Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study[1–3]

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ABSTRACT

Background: Serum 25-hydroxyvitamin D [25(OH)D] concentration has been linked to mortality in several studies, but appropriate cutoffs to define risk categories are under debate.

Objective: We aimed to conduct a repeated-measurements analysis on the association of serum 25(OH)D concentrations with all-cause and cause-specific mortality, with particular attention given to the shape of dose-response relations.

Design: Concentrations of 25(OH)D were measured in n = 9578 baseline and n = 5469 5-y follow-up participants of the ESTHER study, which is a German population-based cohort aged 50–74 y at baseline. Deaths were recorded during 9.5 y of follow-up (median). Restricted cubic splines were used to assess dose-response relations, and Cox regression with time-dependent variables was used to estimate hazard ratios.

Results: During follow-up, 1083 study participants died; of those, 350 individuals died of cardiovascular diseases, 433 individuals died of cancer, and 55 individuals died of respiratory diseases. The overall mortality [HR (95% CI)] of subjects with vitamin D deficiency [25(OH)D concentrations <30 nmol/L] or vitamin D insufficiency [25(OH)D concentrations from 30 to 50 nmol/L) was significantly increased [1.71 (1.43, 2.03) and 1.17 (1.02, 1.35), respectively] compared with that of subjects with sufficient 25(OH)D concentrations (>50 nmol/L)]. Vitamin D deficiency was also associated with increased cardiovascular mortality [1.39 (95% CI: 1.02, 1.89)], cancer mortality [1.42 (95% CI: 1.08, 1.88)] and respiratory disease mortality [2.50 (95% CI: 1.12, 5.56)]. The association of 25(OH)D concentrations with all-cause mortality proved to be a nonlinear inverse association with risk that started to increase at 25(OH)D concentrations <75 nmol/L.

Conclusions: In this large cohort study, serum 25(OH)D concentrations were inversely associated with all-cause and cause-specific mortality. In particular, vitamin D deficiency [25(OH)D concentration <30 nmol/L] was strongly associated with mortality from all causes, cardiovascular diseases, cancer, and respiratory diseases. *Am J Clin Nutr* 2013;97:782–93.

INTRODUCTION

Low vitamin D status, which is a well-known risk factor for osteoporotic diseases (1, 2), has been linked to the occurrence of a variety of other common chronic diseases such as hypertension (3), cardiovascular diseases (CVDs)[4] (4), diabetes mellitus (5), several types of cancer (6), infections (7, 8), and several autoimmune conditions (9). Therefore, vitamin D deficiency might be an underestimated, underlying cause of premature death.

A recent review and meta-analysis of randomized controlled trials (RCTs) concluded that vitamin D_3 supplementation could prevent a number of premature deaths because RCTs showed an overall 6% decrease in total mortality (10). However, this result was not replicated in an individual-patient data meta-analysis of RCTs that supplemented only vitamin D and not in combination with calcium (11). Most currently available RCTs have been of limited value for the endpoint mortality because their primary outcomes were osteoporotic endpoints, and they included participants who do not reflect general population samples (10). Generalizability to the normal population is given in population-based cohort studies, and a recently published meta-analysis of such studies observed an 8% decrease in total mortality per 20-nmol/L increase in 25-hydroxyvitamin D [25(OH)D] concentrations (for ng/mL, divide by 2.496) (12).

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[4] Abbreviations used: CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; ICD-10, International Classification of Diseases, 10th Revision; IOM, Institute of Medicine; LC-MS/MS, liquid chromatography tandem-mass spectrometry; RCT, randomized controlled trial; 25(OH)D, 25-hydroxyvitamin D.

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There are still important, unanswered research questions about the relation of vitamin D and mortality. The recently proposed cutoffs by the US-American Institute of Medicine (IOM) for vitamin D deficiency [25(OH)D concentrations <30 nmol/L) and vitamin D insufficiency (25(OH)D concentrations from 30 to 50 nmol/L) (13, 14) are currently under debate because they are solely based on the association of 25(OH)D concentrations with bone health (15), and it is unknown whether these risk categories are also appropriate for mortality endpoints. A reanalysis of prospective cohort studies suggested a nonlinear association with an optimal range of 25(OH)D concentrations from 75 to 87.5 nmol/L with increasing risk <75 nmol/L and a potential second rise in high 25(OH)D concentrations >112.5 nmol/L (16).

Because of the rapid progress in laboratory analytics of 25(OH)D concentrations since 2008, risk classification needs to be verified in new studies with 25(OH)D immunoassays standardized to the current gold-standard method of liquid chromatography tandem-mass spectrometry (LC-MS/MS) (17–20). Furthermore, to our knowledge, no previous study has used repeated 25(OH)D measurements. Concentrations of 25(OH)D may change during follow-up, which could lead to an underestimation of vitamin D effect. (21). Finally, results for cancer mortality have been very heterogeneous (22, 23), and to our knowledge, no study on the association of 25(OH)D concentrations and respiratory disease mortality has been performed thus far.

Therefore, we conducted a repeated-measurement analysis on the association of LC-MS/MS–standardized 25(OH)D measurements with all-cause, CVD, cancer, and respiratory disease mortality in a large cohort of older adults, with particular attention given to the shape of the dose-response relations and potential cutoffs for risk categories.

SUBJECTS AND METHODS

Study design

This investigation was based on the ESTHER study [Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung (German)], which is an ongoing cohort study, details of which have been reported elsewhere (24). Briefly, 9949 men and women, aged 50–74 y at baseline, were recruited by their general practitioners during a routine health checkup between 2000 and 2002 in the German federal state of Saarland and have been recontacted after 2, 5, and 8 y of follow-up thus far. The distribution of sociodemographic baseline characteristics and common prevalent chronic diseases were similar to the distribution in the respective age categories in the German National Health Survey, which is a representative sample of the German population (24, 25). Thus, this similarity supports the population-based character of the study.

25(OH)D measurements

Blood samples were taken at baseline and the 5-y follow-up at offices of general practitioners, centrifuged, shipped to the study center, and stored at −80°C. The long-term stability of 25(OH)D serum concentrations for ≥10 y is provided under this conditions (26). The automated Diasorin-Liaison analyzer (Diasorin Inc) was used to measure 25(OH)D concentrations in women from stored baseline serum samples in the central laboratory of the University Clinic of Heidelberg in 2006 in the framework of a project on women's health. A within-assay CV of 8–21% and a between-assay CV of 8–34% have been ascribed to the Diasorin-Liaison analyzer (27). The lower detection limit in our laboratory was 15 nmol/L. For a new project, funding was obtained in 2009 to measure 25(OH)D in men from stored baseline serum samples and in the 5-y follow up for both sexes. The Diasorin-Liaison method used for women was unavailable because it had been replaced by the manufacturer by another method in 2007 (27). Therefore, the automated IDS-iSYS analyzer (Immunodiagnostic Systems GmbH) was used for new measurements, and these were performed in the laboratory of the Institute for Experimental Endocrinology, Charité University Medicine, Berlin, Germany. According to the information from the manufacturer, the assay has an intraassay CV <7.3%, an interassay CV <8.9%, and a lower detection limit of 9 nmol/L (27).

