25-Hydroxyvitamin D and Parathyroid Hormone Are Not Associated With Carotid Intima-Media Thickness or Plaque in the Multi-Ethnic Study of Atherosclerosis

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Objective—Observational evidence supports independent associations of 25-hydroxyvitamin D (25-OHD) and parathyroid hormone (PTH) with cardiovascular risk. A plausible hypothesis for these associations is accelerated development of atherosclerosis.

Approach and Results—We evaluated cross-sectional and longitudinal associations of 25-OHD and PTH with carotid intima-media thickness (IMT) and carotid plaques among 3251 participants free of cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. 25-OHD and PTH were measured at baseline by mass spectrometry and immunoassay, respectively. All subjects underwent a carotid ultrasound examination at baseline and 9.4 years later (median, range 8–11.1 years). Multivariable linear and logistic regressions were used to test associations of 25-OHD and PTH with the extent and progression of IMT and the prevalence and incidence of carotid plaque. Mean (SD) 25-OHD and PTH were 25.8 ng/mL (10.6) and 44.2 pg/mL (20.2), respectively. No independent associations were found between 25-OHD or PTH and IMT at baseline (increment of 1.9 μm [95% confidence interval, −5.1 to 8.9] per 10 ng/mL lower 25-OHD; increment of 0.8 μm [95% confidence interval, −3.2 to 4.8] per 10 pg/mL higher PTH) or progression of IMT (increment of 2.6 μm [95% confidence interval, −2.5 to 7.8] per 10 ng/mL lower 25-OHD, increment of 1.6 μm [95% confidence interval, −1.9 to 5.2] per 10 pg/mL higher PTH). No associations were found with the baseline prevalence of carotid plaque or the incidence of new plaques during the study period. We did not observe any interaction by race or ethnicity (White, Chinese, Black, and Hispanic).

Conclusions—The consistent lack of association of vitamin D and PTH with carotid IMT and plaque suggests that these hormones may influence cardiovascular risk through pathways not reflected by carotid atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2013;33:2639-2645.)**

Key Words: atherosclerosis ■ carotid artery diseases ■ carotid intima-media thickness ■ mineral metabolism ■ parathyroid hormone ■ plaque, atherosclerotic ■ vitamin D

Lower circulating concentrations of 25-hydroxyvitamin D (25-OHD) and higher circulating concentrations of parathyroid hormone (PTH) have been associated with an increased risk of cardiovascular events in multiple observational cohorts.[1–3] There are several plausible explanations for these observations; one hypothesis is that insufficient vitamin D and excessive PTH accelerate atherosclerosis. Low circulating 25-OHD concentrations are associated with obesity, impaired glucose metabolism, hypertension, and dyslipidemia in cross-sectional studies and with incident hypertension during long-term follow-up.[4–7] Inflammatory, immunomodulatory, and direct vascular effects of vitamin D have also been implicated.[8–10] PTH may affect cardiovascular disease through the development of hypertension,[11] left ventricular hypertrophy,[12] or endothelial dysfunction.[13]

See accompanying article on page 2467

Our aim was to test associations of serum 25-OHD and PTH concentrations with carotid intima-media thickness (IMT) and plaque, 2 noninvasive markers of arterial injury, including atherosclerosis, that independently predict cardiovascular disease,[14] in a large community-based study. We hypothesized that participants with lower 25-OHD or higher PTH would have larger IMT measurements at baseline, more rapid IMT

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progression during the follow-up period, and greater prevalence and incidence of carotid plaques.

Materials and Methods

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

Results

Participant Characteristics

From 6393 participants with available original IMT measurements, 3251 underwent a second ultrasound for IMT progression and had their baseline IMT remeasured using the images from the baseline ultrasound. Mean age and body mass index (SD) of these participants were 60.4 years (9.4) and 28.2 kg/m^2 (5.2), respectively, and 46.5% were men. They were racially/ethnically diverse, with 39.6% of White, 13.3% of Chinese, 25.8% of Black, and 21.3% of Hispanic subjects. Compared with these, participants who did not have a follow-up carotid ultrasound were older (mean age, 64.0 years) and had a greater prevalence of treated diabetes mellitus (11.5% versus 8.1%), hypertension (48.3% versus 40.6%), and current smoking (14.3% versus 11.5%). Measurements of PTH and 25-OHD were similar in these 2 groups.

