CLINICAL RESEARCH STUDY

THE AMERICAN JOURNAL of MEDICINE®

Relationship between 25-Hydroxyvitamin D and All-cause and Cardiovascular Disease Mortality

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ABSTRACT

BACKGROUND: Observational studies have suggested a strong relationship between 25(OH)D and all-cause and cardiovascular disease mortality. A few studies also have described a nonlinear trend for this relationship in population subgroups, but less is known about this relationship in healthy adults. We examined the presence of a nonlinear relationship between 25(OH)D and all-cause and cardiovascular disease mortality among healthy adults.

METHODS: We examined 10,170 participants (≥18 years of age) using National Health and Nutrition Examination Survey data (2001-2004) combined with National Death Index for vital status information through December 2006. Cox proportional hazard models with spline (single knot at population median of 25[OH]D) were fit to estimate hazard ratios (HRs) for all-cause and cardiovascular disease mortality for each 10-unit increase in serum 25(OH)D. Models were adjusted for demographic and conventional cardiovascular disease risk factors.

RESULTS: Mean age of study participants was 46.6 (20.5) years, while median (interquartile range) 25(OH)D was 21 (15-27) ng/mL. After a median follow-up of 3.8 years (range 2.8-4.9), 509 all-cause and 184 cardiovascular diseases-related deaths were observed. In univariate analysis, 25(OH)D decreased hazards of all-cause (HR 0.59; 95% confidence interval [CI], 0.45-0.77) and cardiovascular disease (HR 0.56; 95% CI, 0.38-0.82) mortality below but not above its population median. In adjusted models, 25(OH)D retained the inverse association for all-cause (HR 0.54; 95% CI, 0.35-0.84) and cardiovascular disease (HR 0.50; 95% CI, 0.26-0.98) mortality below but not above its population median.

CONCLUSIONS: We found an inverse association between 25(OH)D and all-cause and cardiovascular disease mortality in healthy adults with serum 25(OH)D levels of ≤21 ng/mL. Clinical trials for the primary prevention of cardiovascular disease with 25(OH)D supplementation may target healthy adults with serum 25(OH)D levels of ≤21 ng/mL to validate these findings.

 • *The American Journal of Medicine (2013) 126, 509-514*

KEYWORDS: All-cause mortality; Cardiovascular disease mortality; NHANES; 25(OH)D; Vitamin D

Observational studies have reported a strong association between lower serum 25(OH)D levels and an increased risk of all-cause[1-6] and cardiovascular disease mortality.[7-9] Several mechanisms have been proposed to elucidate possible underlying pathways for these associations. For example, lower levels of serum 25(OH)D have been linked with endothelial dysfunction,[10] subclinical atherosclerosis,[11,12] hypertension,[13,14] upregulation of renin-angiotensin-aldosterone system (RAAS), hypertrophy of smooth muscles and left ventricle,[15-17] type 2 diabetes and metabolic syndrome,[18,19] and secondary hyperparathyroidism.[20]

Interestingly, most of the observational studies have examined the associations between 25(OH)D status and all-cause and cardiovascular disease mortality in specific subpopulations, such as individuals with chronic kidney disease,[1,2] hospitalized or nursing home residents,[5,6] or individuals with preexisting cardiovascular disease.[7,9] In comparison with generally healthy populations, these subgroups may not reflect the true status of serum 25(OH)D and its

Funding: Rehan Qayyum was supported by National Institute of Health grant No. 1K23HL105897-01.

Conflict of Interest: None.

Authorship: Both authors had access to the data and a role in writing the original manuscript.

