ORIGINAL RESEARCH

Racial/Ethnic Differences in 25-Hydroxy Vitamin D and Parathyroid Hormone Levels and Cardiovascular Disease Risk Among Postmenopausal Women

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***Background*—**Recent evidence suggests that racial/ethnic differences in circulating levels of free or bioavailable 25-hydroxy vitamin D (25[OH]D) rather than total 25(OH)D may explain apparent racial disparities in cardiovascular disease (CVD). We prospectively examined black-white differences in the associations of total, free, and bioavailable 25(OH)D, vitamin D–binding protein, and parathyroid hormone levels at baseline with incident CVD (including nonfatal myocardial infarction, nonfatal stroke, and CVD death) in postmenopausal women.

***Methods and Results*—**We conducted a case-cohort study among 79 705 postmenopausal women, aged 50 to 79 years, who were free of CVD at baseline in the WHI-OS (Women's Health Initiative Observational Study). A subcohort of 1300 black and 1500 white participants were randomly chosen as controls; a total of 550 black and 1500 white women who developed incident CVD during a mean follow-up of 11 years were chosen as cases. We directly measured total 25(OH)D, vitamin D–binding protein, albumin, parathyroid hormone, and calculated free and bioavailable 25(OH)D. Weighted Cox proportional hazards models were used to examine their associations with CVD risk. Although vitamin D–binding protein and total, free, and bioavailable 25(OH)D were not significantly associated with CVD risk in black or white women, a significant positive association between parathyroid hormone and CVD risk persisted in white women (hazard ratio comparing the highest quartile with the lowest, 1.37; 95% CI, 1.06–1.77) but not in black women (hazard ratio comparing the highest quartile with the lowest, 1.12; 95% CI, 0.79–1.58), independent of total, free, and bioavailable 25(OH)D or vitamin D–binding protein.

***Conclusions*—**Circulating levels of vitamin D biomarkers are not related to CVD risk in either white or black women. Higher parathyroid hormone levels may be an independent risk factor for CVD in white women. **(*J Am Heart Assoc*. 2019;8:e011021. DOI: 10.1161/JAHA.118.011021.)**

Key Words: 25(OH)D • biomarker • cardiovascular disease • parathyroid hormone/calcitonin • vitamin D • women

Low levels of serum vitamin D have been associated with cardiovascular diseases (CVDs) and CVD risk factors,[1,2] whereas mechanistic evidence supports a role of vitamin D in cardiovascular system, including the renin-angiotensin system, vascular inflammation, vascular calcification, and endothelial cell function.[2–4] The higher prevalence of low vitamin D levels among black adults than among whites might be explained by lower cutaneous vitamin D synthesis attributable to higher melanin levels, lower intake of dairy products and other foods fortified with vitamin D, or racial differences in vitamin D

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Accompanying Tables S1 through S3 and Figures S1 through S3 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.011021

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Clinical Perspective

What Is New?

- Controversy exists about the contributions of vitamin D biomarkers to racial/ethnic disparities in cardiovascular disease (CVD) because of lack of prospective evidence.
- This is, to our knowledge, the largest prospective study of black-white differences in vitamin D biomarkers with regard to CVD risk, which provided an in-depth evaluation of interrelations of total 25-hydroxy vitamin D and parathyroid hormone with CVD.
- None of these vitamin D biomarkers explained the higher CVD risk of black women compared with white women in this cohort, although our findings indicated that higher parathyroid hormone may be an independent risk factor for CVD in white women.

What Are the Clinical Implications?

- These findings from our observational study are timely and can augment and complement findings from randomized trials of the effect of vitamin D supplements on CVD and other chronic disease outcomes.

metabolism.[5] Also, blacks have higher parathyroid hormone (PTH) and experience a disproportionately higher burden of CVD than do whites.[5–7] Low total 25-hydroxy vitamin D (25[OH]D) levels appeared to be a stronger risk factor for coronary heart disease (CHD) in whites than blacks,[8] as well as for stroke mortality.[9] However, because of limited data, evidence to support such an association in white and black adults remains inconclusive, and the specific thresholds of circulating 25(OH)D for optimal cardiovascular health are unclear. Furthermore, free or bioavailable 25(OH)D may be a better measure of vitamin D activity than total 25(OH)D. Few studies have specifically examined possible racial/ethnic differences in vitamin D–binding protein (VDBP) or free or bioavailable vitamin D levels in relation to racial disparities in CVD.

Previous studies have documented consistent and substantial white-black differences in both total 25(OH)D and PTH.[5] Existing data suggest that 25(OH)D threshold values for vitamin D sufficiency that maximally suppress PTH secretion may differ by race/ethnicity.[5] PTH, independently or jointly with vitamin D, plays a central role in calcium metabolism, the renin-angiotensin system, endothelial function, and systemic inflammation and may therefore influence cardiovascular system.[2] Furthermore, elevated PTH levels have been linked to various cardiovascular outcomes, including hypertension, cardiac hypertrophy, and CHD.[10,11] A few studies have specifically examined the joint associations of vitamin D biomarkers and PTH on CVD risk[12–17]; however, the evidence has been inconsistent and inconclusive.

To comprehensively examine racial/ethnic differences in the associations of vitamin D biomarker and PTH with CVD risk, we designed and conducted a prospective case-cohort study in the multiethnic WHI-OS (Women's Health Initiative Observational Study) cohort to test the following specific aims in white and black women, separately: (1) whether vitamin D biomarkers, including plasma total 25(OH)D, VDBP, and/or free or bioavailable 25(OH)D, independently predict the risk of CVD; (2) whether PTH independently predicts the risk of CVD; and (3) whether vitamin D biomarkers and PTH jointly predict the risk of CVD.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

Between September 1994 and December 1998, the WHI-OS enrolled 93 676 postmenopausal women, aged 50 to 79 years, at 40 clinical centers throughout the United States. Women of all races and ethnicities were included, with a priority of enrolling at least 20% racial/ethnic minority groups. At study entry (ie, baseline), WHI-OS participants provided written informed consent and completed questionnaires about dietary habits, medical history, physical activity, socioeconomic status, and use of medications and supplements. Each woman had a physical examination, including direct measurement of weight, height, and waist and hip circumference, and provided a blood sample after an overnight fast of at least 12 hours.[18] Women in the WHI-OS provided written informed consent before participation, and the study protocols and procedures were conducted in accordance with recognized ethical guidelines and were approved at the institutional review boards of each participating center.

CVD End Point Ascertainment

CVD outcomes were ascertained by self-report using annual questionnaires and documented by medical records. CHD was defined as acute myocardial infarction (MI) that required overnight hospitalization and was confirmed by electrocardiography, cardiac enzyme elevations, or coronary death. Stroke diagnosis was based on the rapid onset of a neurologic deficit lasting >24 hours or until death, requiring hospitalization and supported by imaging studies when available.[19] The incident CVD outcome included nonfatal MI, CHD death, stroke (ischemic and hemorrhagic), and a composite of major vascular events (nonfatal MI, nonfatal stroke, and CVD mortality).

