A Reverse J-Shaped Association Between Serum 25-Hydroxyvitamin D and Cardiovascular Disease Mortality: The CopD Study

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Context: Cardiovascular disease is the major cause of death in the Western world, but the association between 25-hydroxyvitamin D [25(OH)D] levels and the risk of cardiovascular disease mortality remains unclear.

Objective: The objective of the study was to determine the association between cardiovascular, stroke, and acute myocardial infarct mortality and serum levels of 25(OH)D.

Design: This was an observational cohort study, the Copenhagen vitamin D study, data from a single laboratory center in Copenhagen, Denmark. Follow-up was from 2004 to 2011.

Setting: Serum 25(OH)D was analyzed from 247 574 subjects from the Copenhagen general practice sector.

Participants: Examination of the association 25(OH)D levels and mortality from cardiovascular disease, stroke, and acute myocardial infarct was performed among 161 428 women and 86 146 men.

Main Outcome Measures: A multivariate Cox regression analysis was used to compute hazard ratios for cardiovascular, stroke, and acute myocardial infarct mortality.

Results: Of 247 574 subjects, a total of 16 645 subjects died in the ensuing 0–7 years. A total of 5454 died from cardiovascular disease including 1574 from stroke and 702 from acute myocardial infarct. The 25(OH)D level of 70 nmol/L was associated with the lowest cardiovascular disease mortality risk. Compared with that level, the hazard ratio for cardiovascular disease mortality was 2.0 [95% confidence interval (CI) 1.8–2.1] at the lower extreme (~12.5 nmol/L) with a higher risk for men [2.5 (95% CI 2.2–2.9)] than for women [1.7 (95% CI 1.5–1.9)]. At the higher extreme (~125 nmol/L), the hazard ratio of cardiovascular disease mortality was 1.3 (95% CI 1.2–1.4), with a similar risk among men and women. Results were similar for stroke and acute myocardial subgroups.

Conclusions: In this large observational study, low and high levels of 25(OH)D were associated with cardiovascular disease, stroke, and acute myocardial mortality in a nonlinear, reverse J-shaped manner, with the highest risk at lower levels. Whether this was a causal or associational finding cannot be determined from our data. There is a need for randomized clinical trials that include information on the effects of 25(OH)D levels greater than 100 nmol/L. **(*J Clin Endocrinol Metab* 100: 2339–2346, 2015)**

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Abbreviations: CI, confidence interval; CopD-study, Copenhagen vitamin D study; HR, hazard ratio; ICD-10, *International Classification of Diseases*, 10th revision; MI, myocardial infarction.

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Throughout the world, emphasis has been put on vitamin D supplementation with or without calcium supplementation for more than a decade. The evidence that 25-hydroxyvitamin D level below 25 nmol/L might be associated with an increased risk of falling in the elderly, particularly among those aged 65–74 years, has been shown by Snijder et al (1). Furthermore, the risk reduction of osteoporotic fractures due to calcium and vitamin D supplementation has been shown in several studies and the evidence behind it has been collected in a report from the Institute of Medicine in 2012 (2).

Several other benefits of vitamin D have been proposed and on a low evidence level, vitamin D deficiency has been suggested to affect diabetes, cardiovascular disease, depression, the immune system, and certain cancers (3–5). The association between 25-hydroxyvitamin D levels and cardiovascular disease mortality has been estimated in several cohort studies but with conflicting results (6–30). Some of the studies did not find any association with low levels of 25-hydroxyvitamin D and increased cardiovascular disease mortality risk (7, 10, 12, 14–16, 18, 20, 29), whereas other studies did find higher mortality risk among persons with low 25-hydroxyvitamin D levels (6, 8, 9, 11, 13, 17, 19, 21–28, 30), and some studies even demonstrated an inverse association with increasing cardiovascular disease mortality risk at lower 25-hydroxyvitamin D levels (6, 13, 19, 21, 26, 27, 30).

In the Copenhagen vitamin D study (CopD-study), we have previously shown a reverse J-shaped association between serum 25-hydroxyvitamin D and all-cause mortality. Surprisingly, serum levels above 100 nmol/L were significantly associated to higher all-cause mortality (31) and was confirmed in the National Health and Nutrition Examination Survey (32). Considering that cardiovascular disease is the major cause of death in the Western world, it would be highly relevant to investigate whether there also is a nonlinear relation between 25-hydroxyvitamin D levels and cardiovascular mortality. Thus, in this study we have evaluated the association between cardiovascular disease mortality according to serum 25-hydroxyvitamin D levels based on our data from 247 574 subjects in general practice in Copenhagen (the CopD-study).

