Prospective association of vitamin D concentrations with mortality in postmenopausal women: results from the Women's Health Initiative (WHI)[1–3]

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ABSTRACT

Background: Prospective epidemiologic data on the association between vitamin D and all-cause and cause-specific mortality are limited.

Objective: This study aimed to determine whether 25-hydroxyvitamin D [25(OH)D] concentrations were prospectively and independently associated with cardiovascular disease (CVD), cancer, and all-cause mortality in postmenopausal women.

Design: A substudy in 2429 postmenopausal women within the Women's Health Initiative (WHI) with measured baseline 25(OH)D concentrations were followed for 10 y for death from CVD, cancer, and all-cause mortality. Proportional hazards models were performed to evaluate quartiles of month-adjusted 25(OH)D concentrations, with adjustment for potential confounders. Sequential model building and analysis for multiplicative interaction were performed to evaluate the effects of central adiposity on the association of low 25(OH)D with all-cause mortality.

Results: Of the 2429 women, 224 deaths occurred, with 79 deaths from CVD and 62 deaths from cancer. Multivariate-adjusted HRs that compared quartiles 1 (lowest) to 4 (highest) of 25(OH)D for all-cause mortality (HR: 1.25; 95% CI: 0.80, 1.95), CVD mortality (HR: 1.27; 95% CI: 0.81, 1.99), and cancer mortality (HR: 1.39; 95% CI: 0.88, 2.19) were not significant. There was a potential interaction (P = 0.08) between abdominal obesity and low 25(OH)D concentrations that showed an increased risk of the lowest quartile of 25(OH)D concentrations (HR: 1.85; 95% CI: 1.00, 3.44) with increased mortality in women with a normal waist circumference but no increased risk in women with abdominal obesity (HR: 0.96; 95% CI: 0.52, 1.76).

Conclusion: Body fat distribution may play an important role in the modulation of the effect of low vitamin D concentrations on health. This trial was registered at clinicaltrials.gov as NCT 00000611. *Am J Clin Nutr* 2011;94:1471–8.

INTRODUCTION

Vitamin D, or the sunshine vitamin, is well known for its role in the regulation of calcium and phosphorus metabolism and, therefore, its role in bone health and renal disease (1, 2). Emerging evidence suggested that vitamin D deficiency may be an important risk factor for CVD[4], cancer (particularly gastrointestinal-related cancers), hypertension, diabetes mellitus, multiple sclerosis, and some infectious diseases (3–8). Prospective epidemiologic data on the association between vitamin D and mortality are limited, but recent analyses from the NHANES III mortality follow-up study (9) and the Ludwigshafen Risk and Cardiovascular Health study in Germany (10) suggested a potential relation between vitamin D deficiency and all-cause mortality. The increased risk of vitamin D deficiency with all-cause mortality appeared greater in women than in men and was associated with an increased risk of cardiovascular-, cancer-, infectious disease–, and trauma-related mortality in unadjusted models but only all-cause– and trauma-related mortality in multivariate adjusted models (9). There are plausible biological mechanisms that might explain protective effects of vitamin D on all-cause mortality. Vitamin D receptors are in various organs, and the activation of these receptors have been shown to effect cell differentiation and inhibit proliferation, invasiveness, angiogene-

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[4] Abbreviations used: CaD, calcium and vitamin D; CT, clinical trial; CVD, cardiovascular disease; OS, observational study; 25(OH)D, 25-hydroxyvitamin D; WHI, Women's Health Initiative.

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sis, and metastatic potential, which might affect cancer mortality (11, 12). In addition to the regulation of calcium homeostasis, vitamin D appears to be critical for innate immunity (8) and has been associated with the production of cathelecidin and other antibiotic peptides that may affect infectious disease mortality (13). Despite this biologic plausibility, most CTs, including the WHI have not shown a benefit of vitamin D supplementation (14, 15) on cancer or mortality outcomes.

In summary, although the vitamin D hypothesis appears plausible, it is far from proven from both epidemiologic and biological perspectives, and therefore, additional studies, particularly in women, need to be performed. Indeed, after thorough review of the scientific literature, a recent Institute of Medicine report suggested that the "health benefits beyond bone health benefits often reported in the media were from studies that provided often conflicting results and could not be considered reliable" (16).

