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Plasma Fibroblast Growth Factor 23, Parathyroid Hormone, Phosphorus, and Risk of Coronary Heart Disease

Eric N. Taylor, MD, MSc^{1,3}, Eric B. Rimm, ScD^{1,4}, Meir J. Stampfer, MD, DrPH^{1,4}, and Gary C. Curhan, MD, ScD^{1,2,4}

¹Channing Laboratory, Brigham and Women's Hospital

²Renal Division, Department of Medicine, Brigham and Women's Hospital

³Division of Nephrology and Transplantation, Maine Medical Center, Portland, ME

⁴Department of Epidemiology, Harvard School of Public Health; Boston, MA, USA

Abstract

Background—Fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and phosphorus all have been proposed as plasma biomarkers for the development of coronary heart disease (CHD) in individuals with normal renal function.

Methods—In a nested case-control study of men in the Health Professionals Follow-up Study free of diagnosed cardiovascular disease at blood draw, we prospectively examined associations between plasma FGF23, PTH, and phosphorus and risk of coronary heart disease (CHD). During 10 years of follow-up, 422 men developed nonfatal myocardial infarction or fatal CHD. Controls were selected in a 2:1 ratio and matched for age, date of blood collection, and smoking status.

Results—Mean estimated glomerular filtration rate was 86 mL/min per 1.73 m² in cases and controls. At baseline, there were no statistically significant differences between cases and controls in plasma levels of FGF23, PTH, or phosphorus. After adjusting for matching factors, family history of myocardial infarction, body mass index, alcohol consumption, physical activity, history of diabetes mellitus and hypertension, ethnicity, region, plasma 25-hydroxyvitamin D and other factors, the odds ratios for incident CHD for participants in the highest compared to lowest quartiles were 1.03 (95% CI 0.70-1.52; P for trend 0.84) for FGF23, 1.20 (95% CI 0.82-1.76; P trend 0.99) for PTH, and 0.72 (95% CI 0.51-1.02; P trend 0.13) for phosphorus.

Conclusions—Plasma FGF23, PTH, and phosphorus are not associated with the development of incident CHD in men without chronic kidney disease.

Abnormalities of mineral metabolism are established risk factors for cardiovascular disease (CVD) and death in individuals with chronic kidney disease.¹⁻⁴ More recent data suggest that calcium-phosphorus homeostasis also may play an important role in the development of coronary heart disease (CHD) in individuals with normal renal function. For example, a study of 958 men (most without chronic kidney disease) reported that each standard

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Corresponding author: Eric N. Taylor, MD Division of Nephrology and Transplantation Maine Medical Center 22 Bramhall Street Portland, ME 04102 Phone:(207)662-5672 Fax: (207)662-6306 entaylor@partners.org.

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deviation increase in parathyroid hormone (PTH) was independently associated with a 38% greater risk for cardiovascular mortality.⁵ Higher plasma phosphorus levels also were associated with a higher risk of CVD in individuals with normal renal function.^{6, 7} In a recent analysis of 833 participants in the Heart and Soul Study with stable CHD and preserved kidney function, individuals in the highest tertile of plasma fibroblast growth factor 23 (FGF23), a bone derived protein that promotes renal phosphorus excretion, had an 83% increase in CVD events.⁸

Despite such data, the relations between many biomarkers of mineral metabolism and subsequent CHD in individuals with normal renal function require elucidation. The only prospective study of PTH and CVD to date reported a total of 53 fatal cases that included deaths from a wide variety of diseases (including stroke, pulmonary embolus, and pulmonary hypertension).⁵ Only a minority of participants in the Heart and Soul Study with CVD events had myocardial infarction (MI) (most had heart failure, stroke, or transient ischemic attack).⁸ In contrast to previous reports, several recent large studies found no association (or inconsistent associations) between plasma phosphorus and CVD.⁹⁻¹¹ Of note, two studies reporting positive associations between plasma phosphorus and CVD observed substantially higher rates of smoking in the highest compared to lowest quartiles of phosphorus.^{6, 7} Although smoking is independently associated with higher plasma phosphorus in a dose dependent fashion,^{9, 12} neither study accounted for smoking quantity or duration in multivariate analyses.