Both immunoassays were standardized retrospectively to the gold standard method LC-MS/MS, and details have been described elsewhere (28). In brief, for each of the 2 assays used, random baseline serum samples of 100 study participants were drawn and remeasured with isotope-dilution LC-MS/MS in 2011 in the Department of Clinical Chemistry, Canisius Wilhelma Hospital, Nijmegen, Netherlands. Details of standardization, precision, and comparability to other assays have been described elsewhere (29). The Spearman's rank correlation between measurements with the Diasorin-Liaison analyzer and LC-MS/MS and between the IDS-iSYS analyzer and LC-MS/MS was high ($r = 0.83$ and $r = 0.86$, respectively). Although absolute measurements of the IDS-iSYS analyzer and LC-MS/MS were comparable, values were consistently substantially lower for measurements with the Diasorin-Liaison analyzer than with LC-MS/MS. Therefore, ordinary least-squares linear regression equations were fitted, and results were used for the standardization of 25(OH)D concentrations measured with the Diasorin-Liaison analyzer

$$\begin{aligned}\text{25(OH)D (LC-MS/MS) [nmol/L]} = {} & 0.7989 \\ & \times \text{25(OH)D (Diasorin-Liaison analyzer) [nmol/L]} \\ & + 17.58\ \text{nmol/L}\end{aligned} \quad (1)$$

or with the IDS-iSYS analyzer

$$\begin{aligned}\text{25(OH)D (LC-MS/MS) [nmol/L]} = {} & 0.9526 \\ & \times \text{25(OH)D (IDS-iSYS analyzer) [nmol/L]} \\ & - 0.3222\ \text{nmol/L}\end{aligned} \quad (2)$$

Covariate assessment

Information on sociodemographic characteristics, lifestyle, and diet were obtained by using a comprehensive questionnaire from study participants at baseline. Height, weight, systolic blood pressure, and history of diabetes, coronary artery disease, or hypertension were assessed and documented on a standardized form by general practitioners during the health checkup. Prevalent CVD was defined by physician-reported coronary artery disease or a self-reported history of myocardial infarction, stroke, pulmonary embolism, or revascularisation of coronary arteries (a bypass or stent). Information on a life-time history of cancer [International Classification of Diseases, 10th Revision (ICD-10) codes C00–D48] was provided by the Saarland Cancer Registry. Furthermore, blood and urine samples were taken during the

health checkup, centrifuged, sent to the study center, and stored at −80°C until analysis. Total cholesterol was measured from serum samples on the Beckman Synchron LX, C-reactive protein (CRP) was measured by using turbidimetry, and cystatin C was determined by using immunonephelometry. Chronic kidney disease (CKD) was defined according to an estimated glomerular filtration rate <60 mL $min^{-1} \cdot 1.73 \cdot m^{-2}$ according to the following equation (30):

$$\text{Estimated glomerular filtration rate} = 74.835 \div (\text{CysC}^{1.333}) \quad (3)$$

At the 5-y follow-up, study participants were asked to donate blood at the next visit at the practice of their general practitioner, and procedures of sample preparation, storage, and biomarker analyses were identical to those at baseline.

Mortality ascertainment

Deaths during follow-up between 2000 and 2010 were identified by inquiry at the residents' registration offices, and information about the vital status of 99.9% of cohort participants were obtained. Death certificates were provided by public health departments for 97.7% of subjects who had died. All deaths coded with ICD-10 codes I00–I99 were considered cardiovascular deaths, cancer deaths were defined by ICD-10 codes C00–C99 and D37–D48, and deaths that were due to diseases of the respiratory system were defined by ICD-10 codes J00–J99.

Statistical analyses

Participants of the ESTHER baseline examination ($n = 9949$) were excluded from the investigation if they had a missing 25(OH)D measurement ($n = 369$) or could not be followed up ($n = 2$), which resulted in a total sample size of $n = 9578$ subjects for this analysis.

All analyses where done with LC-MS/MS–standardized 25(OH)D concentrations. Analyses were conducted according to IOM cutoffs by comparing subjects with vitamin D deficiency (<30 nmol/L) and insufficiency (30–50 nmol/L) with subjects with adequate vitamin D concentrations (>50 nmol/L) (13, 14). In a sensitivity analysis, 25(OH)D concentrations >75 nmol/L were used as the reference group, and an additional group with 25(OH)D concentrations from 50 to 75 nmol/L was analyzed because this group is also under debate as having insufficient 25(OH)D concentrations (15). Because there is no consensus about cutoffs for 25(OH)D risk categories, analyses were repeated in an additional sensitivity analysis with 25(OH)D concentration quintiles by using the highest quintile as the reference.

Differences in baseline characteristics in the 3 vitamin D categories were assessed by using the chi-square test (for categorical variables) or Wilcoxon's rank-sum test (for continuous variables). The survival of participants in different vitamin D categories was compared graphically in unadjusted Kaplan-Meier survival curves and statistically with a log-rank test. Furthermore, Cox proportional hazards models were used to estimate HRs (95% CIs) with respect to all-cause, CVD, cancer, and respiratory disease mortality. Three models were built with an increasing number of established all-cause mortality risk factors and determinants of serum 25(OH)D concentrations as covariates. Continuous covariates were modeled with the best-fitting function determined by fractional polynomials with first-order terms (31). In model 1, adjustment was made for age, sex, and month of blood draw (categorical in 2-mo intervals beginning with January and February). Model 2 was the fully adjusted model with additional adjustment for regular intake of multivitamin supplements, fish consumption <1 time/wk, BMI^{-2}, school education (≤9, 10–11, or ≥12 y), physical activity [low (≤1 h/wk of extensive physical activity that caused sweating) or medium/high (>1 h/wk)], smoking (never, former, or current), $(\text{systolic blood pressure})^3$, CKD, log(CRP), and $(\text{total cholesterol})^{-2}$. Model 3 additionally included potential intermediates in the association of 25(OH)D and mortality (ie, a history of diabetes, hypertension, CVD, and cancer).

All Cox proportional hazards models used repeated measurements from the 5-y follow-up by fitting time-dependent variables (32). In sensitivity analyses, analyses were repeated without time-dependent modeling by using only baseline data with *1*) full follow-up of up to 10 y and *2*) up to 5 y of follow-up. Furthermore, we carried out the analyses by using the 5-y follow-up as baseline with 25(OH)D values measured at the 5-y follow-up only. Potential interactions of vitamin D deficiency and insufficiency with covariates were tested for significance by adding pertinent product terms to model 3. Finally, dose-response relations were plotted with restricted cubic splines (33).

In sensitivity analyses to consider the potential bias by reverse causality, analyses on CVD were repeated in subjects without CVD at baseline, and analyses on cancer mortality were repeated by excluding subjects with a life-time history of cancer or who died of cancer in the first 5 y of follow-up.

All statistical tests were 2-sided with an α level of 0.05, and all analyses were conducted with the software package SAS (version 9.2; SAS Institute Inc).

