Vitamin D Status and Risk of All-Cause and Cause-Specific Mortality in a Large Cohort: Results From the UK Biobank

Xikang Fan,[1,*] Jiayu Wang,[1,*] Mingyang Song,[2,3,4] Edward L. Giovannucci,[2,3,5] Hongxia Ma,[1,6,7] Guangfu Jin,[1,6,7] Zhibin Hu,[1,6,7,#] Hongbing Shen,[1,7,#] and Dong Hang[1,7,#]

[1]Department of Epidemiology and Biostatistics, International Joint Research Center on Environment and Human Health, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, China; [2]Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA,US; [3]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA, US; [4]Clinical and Translational Epidemiology Unit and Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, US; [5]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, US; [6]State Key Laboratory of Reproductive Medicine, Center for Global Health, Nanjing Medical University, Nanjing, China; and [7]Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Medicine, Nanjing Medical University, Nanjing, China

ORCiD numbers: 0000-0002-9944-284X (X. Fan); 0000-0002-5608-0738 (J. Wang); 0000-0002-1324-0316 (M. Song); 0000-0002-2462-9693 (H. Ma); 0000-0001-6944-0459 (D. Hang).

Context: Although an inverse association between vitamin D status and mortality has been reported in observational studies, the precise association shape and optimal vitamin D status remain undetermined.

Objective: To investigate the association between vitamin D status and risk of all-cause and cause-specific mortality and estimate optimal serum 25-hydroxyvitamin D [25(OH)D] concentrations.

Design: Prospective cohort study.

Setting: UK Biobank.

Participants: 365 530 participants who had serum 25(OH)D measurements and no history of cardiovascular disease (CVD), cancer, or diabetes at baseline (2006-2010).

Main outcome measures: All-cause and cause-specific mortality.

Results: During a median follow-up of 8.9 (interquartile range: 8.3-9.5) years, 10 175 deaths occurred, including 1841 (18.1%) due to CVD and 5737 (56.4%) due to cancer. The multivariate analyses revealed nonlinear inverse associations, with a decrease in mortality risk appearing to level off at 60 nmol/L of 25(OH)D for all-cause and CVD deaths and at 45 nmol/L for cancer deaths. Compared to participants with 25(OH)D concentrations below the cutoffs, those with higher concentrations had a 17% lower risk for all-cause mortality (hazard ratio [HR]: 0.83, 95% confidence interval [CI]: 0.79-0.86), 23% lower risk for CVD mortality (HR: 0.77, 95% CI: 0.68-0.86), and 11% lower risk for cancer mortality (HR: 0.89, 95% CI: 0.84-0.95).

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*Co-first authors, contributed equally.
#Co-corresponding authors, contributed equally.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; RCT, randomized clinical trial; CVD, cardiovascular disease; BMI, body mass index; VDSP, Vitamin D Standardization Program; ICD, International Classification of Diseases; HR, hazard ratio; CI, confidence interval.

Conclusions: Higher 25(OH)D concentrations are nonlinearly associated with lower risk of all-cause, CVD, and cancer mortality. The thresholds of 45 to 60 nmol/L might represent an intervention target to reduce the overall risk of premature death, which needs further confirmation in large clinical trials. (***J Clin Endocrinol Metab*** **105: e3606–e3619, 2020)**

Key Words: vitamin D, 25-hydroxyvitamin D, mortality, cancer, cardiovascular disease

As an essential micronutrient, vitamin D is mainly derived from biosynthesis in the skin from sun exposure, and some is absorbed from diet and supplement use (1). Beyond its well-established roles in calcium homeostasis and bone health, vitamin D has shown anti-inflammatory, anti-proliferative, anti-oxidative, and immunomodulatory effects in laboratory studies, which may underlie its benefits for various nonskeletal diseases (2).

Supplemental vitamin D has been viewed as a potential strategy for preventing common chronic illness, including cardiovascular disease (CVD) and cancer (3,4). However, clinical data examining the effect of vitamin D supplementation on mortality remain inconclusive. Previous systemic reviews and meta-analyses of randomized controlled trials (RCT) suggested that vitamin D supplementation had a small beneficial effect on all-cause mortality (5-7). In a recent meta-analysis of 52 trials with a total of 75 454 participants, vitamin D supplementation was not associated with all-cause or CVD mortality, but was associated with a 16% lower risk of cancer mortality (8). Indeed, many of the trials had different treatment regimens and dosing intervals (daily, weekly, monthly, or bolus doses) and were limited by relatively short follow-up and small proportions of participants with low enough vitamin D levels to benefit from supplementation.

Previous meta-analyses of prospective cohort studies suggested inverse associations of vitamin D status, assessed by circulating 25-hydroxyvitamin D [25(OH)D] concentrations, with all-cause and/or cause-specific mortality (9-14). However, a large degree of heterogeneity has been observed in the meta-analyses due to variations of the included studies in the duration of follow-up, the categories of 25(OH)D, and the ability to control for confounding variables. Particularly, 25(OH)D concentrations differ noticeably across assay methods (15,16), and the meta-analyses are commonly constrained by a lack of standardized serum 25(OH)D data. More important, no consensus has emerged on the optimal serum 25(OH)D concentrations. According to current guidelines, the recommended concentrations vary from 25 nmol/L to >100 nmol/L (17).

To assess the association between vitamin D status and mortality risk in greater detail, we therefore used the UK Biobank, a large prospective cohort study, with recently released standardized data on baseline biochemistry measurements of serum 25(OH)D, to investigate the associations of 25(OH)D concentrations with mortality from all causes, CVD, cancer, and other causes and estimate the thresholds for serum 25(OH)D with respect to the different outcomes.

Methods

Study population

We included participants from UK Biobank, a prospective cohort study consisting of approximately half a million people (aged 37-73 years) recruited across the United Kingdom between 2006 and 2010 (18). These participants attended 1 of 22 assessment centers in England, Wales, and Scotland, where they completed baseline questionnaires, underwent various physical assessments, and reported medical conditions. During the baseline assessment visit, 45 mL of blood were collected and transported overnight by commercial courier to a central laboratory. Upon arrival, samples were immediately centrifuged and aliquoted into cryotubes as plasma, serum, white cells, and red cells stored in ultra-low temperature archives (19).

In the current analysis, we excluded participants who had a self-reported history of CVD, cancer, or diabetes at the time of blood draw and those who had no available data on 25(OH)D concentrations or covariates. In total, 365 530 participants were included in the final analysis (Fig. 1).