We prospectively examined the independent associations between plasma levels of FGF23, PTH, and phosphorus and the risk of nonfatal MI and fatal CHD among 1259 participants of the Health Professionals Follow-up Study without chronic kidney disease.

METHODS

Source population

The Health Professionals Follow-up Study (HPFS) enrolled 51,529 male dentists, optometrists, osteopaths, pharmacists, podiatrists, and veterinarians who were 40 to 75 years of age in 1986. At baseline, study participants filled out a detailed questionnaire about diet, medical history, and medications. Subsequent questionnaires have been mailed every two years to update information on health-related behaviors, medications, and medical events. In 1993, blood samples were submitted by 18,225 participants. Participants who provided blood were somewhat younger than participants who did not but were otherwise similar.

Study population

Based on the cohort that provided blood samples, and after exclusion of participants with a history of CVD before 1994, we identified 432 participants with incident nonfatal MI or fatal CHD between the date of blood collection and January 31, 2004. We assessed disease status through January 31, 2004 for > 97% of the men. Controls were randomly selected from participants with a blood sample who were alive and who did not have a history of CVD at case ascertainment. For controls, we used a 2:1 ratio and matched on age, month and year of blood collection, and smoking status (risk set sampling).¹³ The research protocol for this study was reviewed and approved by the institutional review board of Brigham and Women's Hospital.

Ascertainment of nonfatal MI and fatal CHD

Study physicians reviewed the medical records of all participants for whom nonfatal MI or fatal CHD was reported during follow-up. Each biennial questionnaire contains a question on whether the man has had "professionally diagnosed ... myocardial infarction (heart

attack)” in the preceding two years. Myocardial infarction was confirmed if it met the World Health Organization's criteria, which include symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes. For approximately 70% of the men who self-reported MI, we confirmed the diagnosis using these methods. Reasons for nonconfirmation of MI were that either no further information was available, typically because the participant did not consent or the hospital did not send the hospital records, or a reported case was rejected based on the medical record information received. We excluded nonconfirmed participants from the control selection process. Deaths were identified from state vital statistics records and the National Death Index or were reported by next of kin or by the postal system. Fatal CHD was confirmed in one of two ways: 1) by hospital records or autopsy or 2) by death certificate if all the following three conditions were met: CHD was listed as the cause of death on the death certificate, CHD was the underlying and most plausible cause of death, and if evidence of previous CHD was available.

Ascertainment of medical history, anthropometric data, and diet and lifestyle factors

Anthropometric data, lifestyle factors, and diet were based on the 1994 questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Nutrient intake was computed based on a validated semiquantitative food frequency questionnaire, which inquires about average food intake during the past year using composition values from the United States Department of Agriculture, supplemented with other data. Physical activity was expressed as metabolic equivalent task-hours based on self-reported types and durations of activities over the previous year. Medical history information was obtained from biennial questionnaires. There have been multiple previous reports on the validity and reproducibility of these collected data and measurements in HPFS.¹⁴⁻¹⁹

Measurement of biochemical variables

Blood samples were collected in three 10-mL liquid EDTA blood tubes, placed on ice packs, stored in polystyrene foam containers, and returned to the HPFS blood storage facility via overnight courier. More than 95% of the samples arrived within 24 hours of collection. Blood samples were centrifuged and aliquoted for storage in the vapor phase of liquid nitrogen freezers (-130°C or colder). Fewer than 15% of the samples were slightly hemolyzed, and few were moderately hemolyzed ($< 3\%$), lipemic ($< 1\%$), or not cooled on arrival ($< 0.5\%$).