Materials and Methods

Analytic sample

The CopD-study database is based on a unique database from the Copenhagen General Practitioners Laboratory and contains data from 2004 to 2010 (31). Briefly, it contains vitamin D measurements from 247 574 citizens from the Copenhagen area and allows prospective, observational cohort studies to be conducted, with a broad age range and distinguishing among men (n = 86 146) and women (n = 161 428). This is probably the largest study nationally and internationally from a single center. The database has been given the acronym the CopD-study, which stands for the Copenhagen vitamin D study (31). In the present study, 3902 subjects were excluded due to delay in registration or missing and/or error in data, leaving a total population of 243 672; of these, 16 645 subjects were registered in the Danish Registry of Causes of death (100% follow-up).

Mortality ascertainment

The personal identification number is unique to every citizen in Denmark and enables matching of individuals to Danish Health Registers. Thus, the CopD database was linked to the Danish Registry of Causes of Death and followed until December 31, 2011. The Danish Registry of Causes of Death provides up to five suspected causes of death, which are classified according to the *International Classification of Diseases*, 10th revision (ICD-10) three-digit codes. All subjects were followed from the date of serum 25-hydroxyvitamin D measurement until the date of emigration, date of death, or December 31, 2011, whichever came first. Data on age, gender, and time of blood sample were included in the analysis. All necessary approvals from the Danish Data Protection Agency were obtained before collecting data.

Serum 25-hydroxyvitamin D assays

Serum 25-hydroxyvitamin D was assessed in serum by two commercially available assays, LIAISON 25-hydroxyvitamin D assay (Diasorin) and OCTEIA 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 (Immunodiagnostic Systems, Ltd) according to the instructions of the manufacturers. Both assays determine the sum of serum 25-hydroxyvitamin D3 and D2. For the LIAISON assay, the interserial coefficient of variation percentage was 12.5% (at level 41 nmol/L). For the OCTEIA assay, the interserial coefficient of variation percentage was 9% (at level 32 nmol/L). Results obtained by the OCTEIA assay were adjusted to results obtained by LIAISON using the following equation: LIAISON = 0.893 × OCTEIA + 0.48. The equation was determined by parallel analysis of 59 human serum samples during a period of 5 days in August 2007. The LIAISON assay was used after August 19, 2007, and until the end of the study. Both assays were subject to external quality control through participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital, London, United Kingdom). The assessment scheme included four distributions annually. Each distribution comprised five samples. The results from Vitamin D External Quality Assessment Scheme through the entire study period (from 2004 to 2010) confirmed the reliability of the assays, and the results deviated less than15% from the method mean.

Statistical analyses

Normally distributed variables were shown as mean (SD), and differences between groups were analyzed using unpaired *t* tests. Nonnormally distributed variables were shown as medians with 5% and 95% percentiles, and Mann-Whitney *U* tests were used to test for differences. Categorical variables were shown as proportions, and the differences were analyzed using χ^2 tests. Values of $P < .05$ were considered statistically significant.

The nonlinear association between cardiovascular disease mortality and serum level of 25-hydroxyvitamin D was analyzed using a Cox proportional hazards model in which serum levels of 25-hydroxyvitamin D were entered in the model as a restricted

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Table 1. Baseline Characteristics of the CopD Study According to 25-Hydroxyvitamin D Level and by Mortality From Cardiovascular Diseases Including Acute Myocardial Infarction and Stroke[a]