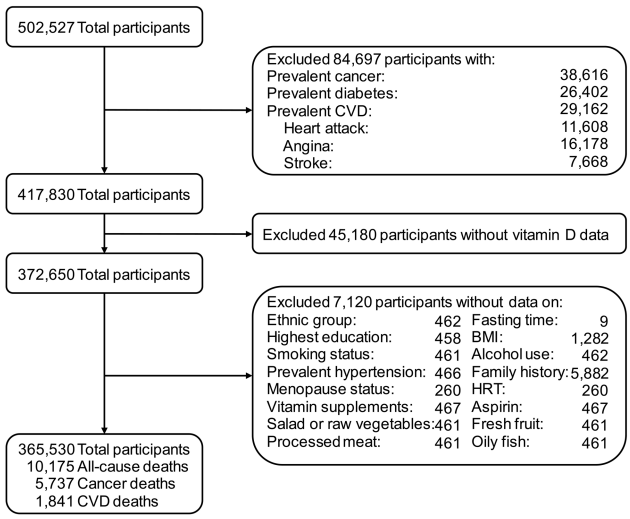

Figure 1. Flow chart of study participants. CVD, cardiovascular disease.

Assessment of 25(OH)D

Details about serum biomarker measurements and assay performances have been described in the online UK Biobank Showcase (http://biobank.ndph.ox.ac.uk/showcase/showcase/docs/serum_biochemistry.pdf). Briefly, serum concentrations of 25(OH)D were measured in UK Biobank's purpose-built facility using a direct competitive chemiluminescent immunoassay method based on DiaSorin Liaison XL Analyzer (Diasorin S.p.A), with a detection range of 10 to 375 nmol/L. The average coefficients of variation of 25(OH)D derived from internal quality control samples of known high, medium, and low concentrations were 5.04%, 5.39%, and 6.14%, respectively. Moreover, the assay of serum 25(OH)D was registered with an external quality assurance scheme (RIQAS Immunoassay Specialty 1) to verify accuracy. The external quality assurance results showed that 100% of participated distributions (n = 108) were good or acceptable.

Ascertainment of mortality outcomes

Dates and causes of death were obtained from death certificates held by the National Health Service Information Centre (England and Wales) and the National Health Service Central Register Scotland (Scotland) from baseline until January 31, 2018 (20). Primary causes of mortality were defined using the 10th revision of the *International Statistical Classification of Diseases* (ICD-10). The primary outcomes of the current study included all-cause mortality and 2 leading cause-specific mortality (ie, mortality due to CVD [ICD-10 I00-I79] and mortality due to cancer [ICD-10 C00-C97]).

Ascertainment of covariates

Information on education degree, lifestyle factors, medical history, medication and supplement use, and dietary intake were collected using a touch-screen, self-completed questionnaire at the baseline assessment visit for UK Biobank. Fasting status were categorized by *yes* or *no* according to fasting time ≥8 or <8 h. Seasons of blood draw were categorized by the months attending assessment centers: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February). Height and body weight were measured by trained nurses at baseline, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Physical activity was measured as total metabolic equivalent task-minutes per week for all activity including walking and moderate and vigorous activity. Further details of covariate measurements can be found in the UK Biobank online protocol (http://www.ukbiobank.ac.uk).

Statistical analysis

Person-time was calculated for each participant from the date of attending an assessment center to the date of death or the date of last follow up (January 31, 2018 for England

Table 1. Baseline characteristics of participants according to deciles of serum 25(OH)D concentrations

Characteristics	Serum 25(OH)D Concentrations		
	Decile 1 (10–22.7 nmol/L)	Decile 5 (41.5–47.2 nmol/L)	Decile 10 (76.7–340.0 nmol/L)
N	36 373	36 952	36 551
Age, mean (SD), y	53.66 (8.05)	55.83 (8.10)	56.48 (8.11)
Follow-up time, mean (SD), y	8.73 (1.11)	8.85 (1.04)	8.86 (1.04)
Female, n (%)	19 739 (54.27)	20 273 (54.86)	20 124 (55.06)
White race, n (%)	30 254 (83.67)	35 505 (96.37)	36 150 (99.14)
College or university degree, n (%)	13 741 (38.27)	12 607 (34.42)	10 486 (28.92)
Smoking status, n (%)			
Never	19 964 (55.14)	21 047 (57.12)	20 004 (54.92)
Previous	10 087 (27.86)	12 344 (33.50)	13 220 (36.29)
Current	6153 (17.00)	3459 (9.39)	3201 (8.79)
Alcohol drinking, n (%)			
Never	3195 (8.81)	1292 (3.50)	828 (2.27)
Previous	1722 (4.75)	1079 (2.92)	955 (2.61)
Current	31 359 (86.45)	34 552 (93.58)	34 742 (95.12)
Body mass index, mean (SD), kg/m^2	28.14 (5.55)	27.31 (4.54)	25.72 (3.74)
Physical activity, mean (SD), MET-min/w	2152.64 (2488.54)	2625.93 (2666.87)	3278.30 (2998.02)
Prevalent hypertension, n (%)	8959 (24.75)	8850 (24.00)	8226 (22.54)
Family history of CVD, n (%)	19 606 (57.39)	20 712 (58.96)	20 326 (58.24)
Family history of cancer, n (%)	11 785 (33.47)	13 068 (36.09)	13 208 (36.73)
Season of blood draw, n (%)			
Spring	15 831 (43.52)	10 843 (29.34)	6079 (16.63)
Summer	2867 (7.88)	9744 (26.37)	15 032 (41.13)
Autumn	4568 (12.56)	9197 (24.89)	11 922 (32.62)
Winter	13 107 (36.03)	7168 (19.40)	3518 (9.62)
Regular vitamin D supplements, n (%)	540 (1.50)	1336 (3.63)	2429 (6.67)
Regular multivitamin supplements, n (%)	4025 (11.15)	8235 (22.37)	11 348 (31.16)
Regular aspirin use, n (%)	2961 (8.29)	3172 (8.68)	3516 (9.70)

The Kruskal-Wallis 1-way analysis of variance test for continuous variables and the Chi-squared test for categorical variables were used to calculate the *P* values across the decile groups of 25(OH)D. The variables listed all had a *P* value < 0.005.
Abbreviations: CVD, cardiovascular disease; MET, metabolic equivalent, SD, standard deviation.