Plasma FGF23 levels were measured using a second-generation C-terminal human enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA). Plasma levels were measured twice in each participant and results averaged. The coefficient of variation was 9.8%. In 20 HPFS participants, we measured FGF23 at two time points (one year apart) and the intra-class correlation was 0.8. We also measured plasma FGF-23 in triplicate specimens from 16 volunteers after immediate processing, after storage on ice for 24 hours, and after storage on ice for 48 hours. The intra-individual correlations between samples were > 0.9 .

Intact PTH was measured by electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN). The coefficient of variation was 8.3%. Phosphorus was measured by a colorimetric assay on the Hitachi 917 analyzer using Roche reagents (Roche Diagnostics, Indianapolis, IN). The coefficient of variation was 8.0%. Assays used to determine plasma levels of 25-hydroxyvitamin D (25[OH]D), creatinine, lipoproteins, and lipids were previously described.²⁰ Estimated GFR was determined using the Modification of Diet in Renal Disease Study formula.

Statistical analysis

We excluded 9 participants with grossly hemolyzed blood samples. The distributions of FGF23 and phosphorus were right skewed and, in addition, several participants had high values of each plasma factor. To minimize the impact of extreme outliers, we excluded participants with FGF-23 and phosphorus plasma levels in the top 1% of each distribution. We excluded 3 participants with PTH >120 pg/mL so that our study did not include participants with primary parathyroid disorders. After these exclusions, our study population consisted of 422 cases (333 nonfatal MI and 89 fatal CHD) and 837 controls.

Differences in continuous variables between cases and controls were analyzed using analysis of variance (for normally distributed variables) or the Wilcoxon rank sum test (for non-normally distributed variables). Differences in categorical variables were compared using the χ^2 test.

The study design was prospective; blood was collected before the diagnosis of nonfatal MI or fatal CHD. To preserve unmatched cases and controls in our study population after exclusions, we used unconditional logistic regression adjusted for matched variables in our primary analyses. In secondary analyses, we also used conditional logistic regression after restricting the study population to matched cases and controls with complete blood results.

We divided plasma levels of FGF23, PTH, and phosphorus into quartiles based on control distributions. For tests of trend, we modeled the median value of quartiles as continuous variables in the regression model. In multivariate models, we adjusted for the following potentially confounding variables: family history of MI before the age of 60 years (yes or no), alcohol intake (nondrinker; 0.1-4.9, 5.0-14.9, 15.0-29.9, or ≥ 30.0 g/d; or missing), BMI (continuous), physical activity (quintiles), history of diabetes mellitus (yes or no) and hypertension (yes or no), ethnicity (white or other), region (Northeast, mid-Atlantic, Midwest, or South), marine ω -3 intake (quintiles), multivitamin use (yes or no), fasting status, and baseline plasma levels of 25(OH)D (4 categories), low- and high-density lipoprotein cholesterol (quintiles), triglycerides (quintiles), uric acid (continuous), and creatinine (continuous).

We calculated 95% confidence intervals for all odds ratios. All P values are two tailed. Data were analyzed by using SAS software, version 9.1 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Baseline characteristics and biomarker levels of cases and controls are displayed in Table 1. As expected, cases had a higher BMI, less alcohol intake, and were more likely to have a family history of MI before the age of 60 years and a history of diabetes mellitus and hypertension. In addition, cases had higher levels of total and low-density lipoprotein cholesterol and triglycerides but lower levels of high-density lipoprotein cholesterol. The estimated glomerular filtration rate (eGFR) was 86 mL/min per 1.73 m² (standard deviation 19 mL/min per 1.73 m²) in cases and controls. As previously reported, plasma 25(OH)D was lower in cases than controls.²⁰ There were no statistically significant differences between cases and controls in plasma levels of FGF23, PTH, or phosphorus.

