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Low serum 25-hydroxyvitamin D concentrations are associated with greater all-cause mortality in older community-dwelling women

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Abstract

Vitamin D deficiency is associated with osteoporosis, poor muscle strength, falls, and fractures. The relationship between serum vitamin D concentrations and mortality in older community-dwelling women has not been well characterized. We hypothesized that women with lower 25-hydroxyvitamin D (25[OH]D) concentrations were at higher risk of mortality. We examined the association between serum 25[OH]D concentrations and all-cause mortality in a prospective, population-based study of 714 community-dwelling women, aged 70 to 79 years, the Women's Health and Aging Studies I and II in Baltimore, Md. The studies were originally designed to evaluate the causes and course of physical disability in older women living in the community. Vital status was determined through follow-up interviews and matching with the National Death Index. During a median of 72 months of follow-up, 100 (14%) of 714 women died. Women in the lowest quartile of 25(OH)D (<15.3 ng/mL or 38.2 nmol/L) were at higher risk of death (hazards ratio, 2.45; 95% confidence interval, 1.12-5.36; $P = .02$) compared to women in the highest quartile (>27.0 ng/mL or 67.4 nmol/L) of 25(OH)D in a multivariate Cox proportional hazards model adjusting for demographics, season, and conventional risk factors. Older community-dwelling women with low 25 (OH)D levels are at an increased risk of death.

Keywords: Aging; Mortality; Survival; Vitamin D; Women
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; HDL, high-density lipoprotein; HR, hazard ratio; MMSE, Mini-Mental State Examination; NHANES, National Health and Nutrition Examination Survey; PTH, parathyroid hormone; WHAS, Women's Health and Aging Study.

1. Introduction

Vitamin D deficiency remains common among adults [1] and is associated with increased risk of osteopenia, osteoporosis, muscle weakness, poor physical performance [2], falls [3], and fractures [4]. Vitamin D plays an important role in the regulation of calcium, phosphorus, bone metabolism, cellular differentiation, immune function, and skeletal muscle performance [5,6]. Human vitamin D status depends on both sunlight exposure and dietary intake of vitamin D-rich foods, such as oily fish, vitamin D-fortified dairy products, and dietary supplements containing vitamin

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D. Despite the tremendous progress that has been made in eliminating most micronutrient deficiencies in the last several decades in industrialized countries, in contrast, vitamin D deficiency remains a problem of public health importance [7]. Epidemiological studies have shown that both sunlight exposure and dietary intake of vitamin D are inadequate in many populations [5].

A recent study showed that patients with low serum 25-hydroxyvitamin D who were undergoing coronary angiography had a higher risk of all-cause and cardiovascular disease mortality [8]. Low 25-hydroxyvitamin D levels were an independent risk factor for all-cause mortality in participants, aged 20 years and older, in the Third National Health and Nutrition Examination Survey (NHANES III) [9]. Although older women are at particularly high risk of vitamin D deficiency [9,10], the relationship between vitamin D deficiency and mortality in older women has not been characterized. We hypothesized that older community-dwelling women with low serum 25-hydroxyvitamin D levels were at higher risk of all-cause mortality. To address this hypothesis, we examined the relationship between serum 25-hydroxyvitamin D and mortality in a population-based sample of community-dwelling women in Baltimore, Md.

2. Methods and materials

2.1. Study subjects

Subjects in this study were women, aged 70 to 79 years, who participated in the Women's Health and Aging Studies (WHAS) I and II, 2 complementary population-based studies designed to evaluate the causes and course of physical disability in older women living in the community. Details of the study methods and sampling design of the WHAS studies were published elsewhere [11,12]. Participants in WHAS I were recruited from an age-stratified random sample of women 65 years or older selected from Medicare enrollees residing in 12 contiguous zip code areas in Baltimore, Md [11]. Women were screened to identify self-reported physical disability that was categorized into 4 domains by report of difficulty with tasks in the following areas: (1) mobility, (2) upper extremity function, (3) higher functioning household management, and (4) self-care. Women's Health and Aging Study I enrolled the one third of most disabled women 65 years or older, that is, those with disability in 2 or more domains. Of the 1409 women who met study eligibility criteria, 1002 agreed to participate in the study in 1992. Sociodemographic and reported health characteristics did not differ between women who participated and those who declined [11]. Standardized questionnaires were administered to the participants in the home by trained interviewers. Mini-Mental State Examination (MMSE) was administered, and an MMSE score of less than 24 was defined as cognitive impairment [13]. Two weeks later, a trained registered nurse conducted an examination of each study participant at home, using a standardized protocol that included physical performance measures and a standardized physical examination. Height and weight were measured, and body mass index (BMI) was calculated as kilogram per square meter. Approximately 75% of the women also consented to phlebotomy performed during a separate visit by a trained phlebotomist.

