead of the 5-year follow-up, and 518 (51.2%) occurred after the 5-year follow-up.

Unadjusted Kaplan-Meier curves and the log-rank test for comparison of Kaplan-Meier estimates showed that the proportion of study participants free of CVD, CHD, or stroke was significantly lower in subjects with vitamin D deficiency than in subjects with sufficient concentrations of 25(OH)D (Figure 1).

People with vitamin D deficiency also showed a significantly increased CVD, CHD, and stroke risk in model 1 after adjustment for age, sex, and season of blood drawn (Table 2) with similar HRs ranging from 1.46 (95% CI = 1.21–1.77) for total CVD to 1.58 (95% CI = 1.16–2.17) for total stroke. The observed associations between serum concentrations of 25(OH)D and the cardiovascular outcomes were much stronger for fatal than for nonfatal events, with HRs showing an ~2-fold increased risk for a fatal and an ~1.3-fold increased risk for a nonfatal cardiovascular outcome.

Even though the association was attenuated after adjustment for potential confounders (with control for smoking and physical activity being responsible for most of this decrease), a statistically significant 27% increased risk for total CVD and a 62% increased risk of fatal CVD persisted for those with vitamin D deficiency (model 2). No significant association was observed for nonfatal CVD events. A significant 36% increased risk of total CHD and

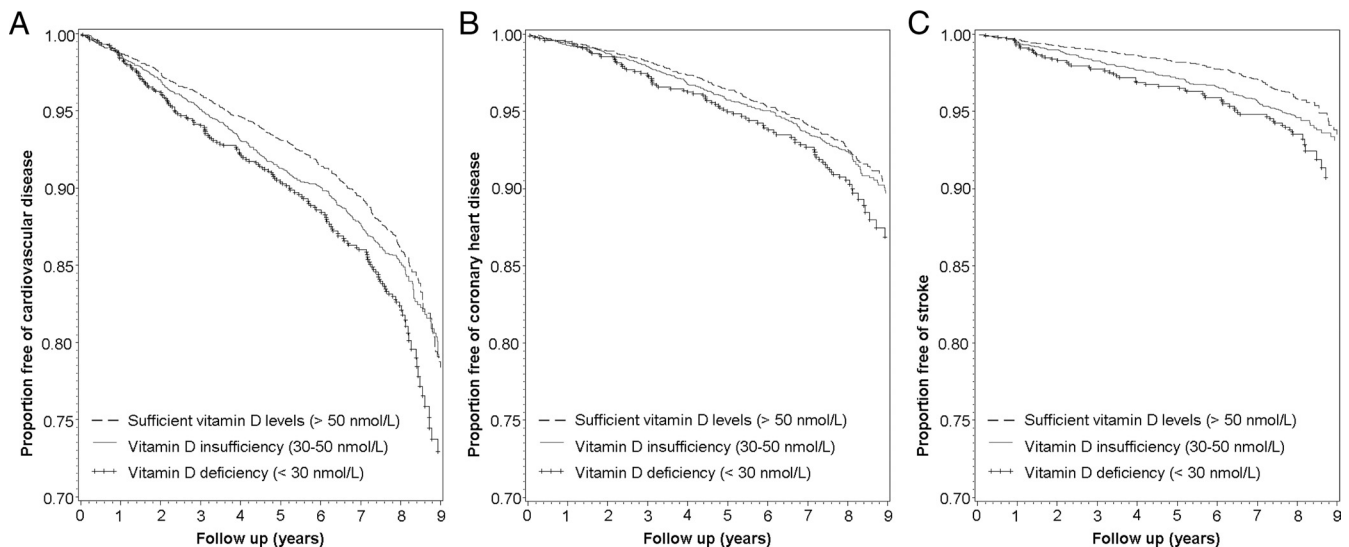
**Table 1.** Baseline Characteristics of the Study Population