Therefore, we examined whether low serum concentrations of 25(OH)D predicted an increased risk of cardiovascular, cancer, and all-cause mortality in a prospective cohort of ethnically diverse postmenopausal women. Because vitamin D concentrations are inversely related with adiposity, we also examined whether adiposity modified the relation between baseline 25(OH)D concentrations and all-cause mortality.

SUBJECTS AND METHODS

Study population

This report represents a post hoc analysis of data collected from 3 nested case-control studies that measured 25(OH)D concentrations in women who participated in a WHI CT and OS. The WHI sample consisted of postmenopausal women aged 50–79 y who were recruited from 40 clinical centers between 1993 and 1998. WHI-OS participants were either ineligible for or chose not to enroll in the WHI-CT (17, 18). WHI-CT participants were randomly assigned to receive postmenopausal hormone therapy, dietary modification, or both, were screened for eligibility, and invited to join the WHI CaD CT at their first or second annual visit. Informed consent was obtained from all participants, and institutional review boards of all collaborating institutions approved the project. A total of 36,282 women were enrolled in the CaD–supplementation trial and were randomly assigned to consume either a placebo or 1000 mg calcium carbonate combined with 400 IU 25(OH)D. Women were allowed to continue their personal use of CaD as long as their vitamin intakes were not >600 IU/d (and, later, 1000 IU/d). Within the CaD trial, 2 case-control studies were conducted to analyze associations between serum concentrations of 25(OH)D and incident colorectal cancer, breast cancer, or hip, spine, or lower-wrist fractures; control subject were matched for age, race-ethnicity, blood draw date, and clinical center at CaD randomization. Participants who self-reported conditions at the WHI-CT baseline that could have affected vitamin D absorption in the gut (ie, a history of ulcerative colitis or Crohn's disease, surgery to remove part of their intestine, or use of a special diet for malabsorption, celiac sprue, or ulcerative colitis) or high blood calcium concentrations were excluded from the case-control studies in the CaD trial. Participants who self-reported incident cancer between WHI-CT baseline and year 1 were also excluded. Only participants who were not randomly assigned to vitamin D supplementation in the CaD case-control study were included in this analysis.

For the OS case-control study, participants who used medications that contained estrogen (up to 1 y before study entry; oral and dermal forms only), androgens (including anabolic steroids, dehydroepiandrosterone, and testosterone), selective estrogen receptor modulators, antiestrogens, or medications for bone loss (including bisphosphonates, calcitonins, and parathyroid hormone) at baseline were excluded from the OS case-control study.

Blood collection and assessment of biomarkers

Fasting blood specimens were collected from all participants at baseline according to a standardized protocol. Participants were instructed to fast for 12 h before collection, take all regular medications except for diabetes medication, take no aspirin or nonsteroidal antiinflammatory drugs for 48 h before the visit except for those medications taken regularly, refrain from smoking for 1 h before the visit, and perform no vigorous physical activity for 12 h before the visit. Aliquots of serum, plasma, and buffy coat were frozen and shipped on dry ice to a central repository and stored at −70°C for future assays in outside laboratories.

Serum 25(OH)D was measured with the DiaSorin Liaison 25(OH)D chemiluminescent radioimmunoassay system (Diasorin) at the Diasorin headquarters with a CV of 11.8%.

Baseline clinical variables

We ascertained all covariates at baseline. Certified WHI-trained staff measured height, weight, waist and hip circumferences, and blood pressure at the baseline visit. Height (in cm) was measured with a wall-mounted stadiometer, and weight (in kg) was measured with a balance-beam scale. BMI was calculated as weight (in kg) divided by height (in m^2). Waist and hip circumferences were determined with a standardized measuring tape. Clinic interviewers recorded current use of prescription medications by inspection at baseline. We used standardized questionnaires to ascertain date of birth, race or ethnicity, education, income, medical and family histories, smoking status, alcohol use, hormone-therapy use, and recreational physical activity. Physical activity was classified on the basis of the frequency and duration of walking and mild, moderate, and strenuous activity in the previous week.

