Independent and Synergistic Associations of Biomarkers of Vitamin D Status With Risk of Coronary Heart Disease

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Objective—To comprehensively evaluate the independent associations and potential interactions of vitamin D–related biomarkers including total and bioavailable 25-hydroxyvitamin D (25OHD), VDBP (vitamin D binding protein), and parathyroid hormone (PTH) with risk of coronary heart disease (CHD).

Approach and Results—We prospectively identified incident cases of nonfatal myocardial infarction and fatal CHD among women in the Nurses' Health Study during 20 years of follow-up (1990–2010). Using risk-set sampling, 1 to 2 matched controls were selected for each case. The analysis of 25OHD and PTH included 382 cases and 575 controls; the analysis of VDBP included 396 cases and 398 controls. After multivariate adjustment, plasma levels of total 25OHD, bioavailable 25OHD, and PTH were not significantly associated with CHD risk. VDBP was associated with a lower CHD risk with an extreme-quartile odds ratio of 0.60 (95% confidence interval, 0.39–0.92; *P* trend=0.02). When examining the biomarkers jointly, a significant, inverse association between 25OHD and CHD was observed among participants with higher PTH levels (*P* for interaction=0.02). The odds ratio (95% confidence interval) comparing the highest quartile of 25OHD to lowest was 0.43 (0.23–0.82; *P* trend=0.003) when PTH levels were above population median (35.3 pg/mL), whereas among the rest of participants the corresponding odds ratio (95% confidence interval) was 1.28 (0.70–2.36; *P* trend=0.43).

Conclusions—Our data suggest that higher 25OHD levels were associated with a lower CHD risk when PTH levels were high, whereas no association was observed for participants with low PTH levels. VDBP but not bioavailable 25OHD was independently associated with lower CHD risk.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37: 2204-2212. DOI: 10.1161/ATVBAHA.117.309548.)**

Key Words: biomarkers ■ myocardial infarction ■ parathyroid hormone ■ risk ■ vitamin D

An increasing number of healthy US adults take vitamin D supplements, primarily for bone health.[1] Vitamin D status has been also implicated in heart health through inhibition of vascular smooth muscle cell proliferation, systemic anti-inflammatory effects, enhancement in insulin secretion and sensitivity, and regulatory effects on the renin–angiotensin–aldosterone system.[2] The most representative measure for vitamin D status is serum 25-hydroxyvitamin D (25OHD) because it reflects the total stored quantity from both endogenous and exogenous sources and has a fairly long circulating half-life.[3] Despite potential pleiotropic cardioprotective mechanisms, prospective observational studies on the association between circulating 25OHD and risk of cardiovascular outcomes have generated mixed results.[3–7]

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The majority of 25OHD and the activated vitamin D are bound to VDBP (vitamin D binding protein), a group-specific component of serum globulin, and transported to target tissues.[8] Beyond transport of vitamin D metabolites, VDBP could also modulate innate immunity and inflammatory responses[9–11] and may be linked to a reduced risk of coronary heart disease (CHD).[12,13] The smaller amount of albumin-bound and the free, unbound 25OHD is often referred to as bioavailable fraction. The free hormone hypothesis suggests that protein-bound hormones are relatively inactive, whereas those liberated from binding proteins are free to exert biological activity.[14] It was previously demonstrated that bioavailable 25OHD was more tightly associated with bone density than

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Nonstandard Abbreviations and Acronyms	
25OHD	25-hydroxyvitamin D
CHD	coronary heart disease
CI	confidence interval
HDL	high-density lipoprotein
OR	odds ratio
PTH	parathyroid hormone
VDBP	vitamin D binding protein

total levels in healthy individuals.[15] Moreover, parathyroid hormone (PTH) is also involved in the regulation of vitamin D metabolism and maintenance of calcium homeostasis. Elevated PTH has been associated with increased CHD risk in a meta-analysis of prospective studies.[16]