Handling of missing values

Multiple imputation was used to adequately deal with missing baseline covariate values. The Markov Chain Monte Carlo method of the SAS procedure PROC MI was used to impute 5 data sets stratified by sex (34). The following variables were used for the imputation model (the percentage of imputed missing values in the study population of $n = 9578$ is given in parentheses): age (0%), education (2.5%), smoking status (2.8%), CVD (0%), cancer (0.2%), diabetes (0.1%), hypertension (0.1%), systolic blood pressure (2.3%), BMI (0.1%), total cholesterol (0.3%), CRP (1.5%), CKD (0.4%), physical activity (0.3%), regular multivitamin intake (2.1%), regular fish consumption (5.8%), 25(OH)D (0%), and season of blood draw (0%). All variables were modeled continuously if possible. From systolic blood pressure, BMI, total cholesterol, and CRP, the logarithm was taken because they were not normally distributed. Categorized variables were modeled according to the groups shown in **Table 1**. Overall, 1108 subjects had at least one missing value from the variables (11.6%). The multiple-imputation assumption (values missing at random) was examined by comparing individuals with complete data with those with incomplete data. Subjects with missing values did not differ from subjects with complete information for most of the baseline characteristics shown in Table 1. However, subjects with missing values, compared with subjects with complete information, had lower mean 25(OH)D concentrations (50.2 compared with 51.3 nmol/L, respectively; $P = 0.01$), a higher mortality rate (160 compared with 120 deaths/100 person-years, respectively; $P < 0.01$), a higher

TABLE 1
Characteristics of the study population at baseline and the 5-y follow-up[1]

	Baseline		5-y follow-up	
Characteristics	n_{total}	Values	n_{total}	Values
Sex (M) [% (n_{char})]	9578	43.8 (4196)	8019	43.3 (3471)
Age (y)	9578	62 ± 6.5[2]	8019	67 ± 6.5
Age ≥65 y [% (n_{char})]	9578	38.7 (3709)	8019	66.1 (5297)
Scholarly education [% (n_{char})]	9337		Not assessed	—
≤9 y		75.0 (7005)		
9–11 y		14.1 (1313)		
≥12 y		10.9 (1019)		
Smoking [% (n_{char})]	9312	—	7900	—
Never		50.5 (4708)		54.1 (4275)
Former		32.5 (3029)		34.9 (2753)
Current		16.9 (1575)		11.0 (827)
Cardiovascular disease [% (n_{char})]	9577	19.5 (1868)	7983	25.6 (2040)
Cancer [% (n_{char})]	9558	8.5 (813)	7970	12.3 (977)
Diabetes [% (n_{char})]	9451	14.7 (1385)	7892	18.1 (1427)
Hypertension [% (n_{char})]	9565	53.2 (5089)	7982	66.5 (5311)
Systolic blood pressure (mm Hg)	9355	140 ± 19.6	Not assessed	—
BMI (kg/m^2)	9565	27.7 ± 4.4	7944	27.7 ± 4.5
Obesity (BMI ≥30) [% (n_{char})]	9565	25.5 (2443)	7944	26.3 (2085)
Total cholesterol (mg/dL)	9554	220 ± 51	4889	239 ± 51
CRP (mg/L)	9441	4.2 ± 8.8	4881	4.6 ± 9.4
Chronic kidney disease [% (n_{char})]	9541	8.8 (836)	Not assessed	—
Low physical activity [% (n_{char})]	9549	67.2 (6412)	7941	55.8 (4428)
Regular intake of multivitamin supplements [% (n_{char})]	9375	14.4 (1354)	7971	11.0 (874)
Fish consumption <1 time/wk [% (n_{char})]	9025	33.5 (3022)	7849	25.7 (2018)
25(OH)D (nmol/L)	9578	51.1 ± 24.6	5469	56.5 ± 26.1
25(OH)D categories [% (n_{char})]	9578	—	5469	—
<30 nmol/L		15.1 (1444)		13.3 (726)
30–50 nmol/L		43.8 (4199)		34.2 (1868)
>50 nmol/L		41.1 (3935)		52.6 (2875)

[1] n_{total} values do not always add up to the total of n = 9578 at baseline and n = 8019 at 5-y follow-up because of missing values. CRP, C-reactive protein; n_{char}, number of participants with the characteristic; n_{total}, number of participants with data for the characteristic; 25(OH)D, 25-hydroxyvitamin D.
[2] Mean ± SD (all such values).

mean age (63 compared with 62 y, respectively; $P < 0.01$), were more frequently insufficiently physically active (73.9% compared with 66.3%, respectively; $P < 0.01$), had diabetes more frequently (18.3% compared with 14.2%, respectively; $P < 0.01$) and higher mean CRP concentrations (4.5 compared with 4.1 mg/L, respectively). These variables described a characteristic lifestyle with low physical activity and adverse diet. The missing-at-random assumption could be assumed because missing values could be explained by other covariates of the imputation model that were also associated with this characteristic lifestyle.

From 9067 survivors at the 5-y follow-up, 8019 subjects (88.4%) sent back the questionnaire, and 5469 subjects (60.3%) provided another blood sample at a follow-up visit with their general practitioner. Therefore, the number of missing values for biomarkers was higher than for other variables and too high to be imputed by, eg, multiple imputation. Furthermore, education, systolic blood pressure, and CKD were not assessed again at the 5-y follow-up. For these variables, baseline values were also used for the 5-y follow-up in time-dependent analyses, with the assumption that the values have not changed. Similarly, missing values in variables that have been assessed again at 5-y follow-up were also filled with values from the baseline examination, with the assuming that they have not changed during the first 5 y of follow-up. If the value was also missing at baseline, the imputed value of the multiple imputation for baseline variables was taken.

All analyses were performed in the 5 imputed data sets, and results of individual data sets were combined by using the SAS procedure PROC MIANALYZE, with the variation between results of the 5 data sets taken into account.

RESULTS

Characteristics of the study population at baseline and the 5-y follow-up are shown in Table 1. At baseline, the mean (±SD) age of the 9578 included study participants was 62 ± 6.5 y, and 4196 (43.8%) of these participants were men. During a median follow-up time of 9.5 y (IQR: 8.9–9.9 y), 1083 study participants died, of whom 350 individuals died of CVDs, 433 individuals died of cancer, and 55 individuals died of respiratory diseases. At the 5-y follow-up, 511 study participants had died, and 8019 participants responded to the questionnaire (response rate in survivors: 88.4%). The burden of risk factors for premature mortality increased considerably in the first 5 y of follow-up with the exception of a decrease in the proportions of current smokers and subjects with low physical activity and low fish consumption (Table 1). The proportion of subjects with vitamin D

deficiency was similar at baseline (15.1%) and the 5-y follow-up (13.3%), but the proportion of subjects with vitamin D insufficiency was higher at baseline (43.8% compared with 34.2%, respectively). To compare subjects with repeated 25(OH)D measurements standardized for season effects on 25(OH)D, 25(OH)D concentrations were z transformed in 2-mo intervals with respect to the months of blood draw. The normally distributed z scores of 25(OH)D concentrations in the 2-mo intervals were recombined thereafter. Season-standardized 25(OH)D z scores from baseline and the 5-y follow-up showed a clear correlation (Spearman's $r = 0.47$, $P < 0.001$), and 71.3% of the study population stayed in the same season-standardized quintile or moved up or down one quintile.

Characteristics of the study population stratified by baseline vitamin D categories are shown in **Table 2**. With only a few exceptions (male sex, history of cancer, and total cholesterol concentrations), the burden of risk factors for premature mortality increased from vitamin D sufficiency over vitamin D insufficiency to vitamin D deficiency.