Outcomes

The identification of fatal events occurred at each center by a routine semiannual and annual follow-up of medical self-reported conditions by family, friends, or post-office response, health care providers, national death index, obituaries, and follow-up of lost participants. All-cause, CVD, and cancer-related mortalities were determined centrally by trained physician adjudicators on the basis of medical records from hospitalizations, emergency room visits, death certificates, and autopsy and coroner's reports when available. The underlying cause of death, as opposed to the immediate or contributing cause of death, was used for the classification of cause-specific mortality. Cardiovascular disease mortality encompassed death from coronary heart disease, cerebrovascular disease, pulmonary embolism, congestive heart failure, and other cardiovascular causes. Cancer mortality encompassed all carcinomas, lymphomas, sarcomas, including metastatic cancer from unknown

primaries, and hematologic malignancies that included blood, bone marrow, and lymph nodes.

Statistical analysis

We combined 3 separate nested case-control studies that included measurements of 25(OH)D concentrations and disease outcomes (CaD and fracture; CaD and breast and colon cancer; and OS and hip fracture) into one population (19). Because both cases and controls were used, and individuals were matched for conditions other than risk of mortality, inverse probability weighting was used to account for the nonrandom selection of the subcohort. Inverse probability weights were determined from a logistic regression model of inclusion in one of the nested case-control studies on age, race, hip, spine, and lower-arm fractures, breast and colorectal cancers, month of blood draw, latitude, and death (20).

Descriptive statistics were calculated for covariates of interest by quartiles of month-adjusted 25(OH)D. The 25(OH)D concentrations were adjusted for the month of blood draw by adding the residuals from locally weighted polynomial regression to the overall mean 25(OH)D concentrations (19). One-factor ANOVA tests were performed to compare means of continuous covariates by 25(OH)D concentrations in quartiles. Chi-square tests were performed to compare quartiles of by 25(OH)D concentrations by categorical variables.

Cox proportional hazards modeling was performed to estimate the HRs of 25(OH) quartiles. Two sets of models were constructed. The first, simpler model was adjusted for age, ethnicity, and an indicator for enrollment in the CaD trial. The following potential confounders with $P \leq 0.10$ in the simpler models on the basis of the clinical literature were included in the full model: age, ethnicity, CaD-trial indicator, smoking status, history of hypertension, systolic blood pressure, history of treated diabetes, CVD, fracture at $\geq$55 y of age, and cancer, waist circumference, weekly alcohol consumption, BMI, and physical activity. Sequential model building was performed by using the significant covariates in the full models to evaluate the additive effects of confounders on the association with all-cause mortality. A likelihood-ratio test was performed to test for an interaction between 25(OH)D and waist circumference $\geq$89 cm to evaluate the impact abdominal obesity on the association of vitamin D and all-cause mortality. Cox proportional hazards modeling was also performed to estimate risk of CVD mortality and cancer mortality by 25(OH)D. Restricted cubic spline analysis was performed to test for nonlinearity of the relation between 25(OH)D and all-cause mortality (21).

RESULTS

In this cohort of 2429 postmenopausal women with >10.5 y of follow-up, 224 deaths occurred, of which 79 deaths were from CVD, and 62 deaths were from cancer. Quartiles of month of blood draw–adjusted 25(OH)D concentrations in nanamoles per liter were quartiles 1 (3.25–36.50 nmol/L), 2 (36.51–49.95 nmol/L), 3 (49.96–65.38 nmol/L), and 4 (65.39–146.67 nmol/L).

At baseline, participants with low vitamin D concentrations (quartile 1) were older, had higher BMIs, higher waist circumferences, were less educated, more likely to be nondrinkers and past drinkers of alcohol, less likely to be white, more likely to be current smokers, more likely to engage in sedentary activity, more likely to have diabetes, and less likely to be receiving vitamin D supplements (**Table 1**).

After adjustment for age, race-ethnicity, and participation in the CT, low vitamin D (quartiles 1 compared with 4) was associated with an increased risk of all-cause mortality (HR: 1.62; 95% CI: 1.11, 2.36) (**Table 2**). After adjustment for potential confounders measured at baseline, including age, race-ethnicity, CT indicator, current smokers, history of hypertension, systolic blood pressure, diabetes, history of CVD, history of fracture, waist circumference, BMI, and physical activity, risk of low vitamin D was attenuated (HR: 1.25; 95% CI: 0.80, 1.95) and lost statistical significance. To better understand this attenuation of risk of low vitamin D concentrations with all-cause mortality in this cohort of postmenopausal women, we performed sequential model building (**Table 3**) because some of the variables may have been in the causal pathway and provided a mechanism by which vitamin D had its effect on mortality. After adjustment for age, race-ethnicity, and CT indicator, the addition if smoking or systolic blood pressure or history of CVD to the model modestly attenuated risk of mortality but the addition of waist circumference to the model significantly attenuated the model to nonstatistical significance.