Previous studies have largely analyzed these vitamin D–related biomarkers separately, and little is known about whether the associations of 25OHD, VDBP, and PTH are mutually independent. In addition, it is unclear whether these biomarkers may interact with each other in relation to CHD risk. Because lower 25OHD levels represent decreased vitamin D stores, whereas elevated PTH levels partly reflect true deficiency or inadequate biological activity of vitamin D[17,18]; we hypothesized that the potential associations of lower vitamin D levels with CHD would be stronger in the presence of concomitant elevated PTH. Therefore, we comprehensively assessed the independent and joint associations of circulating levels of total and bioavailable 25OHD, VDBP, and PTH with risk of CHD in a nested case–control study within a large prospective cohort, the Nurses' Health Study.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Distributions of demographic, lifestyle, and metabolic risk factors according to quartiles of plasma levels of 25OHD, VDBP, and PTH among controls assessed at blood draw are shown in Table 1. Higher circulating 25OHD levels were associated with lower body mass index and higher physical activity levels, diet quality, dietary intake of vitamin D and calcium, and alcohol intake. Participants with higher 25OHD levels were more likely to use postmenopausal hormone and multivitamin. Higher 25OHD levels were also associated with a more favorable lipid profile, including slightly lower total cholesterol and triglycerides as well as higher HDL (high-density lipoprotein) cholesterol. Circulating VDBP was associated with not only younger age and lower body mass index but also lower diet quality and calcium intake. Participants with higher VDBP levels were less likely to have a family history of myocardial infarction. Similar to 25OHD, VDBP also showed an association with an elevated level of HDL cholesterol. In contrast, higher PTH levels were associated with higher body mass index, total cholesterol, low-density lipoprotein cholesterol, and creatinine. Those with higher PTH levels displayed an increased likelihood of having hypertension and hypercholesterolemia. Among controls, total 25OHD was positively correlated with bioavailable 25OHD (r=0.62) and VDBP (r=0.18) but inversely correlated with PTH (r=−0.24), whereas VDBP and PTH were very weakly correlated (r=−0.001; Table 2).

In Table 3, we present the associations of circulating total and bioavailable 25OHD, VDBP, and PTH with risk of developing CHD. In crude models that were adjusted for matching factors (results not shown), total 25OHD and VDBP were associated with lower CHD risk. After further adjustment for body mass index, lifestyle factors, and diet (model 1), the association for 25OHD was attenuated and no longer statistically significant, whereas the association for VDBP remained robust. Compared with the lowest quartile of VDBP, the odds ratio (OR; 95% confidence interval [CI]) in the highest quartile was 0.60 (0.39–0.92; P trend=0.02). PTH and bioavailable 25OHD were not significantly associated with CHD risk, and the multivariate-adjusted OR comparing highest versus lowest quartile was 0.68 (95% CI, 0.46–1.01; P trend=0.06) for PTH and 1.23 (95% CI, 0.79–1.90; P trend=0.19) for bioavailable 25OHD. For total and bioavailable 25OHD as well as VDBP, the associations did not materially change with additional adjustment for clinical factors that could be either confounders or mediators, including baseline history of hypertension or diabetes mellitus, HDL cholesterol, low-density lipoprotein cholesterol, C-reactive protein, and creatinine (results not shown). However, the association for PTH became statistically significant after clinical factors were controlled for; the largest change was seen with additional adjustment for HDL cholesterol and low-density lipoprotein cholesterol. Comparing to the lowest quartile of PTH, the OR (95% CI) in the highest quartile was 0.58 (0.37–0.89; P trend=0.01). Of note, additional mutual adjustment for other biomarkers (model 2) did not appreciably alter any of these associations, with the OR (95% CI) in the highest quartile of 0.98 (0.61–1.58; P trend=0.66) for total 25OHD, 0.55 (0.35–0.87; P trend=0.01) for VDBP, 0.70 (0.44–1.19; P trend=0.13) for PTH, and 0.87 (0.52–1.45; P trend=0.76) for bioavailable 25OHD, compared with the lowest quartile of the biomarkers, respectively.

We next evaluated the potential interactions among these vitamin D–related biomarkers in relation to the risk of CHD (Table 4). We found a significant interaction between PTH and 25OHD on the disease risk (P for interaction=0.02). Among participants who had higher PTH levels, higher 25OHD levels were associated with a lower risk of developing CHD; conversely, we observed a nonsignificant association among participants with lower PTH levels. Comparing the highest quartile of 25OHD to the lowest, the multivariable-adjusted OR (95% CI) was 0.43 (0.23–0.82; P trend=0.003) when PTH levels were above population median (35.3 pg/mL) and 1.28 (0.70–2.36; P trend=0.43) otherwise. Consistent with stratified analyses, when joint categories of 25OHD and PTH levels were evaluated (Figure), inverse associations between 25OHD and CHD risk were present only among participants with high PTH levels, but not among the other participants. However, we did not observe similar interaction using clinical cutoff points of 50 nmol/L for 25OHD and 65 pg/mL for PTH. No significant interaction was observed among other biomarkers.