All-cause mortality

The dose-response relation of 25(OH)D concentrations with all-cause mortality is shown in **Figure 1**A. The restricted cubic-spline curve shows increasing mortality with decreasing 25(OH)D concentrations less than ~75 nmol/L that was significant at 50 nmol/L with an ~1.2-fold higher mortality and at 30 nmol/L with an ~1.6-fold increased mortality.

Overall, survival was significantly lower in subjects with vitamin D deficiency than in subjects with sufficient vitamin D concentrations ($P < 0.01$) and subjects with vitamin D insufficiency ($P < 0.01$) (**Figure 2**A). Furthermore, HRs for subjects with vitamin D deficiency were strongly increased in models adjusted for age, sex, and season (model 1) and were attenuated only slightly by adjusting for conventional risk factors for premature mortality (model 2) and common chronic diseases (model 3) (**Table 3**). In tests for potential interactions of vitamin D categories with risk factors for premature mortality, only the interaction term of vitamin D insufficiency and obesity was significant ($P = 0.03$) with a stronger association in nonobese subjects than in obese subjects (Table 3). Although mortality was much higher in subjects with common chronic diseases, the HRs for all-cause mortality according to 25(OH)D categories were comparable to those in subjects who did not suffer from the diseases.

CVD mortality

The restricted cubic-spline curve of the association of 25(OH)D concentrations and CVD mortality was flatter (Figure 1B) than the curve for all-cause mortality (Figure 1A). Subjects with vitamin D insufficiency did not have a lower survival with respect to CVD mortality than subjects with sufficient vitamin D concentrations (Figure 2B; $P = 0.46$), and this was also mirrored in adjusted HRs close to 1 (**Table 4**). In contrast, survival with respect to CVD mortality was significantly lower in subjects

TABLE 2
Baseline characteristics of study population stratified by 25(OH)D status[1]

	Vitamin D deficiency [<30 nmol 25(OH)D/L]		Vitamin D insufficiency [30–50 nmol 25(OH)D/L]		Sufficient vitamin D status [>50 nmol 25(OH)D/L]	
Characteristics	n_{total}	Values	n_{total}	Values	n_{total}	Values
25(OH)D concentrations (nmol/L)	1444	29.5 (25.6–29.5)[2,]*	4199	36.4 (34.7–44.5)*	3935	65.5 (56.5–80.8)
Sex (M) [% (n_{char})]	1444	41.3 (597)*	4199	32.6 (1368)*	3935	56.7 (2231)
Age ≥65 y [% (n_{char})]	1444	42.2 (609)*	4199	40.6 (1706)*	3935	35.4 (1394)
Scholarly education [% (n_{char})]	1404		4083		3850	
≤9 y		76.1 (1068)		76.7 (3133)*		72.8 (2804)
10–11 y		12.7 (278)		14.2 (581)*		14.4 (554)
≥12 y		11.3 (158)		9.0 (369)*		12.8 (492)
Smoking [% (n_{char})]	1390		4075		3847	
Never		49.1 (683)*		55.4 (2257)*		46.0 (1768)
Former		26.6 (370)*		27.9 (1138)*		39.5 (1521)
Current		24.2 (337)*		16.7 (680)*		14.5 (558)
Cardiovascular disease [% (n_{char})]	1443	22.8 (329)*	4199	18.3 (769)	3935	19.6 (770)
Cancer [% (n_{char})]	1438	8.3 (120)	4187	8.7 (365)	3933	8.3 (328)
Diabetes [% (n_{char})]	1427	18.7 (267)*	4141	14.7 (607)	3883	13.2 (511)
Systolic blood pressure (mm Hg)	1401	140 (130–150)*	4110	140 (130–150)*	3844	140 (125–150)
Hypertension [% (n_{char})]	1442	56.2 (801)*	4193	55.3 (2319)*	3930	49.9 (1960)
BMI (kg/m^2)	1441	27.7 (24.9–30.6)*	4195	27.5 (24.9–30.5)*	3929	26.8 (24.5–29.4)
Obesity (BMI ≥30) [% (n_{char})]	1441	30.4 (438)*	4195	28.5 (1195)*	3929	20.6 (810)
Total cholesterol (mg/dL)	1442	219 (181–252)	4189	223 (189–254)*	3923	219 (188–250)
CRP (mg/L)	1422	2.2 (1.1–4.8)*	4142	2.2 (1.0–4.6)*	3877	2.0 (0.9–4.3)
Chronic kidney disease [% (n_{char})]	1437	9.7 (139)*	4190	9.5 (397)*	3914	7.7 (300)
Low physical activity [% (n_{char})]	1436	74.7 (1072)*	4189	70.3 (2945)*	3924	61.0 (2395)
Daily intake of multivitamin supplements [% (n_{char})]	1412	11.0 (155)*	4094	13.6 (557)*	3869	16.6 (642)
Fish consumption <1 time/wk [% (n_{char})]	1326	35.7 (473)*	3942	34.2 (1349)*	3757	31.9 (1200)

[1] n_{total} values do not always add up to the total because of missing values. Chi-square-test or Wilcoxon's rank-sum test were used for categorical and continuous data, respectively. *$P < 0.05$ for comparisons of characteristics with sufficient vitamin D concentrations as the reference. CRP, C-reactive protein; n_{char}, number of participants with the characteristic; n_{total}, number of participants with data for the characteristic; 25(OH)D, 25-hydroxyvitamin D.
[2] Median; IQR in parentheses (all such values).