	Serum Level of 25-Hydroxyvitamin D, nmol/L							
	All	≤12.5	>12.5 to 25	>25 to 50	>50 to 75	>75 to 100	>100 to 125	>125
All subjects, n	243.672	13 640	41 142	81 158	66 397	29 212	8425	3698
Age, y, mean (SD)	51.3 (20.4)	46.9 (20.4)	48.1 (20.1)	51.3 (20.1)	53.3 (20.2)	53.1 (20.7)	51.8 (21.6)	50.0 (21.3)
Gender, female/male	65.3%/34.7%	60.8%/39.2%	61.6%/38.4%	62.7%/37.3%	67.5%/32.5%	71.3%/28.7%	73.2%/26.8%	74.0%/26.0%
Follow-up, y, median [fifth; 95th percentile]	3.1 [2.0–6.4]	3.3 [1.6–6.7]	3.4 [1.9–6.8]	3.2 [2.0–6.3]	3.1 [2.0–6.0]	3.0.0 [2.0–6.3]	3.0.0 [1.9–6.4]	3.6 [2.0–6.6]
Mortality rate		1.53	1.26	1.10	1.00	1.10	1.44	1.51
Fatal cardiovascular disease, n	5454	382	1026	1786	1305	611	236	108
Age, y, mean (SD)	80.0 (11.2)	78.8 (12.6)	78.3 (12.6)	79.6 (11.1)	80.7 (10.4)	81.8 (10.4)	82.2 (9.6)	81.8 (9.0)
Gender, female/male	61.4%/38.6%	63.9%/36.1%	57.8%/42.2%	56.8%/43.2%	63.5%/36.6%	69.6%/30.4%	72.0%/28.0%	70.4%/29.6%
Follow-up, y, median [fifth; 95th percentile]	1.7 [0.1–4.7]	1.4 [0.1–3.8]	1.7 [0.1–4.5]	1.7 [0.1–4.7]	1.7 [0.2–4.7]	1.7 [0.1–5.1]	1.6 [0.1–4.9]	2.0 [0.1–5.1]
Mortality rate		1.42	1.27	1.12	1.00	1.06	1.43	1.49
Fatal stroke, n	1574	111	273	513	404	179	62	32
Age, y, mean (SD)	80.3 (10.9)	79.9 (10.8)	79.3 (1.8)	80.0 (10.9)	81.0 (10.1)	80.0 (11.5)	82.9 (8.5)	81.3 (8.5)
Gender, female/male	68.4%/31.6%	77.5%/22.5%	65.2%/34.8%	65.9%/34.1%	68.3%/31.7%	72.1%/27.9%	72.6%/27.4%	78.1%/21.9%
Follow-up, y, median [fifth; 95th percentile]	1.7 [0.1–4.8]	1.5 [0.2–3.8]	1.7 [0.1–4.2]	1.7 [0.1–4.9]	1.8 [0.2–4.5]	1.6 [0.1–5.2]	1.7 [0.1–4.3]	1.7 [0.1–5.8]
Mortality rate		1.34	1.09	1.04	1.00	1.01	1.21	1.42
Fatal acute MI, n	702	48	135	240	147	80	36	16
Age, y, mean (SD)	78.9 (11.4)	77.5 (13.2)	76.9 (13.0)	78.9 (11.2)	80.0 (10.5)	81.4 (10.3)	77.8 (9.1)	80.5 (9.9)
Gender, female/male	53.6%/46.4%	4.1%/2.7%	10.1%/9.1%	15.1%/19.1%	12.1%/8.8%	7.1%/4.3%	3.4%/1.7%	1.6%/0.7%
Follow-up, y, median [fifth; 95th percentile]	1.5 [0.1–4.6]	1.4 [0.0–4.0]	1.6 [0.1–4.3]	1.4 [0.1–4.6]	1.6 [0.2–4.9]	1.8 [0.1–5.3]	1.5 [0.1–5.0]	1.8 [0.0–3.8]
Mortality rate		1.59	1.48	1.34	1.00	1.24	1.93	1.95

[a] To convert 25-hydroxyvitamin D level to US units, divide by 2.496 for nanograms per milliliter.

cubic spline with five knots placed at the fifth, 27th, 50th, 73rd, and 95th percentiles of serum 25-hydroxyvitamin D and 70 nmol/L was used as a reference. The models were adjusted for season of blood sampling, age, and gender. All statistical analyses were performed using SAS statistical software (SAS Institute, Inc).

Results

A total of 243 672 subjects with a serum 25-hydroxyvitamin D measurement were included in this study. The mortality data were extracted at the date the study ended, at which point a total of 16 645 subjects had died. The most frequent cause of death was cardiovascular disease (ICD-10; I00–99) (n = 5474). Fatal stroke (ICD-10; I60-I69) (n = 1574) and fatal acute myocardial infarction (MI) (ICD-10; I21) (n = 702) were the main causes of cardiovascular death. The baseline characteristics of the study population stratified by these causes of death and according to the 25-hydroxyvitamin D level are shown in Table 1. Subjects dying from cardiovascular disease tended to be older and had a shorter follow-up (Table 1). Seasonal variation of cardiovascular disease mortality including stroke and acute MI mortality was observed, with increased incidence in winter (data not shown). Mean serum 25-hydroxyvitamin D was 49.5 nmol/L (SD 28.7).

