The Risk of All-Cause Mortality Is Inversely Related to Serum 25(OH)D Levels

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Context and Objectives: Vitamin D plays a key role in maintaining bone health, but evidence for its nonskeletal effects is inconsistent. This study aims to examine the association between serum 25-hydroxyvitamin D [25(OH)D] levels and all-cause mortality in a large general population cohort.

Design, Participants, and Setting: Using the computerized database of the largest health care provider in Israel, we identified a cohort of subjects 20 years old or older with serum 25(OH)D levels measured between January 2008 and December 2009. Vital status was ascertained through August 2011.

Results: Median follow-up was 28.5 months (interquartile range 23.8–33.5 months); 7,247 of 182,152 participants (4.0%) died. Subjects who died had significantly lower serum 25(OH)D levels (mean 44.8 $\pm$ 24.2 nmol/liter) than those alive at the end of follow-up (51.0 $\pm$ 23.2 nmol/liter), $P <$ 0.001. After adjustment for age, gender, ethnicity, and seasonality, the hazard ratio (HR) for all-cause mortality was 2.02 [95% confidence interval (CI) 1.89–2.15] for the lowest serum 25(OH)D quartile (<33.8 nmol/liter) compared with the highest. After further adjustment for comorbidity, use of vitamin D supplements and statins, smoking, socioeconomic status, and body mass index, the HR was 1.81 (95% CI 1.69–1.95). This remained, even after adjustment for serum low-density lipoprotein, high-density lipoprotein, calcium level (corrected for serum albumin levels), and glomerular filtration rate, 1.85 (95% CI 1.70–2.01). The fully adjusted HR associated with being in the second 25(OH)D quartile (33.8–49.4 nmol/liter) was 1.25 (95% CI 1.16–1.34).

Conclusions: All-cause mortality is independently and inversely associated with serum 25(OH)D levels at levels less than 50 nmol/liter. **(*J Clin Endocrinol Metab* 97: 2792–2798, 2012)**

The evidence of the association between vitamin D and a variety of diseases and health indicators has been mounting over recent years (1, 2). Because very few foods naturally contain vitamin D, most of it is gained through food fortification or skin exposure to UVB from the sun (1, 3, 4). Low 25-hydroxyvitamin D [25(OH)D] levels have become more common in modern society because of the decreased sun exposure of the population as a result of reduced outdoor activity and increased sunscreen use, increasing obesity, and changes in food consumption patterns (5).

Historically the function of vitamin D was thought to be limited to keeping normal mineral balance and maintaining bone health (1). In recent years new data suggested that vitamin D receptors are present in most tissues and cells in the body (6–8). In addition, several tissues possess 1α-hydroxylase activity enabling them to produce 1,25(OH)2D locally (6–8). These findings suggest a role for vitamin D in extraskeletal body systems.

Vitamin D has a wide range of suggested biological functions including induction of cell differentiation, inhibition of angiogenesis and cell proliferation, stimulation

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Abbreviations: BMI, Body mass index; CHS, Clalit Health Services; CI, confidence interval; CVD, cardiovascular disease; GFR, glomerular filtration rate; HDL, high-density lipoprotein cholesterol; HR, hazard ratio; LDL, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; SES, socioeconomic status.

of insulin production, inhibition of rennin production, and immune-modulatory proprieties (6, 7, 9–12). Clinical studies have shown that low serum 25(OH)D levels are associated with increased risk of morbidity from all leading chronic diseases namely diabetes mellitus, hypertension, cardiovascular diseases, chronic obstructive pulmonary diseases, and cancer (6, 13–20).

Studies that examined the association between vitamin D and mortality, most of which were not performed in general average risk populations, reached inconsistent results (21–29). Our study was designed to examine the association between serum 25(OH)D level and all-cause mortality in a large cohort of the general Israeli population who underwent testing through their usual medical care services.