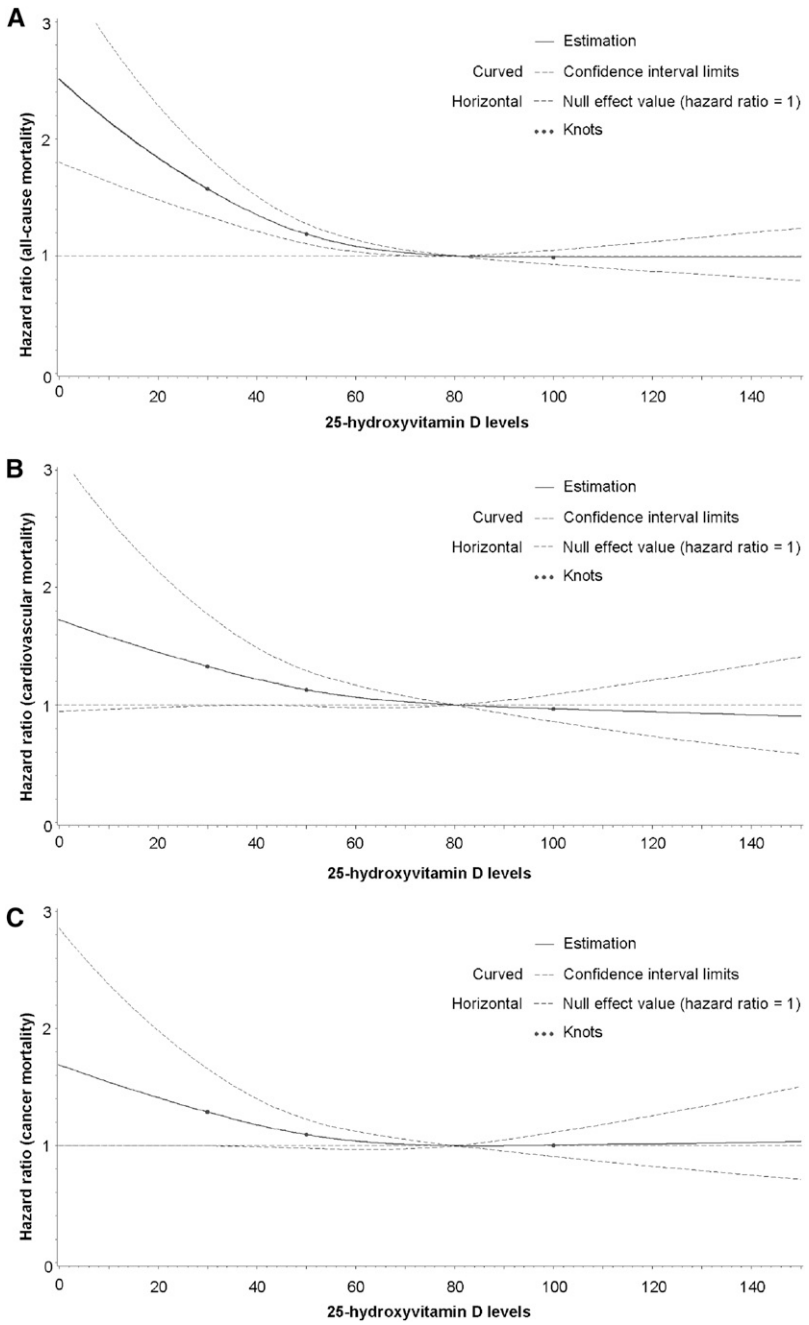

FIGURE 1. Dose-response relation between serum 25-hydroxyvitamin D concentrations and all-cause mortality (A), cardiovascular mortality (B), and cancer mortality (C) in 9578 subjects. Curves were assessed by using restricted cubic splines with knots at 25-hydroxyvitamin D concentrations of 30, 50, and 100 nmol/L, and a 25-hydroxyvitamin D concentration of 80 nmol/L was used as the reference.

with vitamin D deficiency (Figure 2B; $P = 0.02$). However, the association with CVD mortality was comparable to the association with all-cause mortality after the exclusion of subjects with prevalent CVD (1.63; 95% CI: 1.06, 2.52).

Cancer mortality

The dose-response relation of 25(OH)D concentrations with cancer mortality showed a curve that was similar to all-cause mortality but with a flatter run (Figure 1C). As observed for CVD mortality, subjects with vitamin D insufficiency did not have a lower survival with respect to cancer mortality than did subjects with sufficient vitamin D concentrations as determined by using Kaplan-Meier survival curves (Figure 2C; $P = 0.66$) and adjusted HRs (Table 4). In contrast, survival with respect to cancer mortality was significantly decreased in subjects with vitamin D deficiency (Figure 2C; $P < 0.01$), but the association was weaker (1.42; 95% CI: 1.08, 1.88) than that for all-cause mortality (Table 4). The exclusion of subjects with a life-time history of cancer did not alter the HR (1.47; 95% CI: 1.06, 2.03), and the additional exclusion of cancer deaths in the first 5 y of follow-up led to a slight increase in the HR (1.74; 95% CI: 1.23, 2.47).

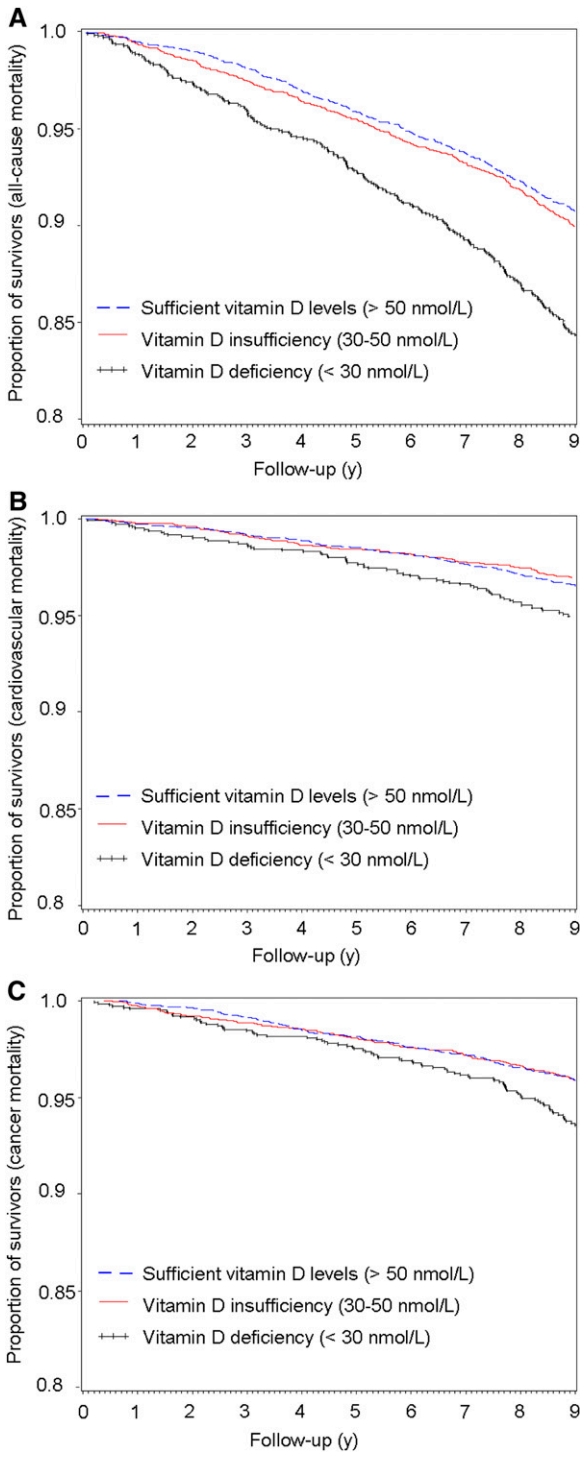

FIGURE 2. Kaplan-Meier survival curves for subjects with vitamin D deficiency (n = 1444), vitamin D insufficiency (n = 4199), and sufficient vitamin D concentrations (n = 3935) with respect to the outcomes all-cause mortality (A), cardiovascular mortality (B), and cancer mortality (C). The log-rank test for comparison of Kaplan-Meier survival curves determined that survival with respect to all outcomes was significantly lower in subjects with vitamin D deficiency than in subjects with sufficient vitamin D concentrations: P-all-cause mortality < 0.01, P-cardiovascular mortality = 0.02, and P-cancer mortality < 0.01. The survival of subjects with vitamin D insufficiency was only significantly lower than for subjects with sufficient vitamin D concentrations for the outcome all-cause mortality ($P < 0.01$) but not for the outcomes cardiovascular mortality ($P = 0.46$) or cancer mortality ($P = 0.66$).

Respiratory disease mortality

Beside CVD and cancer mortality, vitamin D deficiency and insufficiency were furthermore significantly associated with respiratory disease mortality (Table 4).

