Association of Serum 25-Hydroxyvitamin D Concentrations With All-Cause and Cause-Specific Mortality Among Individuals With Diabetes

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OBJECTIVE

The evidence regarding vitamin D status and mortality among people with diabetes is scarce. This study aimed to examine the association of serum 25-hydroxyvitamin D [25(OH)D] concentrations with all-cause and cause-specific mortality among adults with diabetes.

RESEARCH DESIGN AND METHODS

This study included 6,329 adults with diabetes from the Third National Health and Nutrition Examination Survey (NHANES III) and NHANES 2001–2014. Death outcomes were ascertained by linkage to National Death Index records through 31 December 2015. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% CIs for mortality from all causes, cardiovascular disease (CVD), and cancer.

RESULTS

The weighted mean (95% CI) level of serum 25(OH)D was 57.7 (56.6, 58.8) nmol/L, and 46.6% had deficient vitamin D (<50 nmol/L [20 ng/mL]). Higher serum 25(OH)D levels were significantly associated with lower levels of glucose, insulin, HOMA of insulin resistance, HbA_{1c}, blood lipids, and C-reactive protein at baseline (all $P_{trend} < 0.05$). During 55,126 person-years of follow-up, 2,056 deaths were documented, including 605 CVD deaths and 309 cancer deaths. After multivariate adjustment, higher serum 25(OH)D levels were significantly and linearly associated with lower all-cause and CVD mortality: there was a 31% reduced risk of all-cause mortality and a 38% reduced risk of CVD mortality per one-unit increment in natural log-transformed 25(OH)D (both $P < 0.001$). Compared with participants with 25(OH)D <25 nmol/L, the multivariate-adjusted HRs and 95% CI for participants with 25(OH)D >75 nmol/L were 0.59 (0.43, 0.83) for all-cause mortality ($P_{trend} = 0.003$), 0.50 (0.29, 0.86) for CVD mortality ($P_{trend} = 0.02$), and 0.49 (0.23, 1.04) for cancer mortality ($P_{trend} = 0.12$).

CONCLUSIONS

Higher serum 25(OH)D levels were significantly associated with lower all-cause and CVD mortality. These findings suggest that maintaining adequate vitamin D status may lower mortality risk in individuals with diabetes.

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Type 2 diabetes has become a global public health problem, with an estimation of 451 million adults living with diabetes worldwide in 2017 (1). Individuals with diabetes have a two- to fourfold higher risk of developing cardiovascular disease (CVD) and death compared with those without diabetes (2). It is of great importance to identify modifiable factors for the prevention or delay of diabetes complications and premature death.

Vitamin D, a hormone that primarily regulates calcium and phosphate metabolism (3), has been linked to glycemic control and cardiovascular events among diabetes patients (4–8) in whom vitamin D deficiency is particularly common (6,9). Although some epidemiological studies suggested that lower vitamin D concentrations were associated with elevated risk of micro- and macrovascular complications (5–9), the evidence regarding the relationship between vitamin D status and mortality risk among diabetes is limited and somewhat mixed (10,11). For instance, one prospective study of 289 Danish patients with diabetes (196 deaths occurred) found that low 25-hydroxyvitamin D3 [25(OH)D_3] concentrations were associated with an increased risk of all-cause and CVD mortality (10), while another study of 698 Swedish patients with diabetes (only 33 deaths occurred) showed a marginally inverse association of serum 25(OH)D_3 with mortality in men, but not in women (11). Moreover, these existing studies are subject to a few limitations, such as small sample sizes, only assessment of 25(OH)D_3 in absence of total 25-hydroxyvitamin D [25(OH)D] levels, inconsistent definition of vitamin D deficiency, and insufficient adjustment of some important covariates (e.g., dietary factors, lifestyle factors including physical activity, season of vitamin D assessment, and comorbidity). In addition, whether race/ethnicity, smoking, and obesity status could modify the association of interest remains unclear.

To fill these knowledge gaps, we aimed to prospectively examine the associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality in a nationally representative sample of U.S. adults with diabetes.

RESEARCH DESIGN AND METHODS

Study Population

The National Health and Nutrition Examination Survey (NHANES) is a nationally representative study to assess health and nutritional status of the noninstitutionalized civilian population in the U.S. The details of sampling method and data collection have been published elsewhere (12). NHANES was performed by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), and was approved by the institutional review board of the National Center of Health Statistics. All participants provided written informed consent.