Materials and Methods

Study population and data source

The study population was drawn from among the ensurees of the Clalit Health Services (CHS), the largest not-for-profit health care provider in Israel, covering more than half of the Israeli population. CHS's computerized database was established in 1998 and includes among other components a laboratory test results database and a chronic diseases diagnoses database. These databases are considered to be fully valid for most of the components because all of the CHS activities are reported on a centralized computer system covering all of the service suppliers and include laboratories and pharmacies. Data on health habits such as smoking or health-related markers such as body mass index (BMI) or blood pressure levels have been gradually added to the database only in more recent years, and have not reached full coverage yet.

The CHS database was searched for all available serum 25(OH)D test results from January 1, 2008, to December 31, 2009 (431,982 tests in 281,029 subjects). Of these, we selected test results that were performed in subjects who were 20 yr old or older at the time of the test (420,263 tests in 271,176 subjects), reflecting 10.4% of all CHS ensurees at this age. To study laboratory levels with high comparability, only serum 25(OH)D tests that were performed in the two largest CHS laboratories, together performing 67.9% of the tests, were selected for this analysis (285,521 tests). In subjects who performed more than one test during this period, the result of the first test was used (n = 182,152 subjects).

Follow-up

The cohort was followed up for the mortality outcome until August 31, 2011. Vital status was established by matching our data with the National Death Index using a distinct identification number that each resident of Israel has. During the follow-up period, 1757 (~1%) subjects moved from the CHS to another health care provider. Vital status could not be ascertained for these subjects and they were censored at the date of their movement to another health care provider.

Study variables

For the purpose of this study, serum 25(OH)D was classified into predefined categories (<30, 30 to <50, 50 to <75, and ≥ 75 nmol/liter) or into quartiles using two approaches: one based on all measurements and one based on season-specific measurements. Variables studied as possible confounders were demographic variables [age at the time of blood test, gender, and ethnicity (Jews, Arabs)]; season of blood draw [winter (December through February), spring (March through May), summer (June through August), and autumn (September through November)]; and a diagnosis of chronic diseases reflecting leading causes of death [hypertension, diabetes mellitus, any malignancy not including nonmelanoma skin cancer, cardiovascular disease (CVD), ischemic heart disease, cerebrovascular accident, and congestive heart failure]. Recent use of vitamin D supplements and recent use of statins (at yes/no level) were established by searching the pharmacy database for any prescription filled during the last 60 d before enrollment to the study. Smoking status was classified into ever smoking *vs.* never smoking. BMI was calculated [weight (kilograms)/height^2 (meters)]and was classified into four categories (<25, 25 to <30, 30 to <40, and ≥ 40 kg/m^2). Socioeconomic status (SES) was defined based on the SES score of the clinic neighborhood as defined by the Israeli Central Bureau of Statistics. Using the CHS laboratory database, we also searched for concomitant tests of the following serum levels: low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), creatinine, calcium, and albumin. Only blood tests performed ± 6 months from the date of enrollments were selected; if more than one test was available, the nearest blood test to the enrollment date was selected. The glomerular filtration rate (GFR) was estimated by the following Cockcroft-Gault formula: (140 − age in years)(weight in kilograms)(0.85 if females)/(72creatinine in milligrams per deciliter). The corrected serum calcium for albumin was calculated by the following formula: (calcium in milligrams per deciliter) + 0.8(4 − albumin in grams per deciliter).

The 25(OH)D assay

25(OH)D was tested in two central laboratories. Both laboratories used the LIAISON 25-OH vitamin D TOTAL assay (DiaSorin, Stillwater, MN), a competitive two-step chemiluminescence assay. The measuring range is 4.0–150 ng/ml (10–375 nmol/liter), the analytical sensitivity is less than 1.0 ng/ml (2.5 nmol/liter), and the functional sensitivity is less than 4.0 ng/ml (10 nmol/liter). The intraassay precision is up to 5%, and the interassay precision is up to 15%. The specificity for 25-hydroxyvitamin D2 is 104%, and for 25-hydroxyvitamin D3 is 100%. The performance characteristics of the vitamin D assay were checked in the method evaluation process done by Clalit Health Services and were compatible to the manufacturer-generated data. The accuracy of the measurements in the individual laboratory was confirmed by in-house daily quality control monitoring and by the periodic external quality control program (DEQAS, Charing Cross Hospital, London, UK).