Spearman correlation coefficients for plasma biomarkers are displayed in Table 2. Plasma FGF23 and PTH were positively correlated with triglycerides and uric acid and inversely correlated with high-density lipoprotein cholesterol (all P values <0.001). The correlation coefficients between FGF23, PTH, and phosphorus and low-density lipoprotein cholesterol all were ≤ 0.01 and were statistically non-significant.

Multivariate odds ratios for incident CHD (nonfatal MI plus fatal CHD) across quartiles of plasma FGF23, PTH, and phosphorus during 10 years of follow-up are presented in Table 3. The median values of plasma FGF23 and PTH in the highest quartiles were more than twice the median values in the lowest. After adjusting for matching factors (age, month and year of blood collection, and smoking status), family history of MI before the age of 60 years, alcohol intake, physical activity, body mass index, race, multivitamin use, region, marine ω -3 intake, history of diabetes mellitus, history of hypertension, fasting status, and plasma levels of 25(OH)D, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, and creatinine, the odds ratios for incident CHD for participants in the highest compared to lowest quartiles were 1.03 (95% CI 0.70-1.52; P for trend 0.84) for FGF23, 1.20 (95% CI 0.82-1.76; P trend 0.99) for PTH, and 0.72 (95% CI 0.51-1.02; P trend 0.13) for phosphorus.

To compare more extreme values, we also examined sextiles of FGF23, PTH, and phosphorus. We found no independent associations between sextiles of each factor and odds of CHD. For FGF23, PTH, and phosphorus, odds ratios for CHD were similar for participants above and below the median BMI in non-cases and above and below a plasma 25(OH)D level of 20 ng/mL. For analyses presented in Table 3, odds ratios were similar after excluding fatal cases and after excluding controls who underwent coronary revascularization before the end of follow-up. Conditional logistic regression analyses limited to the study population of cases with matched controls yielded similar results. We obtained similar results after excluding current smokers and limiting the analyses to “never” smokers. Finally, we obtained similar results after excluding participants with eGFR $\leq$ 60 mL/min per 1.73 m² and after limiting follow-up to 6 years.

DISCUSSION

In our prospective study, we observed no associations between plasma levels of FGF23, intact PTH, and phosphorus and subsequent risk for incident nonfatal MI or fatal CHD. The odds ratios for CHD associated with phosphorus were less than 1, and the upper bound of the 95% confidence interval excluded a clinically meaningful increase in risk.

Much of the interest in FGF23 as a potential biomarker for CVD was generated by studies in individuals with chronic kidney disease who have circulating levels of FGF23 several orders of magnitude higher than in our study population. For example, hemodialysis patients in the highest quartile of c-terminal FGF23 (FGF23 levels $>$ 4010 RU/mL) had a multivariate odds ratio of 5.7 (95% CI 2.6-12.6) for 1-year all-cause mortality compared to participants in the lowest quartile (FGF23 $<$ 1090 RU/mL).⁴

Recent data suggest that higher FGF23 also may be associated with cardiovascular disease in individuals with normal kidney function (and concomitantly much lower concentrations of plasma FGF23). In a prospective study of 833 individuals in the Heart and Soul Study (mean eGFR $>$ 70 mL/min per 1.73 m²), participants in the highest tertile of plasma FGF23 had an 83% increase in cardiovascular disease events compared to the lowest tertile.⁸ The median plasma level of c-terminal FGF23 in Heart and Soul was 43.1 RU/mL.

There are several possible explanations for the apparent contradiction between the null associations we observed between FGF23 and CHD and the positive associations reported in Heart and Soul. First, unlike our study participants, individuals in Heart and Soul had CHD at baseline. Thus, it is possible that FGF23 is associated with severity of existing CHD but not development of CHD. Second, the majority of CVD events in Heart and Soul were heart failure and stroke or transient ischemic attack. Notably, FGF23 levels were not associated with risk of MI in Heart and Soul. Third, it is possible that the relatively long time of follow-up in our study (10 years) attenuated the magnitude of risk associated with a single plasma

measurement of FGF23. The median follow-up in Heart and Soul was 6 years. However, restriction of our analyses to 6 years of follow-up did not change our results and we demonstrated low within-person variation in FGF23 levels over time.

