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Vitamin D, Parathyroid Hormone and Cardiovascular Mortality in Older Adults: The Rancho Bernardo Study

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Abstract

BACKGROUND—Recent systematic reviews have cast doubt on the association between vitamin D and cardiovascular disease. No prior studies have investigated the association between 25-hydroxyvitamin D (25[OH]D), 1,25-dihydroxyvitamin D (1,25[OH]₂D) and intact parathyroid hormone (iPTH) and cardiovascular disease mortality in a temperate climate.

METHODS—1073 community-dwelling older adults were evaluated in 1997-99; serum levels of 25(OH)D (mean 42ng/mL), 1,25(OH)₂D (median 29pg/mL) and iPTH (median 46pg/mL) were measured; mean estimated glomerular filtration rate (eGFR) was 74mL/min/1.73m². Participants were followed up to 10.4 (mean 6.4) years with 111 cardiovascular disease deaths.

RESULTS—In unadjusted Cox proportional hazards models, higher levels of 1,25(OH)₂D were protective against cardiovascular mortality, while higher levels of iPTH predicted increased risk of cardiovascular death. After adjusting for age alone or multiple covariates, there was no significant association between 25(OH)D, 1,25(OH)₂D or iPTH and cardiovascular mortality; results did not differ by eGFR ≥ 60mL/min/1.73m² or < 60mL/min/1.73m².

CONCLUSIONS—In this prospective study of Caucasian, middle-income, community-dwelling older adults living in sunny southern California, serum levels of 25(OH)D, 1,25(OH)₂D, or iPTH were not independently associated with cardiovascular mortality.

Keywords

aging; cardiovascular mortality; chronic kidney disease; parathyroid hormone; vitamin D

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There is burgeoning public interest in possible wide-ranging health benefits from vitamin D. Nevertheless, two recent systematic reviews concluded that there is no consistent association between vitamin D and cardiovascular disease.^{1,2} In the chronic kidney disease population, estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² is associated with decreased levels of 25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D (1,25[OH]₂D) and increased levels of intact parathyroid hormone (iPTH).³ These abnormalities have been purported as potential mechanisms explaining the association of chronic kidney disease with cardiovascular disease mortality. Even among those with preserved kidney function, multiple mechanisms exist for a vitamin D-cardiovascular disease association. Low serum levels of 25(OH)D are associated with hypertension, diabetes, and insulin resistance;⁴ 25(OH)D and 1,25(OH)₂D can stimulate the secretion and action of insulin,⁵ modulate renin,⁶ inhibit cellular proliferation,⁷ and alter the inflammatory response associated with atherosclerosis.⁸ Levels of iPTH have been associated with pro-sclerotic effects on vascular smooth muscle cells⁹ and with the metabolic syndrome in patients with normal kidney function.¹⁰

Prior prospective studies of the association between serum levels of 25(OH)D or 1,25(OH)₂D with cardiovascular mortality have shown contradictory results.^{11,12,13,14,15} 25(OH)D is the primary storage form of vitamin D and the metabolite that best represents the state of vitamin D sufficiency; 1,25(OH)₂D is the active metabolite under regulation by iPTH and has a shorter half-life. Most previous studies have focused on populations where vitamin D deficiency is common.^{11,14,15,16} The definition of vitamin D deficiency differs among studies; it is unclear whether observed associations at lower levels will persist across a higher range. There is presently little evidence for benefit of vitamin D supplementation in preventing cardiovascular events.^{17,18} Furthermore, although an association between elevated iPTH levels and cardiovascular mortality has been established in dialysis patients,¹⁹ there is only one prior prospective study of iPTH and cardiovascular mortality in community-dwelling adults.²⁰

The present prospective study was designed to determine whether there is a dose-response association between relatively good serum levels of 25(OH)D, 1,25(OH)₂D, and iPTH with cardiovascular mortality in primarily older, middle-income, Caucasian, community-dwelling adults in southern California, where a temperate climate and year-round sunshine would be expected to reduce or prevent vitamin D deficiency.