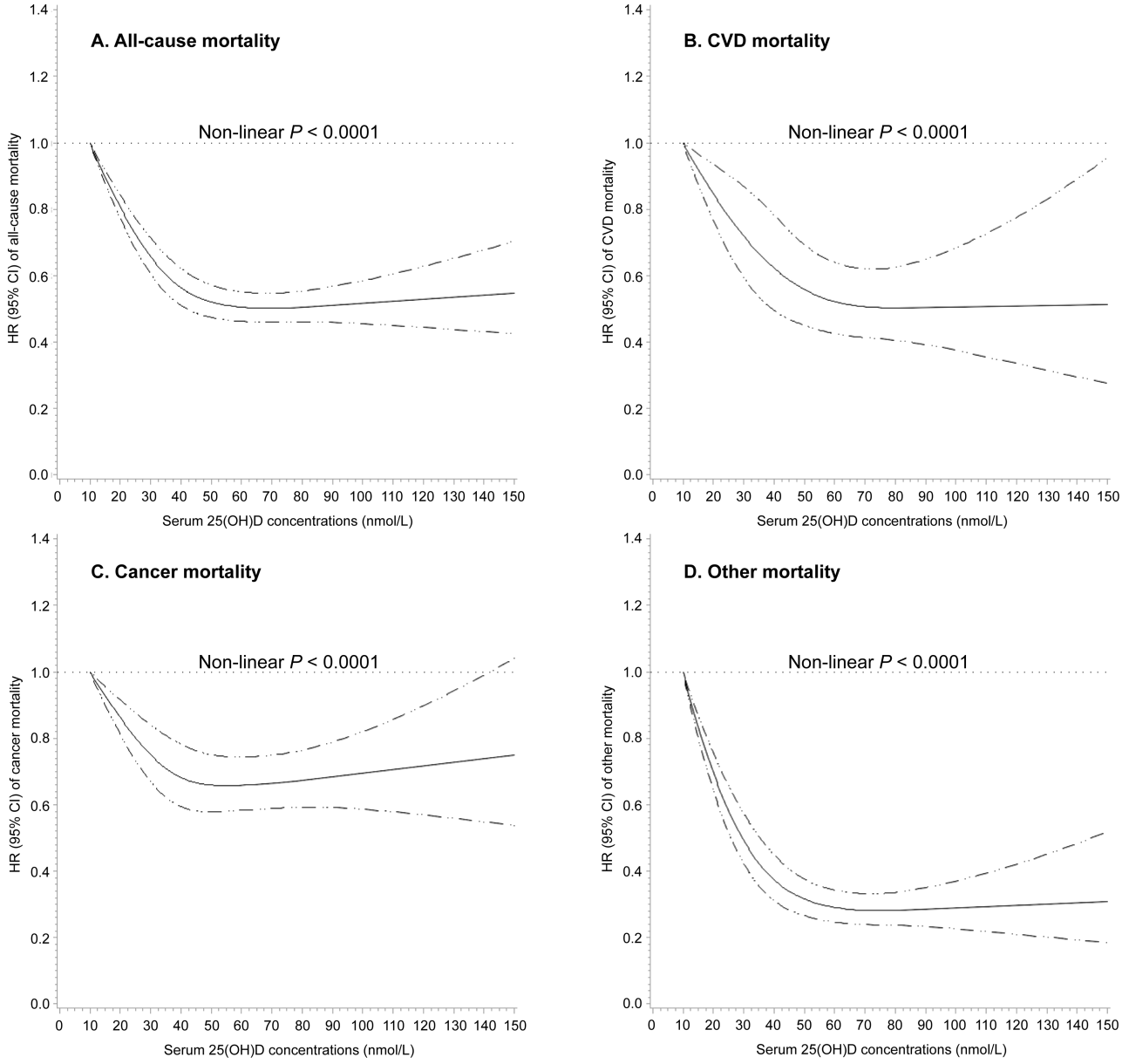

Figure 2. Nonlinear inverse associations of serum 25(OH)D concentrations with all-cause (A), cardiovascular disease (B), cancer (C), and other (D) mortality. The associations were examined by multivariate Cox regression models based on restricted cubic splines. Participants with 25(OH)D concentrations above 150 nmol/L were excluded (n = 140). Solid line represents estimates of hazard ratios and dashed lines represent 95% confidence intervals.

and Wales, and November 30, 2016 for Scotland). We used multivariate cubic regression splines with 4 knots to visually explore nonlinear associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality. A cutoff value was defined as the point where the curve started to level off. A likelihood ratio test was used to compare the model with only the linear term of 25(OH)D concentrations to the model with both the linear and the cubic spline terms, with a P value < 0.05 denoting significant nonlinearity.

The association between 25(OH)D and mortality was analyzed using Cox proportional hazard models. Hazard ratios (HR) and 95% confidence intervals (CI) for each decile of 25(OH)D were calculated, with the lowest decile as the reference. Model 1 was adjusted for age at blood draw, sex, ethnicity, season of blood draw, and fasting status; Model 2 was further adjusted for college or university degree, BMI, smoking status, alcohol drinking, physical activity, family history of CVD/cancer, prevalent hypertension, and, for women, menopause status and hormone replacement therapy; and Model 3 was additionally adjusted for regular use of vitamin D/multivitamin/aspirin, and dietary factors including salad or raw vegetable, fresh fruit, oily fish, and processed meat intake. We also conducted the analyses using defined cutoffs. For site-specific cancers with death counts over 200, we performed secondary analyses to evaluate the association between 25(OH)D and cancer-specific mortality.

Stratified analyses were conducted using cutoffs according to age at blood draw (≤55, >55 years), sex (male, female), season of blood draw (spring, summer, autumn, winter), BMI (<25, 25–30, ≥30 kg/m^2), smoking status (never, former,