Women's Health and Aging Study II was specifically designed to be a companion study for WHAS I and included a cohort of women, aged 70 to 79 years, selected to be representative of the two thirds of least disabled women living in the community [12]. Participants were selected using age-stratified random samples from the same sampling frame as in WHAS I and were screened using the same 4 domains of physical function. Eligible women had either no disability or disability in only one domain. In 1994, 880 women were eligible for WHAS II of which 436 consented to participate. Those agreeing to participate were more highly educated and reported more diseases than those who refused, but they did not differ significantly from nonparticipants by disability characteristics. An interview standardized to that performed in WHAS I including MMSE was administered at the Johns Hopkins Functional Status Laboratory (Johns Hopkins Hospital, Baltimore, MD). Trained technicians then conducted a standardized examination that included a physical examination, assessment of height and weight, and physical performance measures. Phlebotomy was performed in 93% of WHAS II participants following the same protocol as that used in WHAS I.

In both WHAS I and II, demographic characteristics, self-rated health, and information about appetite and eating were measured using standardized questionnaires. Chronic diseases were adjudicated by WHAS coinvestigators based on the questionnaire, physical examination, physician contact, and diagnostic algorithms, as published elsewhere [11]. *Renal insufficiency* was defined as estimated glomerular filtration rate of less than 60 mL/min per 1.73 m^2 using the Modification of Diet in Renal Disease equation of Levey and colleagues [14]. Vital status was determined through follow-up interviews with proxies, obituaries, and matching with the National Death Index for a 6-year period, and causes of death were classified according to the *International Classification of Diseases*, version 9 [15]. Cardiovascular deaths and deaths from cancer and respiratory disease were defined using *International Classification of Diseases*, version 9, codes from 390 to 459, 140 to 239, and 460 to 519, respectively. The Institutional Review Board of the Johns Hopkins University School of Medicine approved the study protocol, and written informed consent was obtained from all participants.

For comparability with the WHAS II cohort age range of 70 to 79 years, we included women from WHAS I only if they were in this age range, yielding 399 participants in WHAS I and 430 participants in WHAS II. Weights were calculated to adjust for sampling probability and response rates. This pooled sample of 829 women has been used in previous analyses of mobility disability [16], obesity and

frailty [17], and undernutrition and frailty [18]. The present study was limited to women who had serum 25-hydroxyvitamin D (25[OH]D) measurements available at baseline, which included 315 women from WHAS I and 399 women from WHAS II.

2.2. Laboratory analyses

Nonfasting blood samples were obtained by venipuncture, and the samples were processed, placed on ice, and sent the same day to the central laboratory of Quest Diagnostics (formerly Corning Clinical Laboratories and MedPath) in Teterboro, NJ, for analyses that included complete blood count, lipid profile, and measurements of selected micronutrients. Serum 25(OH)D was measured using a radioreceptor assay (Nichols Institute Diagnostics, San Juan Capistrano, Calif) [19], with interassay and intraassay coefficients of variation (CVs) of 9.6% and 7.5%, respectively. Serum 1,25-dihydroxyvitamin D was measured with the use of extraction, chromatography, and radioreceptor assay [20] with interassay and intraassay CVs of 10.9% and 7.5%, respectively. Intact serum parathyroid hormone (PTH) was measured using chemiluminescence [21] with intraassay and intraassay CVs of 6.7% and 5.7%, respectively. Serum ionized calcium was measured with ion selective electrodes (Nova 8, Nova Biomedical, Waltham, Mass) with an SD of 0.05 mmol/L for standards. Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by enzymatic methods at Quest Diagnostics. Low-density lipoprotein cholesterol was calculated using the Friedewald equation [22].