METHODS

Study Population

The Rancho Bernardo Study, a cohort of Caucasian, middle-class, community-dwelling adults living in southern California, was established in 1972; the details of the initial study have been published previously.²¹ Between 1997-99, 89% of surviving community-dwelling participants (n=1091) attended a follow-up visit, underwent medical evaluation and provided blood samples. After excluding three participants missing values necessary for calculating eGFR by the abbreviated Modification of Diet in Renal Disease (MDRD) equation,²² 18 participants who did not have samples for measurement of serum levels of 25(OH)D, 1,25(OH)₂D, and iPTH, and two participants with an eGFR <15 mL/min/1.73 m², there remained 1073 participants (411 men and 662 women), median age 76 years, who are the subject of this report. The University of California, San Diego Human Subjects Protections Program approved this study; all participants gave written informed consent.

At the 1997-99 visit, participants completed standard questionnaires including medical history, medications, cigarette smoking, alcohol consumption, and exercise. Participants were asked about a history of physician-diagnosed angina pectoris, myocardial infarction,

stroke, and claudication, and completed the Rose cardiovascular symptom questionnaire.²³ Height and weight were measured using a calibrated stadiometer and balance-beam scale. Systolic and diastolic blood pressures were measured twice in seated subjects after a five-minute rest, using the Hypertension Detection and Follow-up Program protocol.²⁴ Body mass index was calculated as kg/m². Diabetes was defined according to the 1999 World Health Organization criteria:²⁵ fasting plasma glucose ≥ 126 mg/dL (7mmol/L), physician diagnosis of diabetes, or use of diabetes-specific medication (oral or insulin).

Exposures and Outcomes

The primary exposure variables were serum levels of 25(OH)D, 1,25(OH)₂D and iPTH. Blood was obtained by venipuncture after an overnight fast in tubes protected from sunlight. Serum was separated and stored at -70°C within 30 minutes of collection. Serum 25(OH)D (25[OH]D₂+25[OH]D₃) and 1,25(OH)₂D were measured in the research laboratory of Dr. Michael Holick using vitamin D competitive binding protein recognition and chemiluminescence detection.²⁶⁻²⁷ The intra- and inter-assay coefficients of variation for the 25(OH)D assay were 8 and 10% respectively;²⁶ the limit of detection was 5ng/mL (13nmol/L), reference range 10–52ng/mL (25–130nmol/L). For 1,25(OH)₂D, the intra- and inter-assay coefficients of variation were 5–10% and 10–15%, respectively;²⁷ the limit of detection was 4.6pg/mL (12pmol/L). The iPTH values were determined in the same laboratory using a chemiluminescence assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra- and inter-assay coefficients of variation were 6%, reference range 10–65pg/mL (1.1–6.8pmol/L). Seasons were determined from the date of blood draw; we have previously shown that despite year-round sunshine, age-adjusted 25(OH)D levels are lower in this cohort in winter, and higher in fall, and iPTH levels are lower in fall and higher in spring.²⁸ The stability of vitamin D metabolites and iPTH in frozen serum has been well described. Vitamin D metabolites may be protected from degradation by heat or light by binding proteins;²⁹ a recent paper demonstrated no detectable degradation of 25(OH)D in samples stored at -20°C for over 10 years,³⁰ and circulating 25(OH)D has been shown to be extremely stable in stored serum samples, even after many freeze-thaw cycles.³¹ The stability of iPTH in frozen serum has also been shown previously.³²

Serum creatinine was measured by SmithKline Beecham clinical laboratories. eGFR was calculated by the MDRD equation for Caucasians:²²

$$\text{eGFR (mL/min/1.73m}^2\text{)} = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}).$$

The outcome was cardiovascular mortality. Median follow-up was 6.8 years, maximum 10.4 years. Vital status was determined annually by mailed questionnaire until 2008. Death certificates were coded for the underlying cause of death by a certified nosologist using the International Classification of Disease—9th Revision. Cardiovascular deaths were those coded 410–414 (ischemic heart disease), including acute myocardial infarction (410), other acute and subacute forms of ischemic heart disease (411), old myocardial infarction (412), angina pectoris (413), and other forms of chronic ischemic heart disease (414), and also included conduction disorders (426), cardiac dysrhythmias (427), heart failure (428), cerebrovascular disease (430–438), and diseases of arteries, arterioles, and capillaries (440–448).

Statistical Analysis

All measures were normally distributed except serum triglycerides, urine albumin/creatinine ratio, 1,25(OH)₂D, and iPTH, which required log transformation for analyses. A univariate general linear model was used to compare mean values for continuous variables by eGFR

category, calculating a p for trend; the chi square statistic, assessing for a linear by linear association, was used to compare difference in prevalence for categorical variables.³³ Medians for 25(OH)D, 1,25 (OH)₂D, and iPTH were calculated and Kruskal-Wallis tests of difference were used to compare values among eGFR levels.