	n	%
Total	7709	100
25(OH)D, nmol/L		
<30	1114	14.5
30 to <50	3430	44.5
≥50	3165	41.1
Age, y		
<65	5037	65.3
≥65	3135	34.7
Sex		
Women	4574	59.3
Men	3135	40.7
BMI, kg/m <sup>2</sup>		
<25	2243	29.1
25 to ≤30	3616	47.0
≥30	1842	23.9
Smoking status		
Never	3908	52.1
Former	2287	30.5
Current	1311	17.5
Physical activity, h/wk <sup>a</sup>		
Low	5029	65.4
Medium or high	2665	34.6
Total cholesterol, mg/dL		
<200	2359	30.7
≥200	5328	69.3
CRP, mg/L		
≤3	4825	63.6
>3	2759	36.4
Family history of CVD		
No	6225	82.3
Yes	1335	17.7
Fish intake, times/wk		
<1	4773	65.4
≥1	2525	34.6
Multivitamin supplement intake		
Never or irregularly	6457	85.5
Regularly	1097	14.5
Hypertension		
No	4136	53.7
Yes	3561	46.3
DM		
No	6712	88.4
Yes	883	11.6
CKD		
No	7183	93.5
Yes	496	6.5

<sup>a</sup> Medium or high physical activity was defined by doing ≥2 h/wk of light physical activity and ≥2 h/wk of vigorous physical activity, respectively.

a nonsignificant 33% increased risk of total stroke were also observed. Results in models 2 and 3 were comparable, showing that the inclusion of factors that might be mediators of the 25(OH)D effects in the models did not have a strong impact on the results.

In model 3, tests for interactions of vitamin D deficiency and insufficiency with the covariates were statistically significant for high total cholesterol (≥200 mg/dL) and family history of CVD. Additional analyses stratified for fam-



**Figure 1.** Unadjusted Kaplan-Meier curves for subjects with vitamin D deficiency, vitamin D insufficiency, and sufficient vitamin D levels with respect to the outcomes total CVD (A), total CHD (B), and total stroke (C). By log-rank test for comparing subjects with vitamin D deficiency and subjects with sufficient vitamin D levels:  $P$  (CVD) = .001;  $P$  (CHD) = .006;  $P$  (stroke) = .003. By log-rank test for comparing subjects with vitamin D insufficiency and subjects with sufficient vitamin D levels:  $P$  (CVD) = .233;  $P$  (CHD) = .319;  $P$  (stroke) = .054.

ily history of CVD showed a significant association of low levels of 25(OH)D with the development of CVD, CHD, and stroke in the group without family history of CVD but not in the smaller group of patients with such a family history (data not shown). Analyses stratified by high total cholesterol showed no clinically relevant differences among strata (data not shown).

A dose-response analysis between 25(OH)D levels and CVD, CHD, and stroke showed an inverse association for 25(OH)D levels below 75 nmol/L (Figure 2).

Sensitivity analyses accounting for the competing risk of death yielded results similar to those of conventional analyses (Supplemental Table 1). Additional sensitivity analyses excluding events not validated by GPs yielded almost identical results (data not shown).

## Discussion

Our large population-based cohort of older adults showed an association of vitamin D deficiency with an increased risk of CVD, CHD, and stroke and furthermore provides evidence that this association is much stronger for fatal than for nonfatal events.

The observation that 25(OH)D seems to be more strongly associated with cardiovascular mortality than with nonfatal cardiovascular endpoints is in agreement with findings of weaker associations in studies that had not differentiated between fatal and nonfatal outcomes (27, 28) and stronger associations in studies that had exclusively focused on fatal outcomes (13, 15). Our results are also in agreement with a previous study that differentiated between fatal and nonfatal

ischemic heart disease in the same study population (4). A possible explanation for such a finding may be that low vitamin D levels may lead to more severe events and may more strongly affect the course of CVD than its occurrence, eg, by reducing the capacity to cope with CVD events. An alternative explanation might be that the association of 25(OH)D concentrations with mortality is more strongly affected by residual confounding by factors linked to both vitamin D levels and poor health status. For example, low vitamin D levels have been found to be associated with type 2 diabetes (29), poorer cognitive function (30), and CKD (31), which are associated with increased mortality. In epidemiological studies, it is difficult to comprehensively adjust for such conditions reflecting a poor health status. Also the association of vitamin D with risk factors for CVD, such as DM makes it difficult to disentangle the role of vitamin D in the pathogenesis of CVD. However, our estimates adjusted for factors potentially mediating the association between 25(OH)D and CVD tend to suggest that vitamin D is independently associated with CVD events, even if the stronger association with fatal events could also support an interpretation of 25(OH)D concentrations as a marker of poor health (4).