Cardiovascular disease mortality and serum 25-hydroxyvitamin D

In the CopD population, more people had vitamin D levels below 12.5 nmol/L than above 125 nmol/L. Of the subjects who died from cardiovascular disease, stroke, and acute MI, all seemed to be associated with both lower and higher levels of serum 25-hydroxyvitamin D, with a nadir of risk approximately 50–75 nmol/L (Table 1).

The Cox multivariate regression confirmed an inverse association between serum 25-hydrovyvitamin D level below 70 nmol/L mortality risk from cardiovascular disease, stroke, and acute MI ($P < .001$) (Table 2). An inverse association between serum 25-hydrovyvitamin D levels above 70 nmol/L were also found for cardiovascular disease ($P < .001$) and acute MI ($P < .001$) mortality risk but not significant for stroke (Table 2).

A nonlinear association between serum 25-hydroxyvitamin D levels and cardiovascular disease mortality was observed, using restricted cubic spline Cox regression analysis with age as the underlying time scale and estimates adjusted for sex and season (Figures 1–3). For cardiovascular disease mortality, a serum 25-hydroxyvitamin D level of approximately 70 nmol/L was associated with the lowest cardiovascular disease mortality risk. A total of 78.1% of the cohort and 78.2% of the subjects dying from cardiovascular disease had serum 25-hydroxyvitamin D levels below 70 nmol/L. Compared with the reference level of 70 nmol/L, the hazard ratio (HR) of cardiovascular disease mortality at the lower extreme (~12.5 nmol/L) was 2.0 [95% confidence interval (CI) 1.8–2.1], with higher risk for men 2.5 (95% CI 2.2–2.9) than for women 1.7 (95% CI 1.5–1.9). At the higher extreme (~125 nmol/L), the HR of cardiovascular disease mortality was 1.3 (95% CI 1.2–1.4), with similar mortality risk among men 1.3 (95% CI 1.0–1.5) and women 1.3

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Table 2. Cox Multivariate Regression Analysis of Mortality From Cardiovascular Disease, Acute MI, and Stroke per Serum 25-Hydroxyvitamin D Level Below and Above 70 nmol/L

	<70 nmol/L HR (95% CI)	>70 nmol/L HR (95% CI)
Cardiovascular disease		
25-Hydroxyvitamin D per nmol/L	0.986 (0.985–0.988)[a]	1.005 (1.003–1.008)[a]
Sex (male vs female)	1.813 (1.703–1.929)[a]	1.451 (1.279–1.646)[a]
Age per 1 y	1.121 (1.118–1.124)[a]	1.139 (1.132–1.145)[a]
Season (summer vs winter)	1.156 (1.088–1.228)[a]	0.940 (0.839–1.054)
Stroke		
25-Hydroxyvitamin D per nmol/L	0.989 (0.985–0.992)[a]	1.003 (0.999–1.008)
Sex (male vs female)	1.295 (1.147–1.462)[a]	1.170 (0.919–1.488)
Age per 1 y	1.123 (1.118–1.129)[a]	1.129 (1.118–1.141)[a]
Season (summer vs winter)	1.100 (0.982–1.232)	1.186 (0.958–1.469)
Acute MI		
25-Hydroxyvitamin D per nmol/L	0.985 (0.980–0.990)[a]	1.010 (1.005–1.016)[a]
Sex (male vs female)	2.489 (2.098–2.952)[a]	1.808 (1.295–2.525)[a]
Age per 1 y	1.115 (1.107–1.123)[a]	1.121 (1.105–1.138)[a]
Season (summer vs winter)	1.171 (0.988–1.386)	0.706 (0.514–0.970[b]

HRs were estimated in Cox proportional hazard regression models to examine whether the association between cardiovascular disease mortality and covariates differed by serum 25-hydroxyvitamin D levels. The same cutoff point for serum 25-hydroxyvitamin D levels were used. To convert to US units, divide by 2.496 for nanograms per milliliter.

[a] $P < .001$.

[b] $P < .05$.

(95% CI 1.1–1.4) compared with the reference level of 70 nmol/L (Figure 1, A and B).