2.3. Statistical analyses

Continuous variables were compared using Wilcoxon rank sum test. Categorical variables were compared using χ^2

Table 1
Baseline characteristics of women, aged 70 to 79 years, in the WHAS I and II, by quartiles of serum 25(OH)D

Characteristic [a]	25(OH)D quartiles, ng/mL [b]				*P*
	<15.3 (n = 177)	15.3-20.3 (n = 179)	20.4-27.0 (n = 186)	>27.0 (n = 172)	
Age, y	73.0 (71.0, 76.0)	74.0 (71.0, 77.0)	74.0 (72.0, 76.0)	74.0 (72.0, 77.0)	.32
Race, black (%)	39.5	26.8	17.7	11.1	<.0001
Education, <12 y (%)	48.6	45.8	44.1	35.9	.10
Current smoker (%)	14.2	11.2	14.1	8.1	.25
Use of vitamin D supplements (%)	16.3	21.9	26.7	35.6	.0006
BMI (kg/m^2)	28.3 (24.7, 32.6)	27.5 (23.4, 31.5)	27.2 (22.9, 30.4)	25.7 (23.1, 29.5)	.002
Low physical activity (%)	31.2	24.7	19.8	18.9	.03
Total cholesterol (mg/dL)	225 (193, 247)	231 (206, 262)	230 (207, 258)	236 (213, 262)	.009
HDL cholesterol (mg/dL)	51 (42, 63)	52 (42, 65)	54 (44, 65)	53 (44, 67)	.59
Low-density lipoprotein cholesterol (mg/dL)	136 (115, 160)	147 (121, 171)	143 (123, 166)	140 (116, 166)	.17
Triglycerides (mg/dL)	131 (85, 190)	140 (94, 196)	141 (97, 207)	153 (110, 230)	.01
1,25-dihydroxyvitamin D (pg/mL)	38.2 (29.5, 49.2)	39.0 (29.2, 50.8)	39.4 (29.1, 47.8)	39.8 (31.9, 49.0)	.78
PTH (mg/L)	105 (63, 176)	100 (63, 154)	97 (62, 150)	101 (62, 144)	.47
Serum phosphate (mg/dL)	3.6 (3.2, 3.9)	3.6 (3.3, 3.9)	3.7 (3.4, 4.0)	3.6 (3.3, 3.9)	.10
Serum calcium (mg/dL)	9.2 (8.9, 9.4)	9.3 (9.0, 9.6)	9.2 (9.0, 9.5)	9.3 (9.0, 9.5)	.25
MMSE, <24 (%)	7.9	8.9	5.9	2.9	.10
Hypertension (%)	54.6	58.7	44.3	52.9	.05
Angina (%)	17.5	15.6	16.7	12.2	.54
Heart failure (%)	7.9	7.3	3.2	4.1	.14
Peripheral artery disease (%)	13.0	9.5	10.8	13.4	.62
Stroke (%)	3.9	3.9	2.1	1.7	.47
Diabetes mellitus (%)	14.1	16.2	11.3	9.9	.28
Chronic obstructive pulmonary disease (%)	32.2	30.7	23.7	22.1	.08
Depression (%)	20.4	12.8	16.2	16.0	.62
Cancer (%)	11.8	9.5	6.4	10.5	.34
Renal insufficiency (%)	40.9	53.1	48.7	57.0	.02
Season Winter	26.5	32.1	20.4	21.0	<.0001
Spring	13.9	25.6	28.3	32.2	
Summer	24.0	19.8	29.3	26.9	
Autumn	36.2	24.4	25.0	14.4	

[a] Data are given as median and interquartile range or percentage, as indicated.
[b] Quartile cutoffs are equivalent to less than 38.2, 38.2 to 50.7, 50.9 to 64.7, and more than 64.7 nmol/L.