Sensitivity analyses

In analyses with 25(OH)D quintiles (data not shown), results for first and second 25(OH)D quintiles were very close to the shown results for vitamin D deficiency and insufficiency, respectively. Furthermore, HRs were slightly higher for subjects with vitamin D deficiency and insufficiency when the reference group was limited to subjects with 25(OH)D concentrations >75 nmol/L. Compared with this reference group, subjects with 25(OH)D concentrations from 50 to 75 nmol/L had slightly, albeit nonsignificantly, increased risk of all-cause and cardiovascular mortality, whereas risk of cancer mortality was not increased [HRs in model 3: 1.13 (95% CI: 0.91, 1.40), 1.24 (95% CI: 0.86, 1.79), and 1.03 (95% CI: 0.75, 1.42), respectively]. This sensitivity analysis was not conducted for respiratory diseases mortality because of sample-size limitations. In sensitivity analyses without time-dependent modeling that used the baseline or 5-y follow-up 25(OH)D measurements only (and in some analyses only up to 5 y of follow-up duration), the direction or magnitude of effect estimates did not change to any relevant extent.

DISCUSSION

In this large, population-based cohort study with repeated measurements, vitamin D deficiency was strongly associated with all-cause, cardiovascular, cancer, and respiratory disease mortality. The association of 25(OH)D concentrations with all-cause mortality was shown to be a nonlinear inverse association, with mortality starting to increase slightly at 25(OH)D concentrations <75 nmol/L. For subjects with vitamin D deficiency (<30 nmol/L), an 1.7-fold increased mortality was determined. The observed patterns were consistent in men, women, different age groups, and subjects with or without common chronic diseases.

To our knowledge, this is the first study to analyze the association of LC-MS/MS–standardized 25(OH)D concentrations with mortality endpoints to evaluate cutoffs for vitamin D deficiency and insufficiency proposed by the IOM. The results are not directly comparable to previous studies that mostly did not have LC-MS/MS–standardized 25(OH)D concentrations and explored survival differences in 25(OH)D tertiles, quartiles, or quintiles. Nevertheless, the highest mortality was also observed in the group with lowest 25(OH)D concentrations in most (35–44) but not all (45, 46) previously conducted population-based cohort studies (12). The deviation from linearity, with an increased mortality only in 25(OH)D concentrations <75 nmol/L, was in line with a recent meta-analysis of prospective cohort studies by Zittermann et al (16). The only difference was that Zittermann et al (16) reported on a slight, nonsignificant second rise in mortality at ≥112.5 nmol/L that was mainly driven by a study from Sweden (45). In our study, we did not observe any signs for an increased mortality at such high 25(OH)D concentrations, but we could not exclude this possibility because our sample size of subjects with 25(OH)D concentrations that exceeded 112.5 nmol/L was rather small (n = 210; 2.2%). On the

TABLE 3
Associations of vitamin D deficiency and insufficiency with all-cause mortality[1]

Groups	25(OH)D	n_{total}	n_{cases}	IR	Model 1	Model 2	Model 3	*P*-interaction
	nmol/L							
All	<30	1444	238	18.6	1.96 (1.65, 2.33)[2,]*	1.71 (1.43, 2.03)*	1.68 (1.41, 2.01)*	—
	30–50	4199	448	11.7	1.25 (1.08, 1.44)*	1.17 (1.02, 1.35)*	1.17 (1.01, 1.35)*	—
	>50	3935	397	11.0	Ref	Ref	Ref	—
Women	<30	847	113	14.6	1.90 (1.44, 2.50)*	1.73 (1.30, 2.30)*	1.57 (1.18, 2.08)*	0.59
	30–50	2831	238	9.1	1.22 (0.97, 1.55)	1.22 (0.96, 1.54)	1.15 (0.91, 1.46)	0.98
	>50	1704	105	6.6	Ref	Ref	Ref	—
Men	<30	597	125	24.5	2.09 (1.66, 2.61)*	1.80 (1.43, 2.66)*	1.77 (1.40, 2.22)*	—
	30–50	1368	210	17.4	1.29 (1.07, 1.55)*	1.17 (0.97, 1.40)	1.19 (0.99, 1.43)	—
	>50	2231	292	14.5	Ref	Ref	Ref	—
≥65 y of age	<30	609	142	27.1	1.78 (1.42, 2.22)*	1.53 (1.22, 1.92)*	1.41 (1.13, 1.77)*	0.38
	30–50	1706	269	17.8	1.17 (0.97, 1.41)	1.10 (0.92, 1.33)	1.09 (0.90, 1.31)	0.78
	>50	1394	236	19.1	Ref	Ref	Ref	—
<65 y of age	<30	835	96	12.7	2.28 (1.74, 2.97)*	2.06 (1.56, 2.72)*	2.08 (1.58, 2.76)*	—
	30–50	2493	179	7.8	1.38 (1.10, 1.72)*	1.05 (1.05, 1.65)*	1.30 (1.04, 1.63)*	—
	>50	2541	161	6.8	Ref	Ref	Ref	—
Obesity	<30	438	64	16.2	1.38 (0.98, 1.91)	1.27 (0.91, 1.79)	1.17 (0.84, 1.64)	0.67
	30–50	1195	144	13.3	1.18 (0.90, 1.54)	1.10 (0.84, 1.45)	1.07 (0.82, 1.41)	0.03*
	>50	810	110	15.0	Ref	Ref	Ref	—
Nonobese	<30	1003	174	19.7	2.23 (1.82, 2.72)*	1.90 (1.54, 2.33)*	1.90 (1.54, 2.34)*	—
	30–50	3000	304	11.1	1.26 (1.06, 1.49)*	1.18 (1.00, 1.41)*	1.18 (1.00, 1.40)*	—
	>50	3119	285	9.9	Ref	Ref	Ref	—
Diabetes	<30	267	60	26.4	1.61 (1.12, 2.28)*	1.54 (1.07, 2.23)*	1.55 (1.07, 2.23)*	0.95
	30–50	607	126	23.9	1.36 (1.02, 1.82)*	1.31 (0.98, 1.76)	1.32 (0.98, 1.77)	0.17
	>50	511	93	20.6	Ref	Ref	Ref	—
Without diabetes	<30	1160	175	16.8	2.00 (1.64, 2.44)*	1.72 (1.41, 2.11)*	1.69 (1.37, 2.07)*	—
	30–50	3534	315	9.7	1.17 (0.99, 1.39)	1.11 (0.94, 1.31)	1.10 (0.93, 1.30)	—
	>50	3372	301	9.7	Ref	Ref	Ref	—
Hypertension	<30	810	170	24.1	2.01 (1.63, 2.48)*	1.81 (1.47, 2.25)*	1.77 (1.43, 2.19)*	0.21
	30–50	2319	309	14.8	1.24 (1.04, 1.48)*	1.20 (1.00, 1.43)*	1.18 (0.99, 1.41)	0.84
	>50	1960	261	14.7	Ref	Ref	Ref	—
Without hypertension	<30	632	68	11.8	1.78 (1.31, 2.41)*	1.49 (1.09, 2.04)*	1.44 (1.05, 1.97)*	—
	30–50	1874	139	8.1	1.24 (0.97, 1.59)	1.14 (0.89, −1.47)	1.15 (0.90, 1.47)	—
	>50	1970	136	7.4	Ref	Ref	Ref	—
CVD	<30	329	92	33.9	1.97 (1.48, 2.62)*	1.79 (1.33, 2.39)*	1.69 (1.26, 2.26)*	0.87
	30–50	769	164	24.7	1.35 (1.07, 1.72)*	1.27 (1.00, 1.62)*	1.25 (0.98, 1.60)	0.53
	>50	770	146	21.6	Ref	Ref	Ref	—
Without CVD	<30	1114	146	14.5	1.89 (1.52, 2.34)*	1.60 (1.28, 1.99)*	1.61 (1.29, 2.01)*	—
	30–50	3430	284	9.0	1.19 (1.00, 1.43)*	1.11 (0.92, 1.32)	1.09 (0.91, 1.31)	—
	>50	3165	251	8.6	Ref	Ref	Ref	—
Cancer	<30	120	39	41.5	1.83 (1.19, 2.77)*	1.59 (1.04, 2.44)*	1.58 (1.04, 2.43)*	0.46
	30–50	365	85	27.9	1.29 (0.92, 1.81)	1.19 (0.85, 1.67)	1.19 (0.85, 1.66)	0.35
	>50	328	68	23.6	Ref	Ref	Ref	—
Without cancer	<30	1318	196	16.6	1.99 (1.64, 2.40)*	1.73 (1.42, 2.10)*	1.68 (1.38, 2.04)*	—
	30–50	3822	357	10.2	1.23 (1.05, 1.44)*	1.15 (0.98, 1.35)	1.15 (0.98, 1.35)	—
	>50	3605	329	9.9	Ref	Ref	Ref	—
CKD	<30	139	45	39.5	1.49 (1.01, 2.18)*	1.52 (1.02, 2.28)*	1.39 (0.92, 2.08)	0.19
	30–50	379	116	35.6	1.28 (0.96, 1.73)	1.33 (0.99, 1.80)	1.24 (0.91, 1.68)	0.55
	>50	300	81	32.0	Ref	Ref	Ref	—
Without CKD	<30	1298	192	16.5	2.00 (1.65, 2.43)*	1.74 (1.43, 2.12)*	1.75 (1.44, 2.13)*	—
	30–50	3793	331	9.5	1.18 (1.01, 1.39)*	1.13 (0.95, 1.33)	1.13 (0.96, 1.33)	—
	>50	3614	314	9.4	Ref	Ref	Ref	—