In the subgroup of subjects who died from stroke, the mortality was lowest at a serum 25-hydroxyvitamin D level of 75 nmol/L. The corresponding HRs for stroke mortality at the low and high extremes were 1.8 (95% CI 1.6–2.1) and 1.2 (95% CI 1.0–1.5), respectively (Figure 2A). Stroke mortality risk was similar among men and women (Figure 2B). Mortality risk from acute MI was lowest at a serum 25-hydroxyvitamin D level of 70 nmol/L. The HR for acute MI mortality at the lower extreme was 2.1 (95% CI 1.7–2.7) (serum 25-hydroxyvitamin D ~12.5 nmol/L) and 1.6 (95% CI 1.2–2.0) (serum 25-hydroxyvitamin D ~ 125 nmol/L) (Figure 3A). However, for women serum 25-hydroxyvitamin D level around 40 nmol/L was associated with the lowest acute MI mortality risk, at which it was 75 nmol/L (approximately) for men. There was a higher HR for men 2.4 (95% CI 1.7–3.3) than for women 2.0 (95% CI 1.4–2.5) at the lower extreme. At the higher extreme, the HR for acute MI mortality was 1.4 (95% CI 0.9–2.2) for men and 1.7 (95% CI 1.2–2.3) for women, compared with the reference level of 70 nmol/L (Figure 3B).

To account for the possibility that many of the deaths during the first year could be due to illnesses already present at baseline, the data were analyzed excluding from the cardiovascular disease mortality analysis those with death within first and second year of the blood collection. The J shape of the curve remained the same, although the HR for both at the highest and lowest serum 25-hydroxyvitamin D level was slightly decreased (data not shown).

Discussion

In the CopD study, based on 247 574 subjects from a single laboratory handling biochemical analysis from primary health care centers in Copenhagen, we observed 5454 events of cardiovascular disease deaths. Both low and high serum 25-hydroxyvitamin D levels were associated with increased cardiovascular disease mortality as well as mortality from stroke and acute MI. The data exhibited a reverse J-formed association between cardiovascular disease mortality and serum 25-hydroxyvitamin D. The lowest mortality rates were observed at approximately 70 nmol/L, 75 nmol/L, and 70 nmol/L for cardiovascular disease, stroke, and acute MI mortality, respectively, when adjusted for age, sex, and season. The HRs were all higher at lower serum 25-hydroxyvitamin D levels than at higher levels. Thus, a reverse J-shaped association was found. Low 25-hydroxyvitamin D levels have been associated with increased cardiovascular mortality in several studies (6, 8, 9, 11, 13, 17, 19, 21–28, 30), in which some studies have suggested an inverse relation between 25-hydroxyvitamin D levels and cardiovascular disease mortality (6, 13, 19, 21, 26, 27, 30). However, other studies did not find any association with low levels of 25-hydroxyvitamin D and cardiovascular disease mortality risk (7, 10, 12, 14–16, 18, 20, 29).

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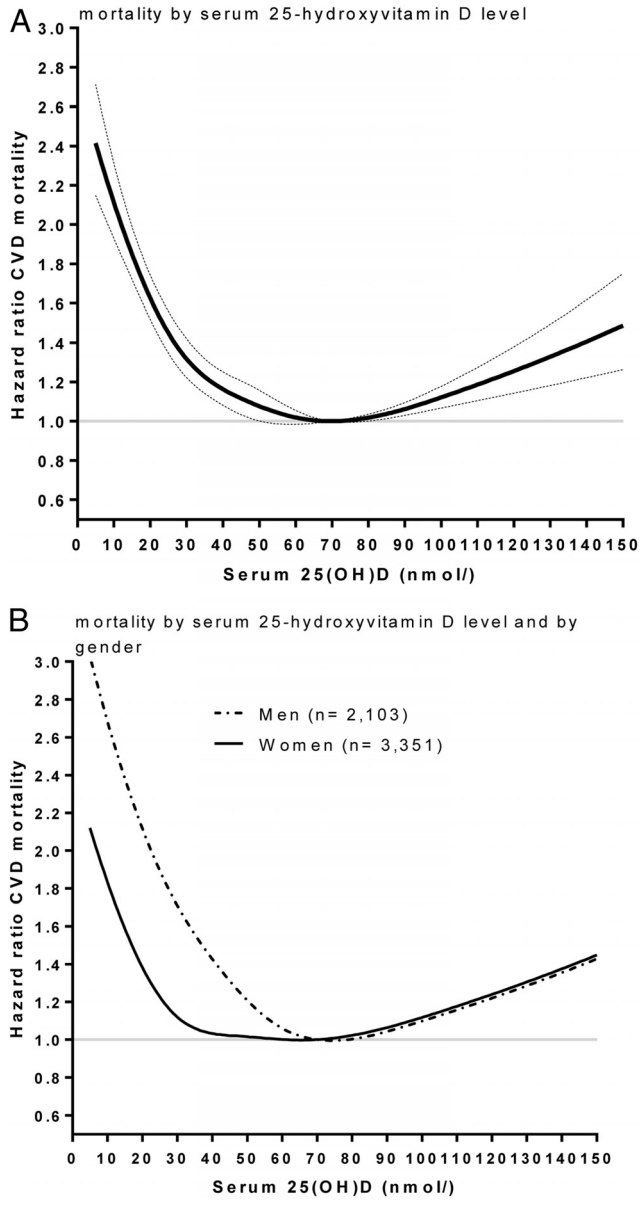

