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ORIGINAL RESEARCH

Fibroblast Growth Factor 23 and Incident Cardiovascular Disease and Mortality in Middle-Aged Adults

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BACKGROUND: Higher circulating fibroblast growth factor 23 (FGF23) associates with greater risk of cardiovascular disease (CVD) and mortality in older adults. The association of FGF23 with cardiovascular outcomes in younger populations has been incompletely explored.

METHODS AND RESULTS: We measured C-terminal FGF23 (cFGF23) and intact FGF23 (iFGF23) in 3151 middle-aged adults (mean age, 45±4) who participated in the year 20 examination of the CARDIA (Coronary Artery Risk Development in Young Adults) study. We used separate Cox proportional hazards models to examine the associations of cFGF23 and iFGF23 with incident CVD and mortality, adjusting models sequentially for sociodemographic, clinical, and laboratory factors. A total of 157 incident CVD events and 135 deaths occurred over a median 7.6 years of follow-up (interquartile range, 4.1–9.9). In fully adjusted models, there were no statistically significant associations of FGF23 with incident CVD events (hazard ratio per doubling of cFGF23: 1.14, 95%CI 0.97,1.34; iFGF23: 0.76, 95%CI 0.57,1.02) or all-cause mortality (hazard ratio per doubling of cFGF23, 1.17; 95% CI, 1.00–1.38; iFGF23, 0.86; 95% CI, 0.64–1.17). In analyses stratified by CVD subtypes, higher cFGF23 was associated with greater risk of heart failure hospitalization (hazard ratio per doubling of cFGF23, 1.52; 95% CI, 1.18–1.96) but not coronary heart disease or stroke, whereas iFGF23 was not associated with CVD subtypes in any model.

CONCLUSIONS: In middle-aged adults with few comorbidities, higher cFGF23 and iFGF23 were not independently associated with greater risk of CVD events or death. Higher cFGF23 was independently associated with greater risk of heart failure hospitalization.

Key Words: cardiovascular disease ■ death ■ fibroblast growth factor 23 ■ heart failure ■ phosphorus

Fibroblast growth factor 23 (FGF23) is a hormone that regulates phosphorus and vitamin D metabolism. Observational studies have shown that higher circulating FGF23 is associated with greater risk of cardiovascular disease (CVD) and death independent of traditional risk factors.[1–10] Prior studies focused primarily on older adults with prevalent CVD. Relatively little is known about the association of FGF23 with CVD and death in younger, healthier adults. In the absence of studies in younger adults with few comorbidities, the utility of FGF23 as a marker of CVD risk across the age spectrum in adults is unclear.

Prior studies were also inconsistent in the assay used to measure FGF23. There are 2 main assays for measuring FGF23—one that exclusively measures the full-length, intact peptide (iFGF23) and another that detects circulating C-terminal cleavage fragments in addition to the intact peptide (cFGF23).[11] Both higher iFGF23 and cFGF23 concentrations associate with greater cardiovascular risk, but the

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CLINICAL PERSPECTIVE

What Is New?

- This study examines the association of C-terminal and intact fibroblast growth factor 23 (FGF23) with cardiovascular disease and death in middle-aged adults with few comorbid conditions.
- The results show no significant associations of either C-terminal FGF23 or intact FGF23 with cardiovascular disease or death in fully adjusted models that account for traditional risk factors and kidney function.
- C-terminal FGF23, but not intact FGF23, was associated with higher risk of incident health failure in fully adjusted models.

What Are the Clinical Implications?

- The results suggest that C-terminal FGF23 may be an independent risk factor for incident heart failure in middle-aged, healthy adults.

Nonstandard Abbreviations and Acronyms

ACR	urine albumin-to-creatinine ratio
CARDIA	Coronary Artery Risk Development in Young Adults
cFGF23	C-terminal fibroblast growth factor 23
FGF23	fibroblast growth factor 23
iFGF23	intact fibroblast growth factor 23

magnitude and strength of these associations differ by assay, generally being greater for cFGF23.[12] The importance of the observed heterogeneity in these associations has been difficult to interpret since few studies measured cFGF23 and iFGF23 in the same cohort,[1] and none did so in a general population cohort. Accordingly, we examined the association of iFGF23 and cFGF23 with incident CVD and death in participants of the CARDIA (Coronary Artery Risk Development in Young Adults) study. We hypothesized that higher FGF23 concentrations would be independently associated with greater risk of incident CVD and death in a relatively healthy cohort of middle-aged adults, and that the strength of these associations would be greater for cFGF23 as compared with iFGF23.

METHODS

Individuals interested in accessing the data set and/or study materials can do so by sending a request to the CARDIA Study Coordinating Center via contact information found on the CARDIA website (www.cardia.dopm.uab.edu).

The CARDIA study is a prospective, population-based cohort designed to examine the early determinants of CVD. The design of this study has been detailed elsewhere.[13] Briefly, between 1985 and 1986, CARDIA recruited 5115 Black or White individuals between the ages of 18 and 30 years from 4 sites in the United States (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). A total of 8 follow-up examinations were conducted at years 2, 5, 7, 10, 15, 20, 25, and 30. All participants provided informed consent, and the institutional review boards at each participating center approved the study.