Cox proportional hazards models were used to generate hazard ratios and 95% confidence intervals (HR; 95% CI)³⁴ separately for each of the three predictor variables (25[OH]D, 1,25[OH]₂D, and iPTH) with adjustment for age, sex, body mass index, systolic blood pressure, low density lipoprotein cholesterol, fasting glucose, exercise, log(urine albumin/creatinine ratio), prevalent cardiovascular disease, season of blood draw, current use of medications (diuretics, calcium channel blockers, beta blockers, angiotensin converting enzyme-inhibitors, calcium supplements, vitamin D supplements, lipid lowering medications), and eGFR. Covariates for multivariate analyses were chosen based on biological plausibility and significant correlations with both the exposure variables and fatal cardiovascular disease. HRs were calculated for one standard deviation (SD) in each predictor variable such that each HR is comparable across variables. The proportional hazards assumption was met.

Statistical tests were 2-tailed, with significance defined as $p < 0.05$. SPSS (SPSS Inc., Base 15.0 for Windows) was used for all analyses. Power calculations revealed that given 80% power, type I error of 0.05, sample size of 1073 and event rate of 0.10, we could detect a HR of 0.75 per SD increase in 25(OH)D, 0.75 per SD increase in $\log(1,25[\text{OH}]_2\text{D})$, and 1.62 per SD increase in $\log(\text{iPTH})$.

RESULTS

Baseline characteristics for the cohort, by level of kidney function, are summarized in Table 1. Ninety percent of participants were over age 60 (mean 74 years). Only 14% of participants had categorically defined 25(OH)D deficiency, $\leq 30\text{ng/mL}$ (75nmol/L), and 3% had a serum 25(OH)D $\leq 20\text{ng/mL}$ (50nmol/L). As shown in Figure 1, age-adjusted median 1,25(OH)₂D decreased ($p < 0.001$) as kidney function declined, but there was no significant decline in 25(OH)D ($p = 0.24$) or rise in iPTH ($p = 0.63$).

Over a median 6.8 (maximum 10.4) year follow-up, there were 266 deaths, including 111 cardiovascular deaths, 71 in those with $\text{eGFR} \geq 60\text{mL/min/1.73m}^2$ and 40 in those with $\text{eGFR} < 60\text{mL/min/1.73m}^2$. Table 2 shows HRs per SD increase for 25(OH)D, $\log(1,25[\text{OH}]_2\text{D})$, and $\log(\text{iPTH})$ as predictors of cardiovascular mortality by eGFR category. In unadjusted analyses, higher levels of 1,25(OH)₂D were protective against cardiovascular mortality but in stratified analyses, this protective effect was observed only in those with $\text{eGFR} < 60\text{mL/min/1.73m}^2$. In unadjusted analyses, higher levels of iPTH predicted increased risk of cardiovascular death, but these associations were no longer statistically significant after adjusting for age. Further multivariable adjustment for the additional covariates indicated in the methods above did not materially change these results.

Testing revealed a significant interaction for 25(OH)D*eGFR ($p = 0.03$), but not 1,25(OH)₂D*eGFR or iPTH*eGFR. In multiply adjusted analyses stratified by kidney function, there was no significant association between any of the predictors and cardiovascular mortality either in those with $\text{eGFR} \geq 60\text{ mL/min/1.73m}^2$ (79%) or $< 60\text{mL/min/1.73m}^2$ (21%) (Table 2).

Repeating analyses using quartiles of 25(OH)D, 1,25(OH)₂D, or iPTH or using dichotomous cutpoints for 25(OH)D ($\geq 30\text{ng/mL}$ [86%] vs. $< 30\text{ng/mL}$ [14%]) or iPTH ($< 65\text{pg/mL}$ [82%] and $\geq 65\text{pg/mL}$ [19%]) did not materially change results (data not shown). Stratifying by 1) creatinine clearance by the Cockcroft Gault equation instead of eGFR by MDRD, 2)

prevalent cardiovascular disease, or 3) urine albumin/creatinine ratio <30mg/g or ≥30mg/g did not change results (data not shown).