Figure 1. HRs for cardiovascular disease mortality by restricted cubic spline Cox regression analysis. Estimates were adjusted for age, sex, and season of blood sampling according to serum 25-hydroxyvitamin D level, with 70 nmol/L as the reference value. A, Cardiovascular disease mortality cases. B, Cardiovascular disease mortality cases by gender. The horizontal gray line corresponds to the normal reference HR of 1.0; values above are associated with increased mortality risk, and values below are associated with decreased mortality risk compared with the reference value. To convert 25-hydroxyvitamin D to US units, divide by 2.496 for nanograms per milliliter.

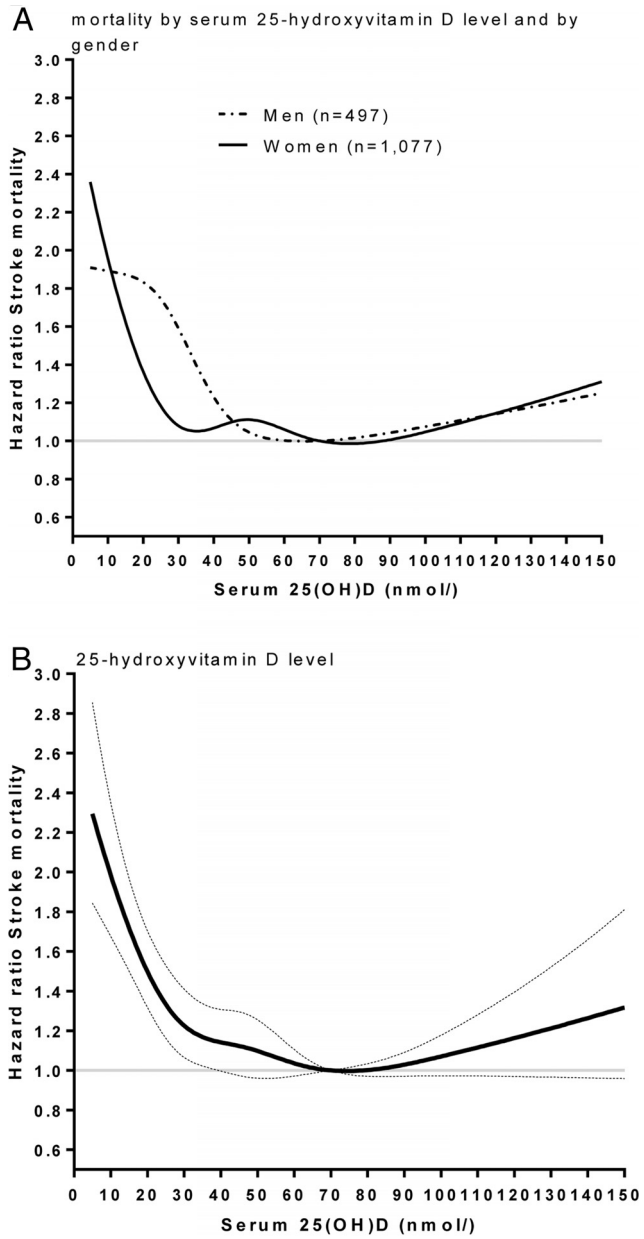

Figure 2. HRs for stroke mortality by restricted cubic spline Cox regression analysis. Estimates were adjusted for age, sex, and season of blood sampling according to serum 25-hydroxyvitamin D level, with 70 nmol/L as the reference value. A, All stroke mortality cases. B, Stroke mortality cases by gender. The horizontal gray line corresponds to the normal reference HR of 1.0; values above are associated with increased mortality risk, and values below are associated with decreased mortality risk compared with the reference value. To convert 25-hydroxyvitamin D to US units, divide by 2.496 for nanograms per milliliter.