To our knowledge, this is the first analysis that used repeated 25(OH)D measurements standardized with LC-MS/MS for estimating HRs for a composite CVD endpoint including fatal and nonfatal events. Repeated 25(OH)D measurements have been used for the investigation of the relationship of circulating concentrations of 25(OH)D and CVD only in the InCHIANTI study (2, 14) and a previous analysis of the ESTHER cohort (13). However, the InCHIANTI study had only 729 study

**Table 2.** Association of Serum Concentrations of 25(OH)D With Cardiovascular Outcomes

Outcome and 25(OH)D, nmol/L	n	Cases	PY	IR	HR (95% CI)		
					Model 1 <sup>a</sup>	Model 2 <sup>b</sup>	Model 3 <sup>c</sup>
Total CVD							
<30	1114	171	6537	26.2	1.46 (1.21–1.77) <sup>d</sup>	1.27 (1.05–1.54) <sup>d</sup>	1.24 (1.02–1.50) <sup>d</sup>
30–<50	3430	448	21 263	21.1	1.24 (1.07–1.43) <sup>d</sup>	1.15 (1.00–1.33)	1.14 (0.99–1.32)
≥50	3165	392	20 374	19.2	Ref	Ref	Ref
Per 25					0.90 (0.84–0.96) <sup>d</sup>	0.94 (0.88–1.00)	0.95 (0.89–1.01)
Nonfatal CVD							
<30	1114	136	6537	20.8	1.35 (1.10–1.66) <sup>d</sup>	1.20 (0.97–1.48)	1.17 (0.94–1.45)
30–<50	3430	383	21 263	18.0	1.24 (1.06–1.44) <sup>d</sup>	1.16 (0.99–1.36)	1.15 (0.98–1.35)
≥50	3165	335	20 374	16.4	Ref	Ref	Ref
Per 25					0.93 (0.87–1.00) <sup>d</sup>	0.97 (0.90–1.04)	0.98 (0.91–1.05)
Fatal CVD							
<30	1114	40	10 064	4.0	2.08 (1.37–3.15) <sup>d</sup>	1.62 (1.07–2.48) <sup>d</sup>	1.55 (1.01–2.37) <sup>d</sup>
30–<50	3430	71	31 447	2.3	1.16 (0.82–1.65)	1.06 (0.74–1.51)	1.05 (0.73–1.49) <sup>d</sup>
≥50	3165	65	29 243	2.2	Ref	Ref	Ref
Per 25					0.70 (0.59–0.84) <sup>d</sup>	0.78 (0.65–0.93) <sup>d</sup>	0.89 (0.66–0.94)
Total CHD							
<30	1114	92	6791	13.6	1.51 (1.17–1.96) <sup>d</sup>	1.36 (1.05–1.77) <sup>d</sup>	1.32 (1.02–1.72) <sup>d</sup>
30–<50	3430	236	22 167	10.7	1.28 (1.05–1.55) <sup>d</sup>	1.20 (0.99–1.47)	1.19 (0.98–1.45)
≥50	3165	208	21 289	9.8	Ref	Ref	Ref
Per 25					0.88 (0.81–0.97) <sup>d</sup>	0.92 (0.84–1.00)	0.92 (0.84–1.01)
Nonfatal CHD							
<30	1114	77	6791	11.3	1.44 (1.09–1.90) <sup>d</sup>	1.33 (1.00–1.76) <sup>d</sup>	1.28 (0.97–1.71)
30–<50	3430	204	22 167	9.2	1.25 (1.01–1.55) <sup>d</sup>	1.19 (0.97–1.48)	1.18 (0.95–1.46)
≥50	3165	179	21 289	8.4	Ref	Ref	Ref
Per 25					0.92 (0.84–1.02)	0.95 (0.87–1.05)	0.96 (0.88–1.06)
Fatal CHD							
<30	1114	16	10 064	1.6	2.00 (1.06–3.77) <sup>d</sup>	1.60 (0.84–3.06)	1.53 (0.80–2.94)
30–<50	3430	32	31 447	1.0	1.32 (0.79–2.22)	1.20 (0.72–2.03)	1.18 (0.70–1.99)
≥50	3165	31	29 243	1.1	Ref	Ref	Ref
Per 25					0.64 (0.48–0.84) <sup>d</sup>	0.70 (0.53–0.92) <sup>d</sup>	0.70 (0.54–0.93) <sup>d</sup>
Total stroke							
<30	1114	64	7171	8.9	1.58 (1.16–2.17) <sup>d</sup>	1.33 (0.97–1.83)	1.31 (0.95–1.81)
30–<50	3430	165	23 298	7.1	1.30 (1.02–1.67) <sup>d</sup>	1.21 (0.94–1.54)	1.20 (0.94–1.54)
≥50	3165	124	22 195	5.6	Ref	Ref	Ref
Per 25					0.86 (0.76–0.96) <sup>d</sup>	0.91 (0.81–1.02)	0.91 (0.81–1.02)
Nonfatal stroke							
<30	1114	55	7171	7.7	1.52 (1.09–2.13)	1.28 (0.91–1.80)	1.26 (0.89–1.77)
30–<50	3430	146	23 298	6.3	1.30 (1.00–1.68) <sup>d</sup>	1.20 (0.92–1.55)	1.19 (0.92–1.55)
≥50	3165	112	22 195	5.1	Ref	Ref	Ref
Per 25					0.86 (0.76–0.97) <sup>d</sup>	0.91 (0.80–1.03)	0.91 (0.81–1.03)
Fatal stroke							
<30	1114	9	10 064	0.9	2.26 (0.92–5.55)	1.91 (0.77–4.79)	1.86 (0.74–4.66)
30–<50	3430	20	31 447	0.6	1.52 (0.72–3.21)	1.46 (0.69–3.07)	1.44 (0.68–3.03)
≥50	3165	12	29 243	0.4	Ref	Ref	Ref
Per 25					0.80 (0.56–1.14)	0.86 (0.60–1.22)	0.86 (0.61–1.23)