DISCUSSION

To our knowledge this is the first prospective study to investigate the role of serum 25(OH)D, 1,25(OH)₂D, and iPTH in the prediction of cardiovascular mortality in a population of older community-dwelling adults with a low prevalence of vitamin D deficiency and a broad range of kidney function. After adjusting for age alone, there was no independent association between serum 25(OH)D, 1,25(OH)₂D, or iPTH with cardiovascular mortality either before or after stratifying for eGFR.

As highlighted in two recent systematic reviews,^{1,2} the results from several prospective studies of the association between 25(OH)D or 1,25(OH)₂D levels and cardiovascular mortality,^{11,12,13,14,15} have been contradictory. Few had measures of the clinically active 1,25(OH)₂D or reported differences by level of kidney function.

Consistent with our null findings, an analysis of 13,331 participants (mean age 45) from the NHANES III cohort found no significant difference in rates of cardiovascular mortality over median 9 years, for the lowest (<18ng/mL) versus the highest (>32ng/mL) quartile of 25(OH)D.¹¹ Their highest quartile of 25(OH)D (>32ng/mL) overlapped with our lowest (4–34ng/mL). In contrast, in a subsequent analysis of this cohort limited to 3408 participants ≥65 (mean 72) years over a median of 7 years, Ginde and colleagues reported participants with 25(OH)D levels <20ng/mL were at higher risk for cardiovascular mortality than those with levels >40ng/mL.¹² These results are compatible with a threshold effect.

In a prospective study of 6,219 participants (mean age 49) without prevalent cardiovascular disease from the Mini-Finland Health Survey, investigators found a protective role for 25(OH)D against total cardiovascular death for the highest quintile of 25(OH)D (median 29ng/mL in men, 27ng/mL in women) versus the lowest (median 9ng/mL in men, 8ng/mL in women) over a median 27 year follow-up.¹⁵ The mean serum 25(OH)D level was 17ng/mL, representing a population with very low 25(OH)D levels. No measure of kidney function was reported.

To our knowledge, only two other studies have reported the longitudinal association between the more clinically active 1,25(OH)₂D and cardiovascular death.^{13,14} Although the role of 1,25(OH)₂D in cardiovascular disease is incompletely understood, reductions in 1,25(OH)₂D may explain some of the increased mortality seen in those with chronic kidney disease. While we did find a protective effect of 1,25(OH)₂D in unadjusted analyses, particularly in those with eGFR <60mL/min/1.73m², this did not persist after adjusting for age or other covariates. Similar to our findings, a prospective study of 226 Japanese patients (mean age 67) with stage 3 or 4 chronic kidney disease (mean eGFR 24mL/min/1.73m²), Inaguma and colleagues found no difference in cardiovascular mortality in those with 1,25(OH)₂D <20pg/mL or >20pg/mL.¹³ In the only other study with measures of both 25(OH)D and 1,25(OH)₂D, Dobnig and colleagues studied 3258 German patients (mean age 62, mean eGFR 81mL/min/1.73m²) referred for coronary angiography over a median 8 years.¹⁴ In adjusted models, there was a significant difference in risk of cardiovascular mortality in those with the two lowest quartiles of 25(OH)D (median 8ng/mL and 13ng/mL) compared with the highest quartile (median 28ng/mL) and in those in the lowest quartile of 1,25(OH)₂D (median 21ng/mL) compared to the highest (median 51ng/mL). Notably, their *highest* quartile of 25(OH)D (median 28ng/mL) corresponded to our *lowest* quartile (median 29ng/mL) whereas 1,25(OH)₂D quartiles in both studies were similar. Their cohort differed from ours in that it consisted of patients selected for prevalent cardiovascular disease with

high rates of cardiovascular disease death, and lower levels of 25(OH)D, likely resulting from differences in sun exposure.

We did not find that baseline iPTH predicted cardiovascular mortality after adjusting for age. To our knowledge, the only prior prospective study to investigate the association between iPTH and cardiovascular mortality was in a community-dwelling population of 958 men (mean age 71, mean eGFR 62mL/min/1.73m²). They found over a median 10 year follow-up, higher plasma iPTH was associated with higher cardiovascular mortality.²⁰ Authors report that regression spline analysis showed a linear increase in cardiovascular mortality with increasing iPTH, however, this risk was significant only in the 4th quartile of iPTH, (~60pg/mL), suggesting a threshold effect.

