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Clinical Research Article

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# Association Between Serum 25-hydroxyvitamin D Concentrations and Mortality Among Adults With Prediabetes

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**Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HEI, Health Eating Index; HOMA-IR, homeostasis model assessment of insulin resistance; ICD-10, International Classification of Diseases, 10th edition; LC-MS/MS, liquid chromatograph-tandem mass spectometry; MET, metabolic equivalent; NHANES, National Health and Nutrition Examination Survey

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## Abstract

**Objectives:** To investigate the association of circulating 25-hydroxyvitamin D [25(OH)D] levels with mortality among adults with prediabetes.

**Methods:** This retrospective cohort study included 15,195 adults with prediabetes (aged ≥20 years) from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 2001-2014. Mortality from all causes, cardiovascular disease (CVD), and cancer was linked to National Death Index mortality data.

**Results:** The median (interquartile range) concentration of serum 25(OH)D was 60.5 (45.3, 77.4) nmol/L, and only 23.1% had sufficient vitamin D (≥75 nmol/L). Elevated serum 25(OH)D concentrations were significantly associated with lower levels of insulin, homeostasis model assessment of insulin resistance, triglyceride, and C-reactive protein, and higher levels of high-density lipoprotein at baseline (all $P_{trend} < 0.05$). During a median follow up of 10.7 years, 3765 deaths (including 1080 CVD deaths and 863 cancer deaths) were identified. Compared with participants with 25(OH)D <30 nmol/L, the multivariate-adjusted hazard ratios and 95% confidence intervals for participants with 25(OH)D ≥ 75 nmol/L were 0.66 (0.53, 0.82) for all-cause mortality ($P_{trend} < 0.001$), 0.66 (0.48, 0.89) for CVD mortality ($P_{trend} = 0.001$), and 0.82 (0.49, 1.35) for cancer mortality

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($P_{trend}$ = 0.32). For per-unit increment in ln-transformed 25(OH)D, there was a 27% lower risk of all-cause mortality and a 34% lower risk of CVD mortality (both $P < 0.01$).
**Conclusions:** These findings suggested that higher serum 25(OH)D concentrations were associated with lower all-cause and CVD mortality among individuals with prediabetes.

**Key Words:** vitamin D, mortality, prediabetes, epidemiology

Prediabetes, characterized by impaired glucose tolerance and/or impaired fasting glucose (1), is a high-risk state for developing diabetes, cardiovascular disease (CVD), and premature death (2-6). The estimated prevalence of prediabetes was 37.5% in US adults (7), and the number of people having prediabetes will reach 472 million by 2030, globally (3). Therefore, it is essential to identify modifiable factors to prevent or delay the development of type 2 diabetes and other adverse health outcomes among individuals with prediabetes.

In addition to the well-documented roles in mineral homeostasis and bone metabolism (8), vitamin D has also possessed anti-inflammatory, antiproliferative, and immunomodulatory properties (9, 10). Although several recent intervention trials found that vitamin D supplementation did not lower CVD risk among participants who had relatively high vitamin D status at baseline (e.g., mean 77 nmol/L in the Vitamin D and Omega-3 Trial (VITAL) (11, 12), the long-term health effects of vitamin D among adults with prediabetes, in whom the prevalence of vitamin D deficiency is relatively high (13), remains unclear. Some epidemiologic studies suggested that low vitamin D status is associated with high insulin resistance, systemic inflammation, increased arterial stiffness, and unfavorable lipid profiles in prediabetes (14-16). In addition, vitamin D supplementation could significantly improve insulin sensitivity and reduce the risk of progressing to diabetes (17, 18). However, it remains unknown whether low vitamin D status is associated with elevated risk of premature death among individuals with prediabetes.

To fill these knowledge gaps, we aimed to prospectively investigate the associations between serum 25-hydroxyvitamin D [25(OH)D] concentrations and risk of all-cause and cause-specific mortality in a nationally representative sample of US adults with prediabetes.

## Materials and Methods

### Study population

The National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention, uses a complex, multistage probability sampling design to obtain nationally representative samples of the civilian noninstitutionalized US population. The details of the questionnaire interviews, physical examinations, and specimen analyses have been described elsewhere (19). The protocol has been approved by the Ethics Review Board of National Center for Health Statistics, and all participants completed written informed consent.

The current study included 15,650 adults (age ≥20 years) with prediabetes from NHANES III (1988-1994) and NHANES 2001-2014. After excluding participants without serum 25(OH)D measurement (n = 442), no data of mortality status (n = 11), or lacking of follow-up time (n = 2), a total of 15,195 participants were finally included in the present analysis. Flowchart of the study participants is shown in Supplemental Figure 1 (20).