Among the 3251 participants with subsequent carotid ultrasound and new readings of baseline IMT, 1033 (31.8%) had 25-OHD <20ng/mL at baseline (Table 1). Despite being younger, these participants had more cardiovascular risk factors (diabetes mellitus, hypertension, smoking, higher body mass index, higher C-reactive protein), but had higher mean estimated glomerular filtration rate, compared with participants with higher 25-OHD concentrations. Racial/ethnic differences were striking, with lower and higher 25-OHD concentrations among Black and White subjects, respectively. The proportion of 25-OHD <20 ng/mL was 15.1%, 23.7%, 60.5%, and 33.0% among White, Chinese, Black, and Hispanic participants, respectively.

Three hundred seventy participants (11.4%) had PTH ≥65 pg/mL. We observed a marked increase in the prevalence

Table 1. Baseline Characteristics of 3251 MESA Participants

	Annualized 25-OH-Vitamin D, ng/mL			PTH, pg/mL			
	<20 (n=1033)	20.0–29.9 (n=1155)	≥30.0 (n=1063)	<33.0 (n=964)	33.0–44.2 (n=962)	44.3–64.9 (n=955)	≥65 (n=370)
Age, y	58.8 (9.1)	60.6 (9.4)	61.6 (9.4)	58.8 (9.1)	60.6 (9.4)	61.6 (9.4)	58.8 (9.1)
Male, n (%)	446 (43.3)	576 (50)	456 (46)	446 (43.3)	576 (50)	456 (46)	446 (43.3)
Race/ethnicity, n (%)							
White	194 (18.8)	472 (41)	621 (58.5)	481 (49.9)	400 (41.6)	318 (33.3)	88 (24)
Chinese	102 (9.9)	195 (16.9)	133 (12.5)	170 (17.6)	149 (15.5)	95 (10)	16 (4.4)
Black	507 (49.2)	225 (19.5)	106 (10)	151 (15.7)	221 (23)	315 (33)	151 (41.3)
Hispanic	228 (22.1)	260 (22.6)	202 (19)	162 (16.8)	191 (20)	226 (23.7)	111 (30.3)
Treated diabetes mellitus, n (%)	106 (10.3)	104 (9)	54 (5.1)	90 (9.4)	66 (6.9)	72 (7.6)	35 (9.8)
Hypertension, n (%)	460 (44.6)	471 (40.9)	310 (36.4)	310 (32.2)	356 (37)	447 (46.9)	205 (56)
Treatment for hypertension, n (%)	376 (36.5)	407 (35.3)	268 (31.2)	268 (27.8)	313 (32.6)	366 (38.4)	167 (45.6)
SBP, mm Hg	126.2 (20.9)	124.1 (20.1)	122.4 (19.4)	119.9 (18.7)	122.6 (19.6)	127.4 (20.2)	131.6 (22)
DBP, mm Hg	73.1 (10.1)	71.8 (10)	70.5 (9.8)	70.5 (9.5)	71.5 (10.2)	72.7 (9.9)	73.4 (10.8)
BMI. kg/m^2	29.9 (5.6)	28 (5)	26.8 (4.5)	26.8 (4.6)	27.9 (5)	28.9 (5.2)	30.7 (6.1)
Total cholesterol, mg/dL	193.3 (36.5)	192.8 (34.4)	195.8 (33.9)	195.4 (34.6)	193.5 (35.1)	194.6 (34.5)	189.4 (35.9)
LDL, mg/dL	118.7 (32.5)	116.4 (30)	116 (29.4)	117.4 (29.4)	117.1 (31.1)	117.8 (30.6)	113.3 (32.5)
HDL, mg/dL	49.8 (14.5)	50 (14.6)	53.7 (15.7)	51.3 (14.4)	50.6 (14.9)	51.4 (15.6)	51.6 (15.6)
Treatment with statins, n (%)	136 (13.2)	187 (16.2)	129 (16.1)	129 (13.4)	133 (13.8)	158 (16.6)	74 (20.2)
Current smokers, n (%)	158 (15.4)	114 (9.9)	101 (9.5)	125 (13)	116 (12.1)	97 (10.2)	35 (9.6)
Former smokers, n (%)	361 (35.1)	415 (36.1)	399 (37.6)	349 (36.2)	343 (35.8)	359 (37.6)	124 (34)
GFR, mL/min per 1.73 m^2	88.9 (16)	86 (15.6)	83 (15.2)	86.4 (14.8)	86.4 (15.6)	86 (15.8)	83.1 (18.2)
Calcium, mg/dL	9.6 (0.4)	9.6 (0.4)	9.7 (0.4)	9.7 (0.4)	9.7 (0.4)	9.6 (0.4)	9.6 (0.5)
Phosphorus, mg/dL	3.7 (0.5)	3.6 (0.5)	3.7 (0.5)	3.8 (0.5)	3.7 (0.5)	3.6 (0.5)	3.5 (0.5)
IL-6, IU/mL	38 (13.5)	40.4 (17.1)	41.9 (23.1)	39.5 (14.9)	39.9 (22.5)	40.4 (13.1)	41.5 (25.3)
CRP, mg/L	1.7 (1.3)	1.4 (1)	1.3 (1)	1.3 (1.1)	1.4 (1.1)	1.5 (1.2)	1.7 (1.1)