Abbreviations: IR, incidence rate per 1000 person-years; PY, person-years; Ref, reference.

<sup>a</sup> Adjusted for age (linear), sex, and season of blood draw (six 2-month intervals).

<sup>b</sup> Adjusted for variables of model 1 and BMI (<25/≥25–<30/≥30 kg/m<sup>2</sup>), smoking (never/former/current), physical activity (low/medium or high), total cholesterol (<200/≥200 mg/dL), CRP (≤3/>3 mg/L), family history of CVD (yes/no), fish consumption (<1/≥1 time/wk), and regular multivitamin supplement intake (yes/no).

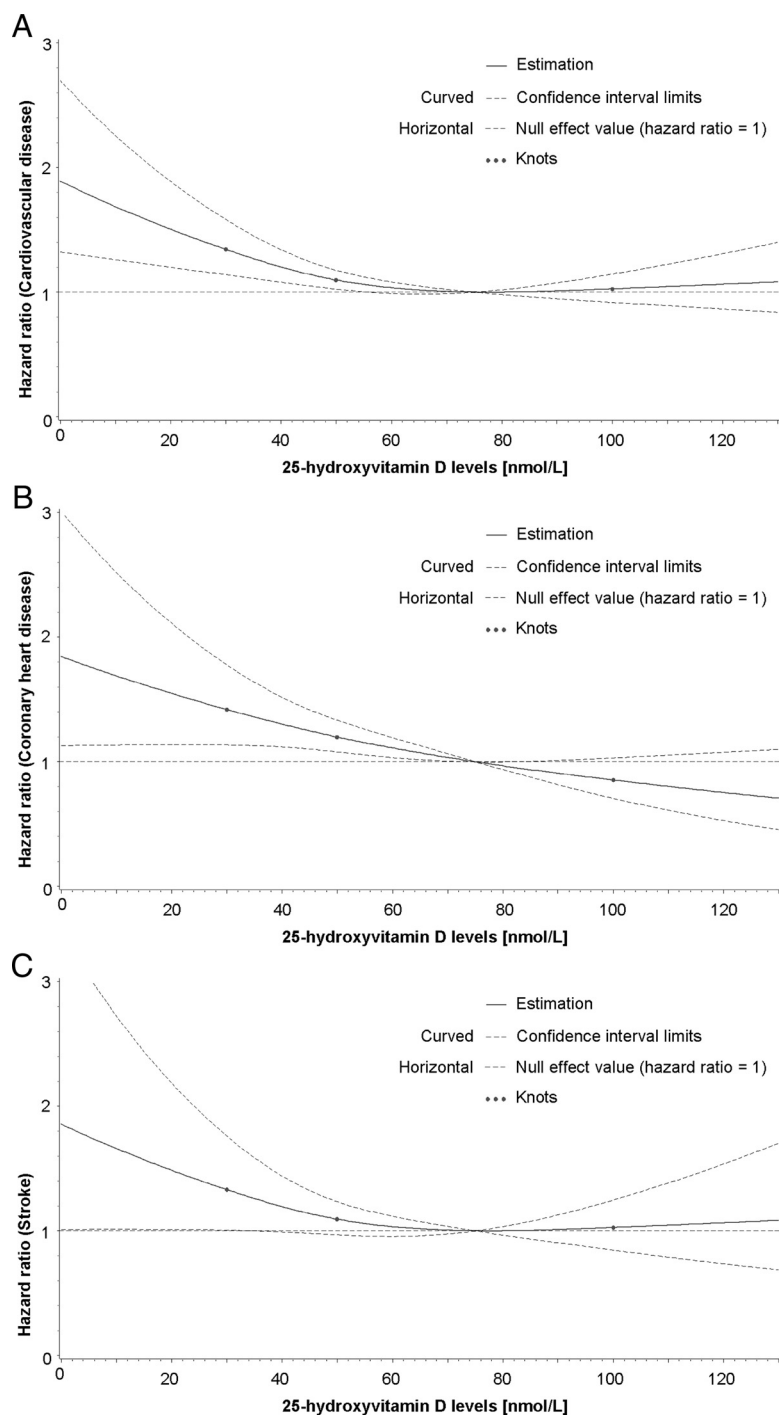
<sup>c</sup> Adjusted for variables of models 1 and 2 and hypertension (yes/no), DM (yes/no), and CKD (yes/no).