A Prospective Case-Cohort Study Design

We designed a case-cohort study of CVD within the WHI-OS for efficiency and to preserve stored blood samples.[20] We included only black and non-Hispanic white women because of their sufficient numbers of incident CVD cases for testing our hypotheses. After excluding those with a history of MI or stroke or receiving dialysis at baseline, case identification and subcohort sampling were performed for the 79 705 eligible women. CVD cases were women with confirmed incidental nonfatal MI, nonfatal stroke, or CVD death, as of the September 2013 database. A subcohort of 1415 black women was randomly selected from 6794 eligible black participants. All incident CVD cases in black women (not included in the subcohort) were included, yielding a final selection of 550 cases/1300 controls for black women. A subcohort of 1619 non-Hispanic white women was selected from 72 911 white eligible participants. A random sample of 1381 incident cases of CVD was selected from a total of 5941 eligible cases among non-Hispanic white women, excluding those who were already part of the subcohort sample, yielding a final selection of 1500 cases/1500 controls for non-Hispanic white women.

Biomarker Measurement

Plasma concentrations of total 25(OH)D, VDBP, PTH, albumin, and creatinine were measured by Dr Nader Rifai's Clinical and Epidemiologic Research Laboratory at Boston Children's Hospital (Boston, MA).[21] Total 25(OH)D was measured by an enzyme immunoassay from Immunodiagnostic Systems Inc (Fountain Hills, AZ). VDBP was measured by a monoclonal ELISA assay from R & D Systems (Minneapolis, MN). PTH was measured by an electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN). The lowest detection limit of this PTH assay is 1.2 pg/mL, and the day-to-day imprecision values at concentrations of 44.3, 161, and 702 pg/mL are 7.1%, 5.0%, and 5.4%, respectively. Albumin was measured by a colorimetric assay, an automated dye-binding method using the Roche P Modular system, and Roche Diagnostics reagents. The averaged coefficients of variation for each analyte were 6.95% for 25(OH)D, 5.46% for VDBP, 3.46% for PTH, 0.74% for albumin, and 1.82% for creatinine. We calculated free 25(OH)D, bioavailable 25(OH)D, and glomerular filtration rate (GFR). Free 25(OH)D was calculated as follows: free 25(OH)D=(total 25[OH]D levels)/[1+($6\times10^3\times$albumin)+($7\times10^8\times$VDBP)].[22] Bioavailable 25(OH)D (free+albumin-bound fraction) was calculated using equations adapted from that of Vermeulen et al.[22] In addition, we estimated GFR (eGFR) as a secondary measure of kidney function, by the equation developed and validated from the Modification of Diet in Renal Disease Study, as recommended by the National Kidney Foundation[23]: eGFR (in mL/min per 1.73 m^2)=$186\times$[creatinine (in mg/dL)$^{-1.154}\times$age$^{-0.203}\times0.742$ (female)$\times1.210$ (black)].

Statistical Analysis

Differences in plasma total and free 25(OH)D, VDBP, and PTH between cases and controls were examined using the Student *t* test for white and black women, separately. Age- and ethnicity-adjusted Pearson's partial correlation coefficients were calculated to evaluate correlations between vitamin D biomarkers among noncases.

All participants were categorized into quartiles according to the distributions of each biomarker in the noncases. We then calculated the hazard ratio (HR) of CVD events and the corresponding 95% CI using a weighted Cox regression model by designating the lowest quartile of each biomarker as the reference group. The weight method was proposed by Barlow et al as the inverse of the sampling probability of the subcohort stratified by race.[24] Age, clinical center, and race/ethnicity were adjusted in model 1. The main models were further adjusted for family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, eGFR, body mass index (BMI), and season of blood draw (model 2). Multivariable models were additionally adjusted for history of hypertension, history of hypercholesterolemia, history of diabetes mellitus, and statin use (model 3). Furthermore, we included each vitamin D biomarker with PTH simultaneously in the same model 3. In addition, we performed the same models for individual CVD end points, including MI, stroke, and CVD mortality. To explore the possible nonlinear dose-response relationship between vitamin D biomarker and CVD risk, we fitted restricted cubic spline proportional hazards regression models with 3 knots at 10, 50, and 90 percentiles of the distribution of each biomarker, controlling for the same covariates in model 3. We also examined whether the associations of vitamin D biomarkers with CVD were modified by race/ethnicity by adding interaction terms to model 2.

To evaluate the effect of the joint relationship of total 25(OH)D and PTH on CVD risk, we divided the study population into 4 subgroups according to 25(OH)D levels (<50 or ≥50 nmol/L) and PTH levels (<65 or ≥65 pg/mL). Compared with women with 25(OH)D ≥50 nmol/L and PTH <65 pg/mL, each subgroup-specific HR was estimated.

Finally, to explore potential effect modifications and to assess the robustness of the results, we stratified the analysis by age (<60 or ≥60 years), BMI (<25 or ≥25 kg/m^2), physical activity (<8 or ≥8 metabolic equivalent h/wk), cigarette smoking status (current smoker or nonsmoker), alcohol consumption (current drinker or nondrinker), hormone therapy use (user or nonuser), seasons of blood draw (winter or summer), family history of CVD (yes or no), history of diabetes

mellitus (yes or no), history of hypertension (yes or no), statin use (user or nonuser), and renal function (eGFR: <90 or ≥90 mL/min per 1.73 m^2).

All *P* values were 2 tailed, using a significance level of 0.05. All analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

Results

Compared with controls, CVD cases had significantly higher age, BMI, waist/hip ratio, and creatinine; had lower levels of physical activity, current alcohol intake, albumin, and eGFR; had less hormone therapy use; and were more likely to have a family history of CVD and a history of diabetes mellitus, hypertension, or high cholesterol among both white and black women (Table 1). The mean levels of total 25(OH)D and VDBP in white women were considerably higher than those in black women, and their differences between cases and controls were statistically significant in white women only (*P*=0.0001 and *P*=0.02, respectively). Black women had higher PTH levels than white women; differences in PTH between cases and controls were significant in both white (*P*<0.0001) and black (*P*=0.03) women.

Among all noncases, total 25(OH)D was moderately correlated with free 25(OH)D (r^2=0.45) and bioavailable 25(OH)D (r^2=0.46) (Table 2). Free 25(OH)D was highly correlated with bioavailable 25(OH)D (r^2=0.99). Both free and bioavailable 25(OH)D were inversely correlated with VDBP, with r^2 ranging from −0.58 to −0.59. The correlations were similar between white and black women.