### Ascertainment of prediabetes

According to the criteria of the American Diabetes Association, prediabetes was defined as individuals without diabetes mellitus, but with fasting plasma glucose level of 100 mg/dL to 125 mg/dL, or 2-hour plasma glucose level of 140 mg/dL to 199 mg/mL, or glycated hemoglobin A1c (HbA1c) level of 5.7% to 6.4%.

### Measurement of serum 25(OH)D

Details of serum 25(OH)D assay methods have been described elsewhere (21). Briefly, the DiaSorin radioimmunoassay kit (Stillwater, MN) was used to determine serum 25(OH)D in NHANES III and NHANES 2001-2006. A standardized liquid chromatograph-tandem mass spectrometry (LC-MS/MS) method was used for serum 25(OH)D measurements since 2007. The original results of serum 25(OH)D concentration in NHANES III and NHANES 2001-2006 were converted by regression to equivalent data from LC-MS/MS. As recommended, we used LC-MS/MS equivalent data for all analysis. The values of concentration below limit of detection were calculated as limit of detection divided by the square root of 2.

### Ascertainment of mortality

Mortality status was acquired from the NHANES Public-use Mortality File, linked to the National Death Index

through December 31, 2015. The disease-specific death was defined on the basis of the International Statistical Classification of Disease, Tenth Revision (ICD-10). Cardiovascular mortality included deaths from heart diseases (ICD-10 codes I00-I09, I11, I13, I20-I51) or cerebrovascular diseases (ICD-10 codes I60-I69). Cancer mortality included deaths from malignant neoplasms (ICD-10 codes C00-C97).

### Assessment of covariates

Questionnaire interviews were conducted to obtain information on demographics (age, sex, race/ethnicity, family income, and educational level), living habits (smoking status, alcohol consumption, and physical activity), and health conditions (history of hypertension, high blood cholesterol, and CVD). Physical examinations were conducted to measure body weight and height at the mobile examination center. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Nonsmokers were defined as individuals who smoked fewer than 100 cigarettes in life. Those who smoked more than 100 cigarettes but did not smoke now were former smokers. Those who smoked now were current smokers. Alcohol consumption was categorized as nondrinkers (0 drink/d), moderate drinkers (<2 drinks/d for males and <1 drink/d for females), and heavy drinkers (≥2 drinks/d for males and ≥1 drink/d for females). Based on total times of physical activities per week and the metabolic equivalents (METs) of those activities, leisure-time physical activity was classified as low (no activity), moderate (1-3 times/wk and METs >6, or 1-5 times/wk and 3 ≤ METs < 6), and vigorous (>3 times/wk and METs > 6, or > 5 times/wk and 3 ≤ METs < 6). Medical conditions were confirmed based on the response of "yes" to the questions "Ever been told by a doctor or other health professional that you had hypertension, high blood cholesterol, heart failure, coronary heart disease, angina, heart attack, or stroke," respectively.

Metabolic biomarkers including plasma glucose, insulin, HbA1c, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, triglyceride, and C-reactive protein (CRP) were determined among participants who provided blood samples. Homeostasis model assessment of insulin resistance (HOMA-IR) was computed by the formula: HOMA-IR = insulin/ ($22.5e^{-\ln \text{glucose}}$) (22). As an indicator of diet quality, the Healthy Eating Index (HEI) scores were calculated from 24-hour dietary recall interviews. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (23).

### Statistical analysis

All analyses were performed using SAS, version 9.4 (Cary, NC). The survey analysis procedures were applied to account for the complex sampling design of NHANES. Pearson-time was calculated from the examination of vitamin D concentrations to the date when death occurred or the end of follow-up (December 31, 2015), whichever came first. We used the generalized linear model to examine the associations between vitamin D concentrations and cardiometabolic biomarkers at baseline. Cox proportional hazards regression model was applied to evaluate the associations of serum 25(OH)D concentrations with all-cause and cause-specific mortality. Given that few participants with 25(OH)D < 25 nmol/L (<5%) in the current study population, vitamin D status was categorized as 4 groups: <30 nmol/L (severe deficiency), 30 to <50 nmol/L (moderate deficiency), 50 to <75 nmol/L (insufficient), and ≥75 nmol/L (sufficient). The linear trend was evaluated by assigning a median value to each category as a continuous variable. Serum 25(OH)D were also analyzed as a continuous variable after ln-transformation (because of the skewed distribution). The multivariate models included age (years), sex (male or female), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other), BMI (<25.0, 25.0 to <30.0, or ≥30.0 kg/m$^2$), the ratio of family income to poverty threshold (< 1, 1:3, or >3), education attainment level (less than high school, high school or equivalent, or college or above), smoking status (nonsmoker, former smoker, or current smoker), alcohol consumption (nondrinker, moderate drinker, or heavy drinker), leisure-time physical activity (low, moderate, or vigorous), and self-reported hypertension, high blood cholesterol, CVD, or cancer. We used multiple imputation for covariates with missing values (<5%).