In the current study, we used data from NHANES III (1988–1994) and seven cycles of NHANES from 2001 to 2014 (vitamin D data were not available in NHANES 1999–2000). Individuals (aged ≥20 years) with diabetes were included in the analysis. Diabetes was defined as self-reported doctor diagnosis of diabetes, use of insulin or oral hypoglycemic medication, fasting glucose ≥7.8 mmol/L in NHANES III (the diagnosis criterion of fasting glucose was 7.8 mmol/L before American Diabetes Association criteria in 1997) or 7.0 mmol/L in NHANES 2001–2014, or glycated hemoglobin A_{1c} (HbA_{1c}) ≥6.5% due to the changes in the diagnostic criteria over time. A total of 7,264 adults with diabetes had measurements of serum 25(OH)D concentrations. After excluding those who were self-reported as pregnant (n = 27) or having cancer (n = 908) at baseline, 6,329 participants were included in the current study.

Measurement of Serum 25(OH)D

In the NHANES III (1988–1994) and NHANES 2001–2006, serum 25(OH)D concentrations were measured by DiaSorin radioimmunoassay kit (Stillwater, MN). Starting from the 2007 to 2008 cycle, serum 25(OH)D concentrations were measured by a standardized liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. Serum 25(OH)D data from NHANES III and NHANES 2001–2006 were converted by regression method to equivalent 25(OH)D measurements from the LC-MS/MS method, and the details have been documented elsewhere (13). As recommended by the CDC, we used the LC-MS/MS–equivalent data for all analyses (13).

Ascertainment of Mortality

Mortality from all causes, CVD, and cancer was ascertained by linkage to the National Death Index through 31 December 2015. The ICD-10 was used to determine disease-specific death. CVD mortality was defined as ICD-10 codes I00–I09, I11, I13, I20–I51, or I60–I69, and cancer mortality was defined as ICD-10 codes C00–C97. Given that data on mortality from stroke (ICD-10 codes I60–I69) were only available until 31 December 2011 in the 2011 version of the National Death Index matched mortality data set, a sensitivity analysis of CVD mortality was conducted up to 31 December 2011 among participants from NHANES III and NHANES 2001–2010.

Assessment of Covariates

Information on age, sex, race/ethnicity, education level, family income, smoking status, physical activity, disease status, and medication use was collected from household interviews using standardized questionnaires. Body weight, height, and alcohol intake were obtained when people participated in the physical examinations at a mobile examination center. BMI was calculated as weight in kilograms divided by height in meters squared. Race/ethnicity was classified as non-Hispanic White, non-Hispanic Black, Mexican American, or other. Education level was categorized as less than high school, high school or equivalent, or college or above. Family income-to-poverty ratio was classified as 0–1.0, 1.0–3.0, or >3.0. Smoking status was classified as never smoker, former smoker, or current smoker. Drinking status was grouped into nondrinker, low-to-moderate drinker (defined as <2 drinks/day in men and <1 drink/day in women), or heavy drinker (defined as ≥2 drinks/day in men and ≥1 drink/day in women). Leisure-time moderate-to-vigorous physical activity was categorized into inactive group (no leisure-time physical activity), insufficiently active group (leisure-time moderate activity 1–5 times per week with metabolic equivalents ranging from 3–6 or leisure-time vigorous activity 1–3 times per week with metabolic equivalents >6), or active group (those who had more leisure-time moderate-or-vigorous activity than above) (14).

In addition, plasma glucose, insulin, HbA_{1c}, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, and C-reactive protein (CRP) were measured at baseline when the participants provided their blood samples. Rigorous procedures were applied throughout blood collection and analysis, and details were described in the NHANES Laboratory/

Medical Technologists Procedures Manual (15). The HOMA of insulin resistance (HOMA-IR) was calculated based on the method of Matthews et al. (16).

Statistical Analysis

Sample weights, clustering, and stratification were incorporated in all analyses because of the complex sampling design of the NHANES, as required to analyze the NHANES data (12). The generalized linear model was applied to examine the associations of serum 25(OH)D levels with cardiometabolic biomarkers at baseline, including plasma glucose, insulin, HOMA-IR, HbA_{1c}, triglycerides, total cholesterol, HDL, LDL, and CRP. Pearson-time was calculated from the date of the examination of serum 25(OH)D to the date of death or the end of follow-up (31 December 2015), whichever came first. The Cox proportional hazards model was used to estimate hazard ratios (HRs) and 95% CIs for the association between serum 25(OH)D concentrations and all-cause and cause-specific mortality. According to the Endocrine Society Clinical Practice Guidelines, vitamin D status was categorized into four groups: severe deficiency (<25.0 nmol/L; to convert from nmol/L to ng/mL, divided by 2.5), moderate deficiency (25.0–49.9 nmol/L), insufficient (50.0–74.9 nmol/L), and sufficient (≥75.0 nmol/L) (17). Serum 25(OH)D concentrations were also analyzed as a continuous variable after natural log transformation. In the multivariate models, we adjusted for age (years), sex (male or female), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other) in model 1. In model 2, we further adjusted for BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), education level (less than high school, high school or equivalent, or college or above), family income-poverty ratio (0–1.0, 1.0–3.0, or >3.0), smoking status (never, former, or current smoker), drinking status (none, low-to-moderate, or heavy drinker), and leisure-time moderate-to-vigorous physical activity (inactive, insufficiently active, or active). In model 3, we further adjusted for duration of diabetes (years); diabetes medication use (none, only oral medication, insulin, or others); HbA_{1c} (<7.0% or ≥7.0%); and presence of hypertension, hypercholesterolemia, or CVD (yes or no). Multiple imputation was performed for covariates with missing values. The linear trend was tested by assigning a median value to each category as a continuous variable.