Table 1. Baseline Characteristics at Blood Draw by Levels of Vitamin D-Related Biomarkers Among Controls*

	Quartiles of 25-Hydroxyvitamin D				
	1	2	3	4	*P* for Trend†
Range	19.8–51.2	51.4–62.9	62.9–75.0	75.0–416.4	…
Age, y‡	62.1±7.3	63.9±8.1	63.4±7.5	62.2±7.8	0.90
White, %	97.9	99.3	100	100	0.02
Body mass index, kg/m^2	26.0±4.8	25.3±4.5	25.6±4.9	24.5±3.4	0.006
Physical activity (MET-h/wk)	14.0±14.5	16.4±14.2	20.5±19.7	18.1±18.0	0.01
Alternate Healthy Eating Index score	36.5±8.9	39.0±9.2	39.5±8.1	39.4±8.1	0.004
Dietary vitamin D intake, IU/d	194.5±105.1	200.4±89.2	228.0±97.3	253.3±115.5	<0.001
Dietary calcium intake, mg/d	673.9±189.3	711.0±195.8	747.2±238.2	767.7±222.0	<0.001
Alcohol, g/d	5.5±7.9	6.8±11.0	8.9±12.7	8.3±10.5	0.01
Never smoker, %	32.2	38.2	34.0	43.1	0.11
Past smoker, %	30.1	41.0	43.1	39.6	0.09
Current smoker, %‡					
1–14 cigarettes/d	18.2	6.3	9.7	9.0	0.04
15–24 cigarettes/d	11.2	9.0	9.0	2.8	0.01
≥25 cigarettes/d	8.4	5.6	4.2	4.9	0.17
Hypertension, %	36.4	37.5	30.6	31.9	0.25
Hypercholesterolemia, %	42.0	48.6	52.1	44.4	0.56
Diabetes mellitus, %	6.3	7.6	5.6	6.3	0.81
Parental MI before age 65 y, %	19.6	20.8	22.2	19.4	0.95
Fasting status, %‡	67.8	73.6	78.5	73.6	0.18
Postmenopausal, %	90.9	91.0	92.4	88.9	0.66
Postmenopausal hormone use, %§	59.2	71.8	66.9	75.0	0.02
Use of aspirin, %	49.7	54.9	54.2	61.1	0.07
Use of multivitamin, %	32.6	52.1	49.6	52.2	0.003
Biomarkers					
25-hydroxyvitamin D, nmol/L	41.3±7.8	57.5±3.5	68.6±3.5	89.8±29.9	<0.001
Vitamin D binding protein, mg/L	241.1±73.3	238.7±61.7	257.3±79.3	267.8±75.4	0.006
Parathyroid hormone, pg/mL	43.1±16.9	39.3±19.2	37.3±12.4	34.5±11.7	<0.001
Total cholesterol, mg/dL	232.1±38.1	223.6±37.4	227.6±47.1	221.5±32.3	0.05
LDL cholesterol, mg/dL	135.8±34.9	127.6±33.6	132.7±36.4	128.0±34.6	0.14
HDL cholesterol, mg/dL	58.5±15.9	62.4±18.6	59.2±17.7	63.9±19.5	0.04
Triglycerides, mg/dL	135.5±101.9	115.2±72.3	125.7±68.7	114.1±55.9	0.05
High-sensitivity C-reactive protein, mg/dL	0.40±0.55	0.36±0.42	0.39±0.44	0.30±0.34	0.10
Creatinine, mg/dL	0.73±0.15	0.74±0.16	0.74±0.15	0.76±0.18	0.10
Albumin, g/dL	4.6±0.31	4.5±0.33	4.6±0.31	4.6±0.30	0.37
Hemoglobin A1c, %	5.7±0.63	5.6±0.51	5.7±0.41	5.6±0.58	0.35

(*Continued*)

Discussion

In this prospective study of healthy US women, although higher plasma levels of 25OHD were correlated with a lower risk profile, they were not independently associated with CHD incidence after multivariate adjustment. VDBP showed a significant association with lower CHD incidence, independent of established demographic, lifestyle, and dietary risk factors. PTH was associated with lower risk of CHD only when clinical biomarkers were further controlled for. In addition, we found that PTH status modified the relation between