Primary Exposure

The primary exposure was FGF23. Given uncertainty as to which assay is best to use for examining CVD and mortality outcomes, both cFGF23 and iFGF23 were measured at the baseline visit (year 20) in the University of Miami Biomarker and Immunoassay Laboratory using established ELISAs (Quidel, intra-assay coefficients of variation <3%).

Outcomes

The primary outcomes were incident CVD events (fatal and nonfatal coronary heart disease [CHD], fatal and nonfatal stroke, hospitalization for heart failure) and all-cause mortality through August 31, 2018. In secondary analyses, we also examined individual CVD event types separately. The methods used to capture CVD events have been reviewed previously.[14–16] Briefly, incident CVD events and deaths were captured through follow-up study examinations or yearly interviews by telephone, mail, or electronically with each participant or designated proxy. During these contacts, participants or proxies were asked about interim hospital admissions, outpatient procedures, or deaths. Medical records were requested for hospitalizations and procedures. In addition, vital status was assessed every 6 months, and medical and other death records were requested as needed after consent was provided by the next of kin. Two physician members of the Endpoints Committee reviewed medical records to adjudicate each possible CVD event or underlying cause of death using specific definitions. If disagreements occurred, the full committee reviewed the case.

Covariates

Covariates were obtained from the year 20 examination, the baseline for this analysis. Age, sex, income, education level, smoking habits, and medication use

were ascertained through standardized questionnaires. Physical activity was determined through a questionnaire and reported as exercise units.[17,18] Diabetes mellitus status was defined as a fasting glucose ≥126 mg/dL or use of diabetic medications. Height and weight were measured with participants wearing light clothing and no shoes, and body mass index (BMI) was calculated as kg/m^2. After resting for 5 minutes, systolic and diastolic blood pressure were measured using a standard automated blood pressure measurement monitor calibrated to a random-zero sphygmomanometer. A total of 3 measurements were obtained, with the average of the second and third measurements used. High-density lipoprotein cholesterol was determined by precipitation with dextran sulfate–magnesium chloride,[19] and total cholesterol and triglycerides were determined enzymatically.[20] We estimated the glomerular filtration rate (eGFR) using the most recent Chronic Kidney Disease Epidemiology Collaboration serum creatinine equation.[21] Urine albumin was measured by nephelometry, and urine creatinine was assessed using the Jaffe method in a single untimed (spot) urine sample collected at the year 20 examination. The urine albumin-to-creatinine ratio (ACR) was expressed as milligrams of albumin per gram of creatinine.

Statistical Analysis

Descriptive statistics were used to compare participant characteristics within the cohort overall and across quartiles of cFGF23 and iFGF23, separately. Incidence rates for CVD events and mortality were calculated by quartiles of cFGF23 and iFGF23. After testing for violation of the proportional hazards assumption, Cox proportional hazards models were used to estimate the hazard ratio of incident CVD events and all-cause mortality as a function of cFGF23 and iFGF23 in sequential, multivariable-adjusted models. In all models, FGF23 was analyzed in quartiles, with the lowest quartile serving as the reference group. In addition, FGF23 was analyzed on a continuous scale after log base 2 transformation (interpreted as "per doubling" of FGF23) since FGF23 was not normally distributed. Age, sex, race, and educational attainment (highest level of education) were adusted in model 1. Model 2 adjusted for variables in model 1 plus diabetes mellitus, smoking status (former, current, never), physical activity score, BMI, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, antihypertensive drug use, and statin use. Model 3 further adjusted for eGFR and ACR. In secondary analyses, we examined the association of cFGF23 and iFGF23 with incident CHD, stroke, and hospitalization for heart failure, separately, using a similar modeling strategy. We examined for effect modification by sex, race, and chronic kidney disease (eGFR <60 mL/min per 1.73 m^2) by testing the statistical significance of a multiplicative interaction term in the models (modeling FGF23 as a categorical variable in quartiles). To capture potentially nonlinear associations of FGF23 with outcomes of interest, Cox regression models with restricted quadratic splines were used to model the associations of FGF23 with risk of incident CVD events and mortality in fully adjusted models. A 2-tailed P value <0.05 was considered statistically significant for all analyses.

RESULTS

Study Population Characteristics

A total of 3404 CARDIA participants had FGF23 concentrations measured at the year 20 visit. After excluding 54 individuals with prevalent CVD at baseline and 199 participants missing comorbidity or laboratory data, a total of 3151 participants were included in the final analytic sample. The median iFGF23 concentration was 66 (interquartile range, 54–86) pg/mL and the median cFGF23 concentration was 79 (interquartile range, 63–111) RU/mL, similar to what has been reported in prior population-based studies. Baseline characteristics of study participants in the cohort overall and stratified by quartiles of baseline cFGF23 concentrations are depicted in Table 1. Characteristics by baseline iFGF23 concentrations are shown in Table S1. In general, participants in the highest quartile of cFGF23 were more likely to be Black women; have a higher BMI; be current smokers; and have lower physical activity, a history of diabetes mellitus and hypertension, lower mean eGFR, and higher median urine ACR. Participants in the highest quartile of iFGF23 were more likely to be White women (46% were men), , and have higher BMI, lower total cholesterol, lower high-density lipoprotein cholesterol, a history of never smoking, more years of education, diabetes mellitus, hypertension, and lower eGFR relative to those in lower quartiles. The correlation between iFGF23 and cFGF23 concentrations was modest (ρ, 0.26; P<0.001).