To examine a dose-response relationship between serum 25(OH)D concentrations and mortality, restricted cubic spline regression with three knots (5th, 50th, and 75th) was used, with the multivariate adjustment mentioned above. Tests for nonlinearity were performed using the likelihood ratio test comparing two models: one with only the linear term and the other with the linear and the cubic spline terms. Stratified analyses were also conducted by age (≤60 or >60 years), sex (male or female), race/ethnicity (White or non-White), smoking status (never, former, or current smokers), BMI (<30.0, or ≥30.0 kg/m^2), physical activity (inactive, insufficiently active, or active), diabetes duration (≤10 or >10 years), and number of self-reported comorbidities (i.e., coronary heart disease, stroke, kidney dysfunction, and retinopathy). The *P* values for the product terms between serum 25(OH)D concentrations and stratification variables were used to estimate the significance of interactions.

Several sensitivity analyses were performed to test the robustness of our findings. First, given the seasonal variation of vitamin D status, we further adjusted for month when blood was drawn (data only available in the NHANES 2001–2014). Second, given that some dietary factors might influence the association of interest (18), dietary supplement use (yes or no), polyunsaturated fatty acid intake (in tertiles), and calcium and magnesium (in tertiles), or healthy eating index (HEI-1995 for NHANES 1988–94 and HEI-2010 for NHANES 2001–2014, both in quartiles) were further adjusted for in the multivariate model. Third, as renal dysfunction could influence circulating vitamin D levels and cardiovascular events, kidney function assessed by estimated glomerular filtration rate (calculated by using the Chronic Kidney Disease Epidemiology Collaboration formula) was further adjusted (19). Fourth, repeated analyses were performed according to quartiles of serum 25(OH)D. Fifth, participants with a history of CVD were further excluded. Sixth, to reduce the potential reverse causation bias, participants who died within 2 years of follow-up were excluded. Seventh, to examine whether the potential mediators, including inflammation, blood lipids, or insulin resistance, could explain the observed association, CRP, LDL, HDL, triglyceride, or HOMA-IR were further adjusted for in the subgroup analyses (available data ranged from 2,361 to 3,884). Finally, considering of the interrelationship of parathyroid hormone (PTH) and vitamin D status, we further adjusted for PTH levels in a subgroup of the study participants (only available in the NHANES 2003–2006, $n = 1{,}053$). All of the analyses were conducted using SAS Survey Procedure software (version 9.4; SAS Institute, Cary, NC). Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Among the 6,329 adults with diabetes (mean age, 56.4 years; 49.8% male), the weighted mean (95% CI) concentration of serum 25(OH)D was 57.7 (56.6, 58.8) nmol/L; 46.6% had deficient vitamin D (<50 nmol/L); and 81.9% had insufficient vitamin D (<75 nmol/L). The baseline characteristics of the study population according to serum 25(OH)D status are shown in the Table 1. Participants who had higher 25(OH)D levels were more likely to be older and non-Hispanic White; were less likely to be obese and current smokers; and had higher education levels, family income, and leisure-time physical activity.

The least squares means of cardiometabolic biomarkers according to serum 25(OH)D concentrations are shown in the Table 2. Higher levels of serum 25(OH)D were significantly associated with lower levels of glucose, insulin, HOMA-IR, HbA_{1c}, total cholesterol, LDL, triglyceride, and CRP and with higher levels of HDL at baseline (all $P_{trend} < 0.05$).