Restricted cubic spline regression with 3 knots (25th, 50th, and 75th) was applied to test a nonlinear association between serum 25(OH)D concentrations and mortality. Stratified analysis was also performed according to age (<60 or ≥60 years), sex (male or female), race/ethnicity (White, or non-White), BMI (<25.0, 25.0 to <30.0, or ≥30.0 kg/m$^2$), education attainment level (less than high school or high school or above), smoking status (nonsmoker, former smoker, or current smoker), alcohol consumption (nondrinker, moderate drinker, or heavy drinker), leisure-time physical activity (low, moderate, or vigorous), blood drawing season (November 1 to April 30 or May 1 to October 31), and renal function (eGFR ≤90 or eGFR >90).

In addition, several sensitivity analyses were also conducted: (1) blood drawing season (data available in

NHANES 2001-2014 only) were further adjusted because large seasonal variation of serum 25(OH)D has been reported previously; (2) dietary supplement use, calcium and magnesium intake, polyunsaturated fatty acid intake, or HEI score were additionally adjusted to account for the potential influence of dietary factors; (3) eGFR was further controlled as renal function was suggested to be associated with circulating vitamin D levels and cardiovascular events; (4) PTH was further included in the multivariate models, given the internal relationship between vitamin D status and PTH level (data available in NHANES 2003-2006 only); (5) analyses were restricted to participants without missing value of covariates, or without history of CVD, or cancer; (6) deaths occurred within the first 2 years of follow-up were excluded to minimize the potential reverse causation bias; (7) the main analyses were repeated according to quartiles of serum 25(OH)D concentrations; and (8) metabolic biomarkers including CRP, HDL-C, low-density lipoprotein cholesterol, triglyceride, or HOMA-IR (data available in subgroup of participants only) were further adjusted to examine whether inflammatory, blood lipids, or insulin resistance might mediate the association of interest. Furthermore, we repeated the main analyses for participants with impaired fasting glucose and participants with impaired glucose tolerance, respectively. Two-tailed $P < 0.05$ was considered to be statistically significant.

## Results

Among 15,195 participants included in the study, there were 7207 (47.4%) who underwent an oral glucose tolerance test, 2807 (18.5%) of which met the diagnosis criterion of prediabetes. The mean age of the study population was 52.3 years, and 53.7% were male. The weighted median (interquartile range) serum 25(OH)D concentration was 60.5 (45.3, 77.4) nmol/L, and 38.9% of adults with prediabetes had deficient vitamin D (<50 nmol/L), and only 23.1% had sufficient vitamin D (≥75 nmol/L). Table 1 shows the baseline characteristics of participants with prediabetes according to serum 25(OH)D concentrations. Participants with higher serum 25(OH)D concentrations tended to be older and non-Hispanic White, were less likely to be current smokers and obese, and had higher family income, education attainment level, and leisure-time physical activity. In addition, participants with higher serum 25(OH)D concentrations were significantly associated with lower levels of insulin, HOMA-IR, triglyceride, and CRP, and higher levels of HDL (all $P_{trend} < 0.05$), as shown in Table 2.

During a median follow-up of 10.7 years (interquartile range, 5.5-22.1 years), a total of 3765 deaths were identified, including 1080 CVD deaths and 863 cancer deaths. Table 3 shows the associations between serum 25(OH)D concentrations and all-cause and cause-specific mortality. Compared with participants with serum 25(OH)D levels <30 nmol/L, the multivariate-adjusted hazard ratios (95% confidence intervals) for those with serum 25(OH)D levels ≥ 75 nmol/L were 0.66 (0.53-0.82) for all-cause mortality ($P_{trend} < 0.001$), 0.66 (0.48-0.89) for CVD mortality ($P_{trend} = 0.001$), and 0.82 (0.49, 1.35) for cancer mortality ($P_{trend} = 0.32$). Figure 1 shows a linear relationship between 25(OH)D levels (range, 17.3-130 nmol/L) and all-cause mortality and CVD mortality ($P_{linearity} < 0.001$). For per 1-unit increment in ln-transformed 25(OH)D level, there was a 27% lower risk of all-cause mortality and a 34% lower risk of CVD mortality (Table 3).