25-OHD indicates 25-hydroxyvitamin D; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL, high-density lipoprotein; IL-6, interleukin-6; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; PTH, parathyroid hormone; and SBP, systolic blood pressure.

of hypertension with increasing PTH concentrations and an expected inverse correlation between PTH and glomerular filtration rate. Black and Hispanic participants were more likely to have higher PTH concentrations. The proportion of high PTH concentrations (≥65 ng/mL) was higher among Black and Hispanic participants (18.0% and 16.1%) than among White and Chinese participants (6.8% and 3.7%). During follow-up, the prevalence of treatment for traditional cardiovascular risk factors increased. This increase did not differ by 25-OHD or PTH status at baseline. For example, the prevalence of statin use from baseline to examination 5 did not increase more for participants with 25-OHD <20 ng/mL (18.9%) than for those with 25-OHD >30 ng/mL (23.9%).

Carotid IMT and Plaque

At baseline, the means (SD) of the common carotid artery (CCA) maximum IMT and internal carotid artery (ICA) maximum IMT were 927 μm (SD, 210 μm) and 906 μm (SD, 399 μm), respectively. Median (range) time between ultrasound examinations was 9.4 years (8.0–11.1 years). Mean (SD) changes in CCA-IMT and ICA-IMT between ultrasound examinations were 137 μm (SD, 140 μm) and 164 μm (SD, 276 μm), respectively. At least 1 carotid plaque was found among 1525 of 3246 participants at baseline (47.0%). Among participants without plaques at baseline, 698 (40.6%) had developed a carotid plaque at the time of the second ultrasound. Mean plaque scores were 1.08 (SD, 1.61) at baseline and progressed by a mean of 1.18 (SD, 1.45) during the study period.

25-OHD, IMT, and Plaque

At baseline, lower 25-OHD concentrations were associated with modestly greater CCA and ICA-IMT in demographic-adjusted analyses (Table 2, left side, model 1). However, in models further adjusted for confounders, we found no independent association of 25-OHD with CCA or ICA-IMT or their change over time (Table 2, top and middle rows, model 2). Adjustment for body mass index was responsible for most of the attenuation observed from model 1 to model 2. The precision of the null estimates ruled out clinically meaningful associations: the adjusted mean differences in baseline CCA-IMT and its change over time, per 10 ng/mL lower 25-OHD, were 1.9 μm (95% confidence interval [CI], −5.1 to 8.9) and 2.6 μm (95% CI, −2.5 to 7.8), respectively. In addition, 25-OHD concentrations were not associated with the

Table 2. Cross-Sectional and Longitudinal Associations of Serum 25-OHD Concentration With Carotid Intima-Media Thickness and Plaque