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0002-9343/$ -see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.amjmed.2012.11.021

impact on the prevention of all-cause or cardiovascular disease mortality. In addition, a number of studies have used a quartile-based analytic approach to estimate associations between 25(OH)D status and all-cause and cardiovascular disease mortality, which could possibly obscure findings at the extremes of 25(OH)D status in respective cohorts.[21] Moreover, the cutoff values for quartiles across these studies were not uniform; mean serum 25(OH)D levels in the lowest quartiles ranged from 5.6 to 17.8 ng/mL.[1-6,9]

More recently, studies have suggested a nonlinear trend in the relationship between serum 25(OH)D and mortality, with increased risks observed at both its higher and lower serum levels.[3,22-24]

Our aim for this study was to examine an association between vitamin D and all-cause and cardiovascular disease mortality in healthy adults. We hypothesized that in a large community-based cohort of healthy and asymptomatic adults, the association between 25(OH)D and all-cause and cardiovascular disease mortality is nonlinear such that an increase in 25(OH)D above its population median levels may have no impact on the reduction of the risks for all-cause or cardiovascular disease mortality. To study this hypothesis, we evaluated the association of 25(OH)D with all-cause and cardiovascular disease mortality using continuous National Health and Nutrition Examination Survey (NHANES) data for years 2001-2004 linked with National Death Index (NDI) mortality files through December 2006.

CLINICAL SIGNIFICANCE

- In healthy adults, there exists an inverse association between 25(OH)D and all-cause and cardiovascular disease mortality.
- In healthy adults, there seems to be no additional protection against all-cause or cardiovascular disease mortality once serum 25(OH)D levels rise above 21 ng/mL.
- We suggest that clinical trials for the primary prevention of cardiovascular disease with 25(OH)D supplementation may target healthy adults with serum 25(OH)D levels of ≤21 ng/mL.

METHODS

We used publicly accessible data from the continuous NHANES, which is an ongoing, multistage probability sample, cross-sectional survey designed to assess health and nutritional status of the civilian, noninstitutionalized population of the US. Detailed interviews, physical examinations, and serum samples were obtained from more than 10,000 individuals from the survey conducted between 2001 and 2004. Details of the sampling procedures and data collection techniques have been described previously and are available online (http://www.cdc.gov/nchs/nhanes/about_nhanes.htm; accessed September 8, 2012). In order to create a larger sample, data from 2 2-year cycles of the continuous NHANES were combined for years 2001-2002 and 2003-2004. Sample weights were constructed with rescaling of weights such that the sum of weights matched survey population at the midpoint of each survey period.

Demographic information was ascertained from self-reported responses to the questionnaire administered by trained interviewers. Body mass index (BMI) was calculated by dividing body weight in kilograms with height in meters squared. Obesity was defined as a BMI of ≥30 kg/m^2, and overweight if BMI was ≥25 kg/m^2 but <30 kg/m^2. Blood pressure was recorded up to 4 readings. Hypertension was defined as mean systolic blood pressure ≥140 mm Hg, mean diastolic blood pressure ≥90 mm Hg, a diagnosis of hypertension, or current use of antihypertensive medications. Glomerular filtration rate was calculated using Modification of Diet in Renal Disease equation.[25] Serum glucose was measured using Beckman Synchron LX20 (Beckman Coulter, Brea, Calif) test on refrigerated specimen, and cholesterol was measured enzymatically in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. Participants were categorized as smokers if they were currently smoking or had ever smoked >100 cigarettes. Beckman Coulter method was used for counting white blood cells in combination with automatic diluting and mixing device for sample processing.

DiaSorin (Saluggia, Italy) radio immunoassay, a 2-step procedure, was used to assay 25(OH)D. The first procedure involved extraction of 25(OH)D and other hydroxylated metabolites from serum with acetonitrile. Then the treated samples were assayed using an equilibrium radio immunoassay procedure. Updated and adjusted data files were used for 25(OH)D to address assay drift. Serum C-reactive protein was quantified by latex-enhanced nephelometry using a Behring Nephelometer II Analyzer.[26]

Data on mortality status were obtained using probabilistic match between NHANES and the NDI death certificate records. NDI provided follow-up information from the date of survey participation to December 31, 2006. The underlying cause of death was coded according to the International Classification of Diseases, Tenth Revision. We defined cardiovascular disease mortality broadly and included deaths due to major cardiovascular diseases (I00-I78), diseases of the heart (I00-I09, I11, I13, I20-I51), other heart diseases (I26-I51), essential (primary) hypertension and hypertensive renal disease (I10, I12), cerebrovascular diseases (I60-I69), atherosclerosis (I70), other diseases of the circulatory system (I71-I78), and other disorders of the circulatory system (I80-I99).