In subgroup/sensitivity analyses shown in the online repository (20), the results were similar when analyses were stratified by age, sex, ethnicity/race, BMI, smoking status, alcohol consumption, leisure-time physical activity, blood drawing season, or renal function, although some of the associations did not reach statistical significance largely because of reduced power (Supplemental Table 1). No significant interactions were observed between serum 25(OH)D and these stratified variables. In sensitivity analyses, the results did not significantly change when further adjustment of season of blood draw (Supplement Table 2), or dietary supplement use, calcium and magnesium intake, and polyunsaturated fatty acid intake (model 2, Supplement Table 3), or HEI score (model 3, Supplement Table 3), or renal function (model 4, Supplemental Table 3), or PTH (Supplemental Table 4). Consistent results were demonstrated when excluding participants with missing value of covariates, or excluding participants with history of CVD or cancer (Supplemental Table 5), or excluding death occurred within 2 years of follow up (Supplemental Table 6). Similar results were observed when serum 25(OH)D was categorized as quartiles (Supplemental Table 7). When CRP, blood lipids, or HOMA-IR were further adjusted for in a subgroup of participants, the association between serum 25(OH)D and mortality did not materially change (Supplemental Table 8).

## Discussion

In a nationally representative sample of adults, we, for the first time, found that higher serum vitamin D concentrations were significantly and linearly associated with lower risk of all-cause and CVD mortality among individuals with prediabetes. In addition, higher serum 25(OH)D concentrations were linked to a favorable lipid profile, lower levels of insulin, HOMA-IR, and CRP. A series of stratified

**Table 1.** Baseline characteristics of participant with prediabetes according to serum 25(OH)D concentrations in NHANES III and NHANES 2001-2014

| Characteristics | Serum 25(OH)D concentrations, nmol/L | | | | Total |
|---|---|---|---|---|---|
| | <30.0 | 30.0 to < 50.0 | 50.0 to < 75.0 | ≥75.0 | |
| No. | 1461 (9.6) | 4457 (29.3) | 5774 (38.0) | 3503 (23.1) | 15195 |
| Age (mean ± SE), y | 49.5 ± 0.6 | 51.0 ± 0.5 | 51.9 ± 0.4 | 54.7 ± 0.5 | 52.3 ± 0.3 |
| Male | 615 (42.1) | 2270 (50.9) | 3394 (58.8) | 1878 (53.6) | 8157 (53.7) |
| Race/ethnicity | | | | | |
| Non-Hispanic White | 246 (16.8) | 1281 (28.7) | 2829 (49.0) | 2415 (68.9) | 6771 (44.6) |
| Non-Hispanic Black | 845 (57.8) | 1556 (34.9) | 959 (16.6) | 312 (8.9) | 3672 (24.2) |
| Mexican American | 233 (15.9) | 1071 (24.0) | 1259 (21.8) | 386 (11.0) | 2949 (19.4) |
| Other | 137 (9.4) | 549 (12.3) | 727 (12.6) | 390 (11.1) | 1803 (11.9) |
| BMI, kg/m$^2$ | | | | | |
| <25.0 | 335 (23.4) | 1037 (23.5) | 1422 (24.9) | 1191 (34.4) | 3985 (26.5) |
| 25.0-29.9 | 406 (28.4) | 1562 (35.4) | 2227 (39.0) | 1333 (38.5) | 5528 (36.8) |
| ≥30.0 | 689 (48.2) | 1808 (41.0) | 2063 (36.1) | 942 (27.2) | 5502 (36.6) |
| Family income to poverty ratio | | | | | |
| <1 | 364 (27.0) | 963 (23.8) | 1061 (20.1) | 517 (16.0) | 2905 (20.9) |
| 1:3 | 619 (45.9) | 1833 (45.3) | 2328 (44.2) | 1340 (41.6) | 6120 (44.1) |
| >3 | 365 (27.1) | 1253 (30.9) | 1878 (35.7) | 1368 (42.4) | 4864 (35.0) |
| Education level | | | | | |
| Less than high school | 732 (50.4) | 2231 (50.3) | 2624 (45.6) | 1288 (36.9) | 6875 (45.4) |
| High school or equivalent | 310 (21.3) | 855 (19.3) | 1108 (19.3) | 717 (20.5) | 2990 (19.8) |
| College or above | 410 (28.2) | 1349 (30.4) | 2023 (35.2) | 1489 (42.6) | 5271 (34.8) |
| Smoking status | | | | | |
| Nonsmoker | 733 (50.2) | 2246 (50.4) | 2833 (49.1) | 1631 (46.6) | 7443 (49.0) |
| Former smoker | 379 (26.0) | 1068 (24.0) | 1369 (23.7) | 804 (23.0) | 3620 (23.8) |
| Current smoker | 348 (23.8) | 1141 (25.6) | 1569 (27.2) | 1064 (30.4) | 4122 (27.1) |
| Alcohol consumption | | | | | |
| Nondrinker | 648 (48.1) | 1920 (46.7) | 2268 (42.1) | 1302 (39.8) | 6138 (43.5) |
| Moderate drinker | 569 (42.2) | 1889 (46.0) | 2702 (50.2) | 1683 (51.4) | 6843 (48.5) |
| Heavy drinker | 131 (9.7) | 301 (7.3) | 414 (7.7) | 288 (8.8) | 1134 (8.0) |
| Leisure-time physical activity | | | | | |
| Low | 809 (55.9) | 2082 (47.3) | 2413 (42.3) | 1402 (40.3) | 6706 (44.6) |
| Moderate | 406 (28.1) | 1410 (32.0) | 1933 (33.9) | 1087 (31.3) | 4836 (32.2) |
| Vigorous | 231 (16.0) | 913 (20.7) | 1362 (23.9) | 987 (28.4) | 3493 (23.2) |
| Medical condition | | | | | |
| High blood pressure | 549 (37.6) | 1636 (36.7) | 2072 (35.9) | 1453 (41.5) | 5710 (37.6) |
| High cholesterol level | 330 (22.6) | 1161 (26.0) | 1803 (31.2) | 1395 (39.8) | 4689 (30.9) |
| Cardiovascular disease | 137 (9.4) | 396 (8.9) | 576 (10.0) | 453 (12.9) | 1562 (10.3) |
| Cancer | 78 (5.3) | 349 (7.8) | 589 (10.2) | 544 (15.5) | 1560 (10.3) |