We also evaluated the relation of vitamin D concentrations by using quintiles, as a continuous variable and as a cubic spline. The quintile analysis and treatment of vitamin D as a continuous variable did not lead to any different conclusions than those already shown. The test for nonlinearity of the relation between 25(OH)D and all-cause mortality was not significant ($P = 0.69$).

For CVD mortality (**Table 4**), an increased risk of low vitamin D was shown in the analysis adjusted for age, race-ethnicity, and CT (HR: 1.92; 95% CI: 1.03, 3.58), but similar to all-cause mortality, this risk was attenuated to nonsignificance (HR: 1.27; 95% CI: 0.81, 1.99) once adjusted for abdominal obesity and other CVD risk factors. For cancer mortality, the analysis adjusted for age, race-ethnicity, and CT revealed a trend toward increased risk (HR: 1.49; 95% CI: 0.73, 3.02) which likewise was attenuated with adjustment for potential confounding (HR: 1.39; 95% CI: 0.88, 2.19) (**Table 5**).

Because of the significant association between abdominal obesity and vitamin D concentrations and the significant attenuation of risk of low vitamin D and all-cause mortality once abdominal circumference was adjusted for, we performed an interaction analysis on the basis of abdominal circumference by using the clinical cutoff of >89 cm (35 inches) used to define abdominal obesity in the National Heart, Lung, and Blood Institute Obesity Education Initiative (**Table 6**). In women with a normal waist circumference (<89 cm), low vitamin D (quartile 1 compared with quartile 4) was associated with statistically borderline ($P = 0.051$) increased risk of all-cause mortality (HR: 1.85; 95% CI: 1.00, 3.44), whereas no association was shown in women with an increased waist circumference by using the same comparisons (HR: 0.96; 95% CI: 0.52, 1.76). Formal testing for statistical interaction between waist circumference and 25(OH)D concentrations on all-cause mortality was not shown to be significant ($P = 0.08$).

DISCUSSION

This study questioned the validity of the vitamin D hypothesis regarding the health benefits beyond bone health in postmenopausal

TABLE 1
Baseline characteristics by quartile of month-adjusted 25-hydroxyvitamin D concentrations[1]