Historically, interest in the potential effect of PTH on CHD risk was generated by the results of studies reporting increased rates of all cause and cardiovascular mortality in individuals with primary hyperparathyroidism.^{21, 22} Similarly, the higher PTH levels in individuals with chronic kidney disease led to speculation that PTH was a “uremic toxin” partly responsible for the high rates of CVD death in individuals with impaired kidney function.^{2, 3}

To our knowledge, only one prospective study to date has reported an association between plasma PTH (independent of simultaneously measured plasma 25[OH]D) and CVD in a population of individuals with predominantly normal kidney function.⁵ In 958 male participants of the Uppsala Longitudinal Study of Adult Men (ULSAM) followed for a median of 9.7 years, each standard deviation increase in PTH was independently associated with a 38% greater risk for cardiovascular mortality.⁵

In contrast to ULSAM, we did not identify an association between plasma PTH and subsequent CVD. However, the numbers and definitions of outcomes in our study were substantially different than in ULSAM. The ULSAM investigators observed only 53 incident cases of cardiovascular mortality. In addition, the ICD-9 and ICD-10 administrative codes used to classify death as cardiovascular in ULSAM encompassed a wide array of potentially disparate diseases, including stroke, pulmonary embolus, pulmonary hypertension, and rheumatic heart disease. Our 422 cases of non-fatal MI and fatal CHD were confirmed by medical record review.

Hyperphosphatemia is an established risk factor for cardiovascular risk and mortality in individuals with chronic kidney disease.^{1, 3} and the impact of plasma phosphorus on mortality may be greater with higher plasma calcium levels.¹ Data also suggest that higher levels of phosphorus are associated with adverse cardiovascular outcomes in individuals with normal renal function.^{6, 7} In the Framingham Offspring Study, the multivariate relative risk of incident CHD for individuals in the highest quartile of plasma phosphorus compared to the lowest quartile was 1.55 (95% CI 1.16-2.07).⁶ Plasma calcium was not associated with CHD, and the CHD risk associated with phosphorus did not vary by plasma calcium level. In secondary analyses of the Cholesterol And Recurrent Events (CARE) study, higher levels of plasma phosphorus (even in ranges considered normal) were associated with higher rates of cardiovascular events and death.⁷

In contrast to the Framingham Offspring Study and CARE, several recent large studies found no or inconsistent associations between plasma phosphorus and CVD. In 15,732 participants of the Atherosclerosis Risk in Communities Study (ARIC) with mean follow-up of 12.6 years, higher plasma phosphorus was associated with increased risk for stroke and death but not CHD.⁹ Although a subsequent analysis of ARIC reported that plasma phosphorus was associated with mortality in men but not women, there was no statistically significant variation by gender for associations between phosphorus and CHD.¹⁰ A secondary analysis of 7259 postmenopausal women in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial reported no associations between plasma phosphorus and incident CHD or stroke during 4 years of follow-up.¹¹

We also observed no association between plasma phosphorus and risk of incident CHD. A potentially important difference between our study and the Framingham Offspring Study and CARE is the large number of smokers in the latter studies. In Framingham Offspring, 44.8% of participants in the highest quartile of phosphorus were current smokers compared to 27.4% in the lowest quartile, and in CARE 40.1% of participants in the highest quartile of

phosphorus were current smokers compared to 6.9% in the lowest. Because current smoking is independently associated with higher levels of plasma phosphorus in a dose dependent fashion,^{9, 12} it is possible that adjusting for smoking as a binary variable without considering quantity or duration of smoking (as in Framingham Offspring and CARE) permits residual confounding by tobacco use in multivariate analyses of phosphorus and cardiovascular disease. Of note, over 50% of participants in ULSAM (a cohort in which a positive association was observed between phosphorus and cardiovascular mortality) were current smokers.²³