After adjustment for age, clinical center, and ethnicity, total 25(OH)D, VDBP, and PTH were inversely and significantly associated with CVD risk in all participants (Table 3). Further adjustment for physical activity, cigarette smoking, alcohol intake, educational levels, season of blood draw, and family history of CVD attenuated these associations; the HRs comparing the highest quartile with the lowest quartile were 0.68 (95% CI, 0.54–0.86; *P* for trend=0.001) for total 25(OH)D; 0.77 (95% CI, 0.60–0.99; *P* for trend=0.006) for VDBP; and 1.45 (95% CI, 1.19–1.76; *P* for trend <0.0001) for PTH. Additionally controlling for BMI, postmenopausal hormone therapy use, history of hypertension, diabetes mellitus, high cholesterol, and statin use, and eGFR levels attenuated the associations of total 25(OH)D and VDBP toward the null but did not materially alter the results for PTH. These associations were similar in white women; none of these biomarkers was significantly associated with CVD among black women. When each vitamin D biomarker was mutually adjusted, PTH remained significantly associated with CVD in all women and white women (Table 3).

In the spline regression analysis, the nonlinear relationship between total 25(OH)D and CVD was apparent among white and black women (*P* for nonlinearity=0.02 and 0.09, respectively) (Figure[A]). The inverse association between total 25(OH)D levels and CVD risk was only seen among women with high levels of 25(OH)D (approximately >55 ng/mL for white women and >35 ng/mL for black women). A nonlinear relationship between PTH and CVD was also observed in both white and black women (*P* for nonlinearity=0.001 and 0.02, respectively) (Figure[B]). The higher PTH levels were associated with increased risk of CVD in women with PTH levels >40 pg/mL for white women and >65 pg/mL for black women. Such positive associations of PTH with CVD seemed stronger in white women than in black women. However, the *P* values for the interaction of race/ethnicity with 25(OH)D and PTH were not statistically significant for CVD (*P* for interaction >0.05). In addition, free and bioavailable 25(OH)D showed a reverse J-shaped relationship with CVD risk among white women (*P* for nonlinearity <0.0001 for both; Figures S1 and S2). There were no associations between VDBP and CVD in white and black women (*P* for nonlinearity >0.05 for both) (Figure S3).

For the joint associations with women with high 25(OH)D and low PTH as the referent group, we found that high levels of PTH (≥65 pg/mL) were consistently and significantly associated with increased CVD risk, regardless of total 25(OH)D levels; multivariate-adjusted HRs were 1.87 (95% CI, 1.33–2.62) for 25(OH)D <50 nmol/L and 1.61 (95% CI, 1.05–2.49) for 25(OH)D ≥50 nmol/L (Table 4). The association was slightly stronger among women with high PTH and low 25(OH)D levels, indicating a possible synergistic association between 25(OH)D and PTH with CVD risk. Similar patterns were observed for white women, but not for black women.

We found no significant associations between baseline 25(OH)D levels and each individual CVD outcome, including MI, stroke, and CVD mortality (Tables S1 through S3). However, PTH was significantly and inversely associated with CVD mortality; the multivariate-adjusted HR was 1.17 (95% CI, 1.08–1.26; *P* for linear trend=0.02). The positive associations remained similarly significant among white women only.

Finally, we observed no significant interactions by age, BMI, physical activity level, alcohol consumption, cigarette smoking, hormone therapy use, season of blood draw, family history of CVD, history of diabetes mellitus, history of hypertension, statin use, or renal function on the associations of vitamin D biomarkers and PTH with CVD risk (data not shown).

Discussion

In this large, prospective case-cohort study performed within an ethnically diverse cohort of US postmenopausal women without apparent CVD at baseline, we found no significant associations between VDBP or total, free, or bioavailable 25

Table 1. Baseline Characteristics by Ethnicity and CVD Status in a Case-Cohort Study From the WHI-OS

	US White Women			US Black Women		
Variables	Cases (n=1500)	Controls (n=1500)	*P* Value	Cases (n=550)	Controls (n=1300)	*P* Value
Age, y	69 (64, 73)	63 (57, 68)	<0.0001	65 (59, 71)	61 (56, 66)	<0.0001
BMI, kg/m^2	27.9±6.2	26.8±5.4	<0.0001	31.3±6.8	30.6±6.5	0.04
Waist/hip ratio, cm	0.83±0.08	0.80±0.07	<0.0001	0.84±0.1	0.82±0.08	<0.0001
Family history of CVD, n (%)	853 (60.3)	741 (52.2)	<0.0001	242 (48.8)	498 (42.4)	0.02
Physical activity, MET h/wk	8 (2.5, 17.1)	10.5 (3.8, 21)	<0.0001	5 (0.8, 13.9)	6.5 (1.3, 15.8)	0.06
Sitting time, n (%)						
<5 h	481 (32.3)	515 (34.5)	0.57	222 (41.1)	498 (38.8)	0.28
5–10 h	643 (43.2)	603 (40.4)		188 (34.8)	451 (35.1)	
≥10 h	366 (24.6)	375 (25.1)		130 (24.1)	335 (26.1)	
Smoking status, n (%)						
Never	705 (48.0)	720 (48.6)	0.02	256 (47.3)	631 (49.3)	0.04
Past	654 (44.5)	684 (46.2)		203 (37.5)	515 (40.2)	
Current	110 (7.5)	77 (5.2)		82 (15.2)	134 (10.5)	
Alcohol status, n (%)						
Never	166 (11.1)	129 (8.7)	0.01	102 (18.7)	248 (19.3)	<0.0001
Past	328 (22)	253 (17)		208 (38.1)	402 (31.2)	
Current	995 (66.8)	1103 (74.2)		236 (43.2)	637 (59.5)	
<1 Drink/d	792 (53.2)	911 (61.3)		219 (40.1)	567 (44.1)	
≥1 Drink/d	203 (13.6)	192 (12.9)		17 (3.1)	70 (5.4)	
History of diabetes mellitus, n (%)	159 (16.7)	84 (7.7)	<0.0001	84 (29.1)	115 (18.4)	<0.0001
History of hypertension, n (%)	747 (50.7)	414 (28.1)		366 (68.0)	679 (52.8)	
Untreated hypertension, n (%)	173 (11.7)	109 (7.4)	<0.0001	57 (10.6)	122 (9.5)	<0.0001
Treated hypertension, n (%)	574 (39.0)	305 (20.7)		309 (57.4)	557 (43.3)	
History of high cholesterol, n (%)	274 (18.6)	209 (14.2)	0.03	103 (19.4)	194 (15.2)	0.001
Hormone therapy use, n (%)	843 (56.2)	961 (64.1)		198 (36.2)	537 (41.2)	
Past use	276 (18.4)	230 (15.3)	0.02	77 (14.1)	170 (13.1)	<0.0001
Current use	567 (37.8)	731 (48.8)		121 (22.1)	367 (28.3)	
Statin use, n (%)	318 (21.2)	214 (14.3)	0.04	99 (18)	186 (14.3)	<0.0001
Educational levels, n (%)						
High school graduate/GED or less	357 (23.8)	306 (20.4)	0.46	158 (28.7)	344 (26.5)	<0.0001
Post high school	597 (39.8)	514 (34.3)		208 (37.8)	486 (37.4)	
College graduate or higher	546 (36.4)	680 (45.3)		184 (33.5)	470 (36.2)	
Geographical latitudes, n (%)						
Southern: <35° north	415 (27.7)	447 (29.8)	0.15	191 (34.7)	426 (32.8)	0.25
Middle: 35°–40° north	427 (28.5)	438 (29.2)		196 (35.6)	428 (32.9)	
Northern: >40° north	658 (43.9)	615 (41.0)		163 (29.6)	446 (34.3)	
Seasons for blood drawing, n (%)						
Spring	433 (28.9)	437 (29.3)	0.43	150 (27.3)	382 (29.8)	0.95
Summer	422 (28.2)	432 (28.9)		143 (26)	348 (27.1)	
Autumn	336 (22.4)	325 (21.8)		122 (22.2)	281 (21.9)	
Winter	306 (20.4)	300 (20.1)		134 (24.4)	272 (21.2)	