[1] Cox proportional hazards regression in 5 imputations created by multiple imputation. Model 1 was adjusted for age, sex, and season of blood draw (categorical in six 2-mo intervals beginning with January and February). Model 2 was adjusted as for variables of model 1 and for regular intake of multivitamin supplements, fish consumption <1 time/wk, BMI^{-2}, scholarly education (≤9, 10–11, or ≥12 y), physical activity (low or medium/high), smoking (never, former, or current), (systolic blood pressure)^3, chronic kidney disease, log(serum C-reactive protein concentrations), and $(\text{total cholesterol})^{-2}$. Model 3 was adjusted as for variables of model 2 and for diabetes, hypertension, cardiovascular disease, and cancer. Subgroup analyses for these diseases were carried out in subjects who had the disease at baseline, and therefore, model 3 was not adjusted for the respective disease of the subgroup analysis. *$P < 0.05$. CKD, chronic kidney disease; CVD, cardiovascular disease; IR, incidence rate per 1000 person-years; n_{cases}, incident case numbers; n_{total}, sample size with imputed missing values in imputed data set no. 1; Ref, reference category; 25(OH)D, 25-hydroxyvitamin D.

[2] HR; 95% CI in parentheses (all such values).

TABLE 4
Associations of vitamin D deficiency and insufficiency with disease-specific mortality[1]

Outcomes	25(OH)D	n_{total}[2]	n_{cases}[2]	IR	Model 1	Model 2	Model 3
	nmol/L						
CVD mortality	<30	1439	71	5.6	1.60 (1.17, 2.15)[3,]*	1.39 (1.02, 1.89)*	1.29 (0.94, 1.76)
	30–50	4188	137	3.6	1.03 (0.81, 1.32)	0.96 (0.74, 1.23)	0.94 (0.73, 1.21)
	>50	3927	142	3.9	Ref	Ref	Ref
Cancer mortality	<30	1439	90	7.0	1.64 (1.25, 2.14)*	1.42 (1.08, 1.88)*	1.42 (1.08, 1.87)*
	30–50	4188	172	4.5	1.11 (0.89, 1.38)	1.05 (0.84, 1.31)	1.04 (0.83, 1.29)
	>50	3927	171	4.7	Ref	Ref	Ref
Respiratory disease mortality[4]	<30	1439	13	1.0	3.34 (1.50, 7.33)*	2.50 (1.12, 5.56)[5,]*	Not calculated[5]
	30–50	4188	26	0.7	2.24 (1.18, 4.39)*	1.97 (1.02, 3.79)[5,]*	
	>50	3927	16	0.4	Ref	Ref	

[1] Cox proportional hazards regression in 5 imputations created by multiple imputation. Model 1 was adjusted for age, sex, and season of blood draw (categorical in six 2-mo intervals beginning with January and February). Model 2 was adjusted as for variables of model 1 and for regular intake of multivitamin supplements, fish consumption <1 time/wk, BMI^{-2}, scholarly education (≤9, 9–11, or ≥12 y), physical activity (low or medium/high), smoking (never, former, or current), (systolic blood pressure)[3], chronic kidney disease, log(serum C-reactive protein concentrations), and $(\text{total cholesterol})^{-2}$. Model 3 was adjusted as for variables of model 2 and for diabetes, hypertension, cardiovascular disease, and cancer. Subgroup analyses for these diseases were carried out in subjects who had the disease at baseline, and therefore, model 3 was not adjusted for the respective disease of the subgroup analysis. $*P < 0.05$. CVD, cardiovascular disease; IR, incidence (mortality) rate per 1000 person-year; n_{cases}, incident case numbers; n_{total}, sample size with imputed missing values in imputed data set no. 1; Ref, reference category; 25(OH)D, 25-hydroxyvitamin D.

[2] Sample sizes and deaths do not add up to the total of 1083 deaths because of 24 excluded deceased subjects with an unknown cause of death. Subjects who died of another cause than the analyzed one were treated as noncases.

[3] HR; 95% CI in parentheses (all such values).

[4] Allocated to the following International Classification of Diseases, 10th Revision code groups: J09–J18 (influenza and pneumonia; $n = 4$;), J40–J47 (chronic lower respiratory diseases; $n = 36$), J60–J70 (lung diseases as a result of external agents; $n = 6$), J80–J84 (other respiratory diseases principally affecting the interstitium; $n = 7$), and J95–J99 (other diseases of the respiratory system; $n = 2$).

[5] Because of a limited number of respiratory deaths, adjustment for respiratory disease mortality has been restricted to the covariates age, sex, season, smoking, and physical activity, and model 3, which adds additional covariates, was not calculated.

contrary, prevalences of vitamin D deficiency (15.1%) and vitamin D insufficiency (43.8%) according to IOM thresholds were high in our cohort, and this finding was consistent with results from other cohorts with older adults from Germany and neighboring countries that used similar cutoffs (47–50).