To address the hypothesis that the inverse association between 25(OH)D with all-cause and cardiovascular disease

mortality is different below and above the population median of 25(OH)D in this large cohort of asymptomatic adults, we introduced spline in the regression models with a single knot at 21 ng/mL, that is, the population median of 25(OH)D. The assumption was that the inverse association between 25(OH)D and mortality is present only up to, but not above, the population median of serum 25(OH)D.

We excluded individuals of age <18 years or with previous cardiovascular disease from the analysis. Assessment of previous cardiovascular disease was based on self-reporting by the participants. For example, participants who said yes to the questions such as "ever told you had a heart attack" or "ever told you had a stroke" were excluded from the analysis. Multivariable models were adjusted for ethnic background, sex, obesity, hypertension, serum glucose, smoking status, C-reactive protein, total cholesterol, and renal function. Selected variables were log-transformed to meet assumptions of residual normality. The hazard ratios of 25(OH)D for all-cause and cardiovascular disease mortality were reported for every 10-unit increase in 25(OH)D both below and above its population median, that is, 21 ng/mL.

All analyses were performed with adjustments for the complex survey sampling method of NHANES data. In continuous NHANES, primary sampling units represent variance (sampling units used to estimate sampling error) units. These sampling weights were assigned to each person, reflecting adjustment for the unequal probability of selection, nonresponse, and adjustments to independent population controls. Participants were oversampled from certain population subgroups, such as African and Mexican Americans, to ensure reliability and precision of estimates. Masked variance units were constructed to protect the confidentiality of data.

Analyses were performed on Stata/IC version 10.1 (Stata Corp LP, College Station, Tex), while survey-specific commands were used to calculate *t*-statistic to assess statistically different means and chi-squared statistic to assess statistically different proportions between the groups. Adjusted coefficients and their 95% confidence intervals (CIs) were estimated using univariate and multivariable Cox proportional hazards models. A *P*-value of <.05 was considered statistically significant.

RESULTS

After excluding participants who were younger than 18 years (n = 9548) or had values missing on 25(OH)D (n = 1443), the study sample comprised 10,170 participants who were free of cardiovascular disease. The mean (SD) age of the study participants was 46.6 (20.5) years, while median (interquartile range) serum 25(OH)D was 21 (15-

Table 1 Population Characteristics

	Vitamin D (ng/mL)		
Covariates	≤21 (5237)	>21 (4933)	*P* Values
Age (years)	44.6	45.2	.17
Mean (95% CI)	(43.8-45.4)	(44.3-46.1)	
Females, n (%)	2795 (55)	2470 (49.3)	<.05
Mortality, n (%)			
All cause	297 (5.7)	216 (4.8)	<.05
Cardiovascular	109 (2.1)	77 (1.6)	.013
Ethnic origin, n (%)			
Non-Hispanic white	1742 (55.2)	3445 (85)	<.05
Mexican American	1358 (10.5)	852 (5.4)	<.05
Non-Hispanic black	1686 (21)	334 (2.9)	<.05
Other Hispanics	193 (5.5)	175 (4.1)	.06
Other race	258 (7.8)	127 (2.8)	<.05
HTN, n (%)	2054 (37)	1741 (32)	<.05
CRP mg/dL	0.5	0.4	<.05
Mean (95% CI)	(0.4-0.6)	(0.3-0.5)	
Serum glucose (mg/dL)	97.7	92.2	<.05
Mean (95% CI)	(96.2-99.1)	(91.4-93.1)	
Obesity	1864 (39.3)	1134 (24.2)	<.05
Smoker (Current and ever)	897 (22.3)	771 (19.6)	.04
GFR mL/min/m^2	101.4	94.4	<.05
Mean (95% CI)	(99.8-103)	(93.2-95.7)	
Total cholesterol (mg/dL)	200.2	201.2	.37
Mean (95% CI)	(198.1-202.3)	(199-203)	