Means or percentages have been accounted for survey weights; values are numbers (percentages).
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; NHANES, National Health and Nutrition Examination Survey; SE, standard error.

analysis and sensitivity analyses showed the robustness of the results.

Numerous observational studies suggested that higher circulating vitamin D levels were associated with a lower risk of CVD events and mortality (24-27), although evidence from intervention trials demonstrated inconsistent findings (28, 29). For incidence, in the VITAL, consisting of 25,871 healthy US adults, daily vitamin D3 supplementation (2000 IU) for 5 years did not significantly reduce the risk of CVD events and all-cause mortality (11), although a secondary analysis of the VITAL found a significant reduction in advanced cancer incidence with vitamin D supplementation (30). In addition, monthly high-dose vitamin D3 supplementation (100,000 IU) for 3.3 years did not lower the incidence of CVD among 5108 New Zealand adults in the Vitamin D Assessment Study (12). Although observational studies are prone to confounding factors, these randomized trials also had some limitations. For example, the beneficial effect of vitamin D could be more pronounced in people with poor vitamin D status (31), but

**Table 2.** Least-square means of cardiometabolic markers according to serum 25(OH)D concentrations among those with prediabetes in NHANES III and NHANES 2001-2014

| | Serum 25(OH)D concentrations, nmol/L | | | | |
|---|---|---|---|---|---|
| | <30.0 | 30.0 to < 50.0 | 50.0 to < 75.0 | ≥75.0 | $P_{trend}$ |
| Glucose (n = 11 493, mmol/L) | 5.68 ± 0.03 | 5.68 ± 0.02 | 5.69 ± 0.02 | 5.69 ± 0.02 | 0.58 |
| Insulin (n = 11 383, uU/mL) | 14.27 ± 0.42 | 14.28 ± 0.35 | 13.61 ± 0.33 | 13.39 ± 0.37 | 0.02 |
| HOMA-IR (n = 11 369) | 5.66 ± 0.02 | 5.67 ± 0.01 | 3.50 ± 0.09 | 3.44 ± 0.10 | 0.03 |
| HbA1c (n = 15 185, %) | 5.66 ± 0.02 | 5.67 ± 0.01 | 5.67 ± 0.01 | 5.68 ± 0.02 | 0.45 |
| Total cholesterol (n = 15 107, mg/dL) | 201.9 ± 2.0 | 205.3 ± 1.6 | 203.9 ± 1.4 | 201.4 ± 1.6 | 0.07 |
| HDL-C (n = 15 075, mg/dL) | 52.33 ± 0.60 | 52.25 ± 0.46 | 52.89 ± 0.46 | 54.67 ± 0.58 | <0.001 |
| LDL-C (n = 8484, mg/dL) | 116.4 ± 2.4 | 121.1 ± 1.7 | 120.0 ± 1.78 | 119.2 ± 1.7 | 0.71 |
| Triglyceride (n = 11 476, mg/dL) | 155.7 ± 8.3 | 159.9 ± 4.3 | 149.9 ± 3.5 | 142.9 ± 4.6 | 0.002 |
| CRP (n = 11 882, mg/dL) | 0.70 ± 0.05 | 0.63 ± 0.04 | 0.60 ± 0.03 | 0.56 ± 0.03 | 0.002 |