During 55,126 person-years of follow-up, 2,056 deaths were documented, including 605 CVD deaths and 309 cancer deaths. After multivariate adjustment including lifestyle factors, BMI, diabetes duration, diabetes medication use, and presence of chronic diseases, higher serum 25(OH)D concentrations were significantly associated with lower all-cause and CVD mortality (Table 3). The multivariate-adjusted HRs and 95% CIs from lowest to highest serum 25(OH)D categories (<25.0, 25.0–49.9, 50.0–74.9, and ≥75.0 nmol/L) were 1.00 (reference), 0.70 (0.55, 0.89), 0.56 (0.43, 0.75), and 0.59 (0.43, 0.83), respectively, for all-cause mortality ($P_{trend} = 0.003$); 1.00 (reference), 0.62 (0.40, 0.96), 0.46 (0.29, 0.73), and 0.50 (0.29, 0.86), respectively, for CVD mortality ($P_{trend} = 0.02$); and 1.00 (reference),

Table 1—Baseline characteristics of participants with diabetes according to serum 25(OH)D concentrations in NHANES III and NHANES 2001–2014

	Serum 25(OH)D concentrations (nmol/L)*				
	Total	<25.0	25.0–49.9	50.0–74.9	≥75.0
Number of participants	6,329	408	2,542	2,234	1,145
Age (mean ± SE), years	56.4 ± 0.3	56.0 ± 0.8	55.5 ± 0.5	56.5 ± 0.6	58.0 ± 0.6
Female	3,178 (50.2)	245 (60.0)	1,346 (53.0)	1,010 (45.2)	577 (50.4)
Race/ethnicity					
Non-Hispanic White	2,169 (34.3)	60 (14.7)	604 (23.8)	904 (40.5)	601 (52.5)
Non-Hispanic Black	1,771 (28.0)	225 (55.1)	883 (34.7)	451 (20.2)	212 (18.5)
Mexican American	1,540 (24.3)	94 (23.0)	743 (29.2)	535 (23.9)	168 (14.7)
Other	849 (13.4)	29 (7.1)	312 (12.3)	344 (15.4)	164 (14.3)
BMI, kg/m^2					
<25.0	1,006 (16.3)	64 (16.2)	364 (14.7)	376 (17.2)	202 (18.1)
25.0–29.9	1,943 (31.4)	84 (21.3)	702 (28.4)	766 (35.0)	391 (35.0)
≥30.0	3,230 (52.3)	247 (62.5)	1,410 (56.9)	1,048 (47.9)	525 (47.0)
Drinking status					
Nondrinker	1,989 (34.0)	113 (31.0)	820 (35.1)	695 (33.3)	361 (33.7)
Low-to-moderate drinker	3,566 (60.9)	226 (62.1)	1,403 (60.1)	1,288 (61.8)	649 (60.5)
Heavy drinker	300 (5.1)	25 (6.9)	112 (4.8)	101 (4.8)	62 (5.8)
Smoking status					
Never smoker	3,066 (48.5)	190 (46.6)	1,222 (48.2)	1,121 (50.2)	533 (46.6)
Ever smoker	2,089 (33.0)	105 (25.7)	788 (31.1)	745 (33.3)	451 (39.4)
Current smoker	1,167 (18.5)	113 (27.7)	525 (20.7)	368 (16.5)	161 (14.1)
Education levels					
Less than high school	3,119 (50.2)	207 (51.8)	1,352 (54.5)	1,105 (50.1)	455 (40.3)
High school or equivalent	1,207 (19.4)	72 (18.0)	448 (18.1)	412 (18.7)	275 (24.4)
College or above	1,887 (30.4)	121 (30.3)	680 (27.4)	687 (31.2)	399 (35.3)
Family income-poverty ratio					
≤1.0	1,514 (25.5)	124 (32.5)	638 (26.8)	525 (24.8)	227 (21.5)
1.0–3.0	2,672 (45.0)	165 (43.2)	1,084 (45.5)	939 (44.4)	484 (45.9)
>3.0	1,747 (29.4)	93 (24.3)	661 (27.7)	650 (30.7)	343 (32.5)
Leisure-time physical activity					
Inactive	2,822 (51.7)	215 (63.8)	1,098 (52.0)	937 (48.1)	572 (53.8)
Insufficiently active	1,709 (31.3)	88 (26.1)	676 (32.0)	657 (33.7)	288 (27.1)
Active	931 (17.0)	34 (10.1)	338 (16.0)	355 (18.2)	204 (19.2)
Duration of diabetes					
≤3 years	2,897 (47.4)	179 (45.4)	1,197 (48.8)	1,062 (49.1)	459 (41.7)
3–10 years	1,434 (23.5)	87 (22.1)	567 (23.1)	512 (23.7)	268 (24.3)
>10 years	1,782 (29.2)	128 (32.5)	691 (28.1)	589 (27.2)	374 (34.0)

Continued on p. 354

0.69 (0.38, 1.25), 0.61 (0.32, 1.16), and 0.49 (0.23, 1.04), respectively, for cancer mortality ($P_{trend} = 0.12$). Figure 1 shows a dose-response relationship between serum 25(OH)D concentration (ranged from 10–130 nmol/L) and all-cause and CVD mortality. After multivariate adjustment, a linear relationship was demonstrated (P linearity <0.001); each one-unit increment in the natural log-transformed 25(OH)D level was associated with a 31% reduced risk of all-cause mortality and a 38% reduced risk of CVD mortality (Table 3). No linear association was observed between serum 25(OH)D concentration and cancer mortality (Supplementary Fig. 1).