CRP = C-reactive protein; GFR = glomerular filtration rate; HTN = hypertension, defined as average systolic blood pressure (BP) >140 mm Hg or average diastolic BP >90 mm Hg or Individuals ever told they have HTN, or if participants were taking an antihypertensive; Obesity = body mass index >30 kg/m^2.

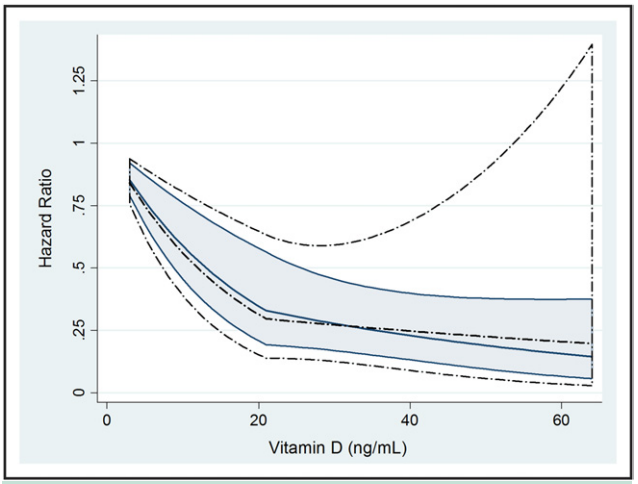

Figure Graph reflecting relationship in the risk (measured as hazard ratio with 95% confidence interval) of all-cause (**solid lines**) and cardiovascular disease (**dotted lines**) mortality with increasing serum 25(OH)D levels (using a single knot at population median of 25[OH]D levels, ie, 21 ng/mL), from the univariate Cox regression models.

27) ng/mL. Study sample included 5265 (52%) females, while 5187 (51%) participants were non-Hispanic whites.

The mean (SD) and median (range) of serum 25(OH)D were 21.9 (8.4) and 22 (16-27) ng/mL, respectively, in men, and 21.7 (9.9) and 21 (14-28) ng/mL, respectively, in women. The mean (25.7 ng/mL) serum 25(OH)D levels were significantly higher in non-Hispanic whites as compared with non-Hispanic blacks (14.7 ng/mL); $P < .001$. A total of 509 all-cause and 184 cardiovascular disease-related deaths were observed after a median follow-up of 3.8 years (range: 2.8-4.9 years).

Table 1 shows distribution of selected variables when data were divided at the population median of 25(OH)D, that is, 21 ng/mL. Mean age and total serum cholesterol levels (44.6 years and 200.2 mg/dL) below and (45.2 years and 210.2 mg/dL) above median of 25(OH)D were statistically similar (both *P*-values $> .05$). More than 50% of women had serum 25(OH)D $\leq$21 ng/mL ($P < .0001$). We found 297 deaths from all-cause among individuals with median serum 25(OH)D of $\leq$21 ng/mL, and 216 deaths from all-cause among individuals with serum 25(OH)D of $>$21 ng/mL ($P < .05$). Additionally, there were 109 deaths among individuals with median serum 25(OH)D of $\leq$21 ng/mL, and 77 deaths among individuals with serum 25(OH)D of $>$21 ng/mL from cardiovascular disease ($P < .05$).