	Cross-Sectional Analyses				Longitudinal Analyses			
		Unadjusted Mean IMT, μm (SD)	Adjusted Difference, μm (95% CI)			Unadjusted Mean Change, μm (SD)	Adjusted Difference, μm (95% CI)	
25-OHD	n		Model 1	Model 2	n		Model 1	Model 2
		Baseline CCA-IMT				Change in CCA-IMT*		
≥30.0 ng/mL	1047	924 (221)	Ref.	Ref.	876	133 (127)	Ref.	Ref.
20.0–29.9 ng/mL	1132	923 (207)	1.3 (−15.1 to 17.7)	−7.2 (−23.3 to 8.9)	931	136 (134)	3.2 (−9.1 to 15.6)	0.1 (−12.3 to 12.5)
<20 ng/mL	1003	936 (204)	23.0 (5.5 to 40.5)	7.5 (−10.0 to 24.9)	776	144 (162)	10.0 (−4.9 to 24.8)	4.6 (−10.6 to 19.8)
P value†			<0.02	0.59			<0.05	0.32
		Baseline ICA-IMT				Change in ICA-IMT*		
≥30.0 ng/mL	838	907 (397)	Ref.	Ref.	514	168 (285)	Ref.	Ref.
20.0–29.9 ng/mL	868	917 (429)	29.7 (−7.8 to 67.3)	19.9 (−18.9 to 58.6)	507	167 (287)	7.8 (−27.0 to 42.9)	1.9 (−34.9 to 38.6)
<20 ng/mL	713	890 (362)	38.3 (−0.7 to 77.3)	20.2 (−19.8 to 60.3)	387	156 (248)	7.2 (−31.7 to 38.8)	−2.4 (−43.7 to 38.8)
P value†			<0.03	0.25			0.66	0.95
	n	Unadjusted Prevalence (%)	Adjusted Odds Ratio (95%CI) Model 3	Model 4	n	Unadjusted Cumulative Incidence (%)	Adjusted Odds Ratio (95%CI) Model 3	Model 4
		Baseline prevalence of carotid plaque				Incidence of a new carotid plaque*,‡		
≥30.0 ng/mL	1048	47.00%	1.0 (ref)	1.0 (ref)	556	41.20%	1.0 (ref)	1.0 (ref)
20.0–29.9 ng/mL	1140	48.10%	1.19 (0.99 to 1.42)	1.17 (0.98 to 1.41)	592	42.70%	1.14 (0.89 to 1.45)	1.16 (0.90 to 1.48)
<20 ng/mL	1024	44.30%	1.15 (0.94 to 1.41)	1.09 (0.88 to 1.35)	570	37.90%	1.04 (0.78 to 1.37)	1.04 (0.78 to 1.39)
P value†			0.28	0.75			0.73	0.67

Linear model 1 adjusted for sex, race, study field center, education, income, and time between the 2 ultrasounds. Linear model 2 further adjusted for physical activity, smoking, BMI, LDL, HDL, use of statins, and GFR. Logistic model 3 adjusted for age, sex, race, site, education, income, and time between the 2 ultrasounds. Logistic model 4 further adjusted for physical activity, smoking, BMI, LDL, HDL, use of statins, and GFR. 25-OHD indicates 25-hydroxyvitamin D; BMI, body mass index; CCA-IMT, intima-media thickness of the common carotid artery; CI, confidence interval; GFR, glomerular filtration rate; HDL, high-density lipoprotein; ICA-IMT, intima-media thickness of the internal carotid artery; and LDL, low-density lipoprotein.

*Between the 2 carotid ultrasounds (9.4 y).

†*P* value generated evaluating 25-OHD as a continuous variable.

‡Among those without carotid plaques at baseline.

Table 3. Cross-Sectional and Longitudinal Associations of Parathyroid Hormone Concentration With Carotid Intima-Media Thickness and Plaque