Continued

Table 1. Continued

Variables	US White Women			US Black Women		
	Cases (n=1500)	Controls (n=1500)	*P* Value	Cases (n=550)	Controls (n=1300)	*P* Value
Ultraviolet B, n (%)						
0.4–0.5 W/m^2	318 (21.2)	331 (22.1)	0.06	160 (29.1)	450 (34.6)	0.58
0.5–1.0 W/m^2	661 (44.1)	633 (42.2)		217 (39.5)	456 (35.1)	
1.1–1.9 W/m^2	521 (34.7)	536 (35.7)		173 (31.5)	394 (30.3)	
Vitamin D biomarkers						
Total 25(OH)D levels, nmol/L	62.1±20.2	64.9±20.2	0.0001	45.3±16.4	45.4±16.3	0.90
Free 25(OH)D levels, nmol/L	8.29±3.94	8.45±3.82	0.28	10.29±5.05	10.29±5.27	1.00
Bioavailable 25(OH)D, nmol/L	3.26±1.57	3.35±1.53	0.10	3.94±1.94	4.03±2.06	0.38
VDBP levels, ng/mL	240.6±77.4	247.1±78.6	0.02	136.7±70.8	138.3±69.4	0.66
PTH, pg/mL	38.8 (30.5, 48.9)	35.6 (28.4, 44.2)	<0.0001	41.8 (32.6, 55)	40.2 (31.4, 51.7)	0.03
Albumin, g/dL	4.34±0.25	4.38±0.24	<0.0001	4.24±0.29	4.33±0.25	<0.0001
CVD core biomarkers						
Creatinine, mg/dL	0.75±0.18	0.73±0.13	<0.0001	0.86±0.32	0.79±0.17	<0.0001
eGFR, ml/min/1.73m^2	80.7±18.5	83.8±16.8	<0.0001	87.2±24.1	93.6±21.5	<0.0001
hs-CRP, mg/L	3 (1.4, 6.8)	2.2 (0.9, 4.9)	<0.0001	4.2 (1.9, 9.4)	3.3 (1.4, 7.2)	<0.0001
Glucose, mg/dL	95 (89, 104)	93 (88, 99)	<0.0001	97 (88, 118)	94 (87, 105)	<0.0001
Insulin, μIU/mL	7.9 (5.3, 12.2)	6.6 (4.6, 10)	<0.0001	9.4 (5.9, 14.5)	9.1 (5.6, 13.7)	0.11
HOMA-%B	85.9 (59.9, 125)	81.6 (57.3, 117)	0.02	87.8 (57.9, 141)	98.8 (63.1, 148)	0.01
HOMA-IR	1.9 (1.2, 3.1)	1.5 (1, 2.4)	<0.0001	2.4 (1.4, 4.2)	2.1 (1.3, 3.6)	0.002

Continuous variables are presented as mean±SD, or, for non-normally distributed variables, median (interquartile range). 25(OH)D indicates 25-hydroxy vitamin D; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GED, General Education Development; HOMA-%B, homeostatic model assessment of pancreatic β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; MET, metabolic equivalent; PTH, parathyroid hormone; VDBP, vitamin D–binding protein; WHI-OS, Women's Health Initiative Observational Study.

(OH)D and incident CVD in either black or white women. None of these vitamin D biomarkers explained the higher CVD risk of black women compared with white women in this cohort, although our findings indicated that higher PTH may be an independent risk factor for CVD in white women, regardless of vitamin D biomarkers as well as known CVD risk factors.

A large body of observational studies have linked low total 25(OH)D (<60 nmol/L) to increased risk for CVD,[1,2,4] although previous work has mainly assessed total 25(OH)D. Accumulating evidence suggests that free and bioavailable 25(OH)D may better reflect the bioactivities of vitamin D in tissues, particularly in bone metabolism.[25] However, there remains much debate over the validity of novel vitamin D biomarkers, especially VDBP, for assessing vitamin D status and/or physiologic functions in various populations, given mixed results on the relationship of VDBP and albumin or calculated free vitamin D to CVD.[26,27]

In our study, VDBP was also measured using a monoclonal ELISA, which has been criticized for being subject to differential binding by genotype.[28] As a tissue-specific and polymorphic glycoprotein,[29] VDBP has several isoforms with different distributions and binding affinities by race. In addition, the amounts of circulating vitamin D metabolites available for target tissues after binding to VDBP also appear to vary by race.[28] Nevertheless, our prospective findings of the modest association between elevated VDBP and low CVD risk did not persist after adjusting for known CVD risk factors. In a multiethnic case-cohort study within the MESA (Multi-Ethnic Study of Atherosclerosis) involving 538 CHD cases over 12 years of follow-up and 999 randomly selected controls, VDBP measured using the liquid chromatography–tandem mass spectrometry method did not vary substantially by race or ethnicity and was associated with CHD events in all racial and ethnic groups. Total and bioavailable 25(OH)D were associated with incident CHD among whites but not among blacks.[26] Taken together, the evidence suggests that

Table 2. Age-Adjusted Spearman Correlation Coefficients for Vitamin D Biomarkers and CVD Biomarkers Among Controls