In univariate regression models with spline (single knot at 21 ng/mL [ie, population median] of 25[OH]D), we observed a 41% reduction in the risk of all-cause mortality for each 10-ng/mL change in serum 25(OH)D up to 21 ng/mL (hazard ratio [HR] 0.59; 95% CI, 0.45-0.77) (**Figure**). An increase in 25(OH)D beyond 21 ng/mL was not associated with any statistically significant reduction (HR 0.83; 95% CI, 0.65-1.06) in the risk of all-cause mortality. In the multivariable models, the inverse relation between 25(OH)D and all-cause mortality remained significant (HR 0.54; 95% CI, 0.35-0.84) below but not above its population median (HR 0.83; 95% CI, 0.63-1.11) of 21 ng/mL (**Table 2**).

Similarly, with univariate regression model using single spline, we observed 44% reduction in the risk of cardiovascular disease mortality for each 10-ng/mL change in 25(OH)D up to 21 ng/mL (HR 0.56; 95% CI, 0.38-0.82) (**Figure**). An increase in serum 25(OH)D levels above 21 ng/mL was not associated with reduction in the risk of cardiovascular disease mortality (HR 0.91; 95% CI, 0.56-1.5). In the multivariable models, the inverse relation between 25(OH)D and cardiovascular disease mortality remained significant below (HR 0.50; 95% CI, 0.26-0.98) but not above its population median (HR 0.83; 95% CI, 0.47-1.47) of 21 ng/mL (**Table 2**).

DISCUSSION

From this large community-based, nationally representative cohort of adult population of the US, we report an inverse and nonlinear association between serum 25(OH)D and all-cause and cardiovascular disease mor-

Table 2 Hazard Ratios and 95% CIs for All-cause and Cardiovascular Mortality per 10 ng/mL Change in 25(OH)D from Univariate and Multivariate Cox Regression Models

	Vitamin D $\leq$21 ng/mL		Vitamin D $>$21 ng/mL	
Cox Regression Models	Hazard Ratio	95% CI	Hazard Ratio	95% CI
All-cause mortality				
Univariate	0.59	0.45-0.77	0.83	0.65-1.06
Multivariate	0.54	0.35-0.84	0.83	0.63-1.11
Cardiovascular mortality				
Univariate	0.56	0.38-0.82	0.91	0.56-1.5
Multivariate	0.50	0.26-0.98	0.83	0.47-1.5

CI = confidence interval.

Multivariate models were adjusted for race, age, sex, hypertension, smoking status, C-reactive protein, obesity, total cholesterol, renal function, and serum glucose.

tality. We found that, independent of demographic and conventional cardiovascular disease risk factors, an increase in serum 25(OH)D up to 21 ng/mL is associated with significant reduction in the risks of all-cause and cardiovascular disease mortality among healthy adults. We further report that an increase in serum 25(OH)D levels above 21 ng/mL offers statistically insignificant reduction in the risk of all-cause or cardiovascular disease mortality in healthy adults.

A number of mechanisms have been proposed to explain the association between lower serum 25(OH)D and all-cause and cardiovascular disease mortality.[10,12,14-19] Several cells types that are actively involved in the pathogenesis of cardiovascular disease have vitamin D receptors. For example, vascular smooth muscle cells express vitamin D receptors, and stimulation of these receptors by vitamin D results in inhibition of smooth muscle cell proliferation.[27,28] Vitamin D can regulate RAAS by suppressing the expression of the renin gene.[29] In fact, it is known that disruption of the vitamin D receptor gene results in the overstimulation of RAAS that leads to higher blood pressure and cardiac muscle hypertrophy.[30] In addition to exerting anticoagulant effects in cultured monocytic cells,[31] the active form of vitamin D can regulate production of inflammatory markers such as interleukin-2, interferon-gamma, and transferrin receptor expression at the messenger RNA level.[32] This demonstrates that vitamin D, through its receptors, plays a critical role in the maintenance of antithrombotic and inflammatory homeostasis.