Association of cFGF23 With Incident CVD

A total of 157 incident CVD events were observed over a median of 7.14 (interquartile range, 2.71–9.23) years of follow-up. Table 2 depicts associations of baseline cFGF23 concentrations with incident CVD. The crude incidence rate of CVD was greater with higher quartiles of cFGF23. In models adjusted for age, sex, race, and educational achievement, higher cFGF23 concentrations were associated with greater risk of incident CVD (Table 2: cFGF23 quartile 4

Table 1. Baseline Characteristics of the Study Population by Quartiles of C-Terminal Fibroblast Growth Factor 23 Concentrations

	Overall	Quartile 1	Quartile 2	Quartile 3	Quartile 4
		(<63.2 RU/mL)	(63.2–79.7 RU/mL)	(79.8–110.4 RU/mL)	(>10.4 RU/mL)
N	3152	785	787	789	791
Age, y, mean (SD)	45.2 (3.6)	45.1 (3.6)	45.3 (3.6)	45.2 (3.6)	45.2 (3.7)
Male sex, %	44	55	53	46	25
Black race, %	46	44	43	42	54
BMI, kg/m^2, mean (SD)	29.3 (6.8)	27.7 (5.6)	28.5 (6.0)	29.8 (6.7)	31.2 (8.1)
SBP, mm Hg, mean (SD)	115.6 (14.4)	115.0 (13.1)	114.9 (13.3)	115.7 (14.8)	116.8 (16.1)
DBP, mm Hg, mean (SD)	72.1 (11.0)	71.0 (10.4)	71.3 (10.7)	72.2 (10.8)	73.9 (11.8)
Total cholesterol, mg/dL, mean (SD)	186.1 (34.7)	183.9 (32.1)	188.6 (36.3)	187.4 (35.2)	184.5 (34.9)
HDL-C, mg/dL, mean (SD)	54.3 (16.6)	55.9 (16.3)	54.3 (17.1)	52.9 (16.2)	54.1 (16.7)
Highest level of education, y, mean (SD)	15.5 (2.5)	15.6 (2.6)	15.7 (2.6)	15.6 (2.5)	15.2 (2.4)
Smoking, %					
None	61.4	64.3	65.1	59.6	56.8
Former	19.5	19.9	19.4	20.7	18.0
Current	19.1	15.8	15.5	19.8	25.2
Physical activity intensity score, median (IQR)	279 (129–492)	325 (170–532)	312 (147–525)	273 (125–504)	220 (86–424)
Comorbidities, %					
Diabetes mellitus	8.1	5.3	5.6	8.1	13.4
Hypertension	20.4	16.3	16.5	18.6	30.2
eGFR, mL/min per 1.73m^2, mean (SD)	98.5 (17.0)	100.0 (15.8)	98.2 (16.5)	97.1 (16.3)	98.7 (18.9)
<60 mL/min per 1.73m^2, %	0.70	0.25	0.25	0.25	2.02
ACR, mg/g, median (IQR)	4.5 (3.1–7.6)	4.3 (3.0–6.9)	4.2 (2.9–6.6)	4.4 (3.1–7.4)	5.4 (3.5–10.7)
≥30 mg/g, %	5.8	4.3	4.3	4.7	9.7

ACR indicates albumin-to-creatinine ratio; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; RU, relative unit; and SBP, systolic blood pressure.

compared with quartile 1; hazard ratio [HR], 1.85; 95% CI, 1.13–3.02). Following further adjustment for diabetes mellitus, smoking status, physical activity score, BMI, systolic blood pressure, lipids, antihypertensive drug use, and statin use, the associations were attenuated and no longer statistically significant (HR comparing quartile 4 to quartile 1, 1.08; 95% CI, 0.67–1.74). Systolic blood pressure was the variable most responsible for the attenuation of the effect estimate. Adjustment for eGFR and ACR attenuated the effect estimates further (model 3 in Table 2). When analyzed on a continuous scale, there was no

Table 2. Hazard Ratio (95% CI) of Incident Cardiovascular Disease Events as a Function of Baseline C-Terminal FGF23 Concentrations

	Events	Crude IR (95% CI)*	Model 1	Model 2	Model 3
FGF23 categories					
<63.2 RU/mL	35	3.9 (2.8–5.4)	reference	reference	reference
63.2–79.7 RU/mL	34	3.8 (2.7–5.3)	1.00 (0.62–1.61)	0.92 (0.57–1.48)	0.89 (0.55–1.44)
79.8–110.4 RU/mL	39	4.3 (3.1–5.9)	1.18 (0.75–1.87)	0.91 (0.57–1.46)	0.87 (0.54–1.40)
>110.4 RU/mL	49	5.5 (4.2–7.3)	1.65 (1.05–2.58)	1.08 (0.67–1.74)	0.99 (0.61–1.60)
P for trend†			0.01	0.54	0.81
Per doubling of FGF23			1.30 (1.12–1.50)	1.17 (1.00–1.38)	1.14 (0.97–1.34)

FGF23 indicates fibroblast growth factor 23; IR, incidence rate; and RU, relative unit.

Model 1 is adjusted for age, sex, race, and educational attainment.