Consistent results were observed when analyses were stratified by age, sex, race/ethnicity, smoking status, BMI, physical activity, diabetes duration, and number of self-reported comorbidities (Supplementary Table 1). No significant interactions were detected between serum 25(OH)D levels and these stratifying variables (all $P_{interaction} > 0.05$).

In the sensitivity analyses, the results were largely unchanged when CVD mortality was ascertained up to 31 December 2011. Similar results were observed when we further adjusted for the month when blood was drawn (Supplementary Table 2); or dietary supplement use, polyunsaturated fatty acid intake, and calcium and magnesium intake (model 2, Supplementary Table 3); or healthy eating index (model 3, Supplementary Table 3); or estimated glomerular filtration rate (model 4, Supplementary Table 3). Consistent results were demonstrated when serum 25(OH)D concentrations were categorized into quartiles (Supplementary Table 4). The results did not significantly change when we excluded participants with presence of CVD (Supplementary Table 5) or those who died within 2 years of follow-up (Supplementary Table 6). When further adjusting for LDL, HDL, triglyceride, or HOMA-IR, the results did not materially change, although the association between 25(OH)D levels and mortality was slightly attenuated with further adjustment of CRP (Supplementary Table 7). The inverse association was observed when PTH was further adjusted for in a subgroup of the study participants (n = 1,053), although the results did not reach statistical significance which could be largely due to reduced power (Supplementary Table 8).

Table 1—Continued

	Serum 25(OH)D concentrations (nmol/L)*				
	Total	<25.0	25.0–49.9	50.0–74.9	≥75.0
Medication use					
No insulin or pills	2,374 (38.1)	151 (37.5)	1,024 (41.0)	827 (37.5)	372 (32.9)
Only diabetes pills	2,408 (38.6)	147 (36.5)	872 (34.9)	899 (40.7)	490 (43.4)
Only insulin	957 (15.3)	63 (15.6)	427 (17.1)	308 (13.9)	159 (14.1)
Pills and insulin	497 (8.0)	42 (10.4)	173 (6.9)	174 (7.9)	108 (9.6)
HbA_{1c}					
<7.0%	3,369 (53.6)	191 (47.0)	1,248 (49.4)	1,219 (55.1)	711 (62.4)
≥7.0%	2,914 (46.4)	215 (53.0)	1,277 (50.6)	994 (44.9)	428 (37.6)
Self-reported diseases					
Hypertension	3,781 (59.7)	272 (66.7)	1,461 (57.5)	1,273 (57.0)	775 (67.7)
Hypercholesterolemia	2,852 (45.1)	176 (43.1)	1,022 (40.2)	1,019 (45.6)	635 (55.5)
CVD	1,398 (22.1)	109 (26.7)	545 (21.4)	467 (20.9)	277 (24.2)

Data are numbers (percentages) unless otherwise indicated. All estimates accounted for complex survey designs. *Vitamin D status was categorized into four groups: severe deficiency (<25.0 nmol/L), moderate deficiency (25.0–49.9 nmol/L), insufficient (50.0–74.9 nmol/L), and sufficient (≥75.0 nmol/L) according to the Endocrine Society Clinical Practice Guidelines.

CONCLUSIONS

In a large prospective cohort study of U.S. adults with diabetes, we found that higher concentrations of serum 25(OH)D were significantly associated with lower all-cause and CVD mortality. The association was independent of traditional risk factors, including dietary and lifestyle factors, BMI, diabetes duration, and diabetes medication use. A variety of sensitivity analyses and stratified analyses demonstrated the robustness of these findings. In addition, higher serum 25(OH)D concentrations were associated with lower levels of inflammatory biomarker and HOMA-IR as well as a better lipid profile. The association between vitamin D and mortality among diabetes was slightly attenuated when further adjusting for CRP.

Although numerous epidemiological studies have shown that vitamin D deficiency is associated with increased risk of CVD events and mortality (20–22), recent randomized clinical trials have found no effect of vitamin D supplementation on cardiometabolic disease prevention (23–26). For instance, in a large-scale, randomized, placebo-controlled trial including 25,871 participants, Manson et al. (24) found that supplementation with vitamin D3 at a dose of 2,000 IU/day for 5 years among initially healthy U.S. adults did not lower incidence of invasive cancer or a composite of major cardiovascular events, although the enrolled participants had relatively higher levels of 25(OH)D at baseline (mean 77 nmol/L). The discrepancy could be because observational studies are susceptible to uncontrolled confounding, such as physical activity, dietary factors, and presence of chronic diseases, but most of the trials did not prespecify CVD as the primary end point with limited cardiovascular events, and only tested one dose of vitamin D (25). In addition, most previous studies were conducted among general populations or individuals at high risk of CVD. Among patients with diabetes who had a high prevalence of vitamin D deficiency and heightened risk of developing CVD complications (2,6,9), evidence is limited regarding the potential health benefits of vitamin D, particularly with respect to mortality (10,11,27). For example, among 289 Danish diabetes patients, Joergensen et al. (10) found that plasma $25(OH)D_3$ deficiency was associated with an increased risk of all-cause and CVD mortality, although severe vitamin D deficiency was subjectively defined as the lower