	Cross-Sectional Analyses				Longitudinal Analyses			
			Adjusted Difference, μm (95% CI)			Unadjusted Mean Change, μm (SD)	Adjusted Difference, μm (95% CI)	
PTH	n	Unadjusted Mean IMT, μm (SD)	Model 1	Model 2	n		Model 1	Model 2
		Baseline CCA-IMT				Change in CCA-IMT*		
<33.0 pg/mL	945	897 (210)	Ref.	Ref.	797	130 (125)	Ref.	Ref.
33.0–44.3 pg/mL	941	925 (207)	16.8 (–0.3 to 33.8)	11.8 (–4.9 to 28.4)	786	141 (143)	11.9 (–1.8 to 25.7)	10.9 (–2.8 to 24.7)
44.4–64.9 pg/mL	939	950 (216)	19.5 (1.7 to 37.3)	10.4 (–7.6 to 28.3)	735	143 (138)	13.0 (–0.9 to 26.7)	12.4 (–1.7 to 26.4)
≥65 pg/mL	357	955 (198)	20.8 (–2.4 to 44.1)	10.1 (–13.3 to 33.6)	265	131 (154)	1.2 (–19.7 to 22.0)	1.0 (–19.6 to 21.5)
P value†			0.13	0.70			0.37	0.37
		Baseline ICA-IMT				Change in ICA-IMT*		
<33.0 pg/mL	740	881 (361)	Ref.	Ref.	465	170 (300)	Ref.	Ref.
33.0–44.3 pg/mL	737	912 (428)	23.0 (–15.3 to 61.4)	16.4 (–22.0 to 54.8)	420	165 (256)	–5.3 (–41.5 to 30.9)	–9.2 (–45.7 to 27.3)
44.4–64.9 pg/mL	701	936 (425)	29.7 (–10.2 to 69.6)	15.8 (–25.4 to 57.1)	393	170 (292)	–12.8 (–54.1 to 28.5)	–16.8 (–60.3 to 26.7)
≥65 pg/mL	241	875 (335)	–23.3 (–73.3 to 26.7)	–42.3 (–95.1 to 10.5)	130	121 (185)	–65.4 (–112.7 to –18.1)	–66.2 (–116.6 to –15.8)
P value†			0.80	0.61			0.04	0.05
			Adjusted Odds Ratio (95%CI)				Adjusted Odds Ratio (95%CI)	
	n	Unadjusted Prevalence (%)‡	Model 3	Model 4	n	Unadjusted Cumulative Incidence (%)‡	Model 3	Model 4
		Baseline prevalence of carotid plaque				Incidence of a new carotid plaque*‡		
<33.0 pg/mL	952	43.9%	1.0 (ref)	1.0 (ref)	534	38.40%	1.0 (ref)	1.0 (ref)
33.0–44.3 pg/Ml	952	46.9%	1.11 (0.92 to 1.34)	1.12 (0.92 to 1.36)	506	41.10%	1.14 (0.88 to 1.48)	1.14 (0.88 to 1.48)
44.4–64.9 pg/mL	945	50.6%	1.16 (0.95 to 1.41)	1.15 (0.94 to 1.42)	467	43.00%	1.16 (0.89 to 1.52)	1.17 (0.89 to 1.55)
≥65 pg/mL	363	41.9%	0.78 (0.60 to 1.02)	0.77 (0.58 to 1.02)	211	39.80%	1.03 (0.73 to 1.47)	1.04 (0.72 to 1.50)
P value†			0.27	0.33			0.98	0.99

Linear model 1 adjusted for sex, race, study field center, education, income, and time between the 2 ultrasounds. Linear model 2 further adjusted for physical activity, smoking, BMI, LDL, HDL, use of statins, GFR, and 25-OHD. Logistic model 3 adjusted for age, sex, race, site, education, income, and time between the 2 ultrasounds. Logistic model 4 further adjusted for physical activity, smoking, BMI, LDL, HDL, use of statins, GFR, and 25-OHD. 25-OHD indicates 25-hydroxyvitamin D; BMI, body mass index; CCA-IMT, intima-media thickness of the common carotid artery; CI, confidence interval; GFR, glomerular filtration rate; HDL, high-density lipoprotein; ICA-IMT, intima-media thickness of the internal carotid artery; LDL, low-density lipoprotein; and PTH, parathyroid hormone.

*Between the 2 carotid ultrasounds.

†*P* value for continuous 25-OHD.

‡Among those without carotid plaques at baseline.

prevalence and incidence of carotid plaque (Table 2, lower rows). No cross-sectional or longitudinal associations with the baseline carotid plaque score or its change over study time were observed: adjusted odds ratio per 10 ng/mL lower 25-OHD 1.00 (95% CI, 0.93–1.08; *P*=0.95) and 1.05 (95% CI, 0.98–1.13; *P*=0.17), respectively.