Model 2 is adjusted for variables in model 1 plus smoking status (former, current, never), physical activity, body mass index, diabetes mellitus, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, and statin use.

Model 3 is adjusted for variables in model 2 plus estimated glomerular filtration rate and urine albumin-to-creatinine ratio.

*Per 1000 person-years of follow-up.

†*P* for linear trend in Cox regression models.

statistically significant association of higher cFGF23 concentrations with incident CVD in the fully adjusted model (HR per doubling of cFGF23, 1.14; 95% CI, 0.97–1.34). Similar findings were observed in fully adjusted spline models (Figure [A]).

Table 3 depicts associations of cFGF23 with incident CVD subtypes (CHD, stroke, and heart failure). There were no significant associations of cFGF23 with incident CHD or stroke in fully adjusted multivariable models. In contrast, each doubling of cFGF23 was associated with a 52% (95% CI, 1.18–1.96) higher risk of hospitalization for heart failure in the fully adjusted model.

Associations of cFGF23 With All-Cause Mortality

Table 4 depicts associations of baseline cFGF23 concentrations with all-cause mortality. Higher cFGF23 concentrations were associated with greater risk of all-cause mortality in models adjusted for age, sex, race, educational attainment, and clinical factors (HR per doubling of FGF23, 1.18; 95% CI, 1.10–1.39), but the association was no longer statistically significant in the fully adjusted model that included eGFR and ACR (HR per doubling of FGF23, 1.17; 95% CI, 1.00–1.38). The associations did not differ in fully adjusted spline models (Figure [B]). The association of cFGF23 quartiles with all-cause mortality differed by race ($P_{interaction}$<0.05), but the magnitude of the difference was small (Table S2).

Associations of iFGF23 With Outcomes

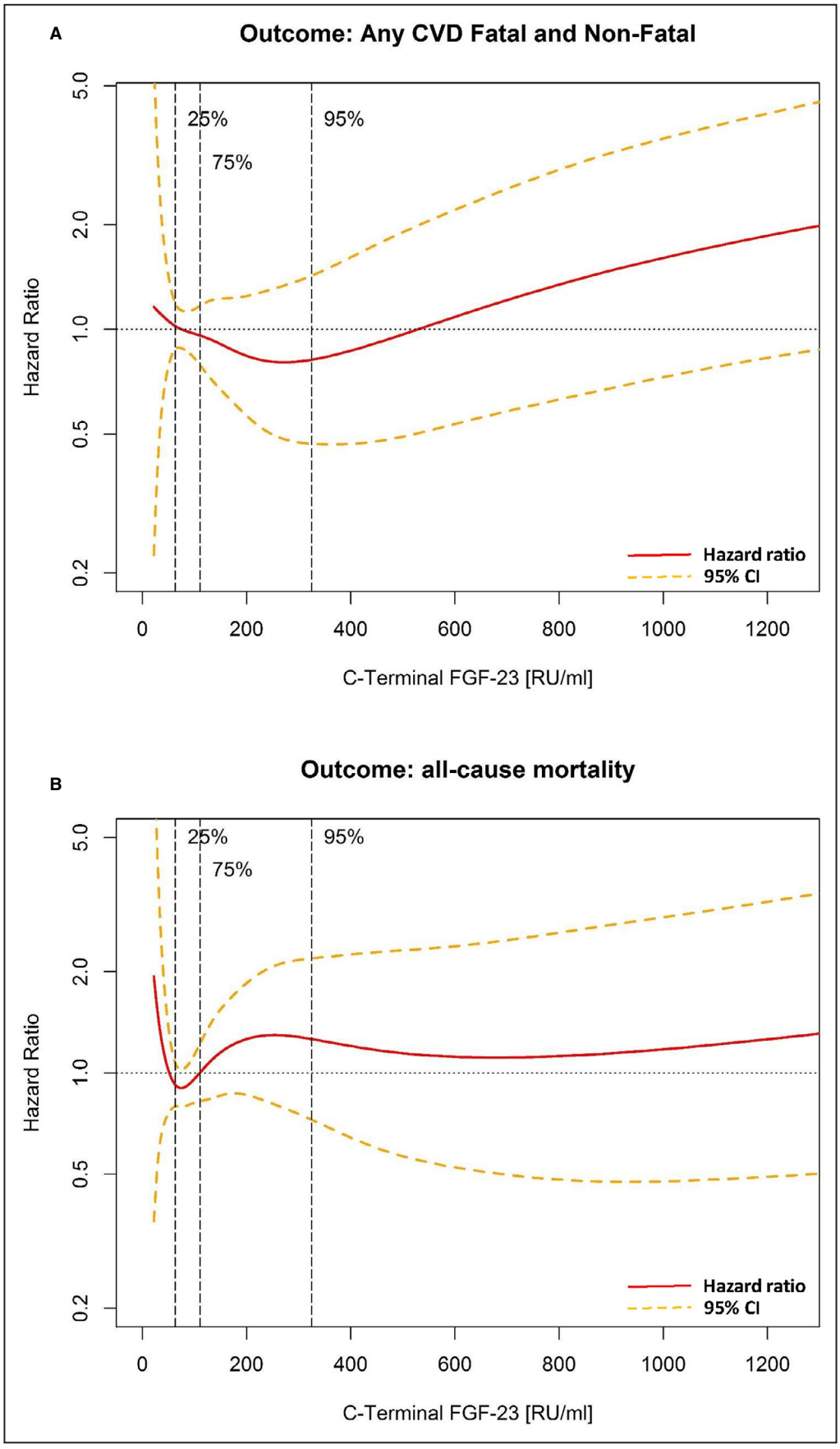
iFGF23 was not associated with overall incident CVD events, specific CVD events, or all-cause mortality (Tables S3 through S5, Figure S1). There was no significant effect modification by sex, race, or chronic kidney disease status when modeling cFGF23 or iFGF23 as categorical variables (*P* for interaction ≥0.10 for all).