In addition to the conflicting results, sufficient data are not available to draw firm conclusions on the relationship between 25-hydroxyvitamin D levels and cardiovascular disease mortality at concentrations at the extremes, especially at the higher extreme. The design of many studies does not allow for analyzing the very low and very high levels of 25-hydroxyvitamin D because they compare reference values with one to four categories. Cutoff levels are a bit arbitrary because the increase in mortality is not continuous and does not start abruptly at a certain 25-hydroxyvitamin D level. We believe that the data need to be analyzed either by a cubic spline analysis or at least by dividing the 25-hydroxyvitamin D levels into categories in which the extreme values are apparent because the association between vitamin D status and mortality might be driven by individuals in the very low and high levels of 25-hy-

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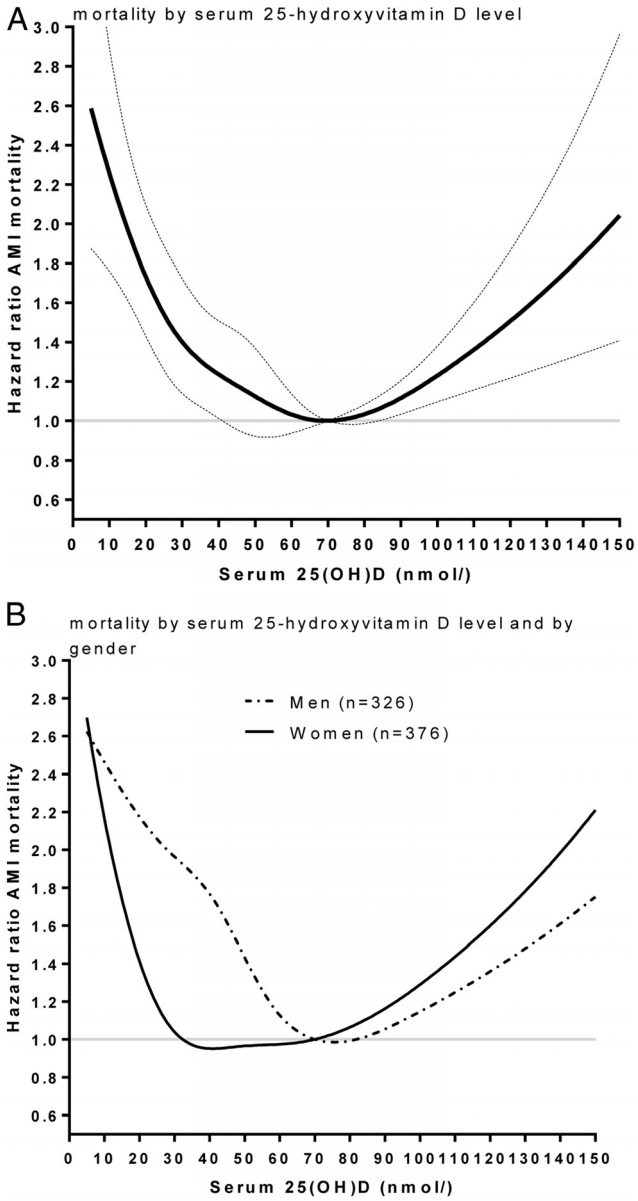

Figure 3. HRs for acute MI mortality by restricted cubic spline Cox regression analysis. Estimates were adjusted for age, sex, and season of blood sampling according to serum 25-hydroxyvitamin D level, with 70 nmol/L as the reference value. A, All acute MI mortality cases. B, Acute MI mortality cases by gender. The horizontal gray line corresponds to the normal reference HR of 1.0; values above are associated with increased mortality risk, and values below are associated with decreased mortality risk compared with the reference value. To convert 25-hydroxyvitamin D to US units, divide by 2.496 for nanograms per milliliter.

droxyvitamin D as stated before (31) and confirmed in other studies (20, 21, 32, 33). However, this may not always be possible because it require a large sample size with sufficient observations in the extremes. Even in relatively large comparative cohort studies, only 210 (2.2%) and 108 (3.4%) had 25-hydroxyvitamin D levels above 112.5 nmol/L and 100 nmol/L, respectively (26, 27). In the present study, 8425 (3.5%) had a serum 25-hydroxyvitamin D level above 100 nmol/L and 3698 (1.5%) had a serum 25-hydroxyvitamin D level above 125 nmol/L.