Table 2. Associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality

	Serum 25(OH)D concentrations, HR (95% CI)											
Cause of Death	**Decile 1 (10.0-22.7 nmol/L)**	**Decile 2 (22.8-29.7 nmol/L)**	**Decile 3 (29.8-35.7 nmol/L)**	**Decile 4 (35.8-41.4 nmol/L)**	**Decile 5 (41.5-47.2 nmol/L)**	**Decile 6 (47.3-53.0 nmol/L)**	**Decile 7 (53.1-59.2 nmol/L)**	**Decile 8 (59.3-66.3 nmol/L)**	**Decile 9 (66.4-76.6 nmol/L)**	**Decile 10 (76.7-340.0 nmol/L)**	***P* for nonlinearity**	**≥Cutoff vs <Cutoff (ref)**
All-cause												
No. of deaths/ person years	1340/317 653	1134/324 482	989/320 918	1001/318 487	944/326 874	1038/323 547	934/322 309	915/322 226	902/324 522	978/323 898		
Model 1	ref	0.74 (0.68-0.80)	0.61 (0.56-0.66)	0.59 (0.54-0.64)	0.52 (0.47-0.56)	0.55 (0.51-0.60)	0.48 (0.44-0.52)	0.46 (0.42-0.50)	0.44 (0.41-0.48)	0.49 (0.44-0.53)	<0.0001	0.77 (0.74-0.81)
Model 2	ref	0.80 (0.74-0.86)	0.68 (0.63-0.74)	0.68 (0.62-0.74)	0.60 (0.55-0.65)	0.65 (0.59-0.70)	0.57 (0.53-0.63)	0.56 (0.51-0.61)	0.53 (0.49-0.58)	0.58 (0.53-0.64)	<0.0001	0.82 (0.78-0.86)
Model 3	ref	0.80 (0.74-0.87)	0.69 (0.63-0.75)	0.68 (0.63-0.74)	0.60 (0.55-0.66)	0.65 (0.60-0.71)	0.58 (0.53-0.63)	0.56 (0.51-0.61)	0.53 (0.49-0.59)	0.58 (0.53-0.64)	<0.0001	0.83 (0.79-0.86)
CVD												
No. of deaths/ person years	259/317 653	218/324 482	177/320 918	199/318 487	172/326 874	189/323 547	169/322 309	158/322 226	139/324 522	161/323 898		
Model 1	ref	0.74 (0.62-0.89)	0.57 (0.47-0.69)	0.60 (0.50-0.73)	0.48 (0.39-0.59)	0.51 (0.42-0.62)	0.44 (0.36-0.54)	0.40 (0.33-0.50)	0.34 (0.28-0.42)	0.40 (0.32-0.49)	<0.0001	0.68 (0.61-0.76)
Model 2	ref	0.81 (0.68-0.97)	0.65 (0.54-0.79)	0.73 (0.60-0.88)	0.59 (0.49-0.72)	0.64 (0.53-0.78)	0.57 (0.47-0.70)	0.53 (0.43-0.66)	0.46 (0.37-0.57)	0.53 (0.43-0.66)	<0.0001	0.76 (0.68-0.85)
Model 3	ref	0.82 (0.68-0.98)	0.66 (0.54-0.80)	0.74 (0.61-0.89)	0.60 (0.49-0.73)	0.65 (0.54-0.79)	0.58 (0.47-0.71)	0.54 (0.44-0.67)	0.46 (0.37-0.58)	0.54 (0.44-0.67)	<0.0001	0.77 (0.68-0.86)
Cancer												
No. of deaths/ person years	645/317 653	613/324 482	534/320 918	551/318 487	533/326 874	591/323 547	552/322 309	565/322 226	570/324 522	583/323 898		
Model 1	ref	0.83 (0.75-0.93)	0.69 (0.61-0.77)	0.68 (0.61-0.76)	0.61 (0.55-0.69)	0.66 (0.59-0.74)	0.60 (0.54-0.68)	0.61 (0.54-0.68)	0.60 (0.53-0.67)	0.62 (0.55-0.70)	<0.0001	0.82 (0.77-0.86)
Model 2	ref	0.89 (0.80-0.99)	0.76 (0.68-0.85)	0.77 (0.69-0.86)	0.70 (0.62-0.79)	0.76 (0.68-0.86)	0.70 (0.63-0.79)	0.71 (0.63-0.80)	0.70 (0.62-0.79)	0.73 (0.65-0.82)	<0.0001	0.88 (0.83-0.93)
Model 3	ref	0.90 (0.80-1.00)	0.77 (0.68-0.86)	0.78 (0.69-0.88)	0.71 (0.63-0.80)	0.78 (0.69-0.88)	0.72 (0.64-0.81)	0.73 (0.65-0.82)	0.72 (0.64-0.81)	0.75 (0.66-0.85)	<0.0001	0.89 (0.84-0.95)
Other causes												
No. of deaths/ person years	436/317 653	303/324 482	278/320 918	251/318 487	239/326 874	258/323 547	213/322 309	192/322 226	193/324 522	234/323 898		

Table 2. Continued

Cause of Death	Serum 25(OH)D concentrations, HR (95% CI)										*P* for nonlinearity	≥Cutoff vs <Cutoff (ref)
	Decile 1 (10.0-22.7 nmol/L)	Decile 2 (22.8-29.7 nmol/L)	Decile 3 (29.8-35.7 nmol/L)	Decile 4 (35.8-41.4 nmol/L)	Decile 5 (41.5-47.2 nmol/L)	Decile 6 (47.3-53.0 nmol/L)	Decile 7 (53.1-59.2 nmol/L)	Decile 8 (59.3-66.3 nmol/L)	Decile 9 (66.4-76.6 nmol/L)	Decile 10 (76.7-340.0 nmol/L)		
Model 1	ref	0.60 (0.52-0.70)	0.52 (0.45-0.61)	0.45 (0.38-0.52)	0.39 (0.33-0.46)	0.41 (0.35-0.48)	0.33 (0.27-0.39)	0.29 (0.24-0.34)	0.28 (0.23-0.33)	0.34 (0.29-0.40)	<0.0001	0.64 (0.58-0.71)
Model 2	ref	0.66 (0.57-0.76)	0.58 (0.50-0.68)	0.51 (0.44-0.60)	0.45 (0.39-0.54)	0.48 (0.41-0.56)	0.38 (0.32-0.46)	0.34 (0.28-0.41)	0.33 (0.27-0.39)	0.39 (0.33-0.47)	<0.0001	0.67 (0.61-0.73)
Model 3	ref	0.66 (0.57-0.76)	0.58 (0.50-0.67)	0.51 (0.43-0.60)	0.44 (0.38-0.52)	0.46 (0.39-0.54)	0.37 (0.31-0.44)	0.33 (0.27-0.39)	0.31 (0.26-0.37)	0.37 (0.31-0.44)	<0.0001	0.66 (0.60-0.73)

Model 1: adjusted for age at blood draw, sex, ethnicity, season of blood draw, and fasting status. Model 2: adjusted for model 1 plus college or university degree, body mass index, smoking status, alcohol drinking, summed metabolic equivalent of task-minutes per week for all activity, family history of CVD or cancer, prevalent hypertension, and, for women, menopause status and hormone replacement therapy. Model 3: adjusted for model 2 plus regular use of vitamin D or multivitamin supplements, regular aspirin use, and dietary factors including salad or raw vegetable intake, fresh fruit intake, oily fish intake, and processed meat intake. The cutoff was 60 nmol/L for all-cause, CVD, and other mortality, and 45 nmol/L for cancer mortality.

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; ref, reference.

current), alcohol drinking (never, former, current), physical activity (≤median, >median), regular vitamin D supplementation (yes, no), and follow-up time (≤5, >5 years) in the fully adjusted model. To investigate potential effect modification by these stratification variables, we used a likelihood ratio test comparing the models with and without interaction terms between 25(OH)D concentrations and each of the stratification variables.

Sensitivity analyses were performed by excluding individuals who died within 2 years after the blood draw and excluding the participants with overall poor self-rated health in baseline questionnaires. We used SAS 9.4 for all analyses. All statistical tests were 2-sided, and $P < 0.05$ was defined as statistically significant.