<sup>d</sup> Statistically significant association ( $P < .05$ ).

participants with repeated measurements and did not include repeated measurements in the multivariate Cox proportional hazards models. Furthermore, the competing risk of death approach allowed us to correct for a possible underestimation, which is especially important for analyses on

nonfatal events. However, no signs of such an underestimation were observed in our analyses.

A recent systematic review that differentiated studies on serum concentrations of 25(OH)D and CVD according to their quality reported a relative risk for the association



**Figure 2.** Dose-response relationship between serum 25(OH)D and incident CVD (A), CHD (B), and stroke (C). Restricted cubic splines with predefined knots at 30, 50, and 100 were used.

of hypovitaminosis D and CVD of 1.67 (95% CI = 1.23–2.28) if all studies were included in the meta-analysis but a relative risk of only 1.27 (95% CI = 1.04–1.56) if only studies graded with good quality were included (2). Our main result of a 27% increased risk of CVD in subjects with vitamin D deficiency in the total population matches the results of the good-quality studies.

In agreement with most other studies, our results did not show an increased CVD risk at higher 25(OH)D levels, even

if we cannot exclude a U-shaped association with our data because only 3.8% of our study participants had 25(OH)D levels above 100 nmol/L. However, a study including 6123 CVD cases showed a generally linear, inverse association between 25(OH)D concentrations ranging from 20 to 60 nmol/L and risk of CVD (1). Although the visual appearance of the spline of the study of Wang et al (28) might seem to suggest a possible U-shaped association between 25(OH)D levels and risk of CVD, more studies with adequate sample size at higher 25(OH)D levels are required to substantiate a potential increase of CVD at very high 25(OH)D levels.

In addition to repeated measurements over time and competing risks analysis, strengths of our analysis include the use of 25(OH)D measurements standardized with LC-MS/MS, the large sample size of the cohort, and high follow-up response rates with respect to the endpoints, with cases confirmed by GPs for the great majority of study participants. However, standardization with LC-MS/MS was based on remeasurements in a randomly selected subsample, and generalizing linear regression equations to the total cohort could have resulted in imprecise individual 25(OH)D levels, especially at the lower and higher end of the measurements spectrum. Nevertheless, potentially imprecise estimates in the extremes of 25(OH)D levels would hardly have affected the main results because they were grouped in the large categories 25(OH)D <30 nmol/L or 25(OH)D >50 nmol/L.

An important limitation of our study is its observational nature, which limits conclusions on causality. Other minor limitations include the self-reported information on history of cardiovascular events, the lack of follow-up measurements of blood pressure and cystatin C, and the lack of PTH measurements; even so, in a study that jointly assessed the impact of 25(OH)D and PTH levels, PTH and 25(OH)D seemed to follow independent pathways (11).

In conclusion, although our results were able to confirm an approximately 27% increased total cardiovascular risk in subjects with vitamin D deficiency, they indicate that the risk increase is much stronger for (and possibly even confined to) fatal CVD events. Further research should aim for elucidating the reasons underlying this pattern, such as a possible specific impact of vitamin D levels on severity or course of CVD.

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