Statistical analysis

Continuous data are presented as mean ± SD or median with the interquartile range as appropriate. Categorical variables are presented as proportions. The unpaired Student *t* test was used to compare means between two groups, and the ANOVA was used to compare means between more than two groups. Com-

parisons of categorical variables between groups were performed with the χ^2 test.

Time to death was calculated from date of blood test drawing until death or end of study date. Distribution of time to death is presented by Kaplan-Meier curves and compared with the log rank test. Proportional hazard regression analysis was used to assess the association between time to death and serum 25(OH)D levels, adjusting for potential confounders. The *P* value for trend was calculated by including the quartiles or the predefined categories of vitamin D as continuous variable in the model. The association was estimated with hazard ratio (HR) with 95% confidence interval (CI), comparing each vitamin D category with highest category as reference.

Adjustment for the potential confounders was assessed using four models. Data on some variables were missing, and the number of subjects included in the multivariate proportional hazard regression models varied according to the variables included in the model. Model 1 included age, gender, ethnicity, and seasonality. Model 2, in addition to covariates in model 1, included use of vitamin D supplements, use of statins, histories of hypertension, diabetes mellitus, cardiovascular disease, and cancer. Model 3, in addition to covariates in model 2, included smoking status, BMI, and socioeconomic status. Model 4, in addition to covariates in model 3, included HDL, LDL, corrected serum calcium for albumin level, and GFR. Model 1 and model 2 included all subjects, whereas model 3 and model 4 included 92.7 and 57.0% of study subjects, respectively.

Two-way interactions were assessed by including the product of the variables in the multivariate model. For all analyses, $P < 0.05$ for the two-tailed tests was considered statistically significant. All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL).

Results

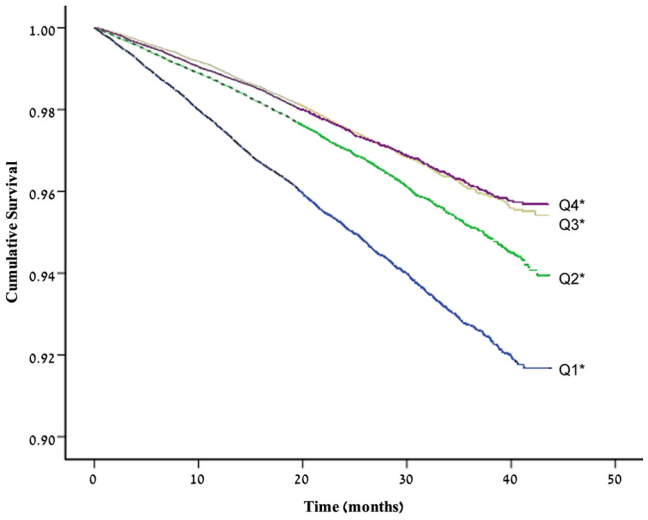
During a median follow-up period of 28.5 months (interquartile range 23.8–33.5 months), a total of 7,247 (4.0%) of the 182,152 cohort participants died. The mean serum 25(OH)D levels was significantly lower (44.8 ± 24.2 nmol/liter) among the subjects who died than among those who are still alive (51.0 ± 23.2).