Results

The median follow-up period was 8.9 years (interquartile range: 8.3-9.5 years). Of 365 530 participants, 10 175 died, including 1841 (18.1%) from CVD, 5737 (56.4%) from cancer, and 2597 (25.5%) from other causes.

Table 1 summarizes the main characteristics of participants by deciles of serum 25(OH)D concentrations. Participants with higher 25(OH)D had a lower BMI and higher levels of physical activity and tended to use vitamin D or multivitamins; they were less likely to be current smokers or have prevalent hypertension. In addition, participants who had their blood draw in summer and autumn were more likely to have higher 25(OH)D concentrations than those in spring and winter.

Figure 2 shows a nonlinear inverse relationship of 25(OH)D concentrations with all-cause and cause-specific mortality of CVD, cancer, and other causes (all *P* values for nonlinearity < 0.0001). Decreasing mortality risk for increasing 25(OH)D concentrations was observed up to around 60 nmol/L for all causes, CVD, and other causes and around 45 nmol/L for cancer, above which there was no further decrease.

Table 2 shows the association between 25(OH)D and all-cause, CVD, cancer, and other mortality. In the fully adjusted models, compared to the lowest decile (10.0-22.7 nmol/L), the other decile groups showed statistically significant HRs ranging from 0.80 to 0.53 for all-cause mortality, 0.82 to 0.46 for CVD mortality, 0.90 to 0.71 for cancer mortality, and 0.66 to 0.31 for other mortality. Compared to participants with 25(OH)D <60 nmol/L, those with ≥60 nmol/L had a 17% lower risk of all-cause mortality (HR: 0.83, 95% CI: 0.79–0.86), 23% lower risk of CVD mortality (HR: 0.77, 95% CI: 0.68-0.86), and 34% lower risk of other mortality (HR: 0.66, 95% CI: 0.60-0.73). For cancer mortality, an 11% lower risk (HR: 0.89, 95% CI: 0.84-0.95) was observed when comparing 25(OH)D ≥45 nmol/L to <45 nmol/L. In cancer-specific analysis,

Table 3. Associations between serum 25(OH)D and cancer-specific mortality

Cause of Death	Serum 25(OH)D Concentrations, HR (95% CI)										*P* for Nonlinearity	≥45 vs <45 (ref) nmol/L
	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10		
Lung cancer												
No. of deaths/ person years	165/317 653	132/324 482	109/320 918	113/318 487	94/326 874	126/323 547	88/322 309	103/322 226	95/324 522	108/323 898		
Fully adjusted model	ref	0.86 (0.68-1.09)	0.73 (0.57-0.93)	0.77 (0.60-0.98)	0.62 (0.47-0.80)	0.82 (0.64-1.04)	0.57 (0.44-0.75)	0.67 (0.51-0.86)	0.59 (0.45-0.77)	0.66 (0.51-0.86)	0.06	0.82 (0.72-0.93)
Colorectal cancer												
No. of deaths/ person years	77/317 653	74/324 482	53/320 918	50/318 487	68/326 874	63/323 547	52/322 309	62/322 226	71/324 522	52/323 898		
Fully adjusted model	ref	0.88 (0.64-1.21)	0.61 (0.43-0.88)	0.57 (0.40-0.82)	0.73 (0.52-1.02)	0.67 (0.47-0.95)	0.55 (0.38-0.79)	0.65 (0.46-0.93)	0.74 (0.52-1.05)	0.56 (0.38-0.82)	0.005	0.87 (0.73-1.03)
Pancreatic cancer												
No. of deaths/ person years	38/317 653	53/324 482	49/320 918	47/318 487	43/326 874	51/323 547	54/322 309	51/322 226	45/324 522	50/323 898		
Fully adjusted model	ref	1.27 (0.83-1.93)	1.14 (0.74-1.75)	1.06 (0.68-1.63)	0.91 (0.58-1.42)	1.05 (0.68-1.63)	1.10 (0.71-1.70)	1.02 (0.66-1.59)	0.88 (0.56-1.40)	1.00 (0.64-1.58)	0.88	0.97 (0.80-1.18)
Lymphatic cancer												
No. of deaths/ person years	51/317 653	45/324 482	44/320 918	45/318 487	36/326 874	39/323 547	53/322 309	57/322 226	43/324 522	66/323 898		
Fully adjusted model	ref	0.78 (0.52-1.16)	0.72 (0.48-1.08)	0.70 (0.46-1.05)	0.52 (0.33-0.81)	0.54 (0.35-0.83)	0.72 (0.48-1.07)	0.76 (0.51-1.14)	0.56 (0.36-0.86)	0.88 (0.59-1.32)	0.007	0.94 (0.77-1.14)
Brain cancer												
No. of deaths/ person years	26/317 653	36/324 482	41/320 918	33/318 487	35/326 874	35/323 547	45/322 309	41/322 226	32/324 522	34/323 898		
Fully adjusted model	ref	1.21 (0.73-2.02)	1.31 (0.80-2.16)	1.02 (0.61-1.72)	1.01 (0.60-1.70)	0.98 (0.58-1.66)	1.23 (0.74-2.04)	1.11 (0.66-1.85)	0.84 (0.49-1.45)	0.89 (0.52-1.54)	0.81	0.85 (0.68-1.07)
Esophageal cancer												
No. of deaths/ person years	41/317 653	24/324 482	23/320 918	31/318 487	22/326 874	23/323 547	30/322 309	21/322 226	26/324 522	25/323 898		
Fully adjusted model	ref	0.56 (0.34-0.92)	0.54 (0.32-0.90)	0.73 (0.45-1.18)	0.49 (0.29-0.84)	0.51 (0.30-0.87)	0.67 (0.41-1.11)	0.48 (0.27-0.83)	0.58 (0.34-0.98)	0.57 (0.33-0.99)	0.03	0.84 (0.64-1.09)
Prostate cancer												
No. of deaths/ person years	18/317 653	24/324 482	20/320 918	22/318 487	31/326 874	26/323 547	24/322 309	21/322 226	24/324 522	27/323 898		
Fully adjusted model	ref	1.07 (0.58-1.97)	0.82 (0.43-1.56)	0.83 (0.44-1.57)	1.04 (0.57-1.88)	0.83 (0.44-1.54)	0.72 (0.38-1.37)	0.62 (0.32-1.21)	0.67 (0.35-1.27)	0.75 (0.39-1.43)	0.19	0.81 (0.61-1.07)
Breast cancer												