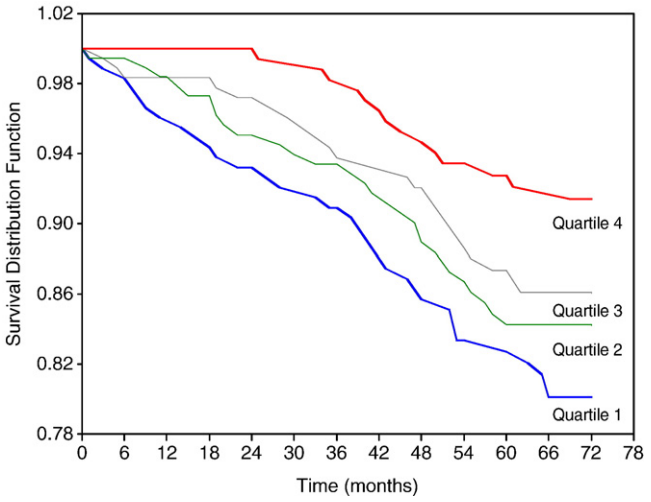

Fig. 1. Survival curves by quartile of serum 25(OH)D for women, aged 70 to 79 years, in the WHAS I and II. $P = .02$ by log-rank test.

tests. *Low physical activity* was defined as the lowest quintile of physical activity scale, based upon a weighted score of kiloJoules expended per week by participant's report [23]. Cox proportional hazards models were used to examine the relationship between 25(OH)D and other covariates with all-cause mortality. Age, race, education, BMI, and other conventional risk factors that have been associated with increased all-cause mortality were included in the final multivariate Cox proportional hazards models. Survival curves were compared using log-rank test. The statistical program used was SAS (SAS Institute, Cary, NC). The level of significance used in this study was $P < .05$.

3. Results

Overall, the median serum 25(OH)D concentration was 20.4 ng/mL (50.9 nmol/L). During a median of 72 months of follow-up, 100 (14%) of the 714 women died. The main causes of death among the women who died were cardiovascular disease (36%), respiratory disease (18%), cancer (15%), other (27%), and unknown (4%). The demographic and disease characteristics of women by quartile of serum 25(OH)D are shown in Table 1. Women in the lower quartiles compared with the upper quartiles of serum 25(OH)D were more likely to be black; not taking in vitamin D supplements; and have a higher BMI, lower physical activity, and lower total cholesterol and triglycerides. Women with lower 25(OH)D levels were more likely to have hypertension and less likely to have renal insufficiency. Women in the lower quartiles of serum 25(OH)D were more likely to have had their blood drawn in autumn and winter.

The proportion of women who died in each quartile of serum 25(OH)D from the lowest to the highest quartile was 19.2%, 13.4%,15.0%, and 8.1%, respectively. The survival curves for women according to quartile of serum 25(OH)D are shown in Fig. 1. Women in the lowest quartile had significantly worse survival than women in the highest quartile of serum 25(OH)D, in multivariate Cox proportional hazards models adjusting for age, race, education, season, and for BMI, smoking, supplement use, physical activity, total and HDL cholesterol, and chronic diseases (Table 2).

No significant relationship was found in univariate Cox proportional hazards models between log serum PTH and mortality (hazards ratio [HR], 1.07; 95% confidence interval [CI], 0.79-1.43; $P = .66$) or between serum 1,25 dihydroxyvitamin D and mortality (HR, 0.99; 95% CI, 0.98-1.01; $P = .19$). Addition of PTH and calcium to the multivariate Cox proportional hazards model that also adjusted for age, race, education, season, BMI, smoking, supplement use, physical activity, total and HDL cholesterol, and chronic diseases did not substantially change the relationship between the highest and lowest quartile of serum 25(OH)D and mortality (HR, 2.87; 95% CI, 1.10-7.49; $P = .03$). Inclusion of serum 1,25 dihydroxyvitamin D to the multivariate Cox proportional hazards model above also did not substantially change the relationship between the highest and lowest quartile of serum 25(OH)D and mortality (HR, 2.68; 95% CI, 1.17-6.12; $P = .019$).