Biomarkers	Total 25(OH)D	Free 25(OH)D	Bioavailable 25(OH)D	VDBP	PTH	Creatinine	hs-CRP	Glucose	Insulin
All noncases (n=2800)									
Total 25(OH)D	1	0.45	0.46	0.34	−0.29	−0.07	−0.07	−0.15	−0.09
Free 25(OH)D		1	0.99	−0.59	−0.15	0.04	−0.04	−0.02*	−0.02*
Bioavailable 25(OH)D			1	−0.58	−0.15	0.04	−0.06	−0.02*	−0.02*
VDBP				1	−0.09	−0.1	−0.001*	−0.12	−0.05
PTH					1	0.27	0.05	0.04	0.07
Creatinine						1	0.04	−0.02*	0.07
hs-CRP							1	0.13	0.06
Glucose								1	0.23
Insulin									1
US white noncases (n=1500)									
Total 25(OH)D	1	0.60	0.60	0.16	−0.27	0.02*	−0.05	−0.15	−0.16
Free 25(OH)D		1	0.99	−0.59	−0.17	0.02*	−0.08	−0.06	−0.03*
Bioavailable 25(OH)D			1	−0.59	−0.17	0.02*	−0.11	−0.06	−0.05
VDBP				1	−0.03*	−0.003*	0.08	−0.09	−0.10
PTH					1	0.22	0.06	0.06	0.08
Creatinine						1	0.03*	−0.07	0.02*
hs-CRP							1	0.12	0.15
Glucose								1	0.34
Insulin									1
US black noncases (n=1300)									
Total 25(OH)D	1	0.65	0.65	0.08	−0.27	0.005*	−0.04*	−0.07	−0.04*
Free 25(OH)D		1	0.99	−0.61	−0.19	−0.007*	−0.03*	−0.03*	−0.04*
Bioavailable 25(OH)D			1	−0.6	−0.19	−0.02*	−0.06	−0.04*	−0.04*
VDBP				1	−0.01*	0.02*	0.02*	−0.02*	0.04*
PTH					1	0.29	0.009*	−0.009*	0.06
Creatinine						1	0.02*	−0.03*	0.07
hs-CRP							1	0.13	0.03*
Glucose								1	0.21
Insulin									1

25(OH)D indicates 25-hydroxy vitamin D; CVD, cardiovascular disease; hs-CRP, high-sensitivity C-reactive protein; PTH, parathyroid hormone; VDBP, vitamin D–binding protein.
*Indicates nonsignificant, $P>0.05$.

differences in VDBP, regardless of assay method, may not explain significant racial and ethnic differences in the association between total 25(OH)D and CVD.

Our results confirm the consistent and substantial white-black differences in total 25(OH)D and PTH,[5] but do not support the hypothesis that racial/ethnic differences in total 25(OH)D contribute significantly to black-white disparities in CVD. In our study, total 25(OH)D was modestly correlated with PTH (r^2=−0.29) in white and black women, consistent with most previous studies.[5,12,30] Previous studies have documented a consistent association between elevated PTH levels and increased risk of CVD outcomes.[10,11] Systematic reviews and meta-analyses of prospective studies showed evidence of substantial between-study heterogeneity in previous studies, such as differences in participant characteristics, PTH assays, baseline PTH levels, CVD ascertainments and end points, and comorbid chronic kidney disease, but none of them influenced the overall association between PTH excess and CVD risk.[10,11] However, it remains unclear whether the PTH-CVD relationship is independent of 25(OH)D

Table 3. HRs for Risk of CVD by Levels of Vitamin D Biomarkers Among Postmenopausal Women

Variable	Model	HR (95% CI)				*P* Value for Trend	$HR_{per\text{-}SD}$ (95% CI)*
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Total 25(OH)D, ranges, nmol/L		11.9–40.0	40.0–53.3	53.3–68.8	68.8–228		
All participants (n=4808)	1†	1 (Referent)	0.75 (0.62–0.92)	0.68 (0.56–0.83)	0.61 (0.49–0.74)	<0.0001	0.84 (0.78–0.91)
	2‡	1 (Referent)	0.86 (0.69–1.07)	0.79 (0.63–0.99)	0.79 (0.62–1.00)	0.07	0.92 (0.85–1.01)
	3§	1 (Referent)	0.90 (0.72–1.12)	0.89 (0.71–1.12)	0.91 (0.72–1.15)	0.55	0.97 (0.89–1.06)
	3+PTH	1 (Referent)	0.94 (0.76–1.18)	0.93 (0.73–1.17)	0.98 (0.77–1.26)	0.96	1.00 (0.91–1.09)