Table 3. Continued

Cause of Death	Serum 25(OH)D Concentrations, HR (95% CI)										*P* for Nonlinearity	≥45 vs <45 (ref) nmol/L
	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10		
No. of deaths/ person years	24/317 653	20/324 482	22/320 918	23/318 487	23/326 874	21/323 547	25/322 309	20/322 226	28/324 522	27/323 898		
Fully adjusted model	ref	0.79 (0.44-1.43)	0.87 (0.48-1.56)	0.90 (0.50-1.61)	0.88 (0.48-1.58)	0.81 (0.44-1.49)	0.97 (0.54-1.75)	0.77 (0.41-1.43)	1.09 (0.60-1.95)	1.07 (0.59-1.94)	0.91	1.01 (0.76-1.34)
Ovarian cancer												
No. of deaths/ person years	20/317 653	27/324 482	19/320 918	27/318 487	12/326 874	21/323 547	20/322 309	14/322 226	23/324 522	30/323 898		
Fully adjusted model	ref	1.22 (0.68-2.19)	0.85 (0.45-1.60)	1.18 (0.65-2.13)	0.51 (0.24-1.05)	0.88 (0.47-1.67)	0.83 (0.44-1.59)	0.57 (0.28-1.16)	0.95 (0.50-1.79)	1.29 (0.70-2.38)	0.13	0.83 (0.62-1.11)

Analyses were adjusted for age at blood draw, sex, ethnicity, season of blood draw, fasting status, body mass index, smoking status, alcohol drinking, summed metabolic equivalent of task-minutes per week for all activity, family history of CVD or cancer, prevalent hypertension, menopause status and hormone replacement therapy (for women), regular use of vitamin D or multivitamin supplements, dietary factors including salad or raw vegetable intake, fresh fruit intake, oily fish intake, and processed meat intake.
Abbreviations: HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease; ref, reference.

participants with 25(OH)D ≥45 nmol/L had an 18% lower risk for lung cancer mortality (HR: 0.82, 95% CI: 0.72-0.93), as compared to those with <45 nmol/L (Table 3).

Figure 3 shows the forest plot results of stratified analyses. The associations of 25(OH)D with all-cause, CVD, cancer, and other mortality were largely consistent across subgroups, with several exceptions. Effect modification by sex, smoking status, physical activity, and follow-up time was observed for all-cause mortality (all *P*s for interaction < 0.05), and the HRs were stronger in males, physically active individuals, current smokers, and those with a follow-up time over 5 years.

Sensitivity analyses showed that the aforementioned associations remained after excluding 964 individuals who died within 2 years after the blood draw (Table 4) or excluding 10 504 individuals who self-rated overall poor health at baseline assessments (Table 5).

Discussion

In this large prospective cohort study, we observed nonlinear inverse associations between serum 25(OH)D concentrations and risk of all-cause and cause-specific mortality. The decreasing risk of all causes, CVD, and other causes mortality appeared to level off at 60 nmol/L of 25(OH)D, and the risk of cancer mortality reached a plateau at around 45 nmol/L. Comprehensive stratified and sensitivity analyses supported the robustness of the observed associations. These findings suggest that 45 to 60 nmol/L of 25(OH)D might represent potential intervention thresholds for reducing premature death risk, which needs to be confirmed in future large RCTs.

Although many observational studies have revealed a nonlinear inverse association between 25(OH)D concentrations and all-cause mortality risk (9,10,12), the precise shape of the 25(OH)D–mortality curve remains unclear. A few studies reported a possible U-shaped or reverse J-shaped curve (14,21,22), while others did not (11,23). A possible explanation for the U-shaped association could be that participants with very high 25(OH)D were taking vitamin D supplements due to poor health, leading to a spurious association between high 25(OH)D concentrations and mortality (24). In addition, initial analysis of NHANES III (the Third National Health and Nutrition Examination Survey) found a reverse J-shaped association between 25(OH)D and all-cause mortality, with a strong inverse association below 40 nmol/L and a weak increased risk above 120 nmol/L (21); however, after standardization of 25(OH)D concentrations using the Vitamin D Standardization Program (VDSP) protocols (https://ods.od.nih.gov/Research/vdsp.aspx), there was no increased nor decreased mortality risk

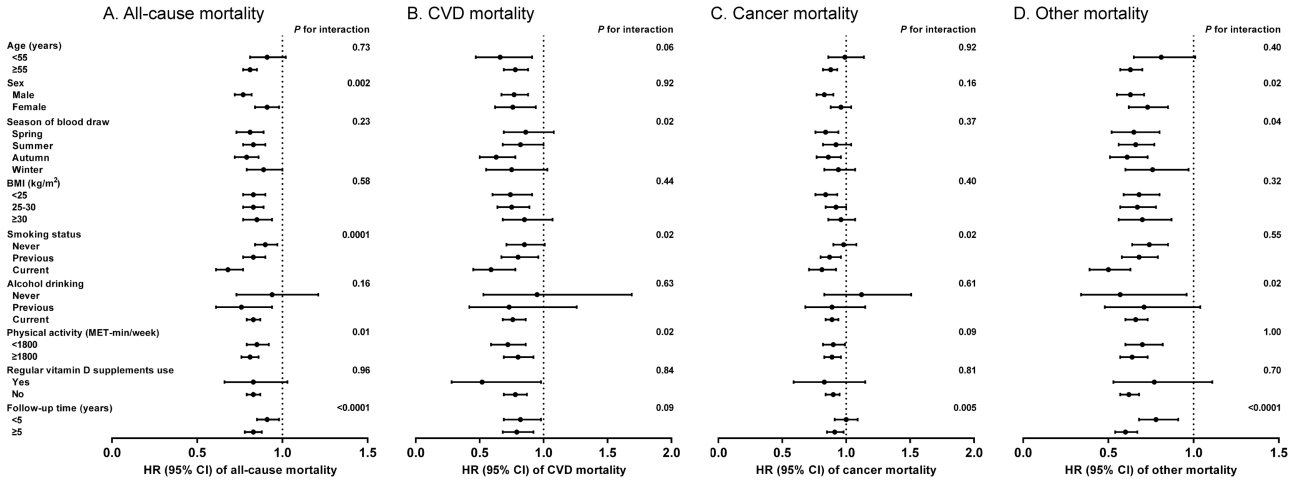

Figure 3. Forest plots of stratified analysis of the associations between serum 25(OH)D concentrations (≥cutoff vs <cutoff [ref]) and the risk of all-cause and cause-specific mortality.

at high 25(OH)D concentrations, which highlighted the importance of standardization methodology when interpreting published results (25). Consistently, in the most recent meta-analysis of 26 916 individuals with VDSP-standardized 25(OH)D data from a European consortium, no apparent excess of mortality risk was observed at high 25(OH)D levels (≥125 nmol/L) (12). In line with the standardized laboratory measurement proposed by VDSP, the UK Biobank used a rigorous protocol to ensure the accuracy and comparability of 25(OH)D measurements. Our analysis indicated a nonlinear curve with a decrease in all-cause mortality risk up to 60 nmol/L, above which the risk plateaued. These findings together support no clear indication of high vitamin D status leading to increased mortality.