4. Discussion

This study shows that community-dwelling older women with low serum 25(OH)D concentrations (lowest quartile, <15.3 ng/mL) had a significantly higher risk of all-cause mortality compared with those with higher serum 25(OH)D concentrations (highest quartile, >27.0 ng/mL), after

Table 2
Relationship between serum 25(OH)D and all-cause mortality in separate multivariate Cox proportional hazards models

Covariates in models	25(OH)D quartiles, ng/mL[a]			
	<15.3	15.3-20.3	20.4-27.0	>27.0
Age	2.66 (1.43-4.97)	1.72 (0.89-3.32)	1.98 (1.04-3.76)	1.00
Age, race, education, season	2.46 (1.28-4.74)	1.67 (0.86-3.26)	1.88 (0.99-3.57)	1.00
Age, race, education, season, BMI, smoking, supplement use, physical activity, total cholesterol, HDL cholesterol, and chronic diseases[b]	2.45 (1.12-5.36)	2.05 (0.97-4.32)	2.25 (1.08-4.69)	1.00

[a] Hazards ratios shown for each quartile of 25(OH)D relative to the highest quartile (reference).
[b] Chronic diseases include hypertension, diabetes, heart failure, stroke, and renal insufficiency.

adjusting for demographic and other risk factors. The present findings from this population-based cohort of aging are consistent with the association between low serum 25(OH)D and mortality that has been described in patients undergoing coronary angiography [8] and in the general population in NHANES III [9]. In addition, a recent meta-analysis suggested that vitamin D supplementation was associated with decreased mortality [24].

The median serum 25(OH)D concentration of about 20 ng/mL (50 nmol/L) among women in the present study is comparable to mean 25(OH)D concentrations described in white and African American women, aged 60 years and older, in NHANES III of 26 ng/mL (65 nmol/L) and 20 ng/mL (50 nmol/L), respectively [25]. The blood samples for vitamin D analyses were collected in NHANES III from 89 survey locations across the United States and during the warmer months in the northern locations, when sunlight is more abundant [25], whereas the blood samples in WHAS I and II were collected throughout the year in Baltimore, Md. Although there is no formal consensus on the optimal levels of 25(OH)D, vitamin D deficiency is often defined as a 25(OH)D level of less than 20 ng/mL (50 nmol/L) [5], which suggests that half of the women in the present study did not have sufficient levels of vitamin D.

A limitation of the study is that serum 25(OH)D was measured using a radioimmunoassay that is considered to overestimate the level of 25(OH)D because the antibody used in the assay also binds 24,25-dihydroxyvitamin D [26]. The same limitation of the assay has been encountered with studies of 25(OH)D in NHANES III [9]. A significant association was found between serum 25(OH)D and mortality in both the present study and NHANES III. The increased variability attributed to the radioimmunoassay would be expected to affect the results toward the null hypothesis. The findings from the present study involving older women cannot necessarily be generalized to older men. It is notable in NHANES III, the association between low serum 25(OH)D, and all-cause mortality in adults aged 20 and older was stronger for women than men [9]. Further studies are needed to examine the relationship between vitamin D status and mortality in older men.

In the present study, the women in the lowest quartile of 25(OH)D also had significantly lower levels of total cholesterol and triglycerides. Whether there is a common biologic mechanism that might explain these associations is not clear; however, 7-dehydrocholesterol is the precursor to both cholesterol and vitamin D.

Several biologic mechanisms have been proposed that could explain a possible causal relationship between vitamin D deficiency and mortality. The active form of vitamin D, 1,25-dihydroxyvitamin D, has pleiotropic effects, and the vitamin D receptor is widely distributed in tissues [5]. Vitamin D plays a role in regulation of the renin-angiotensin axis [27], modulation of cellular proliferation and differentiation [28], cytokine production [29], and atherosclerosis [30,31]. 1,25-dihydroxyvitamin D appears to exert an overall effect upon T cells in blocking the induction of T-helper-1 cytokines, such as interferon-γ, and promoting T-helper-2 responses by enhancing interleukin-4 production [32]. The specific physiologic roles of 1,25-dihydroxyvitamin D in modulating immune responses in humans is still unclear [32]. The role that vitamin D plays in different tissues may account for the associations between vitamin D deficiency and cardiovascular disease [25,33], cancer [34], and mortality [8,9,24].

In conclusion, older community-dwelling women with low serum 25(OH)D levels are at an increased risk of all-cause mortality. Controlled clinical trials are needed to determine whether vitamin D supplementation will improve health outcomes such as cardiovascular disease and mortality in older adults who have insufficient levels of vitamin D.

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