Because some prior published literature in community-dwelling adults suggests an increased risk of cardiovascular mortality only in individuals with vitamin D levels lower than levels observed here,^{11,12} our null results may mean that only larger disruptions in levels of 25(OH)D, 1,25(OH)₂D contribute to cardiovascular mortality. Our null findings are compatible with results from randomized clinical trials of vitamin D supplementation to prevent cardiovascular outcomes^{17,18} although the doses of vitamin D in these trials may have been too low.

Limitations of our study should be noted. The Rancho Bernardo Study participants are Caucasian, typical of suburbs in the 1970s when this cohort was established. They are also middle- to upper-middle class, relatively healthy, and have healthcare. Most importantly, they live in a temperate climate with year-round sun exposure, and thus have little 25(OH)D deficiency.²⁸ These results may not be generalizable to other populations with more vitamin D deficiency, but do yield novel information about potential risks conferred by lesser degrees of vitamin D insufficiency and its importance in warmer, sunnier climates. It is also possible that the higher vitamin D levels in our population are due to differences in lab assay techniques. Competitive binding protein assay may produce higher 25(OH)D results compared to radioimmunoassay and high-performance liquid chromatography;^{35,36} at the time levels were measured, the latter methods were more costly and labor intensive. Levels in Rancho Bernardo may not be directly comparable to studies using different assays. Nevertheless, routine assays accurately rank individuals across the range of 25(OH)D levels,³⁶ and therefore this would not alter the internal validity of our study. Values for 25(OH)D and 1,25(OH)₂D were measured on a single blood specimen, but are known to have diurnal and seasonal variation;^{28,37} this may have weakened a potential association between these measures and cardiovascular death. Nevertheless, nearly all other studies showing an association between vitamin D or iPTH and cardiovascular disease have also used a single measurement at one point in time, and we did adjust for season based on known seasonal variation in vitamin D and iPTH levels in this cohort.²⁸ Our study evaluated only cardiovascular mortality which is one, albeit important, spectrum of cardiovascular risk. Furthermore, cardiovascular mortality was defined only by cause of death information on the death certificate; this limitation is common to epidemiologic studies of mortality. When stratified by eGFR, there were a relatively small number of cardiovascular deaths in each group limiting our power to address the importance of eGFR in this association; small numbers also limited our ability to examine associations with cause-specific cardiovascular mortality with finer granularity. Another limitation, common to all studies of the elderly, is survival bias; persons with more severe kidney disease or lower vitamin D levels may have died or have been too ill to return to the follow-up visit, potentially attenuating any true association.

In conclusion, mild reductions in 25(OH)D or 1,25(OH)₂D or elevations in iPTH were not independently associated with cardiovascular mortality over a 10 year follow-up in older

adults living in a temperate climate with year-round sunshine. These results did not differ by strata of kidney function. Additional prospective studies and randomized controlled trials are needed to further investigate the level of vitamin D and iPTH, if any, necessary to reduce cardiovascular mortality. Different levels of vitamin D may be necessary to reduce bone loss, cancer and diabetes; these associations also await clinical trial confirmation.

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Figure 1. Median age-adjusted serum 25(OH)D, 1,25(OH)₂D, and intact PTH by estimated glomerular filtration rate.