Furthermore, there is still a debate on the threshold for optimal 25(OH)D concentrations. In the systematic reviews by Bischoff-Ferrari et al, the desirable concentrations in relation to various outcomes including mortality began at 75 nmol/L for the entire adult population (26,27), in agreement with the recommendation by the International Osteoporosis Foundation (28) and the Endocrine Society (29). However, based on bone health, the Institute of Medicine considered 50 nmol/L to be sufficient (30). Indeed, there is scare evidence from clinical trials to help determine the optimal concentrations for mitigating mortality risk, and it is difficult to obtain without a multiple-dose design in a large population with a long follow-up. A meta-analysis of 14 prospective cohort studies reported the optimal concentrations in the range of 75 to 87.5 nmol/L for all-cause mortality (14), whereas a following meta-analysis of 32 observational studies suggested above 90 nmol/L (11). Most of the included studies performed statistical analyses on mortality risk according to a few 25(OH)D categories, and the variations in sample size, follow-up duration, and assay methods may contribute to the inconsistency. In our current analysis of a large sample size, a threshold of 60 nmol/L was observed for all-cause mortality, consistent with the results from 2 prospective cohort studies conducted in Norway and Sweden, respectively (31,32). It is noteworthy that the prevalence of 25(OH)D concentrations below 60 nmol/L was 71.1% among the UK Biobank participants. Whether a target ≥60 nmol/L can reduce the overall risk of premature death in this population needs to be confirmed in future clinical trials.

Consistent with most of observational studies and meta-analyses (33,34), we found a nonlinear inverse association between serum 25(OH)D and CVD mortality. Decreasing risk of CVD mortality was previously described up to 75, 80, or 90 nmol/L of 25(OH)D (33,35,36) and in our study, 60 nmol/L, beyond which there was no further decrease. However, data from clinical trials of vitamin D supplementation (7,37) and Mendelian randomization (38-40) did not support the preventive effects of vitamin D against CVD death. Additionally, a trial sequential meta-analysis by Bolland et al suggested that vitamin D supplementation with or without calcium did not reduce skeletal or nonskeletal outcomes including CVD in unselected community-dwelling individuals (6). A possible explanation of the null findings for vitamin D supplementation is the lack of sufficient sample size with low enough vitamin D status. For example, only 13% of participants in the Vitamin D and Omega-3 Trial and 25% in the Vitamin D Assessment study had 25(OH)D concentrations <50 nmol/L at baseline (37,41). The beneficial effects of vitamin D supplementation on CVD mortality may only emerge in those with severe vitamin D deficiency (42). As for Mendelian randomization studies, they assumed a linear, rather than nonlinear, association between 25(OH)D and CVD mortality, thus

Table 4. Sensitivity analyses excluding deaths within the first 2 years of follow-up (n = 964) for the associations between serum 25(OH)D and risk of all-cause and cause-specific mortality

	Serum 25(OH)D Concentrations, HR (95% CI)											
Cause of Death	**Decile 1**	**Decile 2**	**Decile 3**	**Decile 4**	**Decile 5**	**Decile 6**	**Decile 7**	**Decile 8**	**Decile 9**	**Decile 10**	***P* for Nonlinearity**	**≥Cutoff vs <Cutoff (ref)**
All-cause												
No. of deaths/ person years	1210/317 496	1022/324 353	901/320 807	893/318 353	866/326 781	950/323 449	830/322 185	833/322 133	822/324 423	884/323 784		
Fully adjusted model	ref	0.80 (0.74-0.87)	0.69 (0.63-0.75)	0.67 (0.62-0.74)	0.61 (0.56-0.67)	0.66 (0.60-0.72)	0.57 (0.52-0.62)	0.56 (0.51-0.62)	0.54 (0.49-0.59)	0.59 (0.53-0.65)	<0.0001	0.83 (0.79-0.87)
CVD												
No. of deaths/ person years	219/317 496	196/324 353	156/320 807	180/318 353	145/326 781	164/323 449	139/322 185	135/322 133	121/324 423	147/323 784		
Fully adjusted model	Ref	0.87 (0.72-1.06)	0.68 (0.55-0.84)	0.78 (0.64-0.96)	0.59 (0.48-0.74)	0.66 (0.54-0.82)	0.55 (0.44-0.69)	0.54 (0.43-0.68)	0.47 (0.37-0.60)	0.58 (0.46-0.73)	<0.0001	0.78 (0.70-0.88)
Cancer												
No. of deaths/ person years	600/317 496	550/324 353	495/320 807	493/318 353	497/326 781	550/323 449	502/322 185	520/322 133	523/324 423	533/323 784		
Fully adjusted model	Ref	0.87 (0.77-0.97)	0.77 (0.68-0.87)	0.75 (0.67-0.85)	0.72 (0.64-0.81)	0.79 (0.70-0.89)	0.71 (0.63-0.80)	0.73 (0.64-0.83)	0.72 (0.63-0.81)	0.75 (0.66-0.85)	<0.0001	0.91 (0.85-0.96)
Other causes												
No. of deaths/ person years	391/317 496	276/324 353	250/320 807	220/318 353	224/326 781	236/323 449	189/322 185	178/322 133	178/324 423	204/323 784		
Fully adjusted model	ref	0.67 (0.57-0.78)	0.58 (0.49-0.68)	0.49 (0.42-0.59)	0.46 (0.39-0.55)	0.47 (0.40-0.56)	0.36 (0.30-0.44)	0.33 (0.28-0.40)	0.32 (0.26-0.39)	0.36 (0.30-0.44)	<0.0001	0.66 (0.60-0.73)

Analyses were adjusted for age at blood draw, sex, ethnicity, season of blood draw, fasting status, body mass index, smoking status, alcohol drinking, summed metabolic equivalent of task-minutes per week for all activity, family history of CVD or cancer, prevalent hypertension, menopause status and hormone replacement therapy (for women), regular use of vitamin D or multivitamin supplements, dietary factors including salad or raw vegetable intake, fresh fruit intake, oily fish intake, and processed meat intake. The cutoff was 60 nmol/L for all-cause, CVD, and other mortality and 45 nmol/L for cancer mortality.

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; ref, reference.