DISCUSSION

In this study of relatively healthy middle-aged adults, higher FGF23 (cFGF23 or iFGF23) was not consistently associated with greater risk of incident CVD events or mortality in multivariable models adjusted for traditional risk factors and kidney function. In secondary analyses examining CVD subtypes, higher cFGF23, but not iFGF23, was independently associated with greater risk of heart failure hospitalization.

Our findings contrast with multiple prior studies showing robust associations of FGF23 with incident CVD and death in population-based cohorts.[2–4,6–8] There are important differences in the CARDIA study population as compared with these prior studies that may account for these discrepancies. Whereas most of the prior studies focused on older individuals with prevalent CVD risk factors, we examined middle-aged adults (mean age 45) who were free of CVD and had mostly preserved kidney function at their baseline visits. This suggests that higher FGF23 concentrations may be an independent risk factor for CVD events and death in high-risk populations (older individuals with comorbidities), but not in younger, healthier adults. In support of this, a prior study of Framingham offspring participants with a mean age of 58, preserved kidney function, and no baseline CVD showed no statistically significant associations of cFGF23 with incident CVD events in multivariable models adjusted for established risk factors, though a significant association of higher cFGF23 with all-cause mortality was observed.[5] In contrast, another study of middle-aged adults (mean age, 50) free of CVD participating in the European Prospective Investigation of Cancer–Germany cohort showed that higher cFGF23 was independently associated with greater risk of myocardial infarction, stroke, and death.[8] The inconsistency in these results suggests that FGF23 may not be a useful marker of CVD risk in middle-aged adults free of preexisting CVD. Nonetheless, it is notable that systolic blood pressure was the variable most responsible for the attenuation of the association of cFGF23 with cardiovascular events. As prior studies showed that FGF23 associates with the development of hypertension,[22,23] possibly by inducing sodium retention,[24] it is possible that elevated systolic blood pressure may be in the causal pathway between cFGF23 and CVD, partly explaining why cFGF23 was not associated with incident CVD events in multivariable models.

The finding that higher cFGF23 was associated with greater risk of incident heart failure hospitalizations, but not incident CHD or stroke events, is consistent with prior studies showing that higher cFGF23 is a risk factor for heart failure but not atherosclerotic disease events.[3,10] Experimental data have shown that FGF23 can directly induce cardiomyocyte hypertrophy and increase left ventricular mass, supporting the biologic plausibility of an association of cFGF23 with heart failure, but not atherosclerotic disease.[25–27] However, the significant association of cFGF23 with heart failure was not seen for iFGF23. The reasons for this are unclear, but may be related to posttranslational FGF23 processing. Both iron deficiency and inflammation have been shown to increase the transcription of *FGF23*, but this is normally coupled to an increase in the cleavage of the intact peptide, resulting in high circulating cFGF23 concentrations (representing high C-terminal fragments and normal intact peptide) but normal or only slightly elevated iFGF23 (representing just the intact peptide).[11] As such, it is conceivable that elevated cFGF23, but not iFGF23, is


A
Outcome: Any CVD Fatal and Non-Fatal
25%
75%
95%
Hazard Ratio
5.0
2.0
1.0
0.5
0.2
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200
400
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800
1000
1200
C-Terminal FGF-23 [RU/ml]
Hazard ratio
95% CI
B
Outcome: all-cause mortality
25%
75%
95%
Hazard Ratio
5.0
2.0
1.0
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0
200
400
600
800
1000
1200
C-Terminal FGF-23 [RU/ml]
Hazard ratio
95% CI

Figure. Hazard ratios (95% CI) of incident cardiovascular disease (CVD) events (A) and mortality (B) as a function of C-terminal fibroblast growth factor 23 (cFGF23; expressed as relative units per milliliter [RU/mL]).
The models are using restricted quadratic splines adjusted for age, sex, race, and educational attainment, smoking status (former, current, never), physical activity, body mass index, diabetes mellitus, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein cholesterol, statin use, estimated glomerular filtration rate, and urine albumin-to-creatinine ratio. Vertical dashed lines represent 25th, 75th, and 95th percentile of cFGF23 distribution.

associated with heart failure because it is a sensitive marker of iron deficiency, inflammation, or other factors that equally stimulate FGF23 transcription and cleavage. Alternatively, it is possible that high circulating levels of C-terminal fragments could have currently unknown effects that influence the risk of heart failure. These data underscore the importance of accounting for the FGF23 assay used when interpreting the association of FGF23 with heart failure risk in observational studies. They also highlight the potential importance of examining heart failure events separate from atherosclerotic disease events (CHD, stroke) when assessing the association of FGF23 with cardiovascular outcomes.