Another concern is whether the same cardiovascular disease mortality associations can be applied to both women and men. In the present study, there is an inverse association at 50 nmol/L and below for men, at which the slope is not steadily increasing before the serum 25-hydroxyvitamin D less than 30 nmol/L for women. The Institute of Medicine has proposed a second cutoff at 30 nmol/L as a necessary minimum concentration to maintain bone health (34). In the present study, women might have a lower cutoff at 30 nmol/L, but men seem to have a cutoff at 50 nmol/L, at least in regard to cardiovascular disease mortality. Most studies have not found any gender differences investigating the association between cardiovascular disease mortality and 25-hydroxyvitamin D levels by gender (14, 16, 20, 26). However in a study by Rohrmann et al (26), 25-hydroxyvitamin D levels were inversely related to cardiovascular disease mortality in women but not men. A gender-specific association may exist and needs to be further investigated in other studies and for other health outcomes than bone health.

Vitamin D receptors have been found ubiquitously in the human body including in cells of the vessel wall and the heart (35). Additionally, human vascular smooth muscle cells express the 1α-hydroxylase enzyme, enabling the conversion of circulating 25-hydroxyvitamin D to the active hormone 1,25-dihydroxyvitamin D (36). Several mechanisms may explain the link between vitamin D deficiency and cardiovascular diseases and mortality including overexpression of renin (37, 38) and parathyroid hormone (39). Less clear is the reason for the higher levels of vitamin D to be associated with increased cardiovascular disease mortality.

Observational studies such as the present study should be interpreted with caution because they cannot prove causality, and reverse causation may explain the observed associations, and unknown lifestyle factors related to less sunlight exposure or unhealthy diet may be the driving factors explaining the link between vitamin D deficiency and cardiovascular risk factors. Yet at least 94% of the subjects in the present study went to their own general practitioner or to the central laboratory for the general practitioner in Copenhagen to have their serum 25-hydroxyvitamin D level measured and makes confounding by immobility or serious illness unlikely in the present study. However, the lack of information on important covariates such as body mass index, health status, education, smoking, and ethnicity is a limitation of the present study. Additionally, categorization of the study subjects was based on single baseline measurements of serum 25-hydroxyvitamin D, which could lead to misclassification

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because single measurements might not reflect long-term biochemical status. Nevertheless, it has been demonstrated that 25-hydroxyvitamin D concentrations remain relatively stable in repeated-measurement analysis after 5 and 14 years (27, 40).

The major strength of this study was the very large sample size, probably the largest sample size of serum 25-hydroxyvitamin D measurements from a single center, allowing robust analyses of single parameters, such as age, season, and gender. The large number of cardiovascular disease deaths enabled investigation of the association between serum concentrations of 25-hydroxyvitamin D and cardiovascular disease mortality including acute MI and stroke. Another advantage to the CopD study is the personal identification number, which is unique to every citizen in Denmark. In addition to the information on age and gender, the number enables matching of individuals to Danish health registers, including the Danish Causes of Death Register and allows 100% follow-up. Finally, the study is from the primary care and not limited to an elderly and/or hospitalized population.

In conclusion, the present study including 5454 cardiovascular deaths among 247 574 subjects from the primary care showed a reverse J-shaped relationship between serum 25-hydroxyvitamin D and cardiovascular disease mortality, with the lowest mortality rate at 70 nmol/L. High and low levels were also associated with increased mortality from stroke and acute MI. Men tended to be more at risk at lower levels than women. Further investigations specifically aimed at elucidating causal relationships between 25-hydroxyvitamin D levels and cardiovascular disease mortality are needed. The risks and benefits of higher levels of vitamin D have not been well approached in the literature, which calls for more research. Hence, there is a need for randomized clinical trials that also include information on the effects of 25-hydroxyvitamin D levels above 100 nmol/L.

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D.D., H.L.J., and J.C. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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