Table 5. Sensitivity analyses excluding participants with poor self-rated health (n = 10 504) for the associations between serum 25(OH)D and risk of all-cause and cause-specific mortality

	Serum 25(OH)D Concentrations, HR (95% CI)											
Cause of Death	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10	*P* for Nonlinearity	≥Cutoff vs <Cutoff (ref)
All-cause												
No. of deaths/ person years	1127/299 079	1015/311 588	912/310 492	927/309 297	885/318 428	983/316 831	879/315 867	874/316 192	872/318 889	919/317 833		
Fully adjusted model	ref	0.83 (0.76-0.91)	0.72 (0.66-0.79)	0.72 (0.66-0.79)	0.64 (0.59-0.70)	0.70 (0.64-0.76)	0.61 (0.56-0.67)	0.60 (0.55-0.66)	0.59 (0.53-0.64)	0.63 (0.57-0.69)	<0.0001	0.85 (0.81-0.89)
CVD												
No. of deaths/ person years	218/299 079	188/311 588	156/310 492	187/309 297	164/318 428	175/316 831	157/315 867	150/316 192	130/318 889	150/317 833		
Fully adjusted model	Ref	0.82 (0.67-1.00)	0.66 (0.54-0.82)	0.79 (0.64-0.96)	0.65 (0.53-0.80)	0.68 (0.55-0.84)	0.60 (0.49-0.75)	0.58 (0.46-0.72)	0.49 (0.39-0.62)	0.57 (0.46-0.72)	<0.0001	0.78 (0.70-0.88)
Cancer												
No. of deaths/ person years	571/299 079	572/311 588	511/310 492	521/309 297	510/318 428	576/316 831	535/315 867	552/316 192	562/318 889	564/317 833		
Fully adjusted model	Ref	0.92 (0.82-1.04)	0.80 (0.71-0.90)	0.80 (0.71-0.90)	0.74 (0.65-0.84)	0.82 (0.72-0.92)	0.75 (0.66-0.85)	0.77 (0.68-0.87)	0.77 (0.68-0.87)	0.79 (0.69-0.89)	<0.0001	0.91 (0.86-0.97)
Other causes												
No. of deaths/ person years	338/299 079	255/311 588	245/310 492	219/309 297	211/318 428	232/316 831	187/315 867	172/316 192	180/318 889	205/317 833		
Fully adjusted model	ref	0.69 (0.59-0.81)	0.63 (0.53-0.74)	0.54 (0.46-0.65)	0.48 (0.40-0.57)	0.50 (0.42-0.60)	0.39 (0.33-0.48)	0.35 (0.29-0.43)	0.35 (0.29-0.43)	0.40 (0.33-0.48)	<0.0001	0.68 (0.61-0.75)

Analyses were adjusted for age at blood draw, sex, ethnicity, season of blood draw, fasting status, body mass index, smoking status, alcohol drinking, summed metabolic equivalent of task-minutes per week for all activity, family history of CVD or cancer, prevalent hypertension, menopause status and hormone replacement therapy (for women), regular use of vitamin D or multivitamin supplements, dietary factors including salad or raw vegetable intake, fresh fruit intake, oily fish intake, and processed meat intake. The cutoff was 60 nmol/L for all-cause, CVD, and other mortality and 45 nmol/L for cancer mortality.

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; ref, reference.

probably leading to underestimation of any true effect estimates. Furthermore, the common genetic variants used in those studies only explain a small proportion (around 5%) of the variation in serum 25(OH)D levels (43), and the genetic-predicted distribution of 25(OH)D may not extend to low enough concentrations for identifying an association with CVD (44). Laboratory studies suggest that vitamin D may exert its cardiovascular effects including regulating the renin-angiotensin system, inhibiting vascular smooth muscle cell proliferation, and having anti-inflammatory, antifibrotic and antithrombotic properties (45).

With respect to cancer mortality, our observation of an inverse association with 25(OH)D is consistent with previous meta-analyses of observational studies (10,46,47) and a Mendelian randomization study (39). Moreover, meta-analyses of RCTs also found that vitamin D supplementation resulted in a decrease in cancer mortality (7,8,48). However, few studies have explored the threshold for 25(OH)D in relation to cancer mortality. In contrast to an inverse association below 45 nmol/L observed in our analysis, a German population-based cohort study reported optimal 25(OH)D concentrations for cancer mortality at around 75 nmol/L (33). The inconsistency might be partly explained by the differences in numbers of cancer deaths and the association magnitude for different cancers with 25(OH)D. With a much larger number of site-specific cancer deaths, the current study revealed nonlinear inverse associations for lung cancer mortality. In addition, compared to the lowest decile of 25(OH)D, certain higher decile groups were associated with lower risk of colorectal cancer and esophageal cancer mortality. Consistently, a combined analysis of three Danish cohort studies reported that low 25(OH)D concentrations were associated with higher risk of lung cancer mortality (39), and the results from NHANES III suggested an inverse association for colorectal cancer mortality (49,50). Functional evidence from several types of cancer cell lines (including lung and colorectal cancers) and mice xenograft models supports an important role of vitamin D in suppressing cell proliferation and tumor growth, promoting apoptosis and autophagy, and enhancing DNA repair, antioxidant protection, and immunomodulation (51). Vitamin D deficiency may disrupt molecular pathways of these biological activities and therefore promote malignant transformation and metastasis.

Our analysis has several strengths. First, 25(OH)D concentrations were determined by a standard, reliable method, allowing for detailed dose-response analysis and determination of clinically meaningful thresholds. Second, the large sample size and a large number of deaths based on the National Health Service death records provided sufficient power to detect nonlinear associations in the overall population and also allowed for the analyses on site-specific cancer mortality. Third, we were able to adjust for a wide range of demographic, lifestyle, health, and dietary factors. Several limitations also need to be considered. First, reverse causality cannot be excluded. However, all participants with baseline CVD, cancer, and diabetes were removed from the analysis, and our sensitivity analyses supported the robustness of the findings. The stronger associations in participants with a longer follow-up (≥5 years) also argue against reverse causation. Second, given the lack of repeated 25(OH)D measurements, we were unable to analyze the relationship between dynamic 25(OH)D concentrations and mortality. However, existing evidence shows that a single measurement can provide an adequate measure of longer-term vitamin D status (52). Third, since most of the UK Biobank participants were of white origin, the results from this study may not be generalizable to other populations.

Conclusions

The current study indicates that serum 25(OH)D concentrations are nonlinearly associated with lower risk of all-cause mortality and mortality due to CVD, cancer, and other causes. The thresholds of 45 to 60 nmol/L of 25(OH)D might represent a potential target to lower the risk of premature death. RCTs are required to test our hypothesis.

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Additional Information

Correspondence and Reprint Request: Dong Hang, PhD, Department of Epidemiology and Biostatistics, Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, 101 Longmian Avenue, Nanjing 211166, China. E-mail: hangdong@njmu.edu.cn.

Disclosure Summary: The authors have nothing to disclose.

Data Availability: The data sets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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