Table 1. Continued

Quartiles of Vitamin D Binding Protein					Quartiles of Parathyroid Hormone				
1	2	3	4	*P* for Trend†	1	2	3	4	*P* for Trend†
73.8–204.0	204.5–251.4	251.6–302.7	303.2–452.2	…	9.1–28.4	28.5–35.4	35.4–45.4	45.9–210.5	…
66.1±7.3	64.4±7.9	64.6±6.9	61.9±8.4	<0.001	60.3±7.8	63.3±7.2	64.2±7.3	63.8±7.9	<0.001
100	100	100	99	0.18	99.3	99.3	99.3	99.3	0.99
26.9±5.3	25.5±4.9	24.5±3.9	24.8±3.3	<0.001	24.6±4.3	24.9±4.7	25.8±4.6	26.2±4.1	<0.001
17.3±18.1	18.0±17.3	16.8±16.5	18.7±17.3	0.64	18.2±17.9	16.6±15.8	18.9±15.8	15.3±18.0	0.23
40.1±8.5	39.8±8.9	35.9±8.3	38.0±7.4	0.01	38.8±8.5	38.7±8.1	39.1±9.1	37.9±8.9	0.39
217.7±101.8	227.0±105.9	221.7±97.7	213.9±119.9	0.74	220.7±102.9	227.3±96.1	220.9±114.2	207.4±104.6	0.18
752.4±214.6	728.8±203.7	712.0±227.7	688.9±199.9	0.03	751.8±215.3	732.3±208.9	716.8±230.9	699.5±201.5	0.03
5.6±8.2	6.9±9.3	7.0±10.3	7.6±10.5	0.15	6.7±9.4	7.6±11.6	8.1±11.1	7.1±10.7	0.82
40.4	29.0	41.0	37.4	0.89	31.5	39.6	36.8	39.6	0.23
44.4	46.0	36.0	35.4	0.09	39.9	38.9	42.4	32.6	0.32
7.1	10.0	10.0	15.2	0.08	10.5	9.7	9.0	13.9	0.41
3.0	9.0	9.0	9.1	0.13	6.3	9.0	6.3	10.4	0.34
5.1	6.0	4.0	3.0	0.39	11.2	2.8	5.6	3.5	0.02
37.4	32.0	46.0	31.3	0.85	25.2	35.4	36.8	38.9	0.02
55.6	54.0	51.0	43.4	0.08	40.6	43.8	51.4	51.4	0.03
6.1	7.0	3.0	6.1	0.69	8.4	6.3	8.3	2.8	0.11
31.3	22.0	21.0	19.2	0.05	16.1	20.1	22.2	23.6	0.10
78.8	82.0	82.0	68.7	0.10	67.1	73.6	75.0	77.8	0.04
93.9	93.0	93.0	90.9	0.44	83.9	91.7	93.1	94.4	0.002
60.2	73.1	62.4	75.6	0.11	71.7	67.4	70.9	63.2	0.23
40.4	46.0	49.0	51.5	0.11	57.3	59.7	50.0	52.8	0.21
51.1	54.6	40.0	45.9	0.19	52.6	39.4	46.3	48.2	0.76
58.1±14.8	63.3±13.6	65.9±41.5	68.8±19.3	0.003	69.8±19.7	65.9±17.8	62.3±17.0	59.9±34.5	<0.001
156.3±35.7	227.1±13.3	273.5±14.3	345.0±38.4	<0.001	259.3±79.8	250.1±70.5	247.6±66.6	246.4±76.7	0.28
39.2±15.1	39.0±13.2	41.3±22.1	35.8±12.7	0.27	24.0±3.5	31.8±2.1	40.1±2.8	58.3±17.5	<0.001
230.7±44.6	228.2±43.9	228.4±31.4	222.9±42.0	0.19	216.7±37.8	229.1±36.7	228.2±45.6	230.6±34.7	0.01
129.8±36.7	138.7±37.6	132.8±35.8	126.3±34.5	0.35	120.9±32.8	131.9±37.0	132.8±35.8	138.4±32.1	<0.001
58.7±15.5	57.4±14.6	63.8±21.4	64.4±18.3	0.005	62.1±17.9	62.6±19.0	60.8±17.7	58.5±17.6	0.05
116.0±77.8	116.2±56.1	120.7±89.7	128.1±69.6	0.22	115.5±62.4	117.8±59.1	128.0±92.8	129.1±86.9	0.09
0.36±0.42	0.28±0.26	0.28±0.33	0.39±0.41	0.61	0.37±0.56	0.32±0.38	0.39±0.45	0.37±0.37	0.67
0.73±0.13	0.79±0.21	0.74±0.19	0.74±0.14	0.92	0.71±0.12	0.73±0.16	0.75±0.14	0.77±0.21	0.002
4.5±0.26	4.5±0.27	4.5±0.30	4.6±0.32	0.15	4.6±0.30	4.5±0.29	4.5±0.32	4.6±0.35	0.58
5.7±0.69	5.6±0.40	5.6±0.42	5.6±0.49	0.10	5.6±0.56	5.6±0.51	5.7±0.60	5.7±0.47	0.63

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent of task; and MI, myocardial infarction.

*Values are mean±SD for continuous variables or % for categorical variables. n=575 for 25-hydroxyvitamin D and parathyroid hormone and n=398 for vitamin D binding protein.

†*P* for trend was assessed by *F* test and modeling median values in each quartiles continuously for variables expressed as mean±SD or Cochran–Armitage trend test for variables expressed as percentages.