Table 2—Least squares means of cardiometabolic markers according to serum 25(OH)D concentrations among participants with diabetes in NHANES III and NHANES 2001–2014

	Serum 25(OH)D concentrations (nmol/L)				
	<25.0	25.0–49.9	50.0–74.9	≥75.0	P_{trend}
Glucose (n = 3,164), mmol/L	9.5 ± 0.3	9.5 ± 0.2	8.9 ± 0.2	8.5 ± 0.2	<0.001
Insulin (n = 3,331), pmol/L	157.4 ± 17.0	127.4 ± 8.8	120.9 ± 9.0	100.2 ± 11.2	0.004
HOMA-IR (n = 3,117)	13.4 ± 2.0	10.3 ± 1.0	8.8 ± 1.0	6.6 ± 1.3	0.001
HbA_{1c} (n = 5,388), %	7.5 ± 0.1	7.5 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	<0.001
Total cholesterol (n = 5,266), mmol/L	5.22 ± 0.08	5.30 ± 0.04	5.17 ± 0.04	5.00 ± 0.05	<0.001
HDL (n = 5,237), mmol/L	1.33 ± 0.02	1.31 ± 0.01	1.32 ± 0.01	1.36 ± 0.01	0.005
LDL (n = 2,361), mmol/L	2.89 ± 0.09	3.03 ± 0.05	2.92 ± 0.04	2.79 ± 0.05	0.001
Triglyceride (n = 4,812), mmol/L	2.29 ± 0.16	2.34 ± 0.08	2.25 ± 0.08	2.10 ± 0.10	0.03
CRP (n = 3,884), mg/L	9.7 ± 1.0	5.4 ± 0.4	5.2 ± 0.4	5.1 ± 0.5	0.002

The least square (mean ± SE) was estimated using general linear model with adjustment of age (continuous), sex (male or female) and race/ethnicity (non-Hispanic White or other), BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), education level (less than high school, high school or equivalent, or college or above), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), duration of diabetes (≤3, 3–10, or >10 years), and diabetes medication use (none, oral medication and/or insulin).

Table 3—HR (95% CIs) for all-cause and cause-specific mortality according to serum 25(OH)D concentrations among participants with diabetes in NHANES III and NHANES 2001–2014

	Serum 25(OH)D concentrations (nmol/L)					Per one-unit increment in natural log-transformed 25(OH)D
	<25.0	25.0–49.9	50.0–74.9	≥75.0	P_{trend}	
All-cause mortality						
Number of deaths/total	174/408	890/2,542	710/2,234	282/1,145		2,056/6,329
Model 1*	1.00	0.68 (0.52, 0.89)	0.54 (0.40, 0.73)	0.55 (0.40, 0.76)	0.001	0.66 (0.56, 0.78)
Model 2†	1.00	0.72 (0.57, 0.90)	0.59 (0.45, 0.77)	0.61 (0.44, 0.83)	0.005	0.68 (0.58, 0.80)
Model 3‡	1.00	0.70 (0.55, 0.89)	0.56 (0.43, 0.75)	0.59 (0.43, 0.83)	0.003	0.69 (0.58, 0.82)
CVD mortality						
Number of deaths	58	266	202	79		605
Model 1*	1.00	0.62 (0.41, 0.95)	0.47 (0.30, 0.72)	0.52 (0.31, 0.86)	0.03	0.62 (0.47, 0.81)
Model 2†	1.00	0.64 (0.42, 0.98)	0.49 (0.31, 0.76)	0.55 (0.33, 0.92)	0.06	0.62 (0.48, 0.82)
Model 3‡	1.00	0.62 (0.40, 0.96)	0.46 (0.29, 0.73)	0.50 (0.29, 0.86)	0.02	0.62 (0.47, 0.81)
Cancer mortality						
Number of deaths	20	134	117	38		309
Model 1*	1.00	0.62 (0.28, 1.36)	0.54 (0.24, 1.24)	0.43 (0.17, 1.06)	0.09	0.70 (0.43, 1.14)
Model 2†	1.00	0.70 (0.38, 1.30)	0.64 (0.33, 1.23)	0.55 (0.26, 1.14)	0.19	0.79 (0.52, 1.22)
Model 3‡	1.00	0.69 (0.38, 1.25)	0.61 (0.32, 1.16)	0.49 (0.23, 1.04)	0.12	0.74 (0.49, 1.13)