The baseline characteristics of the study participants presented by quartiles of serum 25(OH)D levels are shown in Table

TABLE 1. Baseline characteristics of the study subjects by serum 25(OH)D quartiles (CHS cohort, Israel)

Variables	Quartiles of serum 25(OH)D (nmol/liter)				*P* value
	Quartile 1 (≤33.8)	Quartile 2 (>33.8 to 49.4)	Quartile 3 (>49.4 to 65.2)	Quartile 4 (>65.2)	
N	45,765	45,347	45,671	45,369	
Age (yr), mean (SD)	59.4 (17.4)	61.0 (16.1)	61.1 (16.0)	59.9 (17.1)	<0.001
Gender (%)					<0.001
Females	78.5	73.4	72.2	69.8	
Males	21.5	26.6	27.8	30.2	
Ethnicity (%)					<0.001
Jews	80.6	92.6	95.5	97.0	
Arabs	19.4	7.4	4.5	3.0	
Season (%)					<0.001
Winter-spring	61.6	49.8	43.1	39.5	
Summer-autumn	38.4	50.2	56.9	60.5	
Vitamin D supplements (%)	2.2	2.7	3.4	4.3	<0.001
Statins use (%)	36.1	40.0	41.5	42.2	<0.001
HDL (mg/dl), mean (SD)[a]	52.8 (14.1)	53.7 (14.2)	54.43 (14.3)	54.2 (14.1)	<0.001
LDL (mg/dl), mean (SD)[a]	111.0 (33.5)	110.9 (32.5)	109.2 (31.1)	104.2 (29.5)	<0.001
Calcium (mg/dl), mean (SD)[a]	9.27 (0.51)	9.29 (0.48)	9.29 (0.46)	9.29 (0.45)	<0.001
Creatinine (mg/dl), mean (SD)[a]	0.84 (0.52)	0.84 (0.42)	0.84 (0.36)	0.84 (0.32)	<0.001
Hypertension (%)	45.4	46.0	44.5	42.0	<0.001
Diabetes mellitus (%)	23.8	20.2	17.1	15.3	<0.001
CVD (%)	21.4	19.9	18.9	19.1	<0.001
Cancer (%)	12.5	13.2	13.8	14.0	<0.001
SES (%)[a]					<0.001
Low	23.3	18.4	17.3	16.8	
Middle	37.4	33.2	32.3	31.3	
High	39.3	48.3	50.5	51.9	
Smoking status (%)[a]					<0.001
Ever smoker	22.0	23.8	23.8	24.4	
BMI (kg/m^2) (%)[a]					<0.001
<25	32.1	34.1	38.8	47.4	
25 to <30	43.0	37.9	38.7	35.6	
30 to <40	29.3	25.5	20.9	16.2	
≥40	4.5	2.5	1.5	0.9	
Serum 25(OH)D, mean (SD)	23.2 (6.8)	41.9 (4.5)	57.0 (4.5)	81.4 (16.1)	<0.001

[a] Data were incomplete.



*Quartiles of serum 25(OH)D
Q1 = ≤33.8 nmol/L
Q2 = >33.8 to 49.4 nmol/L
Q3 = >49.4 to 65.2 nmol/L
Q4 = >65.2 nmol/L

FIG. 1. Kaplan-Meier curve for all-cause mortality according to serum 25(OH)D quartiles (CHS cohort, Israel). Q, Quartile.

1. The majority of study participants were females. Compared with those in the highest quartile, subjects in the lowest quartile of serum 25(OH)D level were more likely to be females, to be of Arab origin, and to have their serum 25(OH)D blood test drawn in winter-spring (61.6% compared with 39.5% in the highest quartile). Subjects in higher serum 25(OH)D quartiles had lower BMI and higher socioeconomic class and were more likely to use vitamin D supplements and statins compared with subjects in lower quartiles. The proportion of cardiovascular diseases, diabetes mellitus, and hypertension decreased from lowest to highest quartiles of serum 25(OH)D.