Setting these cutoffs is a bit arbitrary because the increase in mortality is continuous and does not start abruptly at a certain 25(OH)D concentration. However, with the results of the review of Zittermann et al (16) and our large cohort study taken together, evidence accumulates that optimal 25(OH)D concentrations for mortality endpoints begin at >75 nmol/L, which is also in agreement with results for other health outcomes (51). 25(OH)D concentrations from 50 to 75 nmol/L seem to be an interim range in which we observed a 13% higher mortality compared with 25(OH)D concentrations >75 nmol/L. However, this slightly increased mortality was not statistically significant in our study and needs to be confirmed by other studies and for other health outcomes before 25(OH)D concentrations from 50 to 75 nmol/L could be considered to reflect an insufficient vitamin D status. If 25(OH)D concentrations are <50 nmol/L, our study showed, in agreement with other authors, a significantly increased overall mortality (16), which supported the proposal of the IOM to define vitamin D insufficiency or inadequacy at 25(OH)D concentrations <50 nmol/L (13). The IOM also proposed a second cutoff at 30 nmol/L, which is the necessary minimum concentration to maintain bone health, and could be used to define vitamin D deficiency (13). Risk stratification in vitamin D insufficiency and deficiency was strongly supported by our data because mortality was much higher in subjects with 25(OH)D concentrations <30 nmol/L than in subjects with 25(OH)D concentrations from 30 to 50 nmol/L. Furthermore, this differentiation might also be useful for other endpoints such as CVD or cancer because we observed an increased CVD mortality and cancer mortality only in subjects with vitamin D deficiency. A decision between the formerly often used cutoff for vitamin D deficiency at 25 nmol/L and the new one at 30 nmol/L needs additional exploration by taking into account the association of 25(OH)D concentrations with all relevant health outcomes (51, 52).

Another important concern is whether the proposed cutoffs can be identical for men and women, middle-aged and older adults, and subjects with and without common chronic diseases. Our study is in line with other large cohort studies that did not observe a significant sex difference (35, 37, 42, 44). With respect to older adults, our results agree with Hutchinson et al (35) who observed a nonsignificantly weaker association in subjects >65 y of age than in younger subjects but not with Melamed et al (37) who observed no age difference. However, a slightly weaker association in adults aged ≥65 y can be expected because of the depletion of susceptible effect (53, 54). Subjects with low 25(OH)D concentrations who are still alive at the oldest age might be less prone to health hazards form vitamin D deficiency than are more susceptible subjects with vitamin D deficiency who have died already at a younger age and dropped out of the population at risk. Furthermore, comparable absolute differences in mortality translate to lower values of relative mortality at a higher age simply because of the steep increase of mortality at an older age.

Common chronic diseases at an older age, such as hypertension, diabetes, CKD, CVD, and cancer, had no influence on the association of 25(OH)D concentrations and mortality in our study, which was, with few exceptions [CVD in Melamed et al

(37) and diabetes in Ginde et al (55)], in agreement with interaction test results in other prospective cohort studies (35, 37, 43, 55). Obesity was an exception in our study with a weaker association of 25(OH)D and mortality in obese than in nonobese subjects. A similar pattern was observed in a study from Norway (35) but not in 2 US-American studies (37, 42).

Beside all-cause mortality, our study determined significant associations of vitamin D deficiency with CVD, cancer, and respiratory disease mortality. We have previously reviewed the potential mechanisms by which vitamin D deficiency could influence CVD and cancer mortality (12), and Herr et al (56) provided a comprehensive overview on how vitamin D deficiency could play a role in the development of various respiratory diseases. Therefore, we focus on epidemiologic aspects rather than explanatory mechanisms in the following text.

There are several systematic reviews that have summarized epidemiologic studies on the influence of 25(OH)D concentrations on the endpoint CVD mortality (4, 57, 58), and as in our study, a strong inverse association of 25(OH)D and cardiovascular death was consistently observed. In contrast, the results regarding the association of vitamin D and cancer mortality have been more heterogeneous (22, 59–61). Differing results may be explained by 3 challenges for prospective cohort studies that analyze the association of vitamin D and cancer mortality. First, some cancers seem to be more vitamin D sensitive than others (eg, prostate cancer seems to be a cancer that is non–vitamin D sensitive (61, 62), which can deplete the results for a combined cancer endpoint). Second, the longer follow-up period needed for the endpoint cancer mortality could decrease the strength of the association because 25(OH)D concentrations may change over time (23). Third, for a causal relation between 25(OH)D concentrations and cancer mortality, 25(OH)D needs to be measured ahead of the development of cancer so that the cancer cannot influence 25(OH)D concentrations. The second point was considered in our study by the repeated-measurement analysis. The third point was accounted for by excluding subjects with a life-time history of cancer and cancer deaths in the first 5 y of follow-up. The essentially unchanged HR made reverse causality unlikely. We did not analyze site-specific cancer deaths (first point) because of sample-size limitations.

Sample-size restrictions also prohibited more detailed analyses on respiratory disease mortality and it is up to collaborative work in large consortia to explore which specific diseases could have caused the particularly strong associations of inadequate 25(OH)D concentrations with respect to respiratory disease mortality. Most respiratory disease deaths in our study were due to chronic lower respiratory diseases such as asthma and chronic obstructive pulmonary disease that have also been linked to vitamin D deficiency in other studies (56). Our results are further supported by a recent RCT in subjects with chronic obstructive pulmonary disease that determined a significant reduction in exacerbations in the vitamin D–supplementation arm when subjects had vitamin D deficiency at baseline (63).

The main limitation of our prospective cohort study was its observational nature. Despite adjustment for known potential confounders, we could not rule out that low serum 25(OH)D concentrations are only a nonspecific marker for a poor health status, which is confounded by other unconsidered factors.

Strengths of our study were LC-MS/MS–standardized 25(OH)D measurements, repeated measurements after 5 y, the large size of the cohort, and a large number of events identified via an almost complete nation-wide, registry-based follow-up. We observed that 71.3% of study participants remained in the same season-standardized quintile of 25(OH)D concentrations or moved up or down one quintile 5 y after baseline, which resembled the 67.8% of subjects who remained in the same ± 1 season-standardized quintile after 14 y in a large study from Norway (64). Therefore, the relative stable 25(OH)D concentrations during follow-up were the most likely explanation for the comparable results of the repeated-measurement analysis and the conventional approach of using only one-time measurements from the baseline examination.

In conclusion, vitamin D deficiency was strongly associated with all-cause, cardiovascular, cancer and respiratory disease mortality in this large population-based cohort study. The association of 25(OH)D concentrations with all-cause mortality was shown to be a nonlinear inverse association with risk that started to increase slightly at 25(OH)D concentrations <75 nmol/L and was most strongly increased in subjects with vitamin D deficiency. Our results support the importance of additional research on the potential of lowering mortality by ensuring an adequate vitamin D supply.

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The authors' responsibilities were as follows—BS and HB: designed the research; BS: conducted the research, analyzed data, and had primary responsibility for final content of the manuscript; and all authors: wrote the manuscript and read and approved the final manuscript. None of the authors had a conflict of interest.

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