‡Matching factors.

§Among postmenopausal women only.

25OHD and CHD risk, and a significant, inverse association was observed only among women with elevated PTH levels.

Despite experimental evidence supporting a cardioprotective role of vitamin D and potential mechanisms linking vitamin D deficiency and CHD risk,[2] results from prospective investigations remain inconsistent.[3–7] A meta-analysis of prospective studies of 25OHD and cardiovascular event demonstrated a generally linear, inverse association up to 60 nmol/L but revealed no further reductions at higher 25OHD levels.[19] In contrast, 25OHD was not prospectively associated with a comprehensive set of biochemical, electrocardiographic, and echocardiographic measurements of cardiac structure and function among older adults.[20] These discordant findings may be partly explained by the fact that a spectrum of cardiovascular outcomes was considered in prior studies, which may have distinct etiologies with relevance to 25OHD biology. Other sources of heterogeneity may include study population (eg, age, sex, and ethnicity), categories of 25OHD used, and confounders considered. Our findings suggest that previously observed associations are very likely to be confounded by CHD risk factors and lifestyle variables associated with 25OHD. In support of this notion, by using Mendelian randomization approach and eliminating confounding, genetically determined 25OHD was not found to be associated with risk of incident CHD[21] or cardiovascular mortality.[22] Therefore, the causality and the effects of vitamin D supplement on CHD are yet to be clarified in ongoing and future randomized controlled trials. The lack of an association between bioavailable 25OHD and CHD implied that bioavailable 25OHD might not exert higher biological activity on association with disease as expected according to the free hormone hypothesis. In addition, a mean affinity constant between 25OHD and VDBP was used to estimate bioavailable 25OHD,[23,24] without accounting for the potential affinity differences according to VDBP genotype.[25] Additional information on the polymorphisms will be useful for a more accurate estimation of bioavailable 25OHD.

Compared with accumulating epidemiological data on 25OHD, relatively little is known about the effects of VDBP on cardiovascular health. In a recent study, higher plasma levels of VDBP isotopes were associated with increased risk of cardiovascular mortality, but not incident cardiovascular events.[26] However, this study was limited by low statistical power with an inclusion of only 17 cases and did not assess whether the association was independent of other vitamin D–related biomarkers. Our results showed that the observed inverse association between VDBP and CHD remained robust after further adjustment for 25OHD and PTH levels. Experimental evidence suggests that VDBP could modulate immunity by macrophage activation and reduce inflammation and attenuate clot formation through the removal of actin from circulation.[11] Proteomics analyses also revealed that among myocardial infarction patients, VDBP reduced aggregation rate and prolonged coagulation time,[13] and plasma levels of VDBP were inversely correlated with the number of affected arteries.[12] Taken together, our results provide novel evidence that VDBP or its metabolic determinants may reduce CHD risk beyond its link to 25OHD, suggesting that VDBP should be a target for additional experimental, observational, and interventional studies.

Although elevated PTH levels have been linked to hypertension, valvular and myocardial calcifications, as well as left ventricular hypertrophy,[16,27] the independent role of PTH in heart health among people with normal renal function remains controversial. A meta-analysis of 10 prospective studies reported that PTH levels were associated with a 1.45-fold increase (95% CI, 1.24–1.71) in the incidence of cardiovascular events. However, there was considerable between-study heterogeneity about study population, PTH assays, outcome definitions, and adjustment for confounders, with most individual cohorts showing null associations.[28] In contrast to any positive association, PTH levels were inversely associated with incident heart failure, peripheral arterial disease, and cardiovascular mortality in a large US cohort of middle-aged adults.[29] In our analysis, PTH was not significantly associated with CHD in the multivariate model, but the association became significant with further adjustment for some clinical factors in particular HDL and low-density lipoprotein cholesterol. The potentially nonlinear, inverse association was largely driven by the highest PTH quartile. Our findings add to the literature and highlight the need to comprehensively investigate the pathways through which PTH especially in high levels might affect the pathogenesis of CHD.

To our knowledge, this is the first report of a significant interaction between PTH status and 25OHD in relation to CHD risk. Vitamin D regulates intestinal calcium absorption, and the observed interaction between vitamin D and PTH excess is supported by biology because PTH increases in response to endogenous deficiency of vitamin D through negative feedback.[18] A low 25OHD level in the context of elevated PTH may indicate a physiological vitamin D insufficiency, whereas the lack of relation between 25OHD and CHD for those with PTH below the median could be because the lower vitamin D levels with low PTH are not reflecting true deficiency or at least not causing hypocalcaemia and systemic response. The only previous study evaluating the potential interaction between these 2 biomarkers did not detect an additive interaction and the combination of a low 25OHD and PTH excess was not significantly associated with greater risk of cardiovascular outcomes.[17] However, that study focused on older adults with a mean age of 74 years, and a cutoff value of 65 pg/mL for PTH levels was applied to keep consistent with the definition of primary hyperparathyroidism. Further research is warranted to confirm our findings in other cohorts and explore the PTH threshold for associations between 25OHD and cardiovascular outcomes.