Mortality was found to increase when moving from the highest to the lowest 25(OH)D quartile (log rank $P < 0.001$; 3 degrees of freedom) (Fig. 1). In a proportional hazard regression model, the unadjusted HR for all-cause mortality was 1.83 (95% CI 1.62–2.20) for subjects in the lowest quartile compared with the highest quartile (data not shown). After adjusting for age, gender, ethnicity, and seasonality, serum 25(OH)D level independently predicted all-cause mortality with a HR of 2.02 (1.89–2.15), 1.25 (1.16–1.34), and 0.99 (0.92–1.07) for the lowest serum 25(OH)D quartile, second quartile, and third quartile compared with the highest quartile, respectively (model 1, Table 2). Further adjustment for the use of vitamin D supplements, the use of statins, and the existence of major chronic diseases only slightly changes the HR 1.81 (1.69–1.93) for the lowest quartile compared with the highest quartile (model 2, Table 2). The HR remained unchanged after further adjustment for SES, smoking status, and BMI (model 3, Table 2) or after further adjustment for GFR, corrected calcium to serum albumin, HDL, and LDL cholesterol [HR 1.85 (1.70–2.01), 1.28 (1.17–1.40), and 1.02 (0.93–1.12) for the lowest quartile, second, and third quartile compared with highest quartile, respectively (model 4, Table 2)]. Models that included serum 25(OH)D categorized by classical clinical categories also showed a similar association with all-cause mortality that persisted after the most extensive adjustment for the potential confounders (Table 3).

In all model types studied, the significant increase in mortality was confined to the two lower quartiles of 25(OH)D levels of up to 50 nmol/liter, and no mortality disadvantage was seen for people with higher levels.

To evaluate the impact of the missing data on the mortality risk estimates, we fitted the basic model (model 1) separately for those with fully available confounder data (n = 103,879), and those with missing potential confounder data (n = 78,273) and reached similar HR: 2.04 (1.88–2.21) and 1.96 (1.75–2.20), respectively, for those in the lowest quartile compared with the highest quartile.

TABLE 2. Cox proportional hazard models with adjusted HR (95% CI) for all-cause mortality according to serum 25(OH)D quartiles (CHS cohort, Israel)

	Quartiles of serum 25(OH)D (nmol/liter)				
Model	**Quartile 1 (≤33.8)**	**Quartile 2 (>33.8 to 49.4)**	**Quartile 3 (>49.4 to 65.2)**	**Quartile 4 (>65.2)**	***P* for trend**
Deaths/total	2,748/45,765	1,743/45,347	1,404/45,671	1,352/45,369	
Model 1	2.02 (1.89–2.15)	1.25 (1.16–1.34)	0.99 (0.92–1.07)	1.0 (ref)	<0.001
Model 2	1.81 (1.69–1.93)	1.19 (1.10–1.27)	0.98 (0.91–1.05)	1.0 (ref)	<0.001
Model 3	1.81 (1.69–1.95)	1.20 (1.12–1.30)	0.98 (0.90–1.06)	1.0 (ref)	<0.001
Model 4	1.85 (1.70–2.01)	1.28 (1.17–1.40)	1.02 (0.93–1.12)	1.0 (ref)	<0.001

Model 1: adjusted for age, gender, ethnicity, and seasonality.

Model 2: adjusted for use of vitamin D supplements, use of statins, histories of hypertension, diabetes mellitus, CVD, and cancer in addition to covariates in model 1.

Model 3: adjusted for smoking status, BMI, and SES in addition to covariates in model 2.

Model 4: adjusted for HDL, LDL, corrected serum calcium for albumin level, and GFR in addition to covariates in model 3.