*Model 1: adjusted for age (continuous), sex (male or female), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other). †Model 2: further adjusted (from Model 1) for BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), education level (less than high school, high school or equivalent, or college or above), family income-poverty ratio (0–1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), and leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group). ‡Model 3: further adjusted (from Model 2) for duration of diabetes (≤3, 3–10, or >10 years), diabetes medication use (none, only oral medication, insulin, or others), HbA_{1c} (<7% or ≥7%), and self-reported hypertension, hypercholesterolemia, and CVD (yes or no).

10th percentile (<13.9 nmol/L). In another study among 698 Swedish adults with diabetes, only 33 deaths occurred, and a borderline association of serum 25(OH)D_3 with mortality was demonstrated in men but not in women (11). Of note, participants in these previous studies had lower vitamin D levels than those in our study, which is probably due to the higher latitude of Demark and Sweden as well as the fact that these studies only measured 25(OH)D_3 but not total 25(OH)D. Moreover, the existing studies did not take into account some important covariates, such as physical activity, season of vitamin D assessment, or presence of chronic diseases, which could significantly influence vitamin D status and health outcomes. Furthermore, dietary factors including dietary supplement use and intake of polyunsaturated fatty acid, calcium, and magnesium, which might confound the association of vitamin D and mortality among diabetes, have not been examined (18). In our large prospective study using a nationally representative sample of U.S. adults with diabetes, vitamin D deficiency was defined according to the Endocrine Society Clinical Practice Guidelines, and we found a linear dose-response relationship between serum 25(OH)D levels (ranged from 10 to 130 nmol/L) and all-cause and CVD mortality after multivariate adjustment. Although an inverse association between 25(OH)D levels and cancer mortality was also observed in our study, the result did not reach statistical significance, probably due to the limited number of cases. More large prospective studies are needed to confirm these findings.

Given the racial difference in 25(OH)D levels (28), we stratified our analyses by race/ethnicity (white or nonwhite), and similar findings were observed in each strata. Likewise, consistent findings were demonstrated when analyses were further stratified by smoking, obese status, and diabetes duration, and no significant interaction was detected. Interestingly, although the results did not significantly change when blood lipids and HOMA-IR was adjusted for in a subgroup of the study population, the association between serum 25(OH)D concentrations and all-cause mortality among diabetes was slightly attenuated with further adjustment of CRP, suggesting that vitamin D might exert its effect partially through inflammatory pathways.

Although other mechanisms underlying the observed association between vitamin D and mortality among diabetes remain to be elucidated, accumulating evidence from animal and human studies have suggested that vitamin D might be involved in the suppression of the renin-angiotensin-aldosterone system and in improvement in pancreatic β-cell dysfunction and endothelial dysfunction as well as in immunomodulatory activities (3,29,30). In addition, some studies conducted among diabetes patients found that vitamin D could suppress macrophage migration and cholesterol uptake, inhibit foam cell formation, and reverse atherogenic cholesterol metabolism (31,32). Nevertheless, more mechanistic studies are warranted to further illuminate the potential mechanisms through which vitamin D plays a role in the prevention of all-cause and CVD mortality among diabetes patients.

The strengths of the current study include the prospective study design, a relatively large sample size, and the use of a nationally representative sample of U.S. adults with diabetes, which facilitates the generalization of our findings. In addition, given the comprehensive data acquired in the NHANES, we could adjust for a multitude of potential confounding factors including socioeconomic status, race/ethnicity, dietary and lifestyle factors, comorbidities, season of vitamin D assessment, and PTH levels. Several limitations should be considered as well. First, causality cannot be determined because of the observational study design. Second, although some evidence suggested that a single measurement of 25(OH)D