The prospective design, high follow-up rate, and use of plasma biomarkers minimized major sources of bias, such as selection bias or recall bias. We comprehensively evaluated the independent and synergistic associations of biomarkers of vitamin D status with CHD. We controlled for major covariates that could influence biomarker levels and CHD risk as well as metabolic risk factors. Potential limitations of the current analysis, however, should be considered. First, we measured VDBP levels using monoclonal

Table 2. Partial Spearman Correlation Coefficients Between Vitamin D–Related Biomarkers Among Controls*

	Total 25OHD, nmol/L	Bioavailable 25OHD, nmol/L	VDBP, mg/L	PTH, pg/mL
Median	62.6	7.5	250.9	35.9
Total 25OHD	1.00	0.62†	0.18†	–0.24†
Bioavailable 25OHD	…	1.00	–0.59†	–0.18†
VDBP	…	…	1.00	–0.001
PTH	…	…	…	1.00

25OHD indicates 25-hydroxyvitamin D; PTH, parathyroid hormone; and VDBP, vitamin D binding protein.

*n=368. Partial correlation coefficients were adjusted for age at blood draw (years, continuous), smoking status (never, past, or current smoker), fasting status (yes/no), time of blood draw (collection 1: June 1989 to October 1989, November 1989 to March 1990, April 1990 to July 1990, or August 1990 to March 1991; collection 2: March 2000 to July 2000, August 2000 to December 2000, January 2001 to May 2001, or June 2001 to January 2002), body mass index (kg/m^2: <25.0, 25.0–27.4, 27.5–29.9, 30.0–34.9, ≥35.0), postmenopausal status and hormone use (premenopausal or postmenopausal [never, past, or current menopausal hormone use]), physical activity (tertiles), alcohol intake (g/d: 0, 0.1–4.9, 5.0–14.9, ≥15.0), aspirin use (yes/no), myocardial infarction family history (yes/no), and Alternative Healthy Eating Index score (tertiles).

†P<0.001.

sandwich immunoassay (R&D Systems),[30] which has been shown to quantify VDBP differentially by isoform and systematically underestimate VDBP levels among people with Gc1F/Gc1F genotype, compared with using a polyclonal assay or liquid chromatography–tandem mass spectrometry methodology.[31,32] Although the majority (99.1%) of our

Table 3. Odds Ratio (95% Confidence Interval) of Coronary Heart Disease by Levels Of Vitamin D–Related Biomarkers

	Quartiles of Biomarker Levels					
	1	2	3	4	P for Trend	Per SD
Total 25-hydroxyvitamin D, nmol/L						
Median	43.4	57.5	68.5	83.6	…	…
Case/control	119/143	98/144	83/144	82/144	…	…
Model 1*	1.00 (ref)	0.91 (0.63–1.33)	0.76 (0.51–1.13)	0.84 (0.56–1.27)	0.30	0.95 (0.82–1.09)
Model 2†	1.00 (ref)	0.92 (0.60–1.41)	0.75 (0.48–1.16)	0.98 (0.61–1.58)	0.66	1.00 (0.85–1.17)
Vitamin D binding protein, mg/L						
Median	166.3	228.5	270.8	336.7	…	…
Case/control	131/99	101/100	93/100	71/99	…	…
Model 1*	1.00 (ref)	0.76 (0.51–1.14)	0.74 (0.49–1.11)	0.60 (0.39–0.92)	0.02	0.87 (0.75–1.02)
Model 2†	1.00 (ref)	0.71 (0.47–1.09)	0.68 (0.44–1.04)	0.55 (0.35–0.87)	0.01	0.84 (0.71–0.98)
Parathyroid hormone, pg/mL						
Median	24.2	31.9	39.8	53.7	…	…
Case/control	98/143	100/144	101/144	83/144	…	…
Model 1*	1.00 (ref)	0.90 (0.61–1.32)	0.90 (0.61–1.32)	0.68 (0.46–1.01)	0.06	0.94 (0.82–1.07)
Model 2†	1.00 (ref)	0.90 (0.59–1.39)	0.91 (0.58–1.41)	0.70 (0.44–1.19)	0.13	0.96 (0.82–1.11)
Bioavailable 25-hydroxyvitamin D, nmol/L						
Median	5.1	6.8	8.3	11.1	…	…
Case/control	93/92	77/92	102/92	104/92	…	…
Model 1*	1.00 (ref)	0.84 (0.54–1.31)	1.16 (0.76–1.78)	1.23 (0.79–1.90)	0.19	1.10 (0.94–1.29)
Model 2†	1.00 (ref)	0.76 (0.48–1.20)	0.96 (0.61–1.53)	0.87 (0.52–1.45)	0.76	0.99 (0.82–1.19)