Table 1

Baseline Characteristics: The Rancho Bernardo Study 1997–99

Characteristic	All (n=1073)	≥ 90 (n=157; 15%)	eGFR Category		p value
			60–89 (n=683; 64%)	15–59 (n=233; 22%)	
Glomerular Filtration Rate (mL/min/1.73m ²)	74 (19)	105 (12)	75 (9)	50 (8)	<0.001
Age (years)	74 (10)	70 (10)	74 (10)	80 (9)	<0.001
Body Mass Index (kg/m ²)	26 (4)	26 (4)	26 (4)	25 (4)	0.39
Systolic Blood Pressure (mmHg)	136 (21)	132 (20)	135 (20)	142 (23)	<0.001
Diastolic Blood Pressure (mmHg)	74 (10)	74 (8)	74 (9)	72 (12)	0.05
Fasting Plasma Glucose (mg/dL)	102 (20)	104 (30)	101 (16)	102 (20)	0.34
Low Density Lipoprotein (mg/dL)	121 (32)	120 (31)	121 (31)	123 (36)	0.29
High Density Lipoprotein (mg/dL)	60 (19)	61 (20)	60 (18)	61 (19)	0.83
Serum Calcium (mg/dL)	9.2 (0.4)	9.0 (0.4)	9.2 (0.4)	9.3 (0.4)	<0.001
Serum Phosphorus (mg/dL) *	3.4 (0.5)	3.3 (0.5)	3.3 (0.5)	3.4 (0.5)	0.13
25-Hydroxyvitamin D (ng/mL)	42 (14)	42 (11)	42 (15)	41 (13)	0.47
1,25-Dihydroxyvitamin D (pg/mL) †	29 (21–39)	31 (24–43)	30 (22–40)	24 (16–35)	<0.001
Intact Parathyroid Hormone (pg/mL) †	46 (36–60)	46 (38–59)	46 (36–59)	47 (37–63)	<0.001
Women	62%	60%	60%	68%	0.06
Diabetes	9%	6%	8%	14%	0.01
Current smoking	5%	8%	5%	1%	<0.01
Exercise ≥3times per week	72%	75%	74%	66%	0.03
Prevalent Cardiovascular Disease	20%	15%	18%	31%	<0.001
Calcium supplement use	43%	44%	44%	40%	0.42
Vitamin D supplement use	21%	22%	21%	18%	0.30
Lipid lowering medication use	5%	3%	6%	5%	0.50
Angiotensin Converting Enzyme Inhibitor use	11%	6%	10%	16%	<0.01
Beta-blocker use	14%	8%	14%	17%	0.01
Calcium channel blocker use	15%	12%	15%	16%	0.28

* Serum Phosphorus was measured in only a subset of 555 participants

Values for continuous variables are mean (standard deviation) if normally distributed or † median (25–75%ile) if skewed; Values for categorical variables are percentages

p value for trend for univariate general linear model for continuous variables and chi-square linear by linear association for categorical variables
 SI Conversion Factors: To convert 25(OH)D to nanomoles per liter, multiply by 2.496, 1,25(OH)₂D to picomoles per liter, multiply by 2.6, iPTH to nanomoles per liter, multiply by 0.1053

Table 2

Hazard Ratios (95% CI) for Cardiovascular Disease Mortality per Standard Deviation Increase in Serum 25(OH)D, Log(1,25[OH]₂D), and Log(iPTH)

	All (n=1073)	eGFR ≥ 60 (n=840; 78%)	eGFR <60 (n=233; 22%)
25(OH)D			
Unadjusted	0.82 (0.66–1.02)	0.87 (0.68–1.12)	0.73 (0.49–1.10)
Age adjusted	1.01 (0.85–1.21)	1.05 (0.87–1.28)	0.90 (0.61–1.33)
Age, sex, body mass index adjusted	0.93 (0.76–1.14)	0.96 (0.77–1.21)	0.84 (0.56–1.25)
Multiply adjusted*	1.07 (0.86–1.33)	1.25 (0.99–1.57)	0.67 (0.43–1.05)
Log (1,25(OH)₂D)			
Unadjusted	0.76 (0.63–0.90) [‡]	0.86 (0.68–1.09)	0.74 (0.56–0.97) [‡]
Age adjusted	0.85 (0.72–1.01)	0.86 (0.68–1.08)	0.86 (0.64–1.14)
Age, sex, body mass index adjusted	0.87 (0.73–1.03)	0.88 (0.70–1.12)	0.90 (0.67–1.21)
Multiply adjusted*	0.98 (0.80–1.21)	1.04 (0.78–1.39)	0.90 (0.64–1.26)
Log (iPTH)			
Unadjusted	1.25 (1.03–1.52) [‡]	1.19 (0.92–1.54)	1.27 (0.95–1.70)
Age adjusted	1.14 (0.94–1.39)	1.03 (0.78–1.34)	1.30 (0.96–1.78)
Age, sex, body mass index adjusted	1.14 (0.93–1.39)	1.05 (0.81–1.36)	1.30 (0.94–1.79)
Multiply adjusted*	1.10 (0.90–1.36)	1.00 (0.74–1.36)	1.32 (0.95–1.83)

* Multiply adjusted for age, sex, body mass index, systolic blood pressure, low density lipoprotein cholesterol, fasting glucose, exercise, log(urine albumin/creatinine ratio), prevalent cardiovascular disease, season of blood draw, current use of diuretics, calcium channel blockers, beta blockers, angiotensin converting enzyme-inhibitors, calcium supplements, vitamin D supplements, lipid lowering medications and eGFR (only in analyses not stratified by eGFR).

[†] p < 0.05,

[‡] p < 0.01