Least-square (mean ± standard error) was estimated using general linear model with adjustment of age (continuous), sex (male or female), race (non-Hispanic White, non-Hispanic Black, Mexican American, or other), BMI (<25.0, 25.0 to <30.0, or ≥30.0 kg/m$^2$), education (less than high school, high school or equivalent, or college or above), family income to poverty ratio (<1, 1:3, or >3), smoking status (nonsmoker, former smoker, or current smoker), and leisure-time physical activity (low, moderate, or vigorous), and self-reported hypertension (yes or no), self-reported high blood cholesterol (yes or no), self-reported CVD (yes or no), or self-reported cancer (yes or no).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CRP, C-reactive protein; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NHANES, National Health and Nutrition Examination Survey.

**Table 3.** Hazard ratios (95% CIs) for mortality according to serum 25(OH)D levels for participants with prediabetes in NHANES III and NHANES 2001-2014

| | Serum 25(OH)D concentrations, nmol/L | | | | | Per 1-unit increment in ln-transformed 25(OH)D |
|---|---|---|---|---|---|---|
| | <30.0 | 30.0 to <50.0 | 50.0 to <75.0 | ≥75.0 | $P_{trend}$ | |
| All-cause mortality | | | | | | |
| No. deaths/total | 356/1461 | 1206/4457 | 1433/5774 | 770/3503 | | |
| Model 1 | 1 | 0.78 (0.65-0.93) | 0.61 (0.50-0.74) | 0.57 (0.47-0.71) | <0.001 | 0.64 (0.57-0.73) |
| Model 2 | 1 | 0.87 (0.71-1.06) | 0.73 (0.58-0.91) | 0.67 (0.54-0.84) | <0.001 | 0.73 (0.65-0.83) |
| Model 3 | 1 | 0.85 (0.70-1.04) | 0.72 (0.58-0.89) | 0.66 (0.53-0.82) | <0.001 | 0.73 (0.65-0.82) |
| CVD mortality | | | | | | |
| No. deaths | 92 | 371 | 409 | 208 | | |
| Model 1 | 1 | 0.87 (0.61-1.23) | 0.64 (0.48-0.86) | 0.59 (0.44-0.79) | <0.001 | 0.60 (0.48-0.75) |
| Model 2 | 1 | 0.93 (0.64-1.34) | 0.73 (0.53-1.00) | 0.67 (0.49-0.90) | 0.001 | 0.67 (0.54-0.83) |
| Model 3 | 1 | 0.91 (0.63-1.31) | 0.73 (0.54-0.99) | 0.66 (0.48-0.89) | 0.001 | 0.66 (0.54-0.82) |
| Cancer mortality | | | | | | |
| No. deaths | 81 | 273 | 333 | 176 | | |
| Model 1 | 1 | 0.87 (0.54-1.41) | 0.72 (0.43-1.19) | 0.72 (0.43-1.22) | 0.20 | 0.71 (0.52-0.96) |
| Model 2 | 1 | 0.98 (0.60-1.60) | 0.88 (0.53-1.44) | 0.86 (0.51-1.43) | 0.41 | 0.82 (0.61-1.09) |
| Model 3 | 1 | 0.95 (0.59-1.53) | 0.84 (0.52-1.38) | 0.82 (0.49-1.35) | 0.32 | 0.79 (0.60-1.05) |