	Quartile				
	1 (3.25–36.50 nmol/L; n = 608)	2 (36.51–49.95 nmol/L; n = 606)	3 (49.96–65.38 nmol/L; n = 608)	4 (65.39–146.67 nmol/L; n = 607)	P
Age (y)	65.1 ± 7.6[2]	66.5 ± 7.2	65.7 ± 7.3	65.7 ± 7.5	0.02[3]
≥70 y [n (%)]	207 (34.0)	246 (40.6)	205 (33.7)	219 (36.1)	0.05[4]
BMI [n (%)]	28.5 ± 9.9	25.2 ± 12.9	22.4 ± 13.2	21.1 ± 11.8	<0.0001[3]
<25 kg/m^2	117 (20.6)	144 (28.1)	154 (33.2)	207 (44.1)	<0.0001[4]
25 to <30 kg/m^2	190 (33.5)	176 (34.4)	150 (32.3)	165 (35.2)	—
≥30 kg/m^2	261 (46.0)	192 (37.5)	160 (34.5)	97 (20.7)	—
Waist circumference (cm)	91.7 ± 13.8	88.5 ± 12.7	87.2 ± 12.4	82.9 ± 11.9	<0.0001[3]
Systolic blood pressure (mm Hg)	129.7 ± 17.2	128.7 ± 17.5	127.5 ± 17.8	126.6 ± 18	0.01[3]
Education [n (%)]					
Did not finish high school	54 (8.9)	34 (5.7)	32 (5.3)	29 (4.8)	0.01[4]
Finished high school/GED	552 (91.1)	567 (94.3)	569 (94.7)	575 (95.2)	—
Alcohol consumption [n (%)]					
Nondrinker	77 (12.8)	72 (11.9)	83 (13.7)	66 (10.9)	0.0003[4]
Past drinker	132 (21.9)	102 (16.9)	104 (17.2)	103 (17.1)	—
<1 drink/mo	103 (17.1)	78 (12.9)	74 (12.2)	60 (10.0)	—
<1 drink/wk	123 (20.4)	130 (21.6)	121 (20.0)	118 (19.6)	—
1 to <7 drinks/wk	122 (20.3)	149 (24.7)	155 (25.6)	178 (29.5)	—
≥7 drinks/wk	45 (7.5)	72 (11.9)	69 (11.4)	78 (12.9)	—
Alcohol servings/wk	1.63 ± 3.94	2.42 ± 5.02	2.25 ± 4.76	2.58 ± 4.58	0.002[3]
Race-ethnicity [n (%)]					
White	474 (78.0)	536 (88.4)	566 (93.1)	584 (96.2)	<0.0001[4]
Black	80 (13.2)	31 (5.1)	15 (2.5)	3 (0.5)	—
Hispanic	31 (5.1)	14 (2.3)	11 (1.8)	8 (1.3)	—
American Indian	6 (1.0)	3 (0.5)	2 (0.3)	0 (0.0)	—
Asian or Pacific Islander	10 (1.6)	15 (2.5)	9 (1.5)	9 (1.5)	—
Unknown	7 (1.2)	7 (1.2)	5 (0.8)	3 (0.5)	—
HT use [n (%)]					
Never used	311 (52.1)	324 (54.3)	322 (53.5)	293 (48.9)	0.22[4]
Past user	155 (26.0)	150 (25.1)	140 (23.3)	146 (24.4)	—
Current user	131 (21.9)	123 (20.6)	140 (23.3)	160 (26.7)	—
Smoking history [n (%)]					
Never	328 (54.8)	340 (56.6)	341 (56.3)	309 (51.3)	0.001[4]
Past	218 (36.4)	225 (37.4)	228 (37.6)	264 (43.9)	—
Current	53 (8.8)	36 (6.0)	37 (6.1)	29 (4.8)	—
Physical activity [n (%)]					
0 MET-h/wk (inactive)	131 (23.7)	96 (17.5)	91 (16.5)	57 (10.4)	<0.0001[4]
<5 MET-h/wk	158 (28.6)	133 (24.2)	121 (21.9)	91 (16.6)	
5–12 MET-h/wk	131 (23.7)	145 (26.4)	139 (25.2)	136 (24.9)	
>12 MET-h/wk	132 (23.9)	175 (31.9)	201 (36.4)	263 (48.1)	
History of CVD [n (%)]	111 (20.0)	95 (17.4)	116 (20.9)	102 (18.3)	0.88
History of treated diabetes [n (%)]	42 (6.9)	25 (4.1)	21 (3.4)	22 (3.6)	0.01
History of cancer [n (%)]	29 (4.9)	38 (6.4)	35 (5.8)	46 (7.7)	0.23
History of fracture at ≥55 y of age [n (%)]	80 (17.4)	83 (17.6)	97 (20.4)	104 (22.1)	0.28
History of hypertension [n (%)]	97 (16.2)	77 (12.9)	73 (12.1)	61 (10.1)	0.004
History of cholesterol-lowering drugs [n (%)]	74 (12.2)	73 (12.0)	71 (11.6)	54 (8.9)	0.07
Current aspirin use [n (%)]	118 (19.8)	116 (19.4)	139 (23.0)	122 (20.3)	0.55
Geographic region [n (%)]					
Southern: <35° N	178 (29.4)	172 (28.4)	162 (26.6)	181 (29.8)	0.78
Middle: 35–40° N	171 (28.2)	160 (26.4)	171 (28.0)	157 (25.9)	—
Northern: >40° N	257 (42.4)	274 (45.2)	277 (45.4)	269 (44.3)	—
Season of blood draw [n (%)]					
Summer	123 (20.3)	162 (26.7)	201 (33.0)	199 (32.8)	0.25
Fall	113 (18.6)	138 (22.8)	172 (28.2)	169 (27.8)	—
Winter	179 (29.5)	145 (23.9)	114 (18.7)	109 (18.0)	—
Spring	191 (31.5)	161 (26.6)	123 (20.2)	130 (21.4)	—
Received vitamin D supplements [n (%)]	143 (23.6)	287 (47.4)	337 (55.2)	369 (60.8)	<0.0001