US white women (n=2967)	1†	1 (Referent)	0.76 (0.61–0.95)	0.67 (0.53–0.84)	0.68 (0.54–0.85)	0.001	0.84 (0.77–0.92)
	2‡	1 (Referent)	0.78 (0.61–1.00)	0.76 (0.58–0.98)	0.83 (0.64–1.07)	0.22	0.91 (0.83–1.01)
	3§	1 (Referent)	0.87 (0.68–1.12)	0.89 (0.69–1.16)	0.95 (0.73–1.25)	0.86	0.97 (0.88–1.07)
	3+PTH	1 (Referent)	0.88 (0.68–1.13)	0.93 (0.71–1.21)	1.02 (0.78–1.34)	0.73	1.00 (0.90–1.11)
US black women (n=1841)	1†	1 (Referent)	0.87 (0.65–1.16)	0.82 (0.61–1.10)	0.73 (0.55–0.98)	0.04	0.88 (0.77–1.01)
	2‡	1 (Referent)	0.97 (0.69–1.34)	0.97 (0.69–1.36)	0.94 (0.67–1.32)	0.74	0.99 (0.85–1.17)
	3§	1 (Referent)	1.04 (0.74–1.44)	1.09 (0.77–1.53)	1.02 (0.73–1.44)	0.84	1.02 (0.87–1.19)
	3+PTH	1 (Referent)	1.05 (0.75–1.46)	1.10 (0.78–1.56)	1.07 (0.75–1.54)	0.67	1.05 (0.89–1.24)
Free 25(OH)D, ranges, nmol/L		1.63–6.10	6.10–8.22	8.23–11.3	11.3–44.7		
All participants (n=4802)	1†	1 (Referent)	0.91 (0.76–1.09)	0.84 (0.70–1.01)	0.91 (0.76–1.10)	0.33	0.95 (0.89–1.02)
	2‡	1 (Referent)	0.86 (0.67–1.11)	0.99 (0.77–1.27)	0.95 (0.73–1.24)	0.98	1.01 (0.93–1.09)
	3§	1 (Referent)	1.08 (0.89–1.32)	1.03 (0.84–1.26)	1.06 (0.86–1.31)	0.70	1.01 (0.94–1.09)
	3+PTH	1 (Referent)	1.05 (0.85–1.30)	1.09 (0.88–1.34)	1.32 (1.07–1.63)	0.36	1.03 (0.95–1.12)
US white women (n=2965)	1†	1 (Referent)	0.79 (0.63–0.99)	0.80 (0.64–1.00)	0.85 (0.68–1.07)	0.27	0.95 (0.86–1.05)
	2‡	1 (Referent)	1.26 (0.78–2.03)	0.99 (0.73–1.33)	1.15 (0.82–1.62)	0.50	1.02 (0.91–1.15)
	3§	1 (Referent)	0.88 (0.68–1.14)	1.09 (0.84–1.40)	0.97 (0.75–1.26)	0.86	1.03 (0.92–1.16)
	3+PTH	1 (Referent)	0.91 (0.70–1.18)	1.12 (0.86–1.45)	1.05 (0.80–1.37)	0.48	1.06 (0.95–1.19)
US black women (n=1837)	1†	1 (Referent)	1.26 (0.83–1.91)	0.97 (0.75–1.27)	1.04 (0.78–1.39)	0.96	0.97 (0.89–1.06)
	2‡	1 (Referent)	1.26 (0.78–2.03)	0.99 (0.73–1.33)	1.15 (0.82–1.62)	0.50	1.00 (0.90–1.10)
	3§	1 (Referent)	1.07 (0.67–1.72)	0.96 (0.71–1.30)	1.09 (0.77–1.52)	0.67	0.99 (0.89–1.10)
	3+PTH	1 (Referent)	1.08 (0.67–1.73)	0.98 (0.72–1.33)	1.13 (0.79–1.61)	0.53	1.00 (0.90–1.11)
Bioavailable 25(OH)D, ranges, nmol/L		0.59–2.38	2.38–3.25	3.25–4.45	4.45–16.6		
All participants (n=4802)	1†	1 (Referent)	0.89 (0.75–1.06)	0.86 (0.72–1.03)	0.86 (0.71–1.03)	0.96	0.93 (0.87–1.00)
	2‡	1 (Referent)	1.00 (0.82–1.21)	0.99 (0.81–1.21)	0.96 (0.78–1.19)	0.73	0.99 (0.92–1.07)
	3§	1 (Referent)	1.03 (0.85–1.25)	1.05 (0.86–1.29)	0.97 (0.79–1.20)	0.78	0.99 (0.92–1.07)
	3+PTH	1 (Referent)	1.06 (0.86–1.29)	1.08 (0.88–1.33)	1.02 (0.82–1.27)	0.83	1.01 (0.94–1.10)
US white women (n=2965)	1†	1 (Referent)	0.81 (0.65–1.02)	0.81 (0.65–1.02)	0.89 (0.71–1.12)	0.49	0.94 (0.85–1.04)
	2‡	1 (Referent)	0.95 (0.74–1.21)	0.98 (0.76–1.26)	1.07 (0.82–1.39)	0.55	1.01 (0.90–1.14)
	3§	1 (Referent)	1.01 (0.79–1.3)	1.12 (0.87–1.45)	1.09 (0.84–1.42)	0.43	1.02 (0.92–1.15)
	3+PTH	1 (Referent)	1.05 (0.81–1.35)	1.16 (0.89–1.51)	1.18 (0.90–1.55)	0.19	1.05 (0.94–1.18)
US black women (n=1837)	1†	1 (Referent)	1.00 (0.75–1.34)	0.88 (0.65–1.18)	0.91 (0.68–1.22)	0.45	0.94 (0.86–1.02)
	2‡	1 (Referent)	1.06 (0.76–1.46)	0.92 (0.65–1.29)	1.03 (0.73–1.45)	0.98	0.97 (0.87–1.08)
	3§	1 (Referent)	0.98 (0.71–1.35)	0.89 (0.64–1.25)	0.96 (0.69–1.35)	0.79	0.96 (0.86–1.06)
	3+PTH	1 (Referent)	1.00 (0.72–1.39)	0.91 (0.64–1.28)	1.00 (0.70–1.42)	0.94	0.97 (0.86–1.08)
VDBP, ranges, ng/mL		26.1–111	111–196	196–262	262–594		