Model 1: adjusted for age (continuous), sex (male or female) and race (non-Hispanic White, non-Hispanic Black, Mexican American, or other). Model 2: 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 to poverty ratio (<1, 1:3, >3), alcohol consumption (nondrinker, moderate drinker, heavy drinker), smoking status (nonsmoker, former smoker, or current smoker), and leisure time physical activity (low, moderate, or vigorous). Model 3: further adjusted for self-reported hypertension (yes or no), self-reported high blood cholesterol (yes or no), self-reported CVD (yes or no), or self-reported cancer (yes or no).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; NHANES, National Health and Nutrition Examination Survey.

the mean serum 25(OH)D level of participants enrolled in the VITAL was relatively high at baseline (77 nmol/L), and only <25% participants had vitamin D deficiency at baseline in the Vitamin D Assessment Study. Moreover, these trials only tested 1 dose of vitamin D supplementation and had limited power to assess the effect on mortality. So far, evidence from observational studies and clinic trials regarding the role of vitamin D on cancer mortality has been

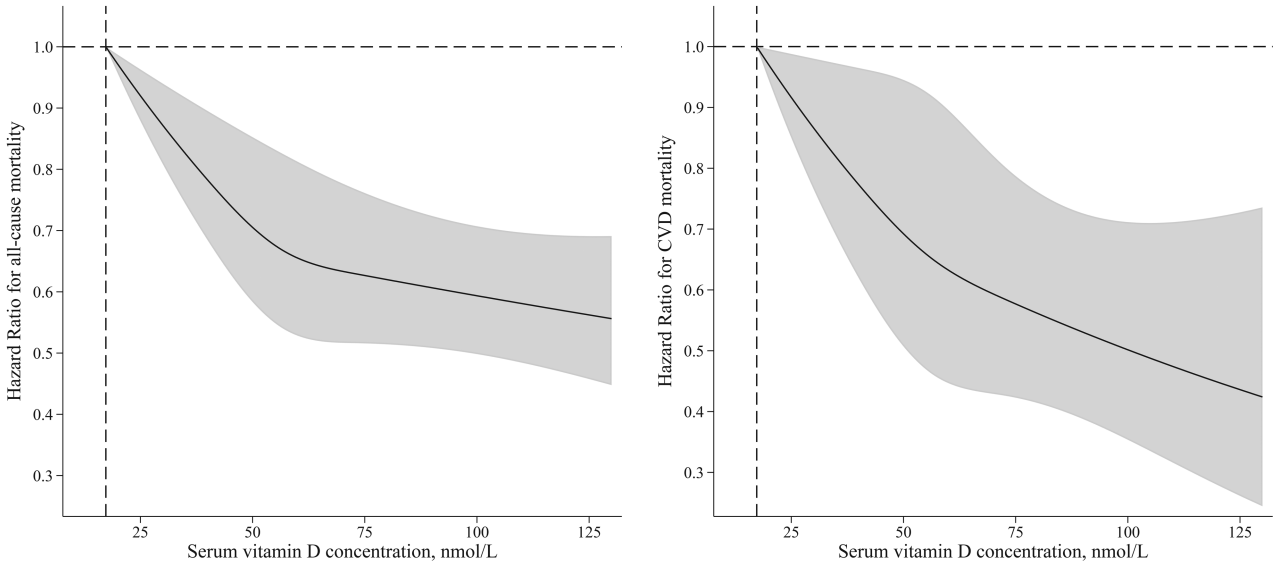


**Figure 1.** Associations between serum 25(OH)D concentrations (17.3-130 nmol/L) with all-cause (left) and CVD mortality (right) among those with prediabetes in NHANES III and NHANES 2001-2014. HRs were adjusted for age (continuous), sex (male or female), race (non-Hispanic White, non-Hispanic Black, Mexican American, or other), BMI (<25.0, 25.0 to <30.0, or ≥30.0 kg/m$^2$), education (less than high school, high school or equivalent, or college or above), family income to poverty ratio (<1, 1:3, or >3), smoking status (nonsmoker, former smoker, or current smoker), and leisure-time physical activity (low, moderate, or vigorous), and self-reported hypertension (yes or no), self-reported high blood cholesterol (yes or no), self-reported CVD (yes or no), and self-reported cancer (yes or no). The solid line represents HRs and the dashed lines represent 95% confidence intervals. Knots were set at 25th, 50th, and 75th. The reference value for serum 25(OH)D was 17.3 nmol/L (HR = 1.0). Both $P_{linearity}$ <0.01. 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CVD, cardiovascular disease; HR, hazard ratio; NHANES, National Health and Nutrition Examination Survey.

limited and inconsistent. In our study, the statistical power seemed limited, and more large well-designed research is warranted to investigate the association of vitamin D with cancer mortality in the future.