*Model 1 was adjusted for age at blood draw (years, continuous), smoking status (never, past, or current smoker), fasting status (yes/no), and time of blood draw (collection 1: June 1989 to October 1989, November 1989 to March 1990, April 1990 to July 1990, or August 1990 to March 1991; collection 2: March 2000 to July 2000, August 2000 to December 2000, January 2001 to May 2001, or June 2001 to January 2002), body mass index (kg/m^2: <25.0, 25.0–27.4, 27.5–29.9, 30.0–34.9, ≥35.0), postmenopausal status and hormone use (premenopausal or postmenopausal [never, past, or current menopausal hormone use]), physical activity (tertiles), alcohol intake (g/d: 0, 0.1–4.9, 5.0–14.9, ≥15.0), aspirin use (yes/no), myocardial infarction family history (yes/no), and Alternative Healthy Eating Index score (tertiles). Additional adjustment for sun exposure (≥8 h/wk outdoor in the summer with sunscreen, ≥8 h/wk outdoor in the summer without sunscreen, or no), dietary vitamin D intake (IU/d, continuous), vitamin D supplement use (IU/d, continuous) or multivitamin use (yes/no) did not appreciably change the results.

†Model 2 was further mutually adjusted for the other biomarkers of interest. We did not simultaneously adjust for total and bioavailable 25-hydroxyvitamin D due to the strong correlation between the two.

Table 4. Odds Ratio (95% Confidence Interval) of Total 25-Hydroxyvitamin D (25OHD), Bioavailable 25OHD, and Vitamin D Binding Protein With Coronary Heart Disease According to Median Level of Parathyroid Hormone

	Quartiles of Biomarkers Levels				*P* for Trend	Per SD	*P* for Interaction
	1	2	3	4			
Total 25-hydroxyvitamin D, nmol/L							
Below median of parathyroid hormone, ≤35.3 pg/mL							
Median	44.8	58.2	67.5	85.2	…	…	…
Case/control	42/56	47/69	47/72	59/86	…	…	…
Model*	1.00 (ref)	1.05 (0.57–1.94)	0.98 (0.53–1.81)	1.28 (0.70–2.36)	0.43	1.10 (0.86–1.40)	0.02
Above median of parathyroid hormone, >35.3 pg/mL							
Median	42.8	57.3	69.1	81.7	…	…	…
Case/control	77/87	51/75	36/72	23/58	…	…	…
Model*	1.00 (ref)	0.89 (0.53–1.50)	0.52 (0.30–0.92)	0.43 (0.23–0.82)	0.003	0.77 (0.58–1.01)	…
Vitamin D binding protein, mg/L							
Below median of parathyroid hormone, ≤35.3 pg/mL							
Median	175.2	228.5	269.3	338.3	…	…	…
Case/control	61/45	51/41	44/44	36/47	…	…	…
Model*	1.00 (ref)	0.86 (0.46–1.61)	0.82 (0.44–1.53)	0.63 (0.33–1.22)	0.17	0.88 (0.69–1.11)	0.86
Above median of parathyroid hormone, >35.3 pg/mL							
Median	158.7	227.2	270.5	334.6	…	…	…
Case/control	69/47	42/51	42/48	31/45	…	…	…
Model*	1.00 (ref)	0.51 (0.28–0.94)	0.61 (0.33–1.12)	0.42 (0.21–0.83)	0.02	0.81 (0.64–1.02)	…
Bioavailable 25-hydroxyvitamin D, nmol/L							
Below median of parathyroid hormone, ≤35.3 pg/mL							
Median	5.3	6.7	8.4	11.5	…	…	…
Case/control	40/38	35/43	57/46	60/50	…	…	…
Model*	1.00 (ref)	0.97 (0.48–1.96)	1.27 (0.65–2.47)	1.48 (0.75–2.93)	0.18	1.21 (0.94–1.56)	0.30
Above median of parathyroid hormone, >35.3 pg/mL							
Median	5.0	6.8	8.2	11.0	…	…	…
Case/control	53/54	42/49	45/46	44/42	…	…	…
Model*	1.00 (ref)	0.83 (0.45–1.52)	0.99 (0.53–1.84)	0.98 (0.52–1.85)	0.92	0.98 (0.80–1.21)	…