Our results of a nonlinear inverse association between 25(OH)D status and all-cause mortality in healthy adults are consistent with few previously reported studies.[3,22] Melamed et al[3] have reported an increased risk of all-cause mortality among women with low (<20 ng/mL) as well as high (>50 ng/mL) levels of serum 25(OH)D using NHANES III data. Similarly, Michaëlsson et al[22] observed an approximately 50% higher total mortality in 1194 elderly men (mean age at baseline, 71 years) with lowest 18.5 ng/mL and highest 39.4 ng/mL serum concentration of 25(OH)D during a median follow-up of 12.7 years. Furthermore, Wang et al[23] from their study of 1739 Framingham Offspring Study participants (all white, mean age 59 years), also have suggested a nonlinear association between 25(OH)D and cardiovascular disease incidence (mean duration of follow-up was 5.4 years). The nonlinearity hypothesis in risk association is further supported by a recent meta-analysis of 14 studies where authors reported 31% reduction in mortality risk when serum 25(OH)D level was increased up to 31 ng/mL above the reference range of 11 ng/mL. Any further increase in 25(OH)D was not associated with decrease in risk of all-cause mortality.[24] In another study of 2878 elderly men recruited from 3 medical centers in Sweden and followed for up to 8.2 years, authors found no increase in survival once serum 25(OH)D rose above 24 ng/mL.[33]

Our findings provide support for the most recent recommendations from the Institute of Medicine in which serum 25(OH)D levels higher than 20 ng/mL were regarded as adequate.[34] In addition, the Committee writing a report on dietary reference intakes for calcium and vitamin D for the Institute of Medicine for 2011 has concluded that the serum concentrations of 25(OH)D above 30 ng/mL are not consistently associated with an increased benefit. The Committee argued against the widely reported deficiency of 25(OH)D; it further stated that the serum 25(OH)D levels of 16 ng/mL cover the requirements of approximately half the population, and levels of 20 ng/mL cover the requirements of at least 98% of the population.[35]

The strengths of our study include its large sample size of middle-aged and elderly men and women. The sampling scheme of the continuous NHANES allows better estimates and adjustments for various ethnic backgrounds. The impact of assay drift over time on 25(OH)D, which could either under- or overestimate associations for clinical outcomes and risk assessment,[36] was not accounted for in the previous national survey data-based studies. We have used updated and adjusted data files from the continuous NHANES 2001-2004 data, which accounts for assay drift as well. However, due to limited data on geographic locations, weather, and latitude, we were unable to analyze the effects of these variables on the association between 25(OH)D and risk of all-cause and cardiovascular disease mortality.

Although results from the observational studies are highly suggestive of a protective association, further evidence on the relationship between vitamin D status and mortality demands randomized controlled trials to prove a definite presence and direction of the association.[37] Based on lack of established benefits of vitamin D supplementation, clinical guidelines by the Endocrine Society do not recommend screening individuals who are not at risk of 25(OH)D deficiency or prescribing 25(OH)D for noncalcemic benefits such as cardiovascular disease protection.[38] A large clinical trial (VITamin D and OmegA-3 TriaL [VITAL]; planned enrollment for about 20,000 individuals) that is projected to provide further evidence on the uncertain state of nonskeletal benefits of vitamin D supplementation, is currently underway. The trial is designed to investigate whether daily supplements of vitamin D (2000 International Units) or fish oil (1 gram of Omega-3 fatty acids) in adults (men >60 and women >65 years of age) reduce risk of death, cancer, or cardiovascular disease mortality among individuals without preexisting cardiovascular diseases.[39]

In conclusion, from this large cohort of asymptomatic adults, independent of traditional cardiovascular disease risk factors, increasing serum 25(OH)D levels offers significant protection against all-cause and cardiovascular disease mortality in a nonlinear fashion. However, once the serum levels of 25(OH)D rise above its population median of 21 ng/mL, it offers no statistically significant protection for all-cause or cardiovascular disease mortality.

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