PTH, IMT, and Plaque

Serum PTH concentrations were not associated with baseline CCA-IMT or its change over time (Table 3, top rows). The adjusted mean difference per 10 pg/mL higher PTH was 0.8 μm (–3.2 to 4.8) and 1.6 μm (95% CI, –1.9 to 5.2), respectively. No association was found between PTH and ICA-IMT at baseline, but participants with higher PTH measurements showed nominally less progression of ICA-IMT between the 2 ultrasounds (–10.6 μm [95% CI, –21.3 to 0.1] per 10 pg/mL increased PTH). After exclusion of one influential outlier with PTH 14.8 pg/mL and IMT progression of 3222 μm, this association was less pronounced (–7.9 μm [95% CI, –17.2 to 1.4] per 10 pg/mL increased PTH). PTH was not independently associated with the prevalence or incidence of carotid plaque (Table 3, lower rows) or with the carotid plaque score at baseline or its change during the study period (adjusted odds ratio per 10 pg/mL higher PTH: 0.97 [95% CI, 0.93–1.01; *P*=0.18] and 0.99 [95% CI, 0.96–1.03; *P*=0.71], respectively).

Additional Analyses

There was no heterogeneity in the associations of 25-OHD and PTH with IMT or its change over time by race/ethnicity (all *P* interaction >0.05; Figure). To confirm that our findings were not influenced by selection bias and, in particular, survivorship bias, we repeated cross-sectional analyses on the 6393 Multi-Ethnic Study of Atherosclerosis (MESA) participants who had baseline measurements of 25-OHD, PTH, and IMT, regardless of the presence of a second carotid ultrasound. Null results were similar, including the absence of effect modification by race/ethnicity.

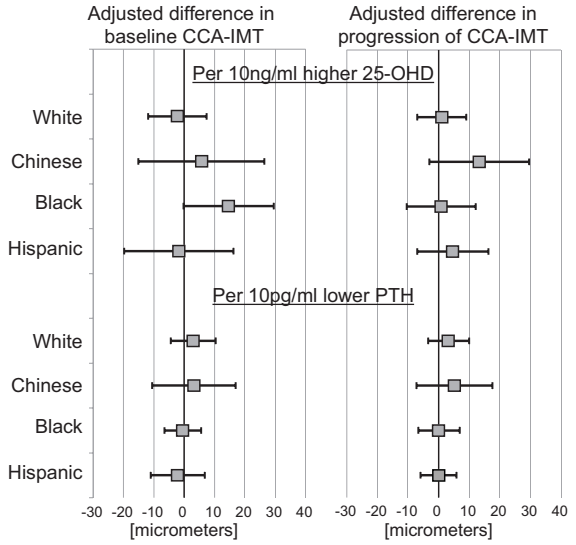

Figure. Associations of 25-hydroxyvitamin D (25-OHD)/parathyroid hormone (PTH) with baseline maximum intima-media thickness of the common carotid artery (CCA-IMT) or its progression in subgroups of race/ethnicity (differences in IMT [in micrometers] per 10ng/mL decrement in 25-OHD or 10 pg/mL increment in PTH). Number of participants for baseline CCA-IMT—white (1261), Chinese (425), black (823), Hispanic (673)/number of participants for progression of CCA-IMT—white (1048), Chinese (360), black (623), Hispanic (549).

Discussion

In this large cohort study of racially and ethnically diverse adults without clinical cardiovascular disease at baseline, we observed no independent associations of serum 25-OHD or PTH concentration with CCA-IMT, ICA-IMT, or carotid plaque. Furthermore, we observed consistent null results for both cross-sectional associations and longitudinal associations evaluating change in IMT and incident plaque during 10 years of follow-up, which have not been reported previously. Estimated magnitudes of association were close to zero, excluded clinically relevant relationships, and did not vary by race/ethnicity. These robustly null results suggest that 25-OHD and PTH do not influence the development of carotid IMT and atherosclerosis in generally healthy adults.

Our null cross-sectional results are in agreement with most previous studies. Five smaller studies have reported a lack of association of 25-OHD with carotid IMT in diverse populations: postmenopausal women recruited from a specialty clinic in Korea,[9] adults from an Amish population,[15] a Dutch population-based study of 600 adults,[16] a community-based study of 900 older Korean adults,[17] and a clinical trial of type 1 diabetes mellitus.[18]

In contrast, 2 clinic-based studies reported positive cross-sectional associations between lower vitamin D and larger IMT. These examined selected populations (type II diabetes mellitus, HIV) and did not exclude participants with known cardiovascular disease, resulting in potential for confounding or bias.[19,20] Our results highlight the important role that confounding can play in analyses of 25-OHD: lower 25-OHD was associated with greater IMT and greater progression of CCA-IMT in models adjusted for demographic variables, as hypothesized, but not with further adjustment for confounding variables. Also, Reis et al[21] studied 654 subjects from a community-based cohort in California, with a mean age of 76 years and a high average 25-OHD (41.5 ng/mL). This study reported an independent association between vitamin D status and ICA-IMT, but not CCA-IMT, which conflicts with our results without a clear explanation. In the same study, PTH was not associated with either ICA-IMT or CCA-IMT.