Study limitations

Our study has limitations. First, the upper bounds of the 95% confidence intervals of odds for incident CHD associated with FGF23 and PTH did not exclude clinically meaningful increases in risk. However, ours is the largest prospective study to date examining independent associations between these factors and incident CHD. Second, the low number of smokers in our study did not allow us to examine the potential impact of quantity and duration of tobacco use on associations between phosphorus and incident CHD. Third, because we used EDTA as a plasma preservative, we could not measure plasma calcium. It is possible that the risk of CHD associated with plasma phosphorus varies by calcium. Finally, our study population was male and predominantly white.

Conclusions

We observed no associations between plasma FGF23, intact PTH, and phosphorus and subsequent incident nonfatal MI or fatal CHD. These data suggest that plasma levels of these factors have limited or no utility as biomarkers for incident CHD in men with normal renal function.

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Table 1

Baseline characteristics of men with incident coronary heart disease and control participants during 10 years of follow-up.

Characteristic	Cases (N =422)	Controls (N =837)	P-value
Age, mean (SD), years	63.6 (8.6)	63.6 (8.6)	0.99
Current smoker, No. (%)	38 (9.0)	71 (8.5)	0.76
Body mass index, mean (SD), kg/m ²	26.1 (3.2)	25.6 (3.4)	0.02
Race/ethnicity, No. (%)			
White	397 (94.1)	792 (94.6)	0.69
Black	0 (0)	2 (0.2)	0.32
Asian	3 (0.7)	4 (0.5)	0.60
Other	22 (5.2)	39 (4.7)	0.67
Region of residence, No. (%)			
South	188 (44.6)	395 (47.2)	0.37
Northeast	134 (31.8)	288 (34.4)	0.35
Midwest	100 (23.7)	151 (18.0)	0.02
Family history of MI before age 60 years, No. (%)	67 (15.9)	92 (11.0)	0.01
Current aspirin use, $\geq$ 2/week, No. (%)	165 (39.1)	291 (34.8)	0.13
History of diabetes mellitus, No. (%)	40 (9.5)	29 (3.5)	<0.001
History of hypertension, No. (%)	159 (37.7)	244 (29.2)	0.002
Fat intake, mean (SD), % energy			
Total	30.4 (6.7)	30.2 (6.9)	0.63
Saturated fat	10.1 (2.8)	10.0 (2.9)	0.34
Marine ω -3	0.12 (0.16)	0.13 (0.20)	0.72
Alcohol consumption, median (IQR), g/d	4.5 (0-14.8)	6.8 (0.9-17.8)	0.007
Multivitamin use, No. (%)	201 (47.6)	404 (48.3)	0.83
Physical activity, median (IQR), MET-h/wk	23.1 (9.6-47.4)	25.9 (11.9-48.8)	0.14
Cholesterol, mean (SD), mg/dL			
Total	212 (39)	203 (35)	<0.001
HDL	42 (11)	46 (13)	<0.001
LDL	135 (35)	126 (31)	<0.001
Triglycerides, mean (SD), mg/dL	167 (103)	143 (102)	<0.001
Creatinine, mean (SD), mg/dL	1.0 (0.2)	1.0 (0.2)	0.84
Uric acid, mean (SD), mg/dL	5.8 (1.3)	5.8 (1.2)	0.85
25(OH)D, mean (SD), ng/mL	23.0 (7.6)	24.4 (8.4)	0.004
Phosphorus, mean (SD), mg/dL	2.8 (0.5)	2.9 (0.6)	0.20
PTH, mean (SD), pg/mL	39.0 (12.8)	38.3 (13.2)	0.36
FGF23, median (IQR), RU/mL	58.6 (47.8-73.7)	57.1 (47.4-72.2)	0.27

Abbreviations: SD, standard deviation; MI, myocardial infarction; IQR, interquartile range; MET-h, metabolic equivalent task-hours; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23.