TABLE 3. Cox proportional hazard models with adjusted HR (95% CI) for all-cause mortality according predefined serum 25(OH)D categories (CHS cohort, Israel)

Model	Serum 25(OH)D nmol/liter				*P* for trend
	<30	30 to <50	50 to <75	≥75	
Deaths/total	2,279/36,159	2,252/56,519	1,906/63,619	810/25,045	
Model 1	1.97 (1.82–2.14)	1.21 (1.11–1.31)	0.99 (0.83–0.98)	1.0 (ref)	<0.001
Model 2	1.76 (1.62–1.91)	1.15 (1.06–1.24)	0.89 (0.82–0.97)	1.0 (ref)	<0.001
Model 3	1.75 (1.61–1.92)	1.15 (1.06–1.26)	0.89 (0.81–0.97)	1.0 (ref)	<0.001
Model 4	1.81 (1.64–2.01)	1.23 (1.11–1.35)	0.92 (0.91–1.03)	1.0 (ref)	<0.001

Model 1: adjusted for age, gender, ethnicity, and seasonality.

Model 2: adjusted for use of vitamin D supplements, use of statins, histories of hypertension, diabetes mellitus, CVD, and cancer in addition to covariates in model 1.

Model 3: adjusted for smoking status, BMI, and SES in addition to covariates in model 2.

Model 4: adjusted for HDL, LDL, corrected serum calcium for albumin level, and GFR in addition to covariates in model 3.

A suggestion of interaction between serum 25(OH)D level and history of diabetes mellitus ($P = 0.069$) was noticed in our data. In the model with the most extensive adjustment, the risk of all-cause mortality in the lowest quartile of serum 25(OH)D level compared with the highest quartile was slightly higher in diabetics patients [2.03 (1.75–2.34) compared with 1.73 (1.56–1.92) in nondiabetic patients]. A suggestion for interaction was also noticed between vitamin D levels and history of cardiovascular diseases ($P = 0.072$). Adjusting for all study covariates, the risk of mortality for those in the lowest quartile compared with the highest quartile was lower 1.75 (1.57–1.95) for subjects with a history of CVD compared with 1.96 (1.72–2.24) for the subjects without a history of CVD.

We reached similar results when we used the season-specific quartiles of serum 25(OH)D levels. In the fully adjusted model, without adjustment for seasonality of blood sampling, the HR was 1.80 (1.66–1.96), 1.20 (1.10–1.31), and 1.02 (0.93–1.12) for the lowest quartile, second, and third quartile compared with highest quartile, respectively. The results were similar when the season of blood sampling was also included in this model: the HR was 1.81 (1.67–1.97), 1.20 (1.10–1.31), and 1.02 (0.93–1.12) for the lowest quartile, second, and third quartile compared with highest quartile, respectively.

Discussion

All-cause mortality was found in our study to be significantly elevated in people who had low serum 25(OH)D levels. This was observed for both people with insufficient levels (<50 nmol/liter) and for people with deficient levels (<30 nmol/liter) in which the risk of death was doubled. These results are in line with the finding of several other smaller studies (22, 24, 26, 27, 30) in which a significant result was reached after adjustment for potential confounders, which in one study was limited to nonsmokers (26). No mortality disadvantage was seen in other studies (21, 28–31).

Our study differs from the others in the very large number of participants stemming from the general population and the extensive power to control for confounders. It is further different in demonstrating effect after a much shorter follow-up period than in other studies. Anderson *et al.* (30) have recently reported a study with similar design to our study using an electronic medical record database that included 41,504 subjects. In line with our findings, Anderson *et al.* observed that even after a shorter follow-up period (average 1.3 ± 1.2 yr), the risk of all-cause mortality was significantly and inversely associated with serum 25(OH)D levels resulting in similar HR to our study. Compared with subjects with serum 25(OH)D levels greater than 30 ng/ml (>75 nmol/liter), the adjusted HR for all-cause mortality was 1.77 ($P < 0.0001$) and 1.20 ($P = 0.003$) for those with serum 25(OH)D levels of 15 ng/ml or less and levels 16–30 ng/ml, respectively.