[1] CVD, cardiovascular disease; GED, general education diploma; HT, hormone therapy; MET-h, metabolic equivalent task hours.
[2] Mean ± SD (all such values).
[3] One-factor ANOVA.
[4] Chi-square test

TABLE 2
Adjusted HRs of risk of all-cause mortality by 25(OH)D quartiles (adjusted for month)[1]

25(OH)D	Univariate HR (95% CI)[2]	*P*-trend	Multivariate HR (95% CI)[3]	*P*-trend
Quartile 1 (n = 608)	1.62 (1.11, 2.36)	0.004	1.25 (0.80, 1.95)	0.39
Quartile 2 (n = 606)	1.40 (0.97, 2.04)	—	1.13 (0.73, 1.75)	—
Quartile 3 (n = 608)	1.03 (0.69, 1.54)	—	1.17 (0.75, 1.81)	—
Quartile 4 (n = 607)	1.00	—	1.00	—

[1] CaD, calcium and vitamin D; 25(OH)D, 25-hydroxyvitamin D.
[2] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, and CaD-trial indicator.
[3] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, CaD-trial indicator, smoking status, history of hypertension, systolic blood pressure, history of treated diabetes, history of cardiovascular disease, history of fracture at ≥55 y of age, history of cancer, waist circumference, BMI, physical activity, and weekly alcohol consumption.

women. Although we showed an association of low vitamin D and all-cause mortality with limited adjustment analyses, these results were attenuated once potential confounders (ie, cigarette smoking, hypertension, diabetes, waist circumference, and physical activity) were accounted for. Much of the risk attenuation appeared to be related to abdominal obesity. Therefore, we evaluated the effect modification by waist circumference and showed a borderline significant interaction, which demonstrated that most of the increased risk appeared in women with normal waist circumferences, and there was no apparent increased risk of low 25(OH)D in women with abdominal obesity.

For both CVD and cancer mortality, where we had smaller sample sizes and, therefore, limited power, we showed a similar risk attenuation of our findings when confounding was adjusted for. Regarding the association between 25(OH)D and all-cause mortality, our findings were similar to the mixed results shown in the Institute of Medicine's report (16). The evaluation of Semba et al (22) of 714 community-dwelling older women (age range: 70–79 y) in the Women's Health and Aging study showed a doubling (HR: 2.45; 95% CI: 1.12, 5.36) of risk in the lowest quartile than in the highest quartile of 25(OH)D, whereas we showed a 25% increased risk (HR: 1.25; 95% CI: 0.80, 1.95) with adjustment for potential confounders. Melamed et al (9), by using a more general population (NHANES III data) and 8.7 y of follow-up, showed a 26% increased risk (RR: 1.26; 95% CI: 1.08, 1.46) when the lowest to highest quartiles of 25(OH)D were compared, which was similar to our risk estimate. Ginde et al (23), by using the same NHANES III cohort but limiting it to older individuals (>65 y of age), showed an 83% (HR: 1.83; 95% CI: 1.14, 2.94) increased risk of participants comparing <25 to 100 nmol/L. Comparisons between studies were difficult because the quartiles used to define low concentrations of 25(OH)D differed by populations, variations in the storage of biologic samples and assays used to determine concentrations of 25(OH)D effected the comparability of 25(OH)D concentrations in each study, and several studies failed to adequately adjust for sun exposure (geography and season) that effected vitamin D concentration, and last confounding adjustment also varied by study.

Because of our risk attenuation with potential confounders, we performed sequential modeling to evaluate risk factors that might have been mediators of the effect of vitamin D on mortality. Smoking, hypertension, and CVD appeared to have only modest effects on the association of vitamin D and all-cause mortality, whereas central adiposity had the greatest impact and attenuated the risk estimate by 17% and, once combined with smoking, hypertension, and CVD, by 29%, which suggested an important role of central adiposity as either a mediator or confounder of this relation. Therefore, we tested for effect modification and showed that women with normal waist circumferences and low vitamin D concentrations had borderline increased risk of all-cause mortality, whereas women with central adiposity did not. It is well known that abdominal obesity is associated with lower vitamin D concentrations, but abdominal obesity is also associated with higher triglycerides, lower HDL cholesterol, metabolic syndrome, insulin resistance, and a proinflammatory state (24–26). Thus, the lack of an association of low vitamin D in women with