Continued

Table 3. Continued

Variable	Model	HR (95% CI)				*P* Value for Trend	$HR_{per\text{-}SD}$ (95% CI)*
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
All participants (n=4811)	1†	1 (Referent)	1.00 (0.81–1.23)	0.90 (0.72–1.11)	0.76 (0.60–0.95)	0.002	0.90 (0.83–0.97)
	2‡	1 (Referent)	1.06 (0.84–1.33)	0.98 (0.77–1.25)	0.85 (0.65–1.10)	0.06	0.93 (0.85–1.01)
	3§	1 (Referent)	1.12 (0.89–1.42)	1.05 (0.81–1.34)	0.93 (0.71–1.21)	0.20	0.96 (0.88–1.05)
	3+PTH	1 (Referent)	1.13 (0.89–1.43)	1.03 (0.80–1.33)	0.92 (0.71–1.21)	0.18	0.96 (0.88–1.05)
US white women (n=2970)	1†	1 (Referent)	0.91 (0.73–1.14)	0.85 (0.68–1.07)	0.78 (0.62–0.98)	0.03	0.90 (0.82–0.99)
	2‡	1 (Referent)	0.96 (0.75–1.22)	0.88 (0.69–1.14)	0.82 (0.63–1.06)	0.10	0.91 (0.82–1.02)
	3§	1 (Referent)	0.91 (0.71–1.17)	0.90 (0.70–1.16)	0.87 (0.67–1.14)	0.32	0.94 (0.85–1.05)
	3+PTH	1 (Referent)	0.88 (0.68–1.14)	0.88 (0.69–1.14)	0.87 (0.67–1.13)	0.31	0.94 (0.84–1.05)
US black women (n=1841)	1†	1 (Referent)	0.94 (0.7–1.25)	1.00 (0.75–1.33)	0.89 (0.69–1.08)	0.22	0.92 (0.81–1.06)
	2‡	1 (Referent)	0.94 (0.68–1.30)	1.04 (0.75–1.45)	0.87 (0.62–1.24)	0.61	0.99 (0.84–1.17)
	3§	1 (Referent)	0.93 (0.67–1.30)	1.08 (0.78–1.50)	0.95 (0.67–1.34)	0.99	1.04 (0.88–1.22)
	3+PTH	1 (Referent)	0.93 (0.66–1.30)	1.07 (0.77–1.49)	0.94 (0.66–1.34)	0.96	1.04 (0.88–1.23)
PTH, ranges, pg/mL		2.36–29.7	29.7–37.4	37.4–47.6	47.6–491		
All participants (n=4806)	1†	1 (Referent)	1.08 (0.89–1.30)	1.22 (1.01–1.46)	1.55 (1.29–1.87)	<0.0001	1.17 (1.11–1.23)
	2‡	1 (Referent)	1.01 (0.82–1.25)	1.12 (0.92–1.38)	1.41 (1.15–1.73)	0.0002	1.17 (1.11–1.25)
	3§	1 (Referent)	1.05 (0.85–1.30)	1.09 (0.88–1.33)	1.30 (1.06–1.60)	0.008	1.12 (1.06–1.19)
	3+Total 25(OH)D	1 (Referent)	1.04 (0.84–1.29)	1.07 (0.87–1.32)	1.28 (1.04–1.59)	0.01	1.12 (1.05–1.19)
	3+Free 25(OH)D	1 (Referent)	1.05 (0.85–1.30)	1.09 (0.88–1.34)	1.32 (1.07–1.63)	0.007	1.12 (1.06–1.19)
	3+Bioavailable 25(OH)D	1 (Referent)	1.06 (0.86–1.29)	1.08 (0.88–1.33)	1.02 (0.82–1.27)	0.01	1.12 (1.05–1.19)
	3+VDBP	1 (Referent)	1.05 (0.85–1.30)	1.09 (0.88–1.34)	1.30 (1.06–1.60)	0.007	1.12 (1.05–1.19)
US white women (n=2969)	1†	1 (Referent)	1.07 (0.84–1.36)	1.11 (0.88–1.41)	1.55 (1.23–1.94)	<0.0001	1.20 (1.12–1.28)
	2‡	1 (Referent)	1.11 (0.86–1.45)	1.03 (0.78–1.34)	1.48 (1.15–1.90)	0.001	1.18 (1.11–1.27)
	3§	1 (Referent)	1.22 (0.93–1.60)	1.07 (0.82–1.40)	1.43 (1.11–1.84)	0.01	1.13 (1.05–1.21)
	3+Total 25(OH)D	1 (Referent)	1.21 (0.92–1.59)	1.05 (0.80–1.38)	1.39 (1.07–1.81)	0.02	1.12 (1.04–1.21)
	3+Free 25(OH)D	1 (Referent)	1.23 (0.94–1.61)	1.08 (0.83–1.42)	1.46 (1.13–1.89)	0.007	1.13 (1.05–1.21)
	3+Bioavailable 25(OH)D	1 (Referent)	1.22 (0.93–1.6)	1.08 (0.82–1.41)	1.44 (1.12–1.87)	0.008	1.13 (1.05–1.21)
	3+VDBP	1 (Referent)	1.22 (0.93–1.59)	1.07 (0.82–1.40)	1.43 (1.11–1.84)	0.008	1.13 (1.05–1.21)
US black women (n=1837)	1†	1 (Referent)	1.16 (0.85–1.56)	1.30 (0.97–1.75)	1.50 (1.12–2.01)	0.005	1.19 (1.05–1.34)
	2‡	1 (Referent)	1.12 (0.79–1.57)	1.24 (0.89–1.74)	1.37 (0.97–1.92)	0.07	1.17 (1.03–1.33)
	3§	1 (Referent)	1.01 (0.71–1.43)	1.12 (0.79–1.58)	1.24 (0.88–1.74)	0.16	1.13 (1.05–1.21)
	3+Total 25(OH)D	1 (Referent)	1.02 (0.72–1.44)	1.14 (0.80–1.63)	1.27 (0.89–1.82)	0.13	1.12 (1.04–1.21)
	3+Free 25(OH)D	1 (Referent)	1.02 (0.72–1.44)	1.13 (0.80–1.60)	1.26 (0.89–1.78)	0.15	1.13 (1.05–1.21)
	3+Bioavailable 25(OH)D	1 (Referent)	1.00 (0.71–1.42)	1.11 (0.78–1.56)	1.22 (0.86–1.73)	0.20	1.13 (1.05–1.21)
	3+VDBP	1 (Referent)	1.00 (0.70–1.41)	1.12 (0.79–1.58)	1.23 (0.87–1.73)	0.16	1.13 (1.05–1.21)