Several explanations could be advanced for the lack of associations between mineral metabolism markers and carotid injury in our study. First, one measure of 25-OHD and PTH may not adequately represent the true average individual status of these hormones because of variability over time. PTH has a substantial within-subject variability,[22,23] but the validity of one measure of 25-OHD is very high, with a correlation of 0.85 between 2 measurements taken 8 months in white and black American subjects.[24] Second, carotid IMT measurement error (which would bias estimates toward the null) cannot be excluded, even with the very good intra- and inter-reader reproducibility measurements used in this study. Third, CCA-IMT may be more closely related to aging and hypertensive medial hypertrophy than atherosclerotic processes.[25] However, ICA-IMT and carotid plaque, which yielded similar results in our analysis, are thought to represent early phenotypes of atherosclerosis. Fourth, more aggressive treatment for cardiovascular risk factors among participants with low 25-OHD or high PTH during follow-up could have attenuated the true associations, but we found that the increase in cardiovascular treatment did not differ by baseline 25-OHD or PTH status. Finally, and most likely in our opinion, our results may suggest that mineral metabolism disturbances affect cardiovascular risk through pathways distinct from carotid atherosclerosis.

Previous experimental and epidemiological evidence supports effects of PTH and 25-OHD on cardiovascular risk that do not involve carotid atherosclerosis. PTH is an independent predictor of cardiovascular mortality in the general population,[3] but its association with incident heart failure appears much stronger than with myocardial infarction.[1] The detrimental effects of PTH on the myocardium (left ventricular hypertrophy, fibrosis, calcifications) or endothelial function may be more important than the effects on arterial wall injury, at least in the carotid arteries.[11–13] Vitamin D may act on cardiovascular risk through several different pathways, such as through an immune or inflammatory modulation or a direct effect on the endothelial or smooth muscle vascular cell.[8,26,27] Of cardiovascular outcomes, lower circulating concentrations of 25-OHD have been most consistently and strongly associated with increased risk of coronary artery disease.[1,28–30] The extent of coronary artery calcium and carotid IMT is only moderately correlated,[31] suggesting that their pathogenesis may differ. Whether 25-OHD influences the development of coronary atherosclerosis, suggested by previous work,[32] needs to be further explored.

Our study design and population bring important strengths to our results. Precise estimates of associations, of utmost importance given the null findings, were possible because of the large sample size and strict quality of the outcome

measures. The possibility of residual confounding was reduced by the well-measured confounding variables and the lack of clinical cardiovascular disease at baseline. Survivorship bias was minimized by showing similar results for cross-sectional associations at baseline between the entire cohort and the subcohort with both ultrasound examinations. Finally, the participants' diversity in race/ethnicity, age range, and sex broadens the generalizability of the results. Study limitations included its observational design, possible measurement error in mineral metabolism and carotid biomarkers, especially for longitudinal IMT and plaque measurements, the use of surrogate markers of carotid atherosclerosis, as well as lack of data on vitamin D supplementation.

In conclusion, data from this large, diverse cohort do not support clinically meaningful relationships of circulating 25-OHD or PTH concentrations with carotid IMT or plaque. If previously observed relationships of these biomarkers with cardiovascular events are causal, pathways other than carotid atherosclerosis are likely responsible.

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Significance

Lower circulating concentrations of 25-hydroxyvitamin D and higher circulating concentrations of parathyroid hormone are associated with increased risk of cardiovascular events, but potential disease pathways are poorly defined. In this study, we measured 25-hydroxyvitamin D and parathyroid hormone in 3251 participants without cardiovascular disease who underwent 2 carotid ultrasounds a mean of 9.4 years apart. 25-hydroxyvitamin D and parathyroid hormone were associated neither with the severity or progression of intima-media thickness nor with the prevalence or incidence of carotid plaques. These null results were observed among all races and ethnicities. The absence of associations suggests that the pathways mediating the increased cardiovascular risk of vitamin D and parathyroid hormone may be independent of carotid atherosclerotic processes.