Strengths of our study include the use of a large biracial cohort with extensive data on confounding variables, detailed cardiovascular phenotyping, and measurements of both cFGF23 and iFGF23. Our study also had limitations. We had relatively few events, and the CIs in the fully adjusted models were relatively wide, indicating that a statistically significant association may have been missed because of lack of adequate statistical power to detect more modest associations. We did not have measures of iron status or inflammation and so could not determine whether these factors were important confounders or in the causal pathway linking cFGF23 with heart failure or explained the relatively modest association between iFGF23 and cFGF23

Table 3. Hazard Ratio (95% CI) of Individual Cardiovascular Disease Subtypes as a Function of Baseline C-Terminal FGF23 Concentrations

	Events	Crude IR (95% CI)*	Model 1	Model 2	Model 3
Coronary heart disease					
FGF23 categories					
<63.2 RU/mL	22	2.4 (1.6–3.7)	reference	reference	reference
63.2–79.7 RU/mL	15	1.6 (1.0–2.7)	0.68 (0.35–1.31)	0.69 (0.40–1.30)	0.66 (0.34–1.27)
79.8–110.4 RU/mL	21	2.3 (1.5–3.5)	1.02 (0.56–1.86)	0.87 (0.47–1.60)	0.81 (0.44–1.50)
>110.4 RU/mL	24	2.7 (1.8–4.0)	1.56 (0.86–2.83)	1.19 (0.64–2.19)	1.00 (0.53–1.88)
P for trend†			0.03	0.27	0.58
Per doubling of FGF23			1.43 (1.17–1.75)	1.32 (1.06–1.64)	1.22 (0.98–1.51)
Stroke					
FGF23 categories					
<63.2 RU/mL	10	1.1 (0.6–2.0)	ref	ref	ref
63.2–79.7 RU/mL	16	1.8 (1.1–2.9)	1.61 (0.73–3.56)	1.67 (0.75–3.71)	1.60 (0.72–3.56)
79.8–110.4 RU/mL	11	1.2 (0.7–2.2)	1.11 (0.47–2.62)	0.80 (0.33–1.94)	0.73 (0.30–1.78)
>110.4 RU/mL	21	2.3 (1.5–3.6)	1.92 (0.88–4.18)	1.25 (0.55–2.84)	1.15 (0.50–2.62)
P for trend†			0.13	0.81	0.94
Per doubling of FGF23			1.31 (1.07–1.61)	1.20 (0.96–1.51)	1.18 (0.95–1.47)
Heart failure					
FGF23 categories					
<63.2 RU/mL	5	0.5 (0.2–1.3)	ref	ref	ref
63.2–79.7 RU/mL	6	0.7 (0.3–1.5)	1.31 (0.40–4.30)	1.23 (0.37–4.06)	1.20 (0.36–3.95)
79.8–110.4 RU/mL	8	0.9 (0.4–1.7)	1.91 (0.62–5.85)	1.62 (0.52–5.05)	1.47 (0.47–4.64)
>110.4 RU/mL	14	1.5 (0.9–2.6)	3.98 (1.39–11.40)	2.90 (0.98–8.64)	2.66 (0.89–7.95)
P for trend†			0.002	0.03	0.04
Per doubling of FGF23			1.63 (1.33–2.01)	1.56 (1.25–1.96)	1.52 (1.18–1.96)

FGF23 indicates fibroblast growth factor 23; IR, incidence rate; and RU, relative unit.
Model 1 is adjusted for age, sex, and race.
Model 2 is adjusted for variables in model 1 plus smoking status (former, current, never), body mass index, diabetes mellitus, and systolic blood pressure.
Model 3 is adjusted for variables in model 2 plus estimated glomerular filtration rate and urine albumin-to-creatinine ratio.
*Per 1000 person-years of follow-up.
†*P* for linear trend in Cox regression models.

Table 4. Hazard Ratio (95% CI) of All-Cause Mortality as a Function of Baseline C-Terminal FGF23 Concentrations

	Events	Crude IR (95%CI)*	Model 1	Model 2	Model 3
FGF23 categories					
<63.2 RU/mL	33	3.6 (2.6–5.1)	reference	reference	reference
63.2–79.7 RU/mL	29	3.2 (2.2–4.5)	0.87 (0.53–1.44)	0.88 (0.53–1.46)	0.88 (0.53–1.46)
79.8–110.4 RU/mL	24	2.6 (1.7–3.9)	0.74 (0.44–1.26)	0.69 (0.40–1.18)	0.70 (0.41–1.20)
>110.4 RU/mL	49	5.4 (4.0–7.1)	1.64 (1.04–2.59)	1.32 (0.82–2.13)	1.27 (0.79–2.06)
P for trend†			0.004	0.06	0.11
Doubling of FGF23			1.29 (1.11–1.50)	1.18 (1.01–1.39)	1.17 (1.00–1.38)

FGF23 indicates fibroblast growth factor 23; IR, incidence rate; and RU, relative unit.

Model 1 is adjusted for age, sex, race, and educational attainment.

Model 2 is adjusted for variables in model 1 plus smoking status (former, current, never), physical activity, body mass index, diabetes mellitus, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein-cholesterol, and statin use.

Model 3 is adjusted for variables in model 2 plus estimated glomerular filtration rate and urine albumin-to-creatinine ratio.

*Per 1000 person-years of follow-up.