TABLE 3
Model building for 25-hydroxyvitamin D and all-cause mortality[1]

	HR (95% CI) of vitamin D Q1 (n = 608) vs Q4 (n = 607)[2]	Change in HR from baseline vitamin D–only model	Percentage change
Baseline season-adjusted vitamin D Qs[3]	1.62 (1.11, 2.36)	0	0
Baseline vitamin D Qs + smoking	1.58 (1.08, 2.30)	0.04	2.47
Baseline vitamin D Qs + SBP	1.59 (1.09, 2.33)	0.02	1.24
Baseline vitamin D Qs + history of CVD	1.52 (1.02, 2.26)	0.10	6.18
Baseline vitamin D Qs + waist circumference	1.33 (0.90, 1.97)	0.29	17.93
Baseline vitamin D Qs + smoking + SBP + history of CVD	1.45 (0.97, 2.15)	0.17	10.51
Baseline vitamin D Qs + smoking + SBP + history of CVD + waist circumference	1.17 (0.77, 1.76)	0.45	27.83

[1] CVD, cardiovascular disease; Q, quartile; SBP, systolic blood pressure.
[2] Estimates obtained from Cox proportional hazards models.
[3] Adjusted for age, race, and clinical trial indicator.

TABLE 4
Adjusted HRs of risk of cardiovascular disease mortality by 25(OH)D quartiles (adjusted for month)[1]

25(OH)D	Univariate HR (95% CI)[2]	*P*-trend	Multivariate HR (95% CI)[3]	*P*-trend
Quartile 1 (n = 608)	1.92 (1.03, 3.58)	0.04	1.27 (0.81, 1.99)	0.33
Quartile 2 (n = 606)	1.35 (0.71, 2.56)	—	1.14 (0.74, 1.78)	—
Quartile 3 (n = 608)	1.27 (0.66, 2.42)	—	1.16 (0.75, 1.80)	—
Quartile 4 (n = 607)	1.00	—	1.00	—

[1] CaD, calcium and vitamin D; 25(OH)D, 25-hydroxyvitamin D.
[2] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, and CaD-trial indicator.
[3] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, CaD-trial indicator, smoking status, history of hypertension, systolic blood pressure, history of treated diabetes, history of cardiovascular disease, history of fracture at ≥55 y of age, history of cancer, waist circumference, BMI, physical activity, and weekly alcohol consumption.

increased waist circumference may be explained by these confounding factors (ie, atherogenic, metabolic, and inflammatory perturbations) overwhelming the modest effects of low vitamin D. Alternatively, because vitamin D is a fat-soluble vitamin, it may be stored in visceral fat and provide an adequate supply of bioavailable vitamin D despite lower serum concentrations. Another potential explanation of our findings may be that abdominal obesity itself or physiologic alterations associated with abdominal obesity may have affected the number or function of vitamin D receptors at important end organs so that the low serum concentrations of vitamin D provided adequate physiologic functioning. If our preliminary findings are replicated, discerning the mechanisms behind this effect modification would be warranted.

Our findings of an increased point estimate of risk of CVD mortality (HR: 1.92; 95% CI: 1.03, 3.58) compared with all-cause mortality (HR: 1.60), which significantly attenuated to nonsignificance once adjustment for known CVD risk factors (HR: 1.27; 95% CI: 0.81, 1.99) was similar to most published cohort studies (4, 9, 10, 23, 27, 28).

Our findings of a moderate but not significant association of low vitamin 25(OH)D and cancer mortality (HR: 1.49; 95% CI: 0.73, 3.02) are consistent with those shown in the NHANES III mortality follow-up study (HR: 1.31; 95% CI: 0.96, 1.81) in the age-, race-, and CT-adjusted analysis. We showed a modest attenuation of this risk once potential confounders were adjusted for (HR: 1.39; 95% CI: 0.88, 2.10), whereas Melamed et al (9) showed a significant attenuation (HR: 0.91; 95% CI: 0.63, 1.31). Both studies suffered from small sample sizes and, thus, an inadequate sample size to truly test this hypothesis. Abnet et al (29) pooled the results of 8 prospective cohort studies and showed no association with esophageal and gastric cancers and serum vitamin D. Yin et al (30) performed a meta-analysis of serum vitamin D and breast cancer and showed a relation between higher concentrations of vitamin D and protection from breast cancer in case-control studies but not in prospective cohort studies. Thus, there appears to be mixed results related the association of 25(OH)D and cancer.