25(OH)D indicates 25-hydroxy vitamin D; CVD, cardiovascular disease; HR, hazard ratio; PTH, parathyroid hormone; VDBP, vitamin D–binding protein.
*HRs represent per-SD increases in biomarker measures among control participants.
†Model 1 adjusted for age, clinical center, and race/ethnicity (for all participants).
‡Model 2 further adjusted for family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, estimated glomerular filtration rate, body mass index, and seasons of blood draw.
§Model 3 further adjusted for history of hypertension, history of hypercholesterolemia, history of diabetes mellitus, and statin use.

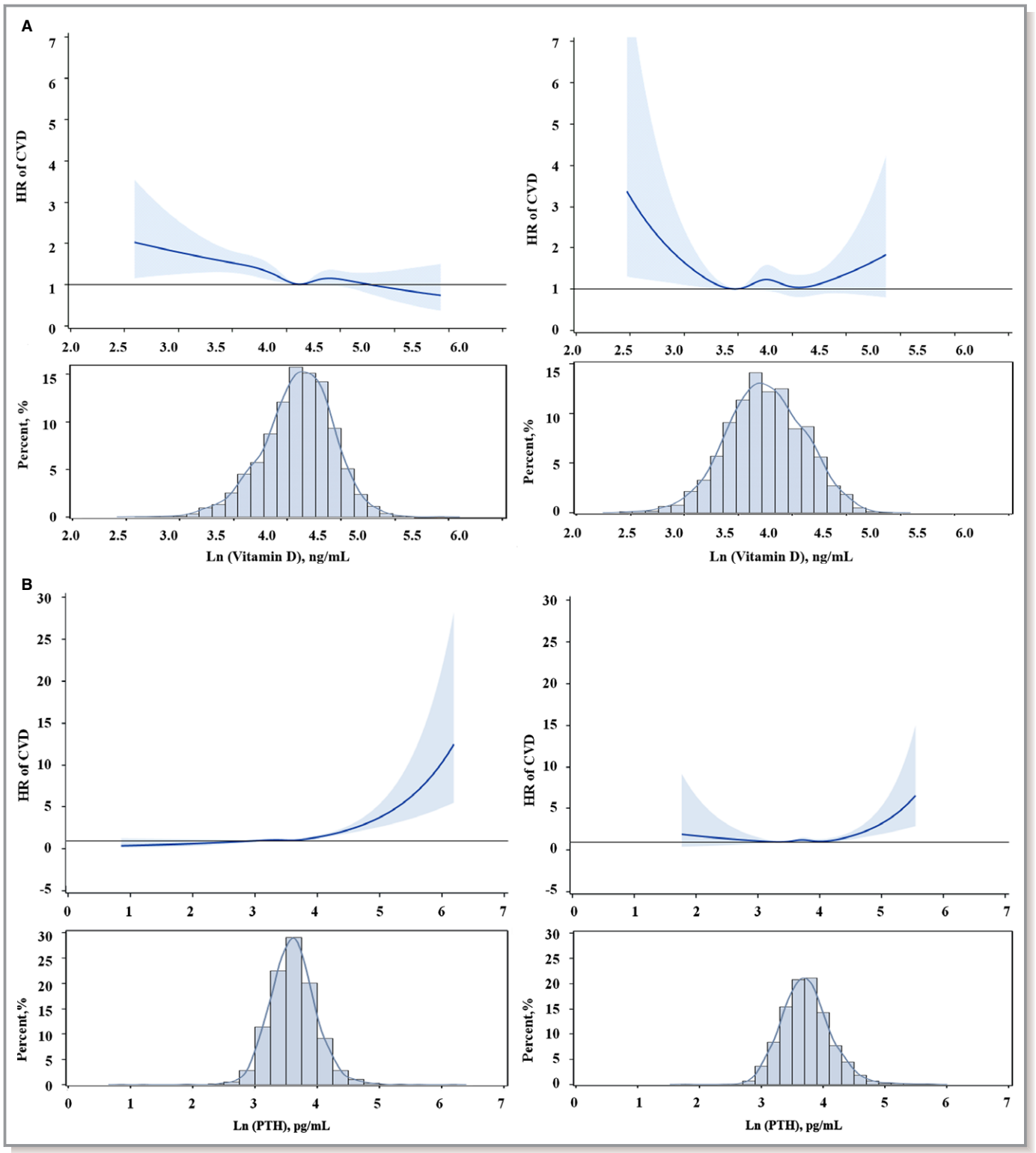

Figure. Race/ethnicity-specific associations of total 25-hydroxy vitamin D (25[OH]D) and parathyroid hormone (PTH) with cardiovascular disease (CVD) events. **A**, Total 25(OH)D and CVD events. **B**, PTH and CVD events. Associations were estimated by proportional hazards regression–based restricted cubic spline analyses among white and black women, separately. We calculated restricted cubic spline with 3 fixed knots at 10%, 50%, and 90% percentiles based on the distribution of each biomarker level. Solid lines with gray areas indicate the hazard ratios (HRs) and 95% CIs. The model was adjusted for age, clinical center, race/ethnicity, body mass index, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, estimated glomerular filtration rate, and season of blood draw.

Table 4. HRs for Risk of CVD by Total 25(OH)D and PTH Levels Among Postmenopausal Women

Model	Total Cases/ Controls	PTH <65 pg/mL		PTH ≥65 pg/mL	
		25(OH)D ≥50 nmol/L	25(OH)D <50 nmol/L	25(OH)D ≥50 nmol/L	25(OH)D <50 nmol/L
All women					
No. (cases/controls)	2021/2781	1148/1527	654/1044	84/59	135/151
1*		1.00 (Referent)	1.37 (1.17–1.60)	1.68 (1.14–2.48)	2.26 (1.68–3.04)
2†		1.00 (Referent)	1.21 (1.02–1.44)	1.61 (1.05–2.49)	1.87 (1.33–2.62)
3‡		1.00 (Referent)	1.09 (0.92–1.31)	1.09 (0.92–1.31)	1.64 (1.17–2.30)
White women					
No. (cases/controls)	1476/1489	985/1121	359/305	68/36	64/27
1*		1.00 (Referent)	1.41 (1.16–1.72)	1.62 (1.03–2.54)	3.41 (2.03–5.76)
2†		1.00 (Referent)	1.25 (1.00–1.57)	1.56 (0.93–2.59)	2.95 (1.70–5.10)
3‡		1.00 (Referent)	1.10 (0.88–1.38)	1.49 (0.92–2.41)	2.39 (1.33–4.29)
Black women					
No. (cases/controls)	545/1292	163/406	295/739	16/23	71/124
1*		1.00 (Referent)	1.14 (0.91–1.44)	1.71 (0.86–3.38)	1.55 (1.08–2.23)
2†		1.00 (Referent)	1.02 (0.78–1.34)	1.89 (0.91–3.94)	1.27 (0.83–1.97)
3‡		1.00 (Referent)	0.98 (0.75–1.28)	1.79 (0.86–3.74)	1.21 (0.79–1.85)

Data are given as HR (95% CI) unless otherwise indicated. 25(OH)D indicates 25-hydroxy vitamin D; CVD, cardiovascular disease; HR, hazard ratio; PTH, parathyroid hormone.
*Model 1 adjusted for age, clinical center, and race/ethnicity (for all participants).
†Model 2 further adjusted for family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, estimated glomerular filtration rate, body mass index, and seasons of blood draw.
‡Model 3 further adjusted for history of hypertension, history of hypercholesterolemia, history of diabetes mellitus, and statin use.

because of limited data. Our positive findings of the PTH-CVD relationship among US white women are consistent with the LASA (Longitudinal Aging Study Amsterdam) (predominantly white men and women)[14] and a US Utah healthcare population[12] showing that PTH was a significant predictor for CVD independent of 25(OH)D. Similarly, the BRHS (British Regional Heart Study), comprising predominantly whites, reported that elevated PTH, but not 25(OH)D, was associated with increased risk of heart failure in British older men with and without CVD.[13] Our findings are less consistent with the Hoorn Study, a Netherlands population-based study of white men and women in which high PTH levels were significantly associated with CVD mortality, but not independent of 25(OH) D levels.[17] In contrast, PTH did not predict CVD mortality in a population of middle-aged and older European men in the EMAS (European Male Aging Study).[16] The ARIC (Atherosclerosis Risk in Communities) Study in the United States found no association of PTH with CHD regardless of renal status or 25(OH)D levels, potentially because of random measurement error caused by time-dependent deterioration of ARIC Study samples.[15]

The interactions of the vitamin D/PTH endocrine system are complex. PTH, independently or jointly with vitamin D, plays a central role in calcium metabolism, the renin-angiotensin system, endothelial function, and systemic inflammation and may therefore influence CVD risk.[2,3] Our results indicate that further investigation of joint associations of vitamin D and PTH with clinically significant end points is important for a better understanding of their relationship with CVD and racial disparities in CVD.

The strengths of our study include its prospective design, the use of the large and well-characterized cohort of white and black women, detailed measures of potential confounders, rigorous adjudication of incident CVD cases, and a comprehensive panel of vitamin D biomarkers. Nonetheless, the present study has several limitations. First, VDBP was measured using a monoclonal ELISA rather than a mass spectrometry assay, although the presumed gold standard mass spectrometry method for directly measuring polymorphic VDBP remains to be validated across diverse populations. Second, single measurement of vitamin D biomarkers with varying coefficients of variation might have biased the results toward the null. However, previous validation data in large cohorts, similar to those of the WHI-OS, have found that a single 25(OH)D measurement is fairly reproducible over 2 to 11 years and reasonably reflects long-term vitamin D status.[15,27] Third, we cannot discount that some associations may be affected by multiple tests and/or residual confounding. It is also possible that the lack of statistically significant interactions by race/ethnicity and racial

differences in the PTH-CVD association may be because of the relatively small sample size of US black women. Finally, because the WHI-OS is an all-female postmenopausal cohort, we were unable to generalize our findings to men without consideration of potential biological differences between the sexes.

In summary, in a large prospective case-cohort study of US postmenopausal women, we found no significant associations of total, free, and bioavailable 25(OH)D and VDBP with CVD risk in either black or white women. However, there appears to be a trend toward increased CVD risk associated with higher PTH levels among white women, independent of vitamin D biomarkers as well as known CVD risk factors. Future longitudinal studies and randomized trials testing vitamin D supplementation with accurate and well-validated quantification of vitamin D–related metabolites are warranted to clarify the relationship between vitamin D and CVD development.

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