†*P* for linear trend in Cox regression models.

concentrations. We analyzed single measurements of FGF23 at the year 20 examination, so we could not determine whether changes in FGF23 more strongly associate with incident CVD events or deaths.[28] The inclusion of only White and Black participants may limit the generalizability of these results to other races and ethnicities in the United States.

In summary, in middle-aged, community-dwelling adults free of CVD, FGF23 was not consistently associated with incident CVD events or death in multivariable adjusted models that accounted for kidney function. We did observe an association of cFGF23 with heart failure events, suggesting that cFGF23 may have some prognostic value for heart failure risk in this population.

ARTICLE INFORMATION

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Supplementary Material

Tables S1–S5
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of the study population by quartiles of intact fibroblast growth factor 23 concentrations.

	Overall	Quartile 1 (< 53.8 pg/ml)	Quartile 2 (53.8-65.5 pg/ml)	Quartile 3 (65.6-86.3 pg/ml)	Quartile 4 (> 86.3 pg/ml)
N	3152	784	784	792	792
Age, mean (SD)	45.2 (3.6)	45.1 (3.6)	45.3 (3.6)	45.2 (3.6)	45.1 (3.7)
Male sex, %	44.4	38.8	42.7	49.9	46.3
Black, %	45.7	49.2	48.1	45.3	40.4
BMI, kg/m², mean (SD)	29.4 (7.1)	28.5 (6.4)	29.0 (6.6)	30.0 (7.9)	29.9 (7.2)
SBP, mmHg, mean (SD)	115.6 (14.4)	115.6 (14.7)	116.5 (14.8)	115.4 (13.5)	114.9 (14.5)
DBP, mmHg, mean (SD)	72.1 (11.0)	72.1 (11.2)	73.0 (11.1)	71.8 (10.5)	71.5 (11.0)
Total cholesterol, mg/dL, mean (SD)	186.1 (34.7)	187.0 (34.6)	187.0 (34.3)	186.7 (35.1)	183.7 (34.8)
HDL-C, mg/dL, mean (SD)	54.3 (16.6)	55.5 (16.3)	55.0 (16.9)	53.8 (16.6)	52.9 (16.6)
Highest level of education, y, mean (SD)	15.5 (2.5)	15.3 (2.5)	15.5 (2.5)	15.5 (2.5)	15.8 (2.6)
Smoking, %					
None	61.4	56.2	57.1	61.9	70.3
Former	19.5	20.2	21.1	18.4	18.4
Current	19.1	23.6	21.8	19.7	11.3
Physical activity intensity score, median (IQR)	279 (129-492)	276 (128-491)	288 (143-514)	281 (127-493)	276 (120-481)
Co-morbidities,%					
Diabetes	8.1	7.5	6.2	8.3	10.3
Hypertension	20.4	19.3	21.0	20.3	21.1
eGFR, ml/min/1.73m2, mean (SD)	98.5 (17.0)	101.2 (16.9)	98.9 (16.8)	97.2 (16.4)	96.6 (17.4)
< 60 ml/min/1.73m2, %	0.70	0.26	0.64	0.63	1.26
ACR, mg/g, median (IQR)	4.5 (3.1-7.6)	4.6 (3.4-8.1)	4.6 (3.2-7.4)	4.4 (3.0-7.3)	4.4 (2.9-7.4)
≥ 30 mg/g, %	5.8	7.0	4.3	5.9	5.8

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio

Table S2. Hazard ratio (95% confidence interval) of all-cause mortality as a function of c-terminal fibroblast growth factor 23 concentrations stratified by race.

	Model 1	Model 2	Model 3
White			
FGF23 Categories			
< 63.2 RU/ml	ref	ref	ref
63.2-79.7 RU/ml	0.44 (0.18-1.08)	0.42 (0.17-1.03)	0.44 (0.18-1.09)
79.8-110.4 RU/ml	1.05 (0.52-2.12)	0.93 (0.46-1.88)	0.92 (0.45-1.87)
> 110.4 RU/ml	1.79 (0.91-3.51)	1.52 (0.77-3.00)	1.37 (0.69-2.74)
Black			
FGF23 Categories			
< 63.2 RU/ml	ref	ref	ref
63.2-79.7 RU/ml	1.70 (0.84-3.43)	1.71 (0.85-3.44)	1.73 (0.85-3.51)
79.8-110.4 RU/ml	0.61 (0.24-1.54)	0.62 (0.25-1.56)	0.64 (0.25-1.64)
> 110.4 RU/ml	1.75 (0.87-3.53)	1.49 (0.74-2.98)	1.33 (0.65-2.71)

Model 1 is adjusted for age, sex, and educational attainment
Model 2 is adjusted for variables in Model 1 plus smoking status (former, current, never), physical activity, body mass index, diabetes, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein-cholesterol, and statin use.
Model 3 is adjusted for variables in Model 2 plus estimated glomerular filtration rate and urine albumin to creatinine ratio.

Table S3. Hazard ratio (95% confidence interval) of incident cardiovascular disease events as a function of baseline intact fibroblast growth factor 23 concentrations.