When interpretating our study results, several strengths and limitations should be noted. First, our study was both prospective in nature and in geographically and ethnically diverse population of postmenopausal women. All outcomes were reliably adjudicated, and many potential confounders were evaluated. Our number of outcomes (n = 224; 10% of the population) with measured 25(OH)D concentrations gave us adequate power to find clinically relevant findings for all-cause mortality but a more limited power to evaluate cause-specific mortality. Accidents and infectious causes of mortality are not adequately evaluated in the WHI cohort. Other potential limitations include assay variation associated with our measurement of 25(OH)D concentrations in 3 different case-control studies that make up this cohort. We adjusted for this variation by adding a study indicator variable to our regression analysis. Potential selection bias associated with the merging of the 3 case-control studies was addressed by using inverse-probability weighing. However, some residual confounding may still have remained because the selected population who had 25(OH)D concentrations may not have truly reflected the entire WHI population in the OSs and CTs. This potential selection bias may have affected our point-estimates results either toward or away from the null. Another potential caveat is that this was only a study in postmenopausal women, and thus, the results could not be generalized to premenopausal women or men.

TABLE 5
Adjusted HRs of risk of cancer mortality by 25(OH)D quartiles (adjusted for month)[1]

25(OH)D	Univariate HR (95% CI)[2]	*P*-trend	Multivariate HR (95% CI)[3]	*P*-trend
Quartile 1 (n = 608)	1.49 (0.73, 3.02)	0.09	1.39 (0.88, 2.19)	0.11
Quartile 2 (n = 606)	1.49 (0.75, 2.96)		1.22 (0.79, 1.89)	
Quartile 3 (n = 608)	0.64 (0.27, 1.51)		1.12 (0.72, 1.72)	
Quartile 4 (n = 607)	1.00		1.00	

[1] CaD, calcium and vitamin D; 25(OH)D, 25-hydroxyvitamin D.
[2] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, and CaD-trial indicator.
[3] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, CaD-trial indicator, education, smoking status, current aspirin use, history of fracture at ≥55 y of age, waist circumference, BMI, physical activity, and use of vitamin D supplements.

TABLE 6
Adjusted HR of risk of all-cause mortality by 25(OH)D quartiles (adjusted for month)[1]

	Multivariate HR (95% CI)[2]	*P*-trend[3]
Waist circumference <89 cm		
25(OH)D		
Quartile 1 (*n* = 272)	1.85 (1.00, 3.44)	0.05
Quartile 2 (*n* = 318)	1.40 (0.74, 2.63)	—
Quartile 3 (*n* = 333)	1.25 (0.67, 2.34)	—
Quartile 4 (*n* = 420)	1.00	—
Waist circumference ≥89 cm		
25(OH)D		
Quartile 1 (*n* = 323)	0.96 (0.52, 1.76)	0.80
Quartile 2 (*n* = 277)	0.95 (0.52, 1.72)	—
Quartile 3 (*n* = 259)	1.05 (0.57, 1.94)	—
Quartile 4 (*n* = 169)	1.00	—

[1] 25(OH)D, 25-hydroxyvitamin D.
[2] Estimates obtained from a Cox proportional hazards model adjusted for age, ethnicity, calcium and vitamin D–trial indicator, smoking status, history of hypertension, systolic blood pressure, history of treated diabetes, history of cardiovascular disease, history of fracture at ≥55 y of age, history of cancer, waist circumference, weekly alcohol consumption, and BMI.
[3] *P*-trend for each stratum of waist circumference = 0.08.

In conclusion, this study adds to the limited but growing number of prospective studies that have examined the relation between low concentrations of vitamin D all-cause and selected-cause mortality. Although an inverse association between 25(OH)D and all-cause and selected-cause mortality was apparent in our study in age-, race-, and month-adjusted analyses; the relation was attenuated to nonsignificance when adiposity and other potential confounding factors were taken into account. The evaluation of a potential effect modification by waist circumference as a measure of abdominal obesity suggested that any potential risk of low vitamin D on all-cause mortality was only in women with normal waist circumferences.

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