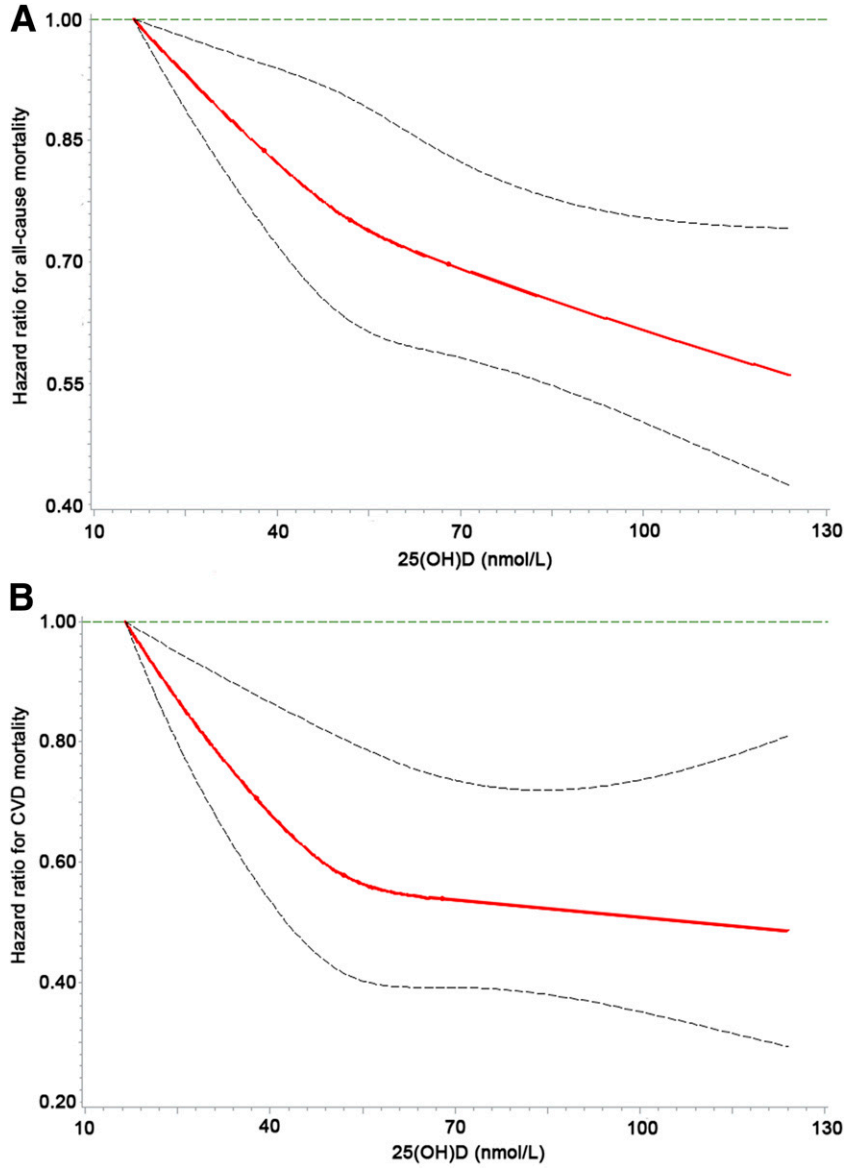

Figure 1—Associations between serum 25(OH)D concentrations with all-cause (*A*) and CVD mortality (*B*) among participants with diabetes in NHANES III and NHANES 2001–2014. HRs were adjusted for age (continuous), sex (male or female), and race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other), BMI (<25.0, 25.0–29.9, or ≥30.0 kg/m^2), education level (less than high school, high school or equivalent, or college or above), family income-poverty ratio (0–1.0, 1.0–3.0, or >3.0), drinking status (nondrinker, low-to-moderate drinker, or heavy drinker), smoking status (never smoker, former smoker, or current smoker), and leisure-time moderate-to-vigorous physical activity (inactive group, insufficiently active group, or active group), duration of diabetes (≤3, 3–10, or >10 years), diabetes medication use (none, only oral medication, insulin, or others), HbA_{1c} (<7% or ≥7%), and self-reported hypertension, hypercholesterolemia, and CVD (yes or no). Both *P* linearity <0.001.

was a reasonable proxy for vitamin D status (33), the current study measured serum 25(OH)D concentrations only once, which would likely underestimate the association of interest (34). Third, the current study did not assess bioavailable vitamin D (nonvitamin D binding protein-bound portion) levels and genetic variants in vitamin D metabolism-related genes (28,35,36). Therefore, more studies are required to explore the role of these factors in the association between 25(OH)D and mortality among diabetes. Fourth, PTH data were only available for a subgroup of the study population, which warrants more investigations in future research. Fifth, the current study did not have detailed information on the severity of diabetes, although the results remained significant when further adjusting for diabetes duration, diabetes medication use, HbA_{1c} levels, and the number of self-reported comorbidities. Sixth, mortality outcomes were ascertained by linkage to the National Death Index with a probabilistic match, which might result in misclassification. However, a prior validation study showing a high accuracy of the method (37). Lastly, the role of confounding by psychosocial stress or genetic susceptibility, residual or unknown confounding, or chance in the current study could not be excluded.

Conclusion

In a nationally representative sample of U.S. adults with diabetes, we found that higher serum 25(OH)D concentrations were significantly associated with lower all-cause and CVD mortality. These findings support the potential benefits of maintaining adequate vitamin D status in the prevention of premature death among individuals with diabetes.

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