*Model was adjusted for age at blood draw (years, continuous), smoking status (never, past, or current smoker), fasting status (yes/no), time of blood draw (collection 1: June 1989 to October 1989, November 1989 to March 1990, April 1990 to July 1990, or August 1990 to March 1991; collection 2: March 2000 to July 2000, August 2000 to December 2000, January 2001 to May 2001, or June 2001 to January 2002), body mass index (kg/m^2: <25.0, 25.0–27.4, 27.5–29.9, 30.0–34.9, ≥35.0), postmenopausal status and hormone use (premenopausal or postmenopausal [never, past, or current menopausal hormone use]), physical activity (tertiles), alcohol intake (g/d: 0, 0.1–4.9, 5.0–14.9, ≥15.0), aspirin use (yes/no), myocardial infarction family history (yes/no), and Alternative Healthy Eating Index score (tertiles). Further adjustment for parathyroid hormone levels (pg/mL, continuous) did not appreciably change the results.

study population is white, among whom the prevalence of Gc1F homozygotes is low,[30,32] indicating our results might not be substantially influenced by these issues, we acknowledge that future studies of VDBP need to use assays that are not affected by VDBP genotype. Second, although the homogeneity of socioeconomic status and ethnicity helps to control for confounding, the results might not be generalizable to other populations. We acknowledge that 25OHD levels and their association with incident CHD vary by race.[33,34] Third, plasma biomarkers were assessed only once at baseline, and changes over time would have caused misclassification and potentially attenuated true associations. Last, although we adjusted for multiple important risk factors for CHD, residual confounding because of unmeasured or imprecisely measured confounding cannot be excluded.

In conclusion, our results indicate that a low 25OHD level in the presence of elevated PTH is a risk factor for CHD among largely healthy US women. High circulating VDBP is independently associated with reduced risk of CHD, whereas PTH and bioavailable 25OHD levels are not significantly associated. Our findings emphasize the importance of simultaneously considering the interrelationships of vitamin D–related biomarkers when evaluating vitamin D status and CHD risk.

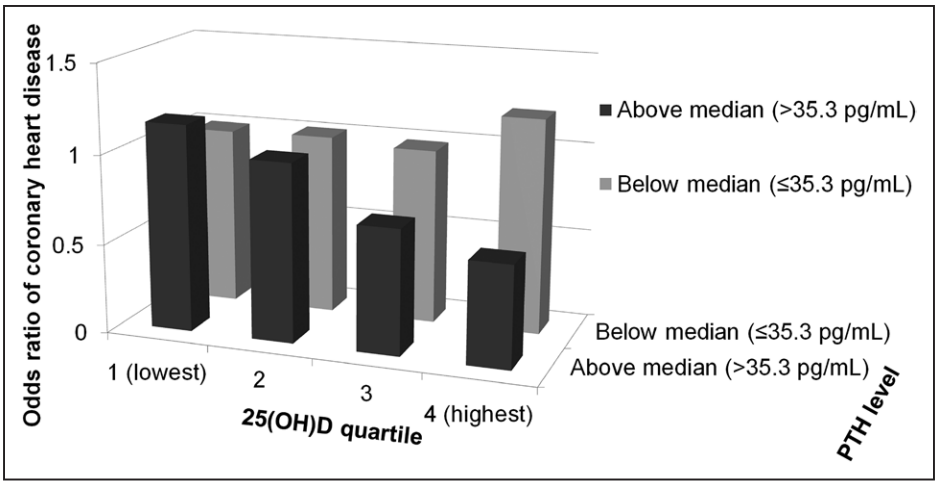

Figure. Joint associations of 25-hydroxyvitamin D (25OHD) and parathyroid hormone with risk of coronary heart disease. Multivariate model was adjusted for age at blood draw, smoking status, fasting status, time of blood draw, body mass index, postmenopausal status and hormone use, physical activity, alcohol intake, aspirin use, myocardial infarction family history, and Alternative Healthy Eating Index score. Participants with a parathyroid hormone level below the median and 25OHD level in the lowest quartile were set as the reference group.

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Disclosures

None.

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Highlights

- In this prospective study of US women, although higher plasma levels of 25-hydroxyvitamin D were correlated with a lower cardiovascular risk profile, they were not independently associated with incidence of coronary heart disease after multivariate adjustment.
- Parathyroid hormone status modified the relation between 25-hydroxyvitamin D and coronary heart disease risk, and a significant, inverse association was observed only among women with elevated parathyroid hormone levels, whereas no association was observed for those with low parathyroid hormone levels.
- Vitamin D binding protein showed a significant association with lower coronary heart disease incidence, independent of established demographic, lifestyle and dietary risk factors.