Of note, the previous studies were mostly conducted among the general population and evidence is limited and inconsistent regarding the potential effect of vitamin D among people with prediabetes, who had a relatively high prevalence of vitamin D deficiency and elevated risk of developing cardiometabolic diseases (2, 13). For example, 2 recent meta-analyses found that vitamin D supplementation among those with prediabetes was associated with a lower risk of diabetes or increased rate of returning to normoglycemia (18, 32). However, some, but not all, small randomized controlled trials in prediabetes found that vitamin D supplementation reduced fasting glucose and HbA1c levels, but did not improve insulin resistance (33-37). In the more recent Vitamin D and Type 2 Diabetes trial consisting of 2423 participants with prediabetes, although daily vitamin D3 supplementation at a dose of 4000 IU did not significantly lower the diabetes incidence than placebo after 2.5 years of follow-up (38), a secondary analysis of Vitamin D and Type 2 Diabetes trial found that participants in vitamin D-treated group who maintained intra-trial 25(OH)D concentrations ≥100 nmol/L had a more than 50% lower risk of developing diabetes, compared with those who maintained 50 to 74 nmol/L (39). In addition, another randomized trial among 162 patients with prediabetes and vitamin D deficiency found that weekly high-dose vitamin D3 supplementation (50,000 IU) for 3 months could significantly improve insulin sensitivity and lower risk of progression to diabetes (17). The inconsistent findings of these intervention trials might be attributable to the differences in sample size, characteristics of the enrolled participants (e.g., vitamin D deficiency or not at baseline), vitamin D supplement dose, or intervention duration. To our knowledge, no study has examined the association between vitamin D status and risk of mortality among prediabetes. In the current prospective study, based on a nationally representative sample of US adults with prediabetes, we found an inverse dose-response (range, 17.3-130 nmol/L) relationship between serum 25(OH)D levels and all-cause and CVD mortality, independent of traditional risk factors. Additionally, race/ethnicity, smoking, and obesity status did not modify the vitamin D–mortality association among prediabetes.

The observed association might be explained by several possible mechanisms. First, vitamin D could be involved in multiple physiological and pathological processes mediated by vitamin D receptor (40), of which the activation in endothelial cells and cardiomyocytes could regulate vascular smooth muscle contractions and vascular tension, resulting in protective effect on endothelial dysfunction and lower production of active oxygen species (41). Second, vitamin D deficiency might

activate the renin-angiotensin axis, followed by a series of changes such as water and salt retention, vasoconstriction, and myocardial fibrosis (42). Third, vitamin D plays an important role in pancreatic endocrine function (43). Some animal studies demonstrated that lacking functional vitamin D receptor was related to impaired oral glucose tolerance, decreased insulin secretion, and reduced levels of pancreatic insulin mRNA (44, 45). Observational studies and randomized trials also observed impaired glucose metabolism and insulin resistance among participants with low vitamin D levels (37, 46-49). Nonetheless, the exact mechanism underlying the vitamin D–mortality association among prediabetes remains to be elucidated, and more mechanistic studies are needed.

The strengths of our study include the prospective study design and the use of a nationally representative sample of US adults. Moreover, we have taken into account a wide range of potential confounders, including season of blood drawing, dietary factors, and cardiometabolic markers (e.g., PTH). However, there are also several limitations that need to be addressed. First, we were unable to establish causal relationship because of the observational study design. Second, serum 25(OH)D levels were only measured at a single time point in the current population, although a previous study tracking 25(OH)D levels for 14 years demonstrated a good proxy for vitamin D status with the use of a single measurement of 25(OH)D in epidemiologic studies (50). Third, we did not evaluate the active form of vitamin D and genetic variants in the vitamin D pathway (51, 52), which warrants more investigations in future. Fourth, self-reported health status might not capture the early stage of cardiometabolic diseases; more detailed clinical information is needed to consider in additional studies. Fifth, given that the current study did not have data on glucose profile of the participants during the follow-up, more studies are warranted to investigate the role of vitamin D on the progression from prediabetes to diabetes and other health outcomes. Finally, residual or unmeasured confounding could not be entirely ruled out.

In conclusion, our study found that higher serum 25(OH)D concentrations were significantly and linearly associated with lower all-cause and CVD mortality among individuals with prediabetes. These findings suggest that maintaining adequate vitamin D status may lower mortality risk in prediabetes.

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## Additional Information

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***Data Availability:*** Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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