Given the short follow-up period in our study, the results suggest that vitamin D status may be associated with short-term mortality, potentially reflecting a promotion process of existing chronic diseases, such as cardiovascular diseases or cancer, rather than initiation of a new disease. This hypothesis is in line with the recent findings of Autier and Gandini (8), who found that vitamin D supplementation affected mortality associated with cancers and cardiovascular diseases but had less of an effect (or not at all) on their incidence. Furthermore, it has been shown that in the setting of a critically ill population, low baseline levels of serum 25(OH)D were found to be a significant predictor of short-term all-cause mortality (32). Vitamin D has immunomodulation properties (12), and thus, subjects with impaired vitamin D status may also be at increased risk of infectious diseases. Because vitamin D can induce cell differentiation, inhibit angiogenesis, and block

cell proliferation (10), a higher level of 25(OH)D is expected to be associated with less tumor progression and formation.

Our data suggest that the increased risk of mortality is limited to serum 25(OH)D levels of below approximately 50 nmol/liter. This threshold was recently suggested to define vitamin D sufficiency according to the Institute of Medicine report on dietary reference intakes for vitamin D and calcium (33, 34). This is also in line with our recent finding that a threshold of 50 nmol/liter was sufficient for serum PTH suppression and prevention of secondary hyperparathyroidism (35). However, the Institute of Medicine report states that scientific evidence supports a key role for vitamin D in maintaining bone health, but the evidence for nonskeletal benefits is inconsistent and inconclusive with regard to causality (33, 34).

Our results suggest that the risk of all-cause mortality associated with vitamin D levels was slightly higher among subjects without known history of CVD disease compared with those with CVD. In accordance with our findings, low serum 25(OH)D levels were shown to be associated with significantly higher all-cause mortality in patients without significant coronary artery disease and serve as important mediators of mortality, even when there is little or no indication of overt vascular disease (36). We also identified a higher risk of all-cause mortality among diabetics with lower 25(OH)D levels, possibly suggesting an interaction between vitamin D and hyperinsulinemia in diabetics.

Although season-specific values of serum 25(OH)D levels are relatively stable in repeated measures over time (37, 38), some studies used the within-season classification into quartiles of serum 25(OH)D instead of the quartiles of all available measurements (26, 36). Wang *et al.* (39) showed that using overall quartiles of serum 25(OH)D with an adjustment for season of blood sampling may be associated with bias away from the null and that using season-specific quartiles did not cause bias away from the null and reduced bias toward the null, irrespective of adjustment for seasonality. In our study, analysis by season-specific quartiles of serum 25(OH)D with and without adjustment for seasonality of blood sampling resulted in a very similar association with mortality. This similarity was due to the high level of agreement between the quartiles classified by the two approaches (81.8%).

Although we have controlled for available potential confounders, many other known or unknown factors may confound the relationship of vitamin D and mortality. Because it is unclear whether low vitamin D levels are simply biomarkers of impaired health status or are the direct cause of disease and mortality, randomized controlled trials with vitamin D supplements are warranted. Currently one such study is underway in subjects with congestive heart failure (40). Our study has other limitations: it relies on a computerized, although highly valid, database that was not specifically designed for the present study. Our study may suffer from selection bias due to the reliance on blood tests that have been ordered by primary care physicians all over the country and could reflect a sicker population. However, the study was performed in a period of increasing interest in assessing vitamin D status in the general population, and the tested population reflects 10.4% of the total ensurees population of CHS at age 20 yr or older. Such a high coverage rate permits defining the study cohort as a population-based cohort. In addition, we suffered from a degree of missing data for various variables used as potential confounders in the models. However, we reached similar result running our basic effects model separately in those with missing covariate data and those with fully available data. The relatively short follow-up period may seem to be a limitation of the study; however, our findings of mortality differences, even after a short follow-up time, could have major practical implications in setting intervention guidelines.

In conclusion, we found vitamin D to be inversely associated with all-cause mortality, with a significant dose-response effect. Subjects with serum 25(OH)D levels less than 50 nmol/liter are particularly at increased risk for mortality. Randomized clinical trials to evaluate the effect of vitamin D supplementation on mortality are warranted to support these findings.

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