Table 2

Correlation coefficients between plasma factors.

	PTH	Phosphorus	Creatinine	25(OH)D	Uric acid	HDL	Triglycerides
FGF23	0.11 (<0.001)	0.10 (<0.001)	0.17 (<0.001)	0.02 (0.47)	0.25 (<0.001)	-0.17 (<0.001)	0.20 (<0.001)
PTH	--	-0.02 (0.41)	0.08 (0.003)	-0.19 (<0.001)	0.20 (<0.001)	-0.10 (<0.001)	0.14 (<0.001)
Phosphorus	--	--	0.03 (0.23)	-0.02 (0.54)	-0.02 (0.40)	0.06 (0.04)	-0.03 (0.34)
Creatinine	--	--	--	0.11 (<0.001)	0.29 (<0.001)	-0.02 (0.42)	0.06 (0.03)
25(OH)D	--	--	--	--	-0.02 (0.41)	0.18 (<0.001)	-0.14 (<0.001)
Uric acid	--	--	--	--	--	-0.14 (<0.001)	0.19 (<0.001)
HDL	--	--	--	--	--	--	-0.55 (<0.001)

Note: Values are Spearman correlation coefficients. P-values are in parentheses.

Abbreviations: See Table 1.

Table 3

Multivariate odds ratios of incident coronary heart disease by quartiles of baseline FGF23, PTH, and phosphorus during 10 years of follow-up.

Biomarker	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
FGF23					
Median, RU/mL	41.3	51.8	63.6	88.4	
No. of cases	99	103	102	118	
Model 1	1.0 reference	1.04 (0.74-1.46)	1.03 (0.74-1.45)	1.19 (0.85-1.66)	0.29
Model 2	1.0 reference	1.05 (0.74-1.49)	1.00 (0.71-1.41)	1.07 (0.76-1.52)	0.74
Model 3	1.0 reference	1.02 (0.70-1.48)	0.98 (0.68-1.42)	1.03 (0.70-1.52)	0.84
PTH					
Median, pg/mL	25.2	32.2	40.6	52.1	
No. of cases	89	129	88	116	
Model 1	1.0 reference	1.45 (1.04-2.03)	0.98 (0.69-1.39)	1.31 (0.93-1.84)	0.50
Model 2	1.0 reference	1.49 (1.06-2.10)	0.98 (0.68-1.42)	1.27 (0.89-1.80)	0.70
Model 3	1.0 reference	1.53 (1.07-2.19)	0.93 (0.63-1.36)	1.20 (0.82-1.76)	0.99
Phosphorus					
Median, mg/dL	2.3	2.7	3.0	3.4	
No. of cases	131	91	97	103	
Model 1	1.0 reference	0.77 (0.56-1.08)	1.02 (0.73-1.42)	0.79 (0.57-1.08)	0.28
Model 2	1.0 reference	0.72 (0.51-1.01)	1.03 (0.73-1.45)	0.76 (0.55-1.05)	0.25
Model 3	1.0 reference	0.73 (0.51-1.04)	1.02 (0.71-1.46)	0.72 (0.51-1.02)	0.13

Model 1 is adjusted for matching factors (age, month and year of blood collection, and smoking status).

Model 2 is adjusted for matching factors (age, month and year of blood collection, and smoking status) and also for family history of MI before the age of 60 years, alcohol intake, physical activity, body mass index, race, multivitamin use, region, marine ω -3 intake, history of diabetes mellitus, history of hypertension, and fasting status.

Model 3 is adjusted for matching factors, for all the variables in Model 2, and for the following biomarkers: FGF-23, PTH, phosphorus, 25(OH)D, HDL cholesterol, LDL cholesterol, triglycerides, uric acid, and creatinine.