	Events	Crude IR (95%CI)*	Model 1	Model 2	Model 3
FGF23 Categories					
< 53.8 pg/ml	46	5.2 (3.9-6.9)	ref	ref	ref
53.8-65.5 pg/ml	33	3.7 (2.6-5.2)	0.69 (0.44-1.07)	0.71 (0.45-1.11)	0.65 (0.41-1.02)
65.6-86.3 pg/ml	46	5.1 (3.8-6.8)	0.95 (0.63-1.43)	0.96 (0.64-1.45)	0.93 (0.61-1.40)
> 86.3 pg/ml	32	3.5 (2.5-5.0)	0.70 (0.45-1.10)	0.76 (0.48-1.20)	0.71 (0.45-1.13)
P for trend			0.27	0.43	0.34
per doubling of FGF23			0.82 (0.63-1.08)	0.86 (0.65-1.13)	0.82 (0.62-1.08)

* per 1000 person-years of follow-up

Model 1 is adjusted for age, sex, race, and educational attainment

Model 2 is adjusted for variables in Model 1 plus smoking status (former, current, never), physical activity, body mass index, diabetes, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein-cholesterol, and statin use.

Model 3 is adjusted for variables in Model 2 plus estimated glomerular filtration rate and urine albumin to creatinine ratio.

Table 4. Hazard ratio (95% confidence interval) of individual cardiovascular disease subtypes as a function of baseline intact fibroblast growth factor 23 concentrations.

	Events	Crude IR (95%CI)*	Model 1	Model 2	Model 3
Coronary heart disease					
FGF23 Categories					
< 53.8 pg/ml	16	1.8 (1.1-2.9)	ref	ref	ref
53.8-65.5 pg/ml	12	1.3 (0.7-2.3)	0.70 (0.33-1.48)	0.71 (0.34-1.50)	0.68 (0.32-1.44)
65.6-86.3 pg/ml	32	3.5 (2.5-5.0)	1.75 (0.96-3.19)	1.85 (1.01-3.38)	1.71 (0.93-3.15)
> 86.3 pg/ml	22	2.4 (1.6-3.7)	1.23 (0.64-2.34)	1.39 (0.72-2.67)	1.21 (0.62-2.35)
P for trend			0.3	0.15	0.33
per doubling of FGF23			1.04 (0.75-1.45)	1.10 (0.79-1.53)	0.99 (0.71-1.37)
Stroke					
FGF23 Categories					
< 53.8 pg/ml	20	2.2 (1.4-3.5)	ref	ref	ref
53.8-65.5 pg/ml	15	1.7 (1.0-2.7)	0.73 (0.38-1.44)	0.70 (0.36-1.37)	0.64 (0.32-1.27)
65.6-86.3 pg/ml	13	1.4 (0.8-2.4)	0.65 (0.32-1.32)	0.70 (0.34-1.40)	0.62 (0.30-1.27)
> 86.3 pg/ml	10	1.1 (0.6-2.0)	0.54 (0.25-1.15)	0.53 (0.25-1.14)	0.47 (0.21-1.02)
P for trend			0.13	0.13	0.08
per doubling of FGF23			0.66 (0.40-1.07)	0.64 (0.39-1.06)	0.58 (0.34-0.97)
Heart Failure					
FGF23 Categories					
< 53.8 pg/ml	8	0.9 (0.4-1.8)	ref	ref	ref
53.8-65.5 pg/ml	10	1.1 (0.6-2.0)	1.22 (0.48-3.09)	1.18 (0.46-2.99)	1.10 (0.43-2.80)
65.6-86.3 pg/ml	8	0.9 (0.4-1.7)	0.91 (0.34-2.42)	0.93 (0.34-2.48)	0.83 (0.31-2.26)
> 86.3 pg/ml	7	0.8 (0.4-1.6)	0.90 (0.33-2.50)	0.84 (0.30-2.35)	0.74 (0.27-2.04)
P for trend			0.70	0.62	0.51
per doubling of FGF23			0.96 (0.55-1.65)	0.92 (0.53-1.60)	0.86 (0.50-1.49)

* per 1000 person-years of follow-up

Model 1 is adjusted for age, sex, race

Model 2 is adjusted for variables in Model 1 plus smoking status (former, current, never), body mass index, diabetes, systolic blood pressure.

Model 3 is adjusted for variables in Model 2 plus estimated glomerular filtration rate and urine albumin to creatinine ratio.

Table S5. Hazard ratio (95% confidence interval) of all-cause mortality as a function of baseline intact fibroblast growth factor 23 concentrations.

	Events	Crude IR (95%CI)*	Model 1	Model 2	Model 3
FGF23 Categories					
< 63.2 RU/ml	46	5.1 (3.8-6.8)	ref	ref	ref
63.2-79.7 RU/ml	29	3.2 (2.2-4.6)	0.58 (0.36-0.93)	0.61 (0.38-0.97)	0.61 (0.38-0.98)
79.8-110.4 RU/ml	35	3.8 (2.7-5.3)	0.72 (0.46-1.11)	0.74 (0.47-1.15)	0.75 (0.48-1.18)
> 110.4 RU/ml	25	2.7 (1.8-4.0)	0.53 (0.33-0.86)	0.59 (0.36-0.97)	0.55 (0.33-0.92)
P for trend			0.03	0.08	0.05
doubling of FGF23			0.83 (0.62-1.11)	0.88 (0.66-1.19)	0.86 (0.64-1.17)

* per 1000 person-years of follow-up

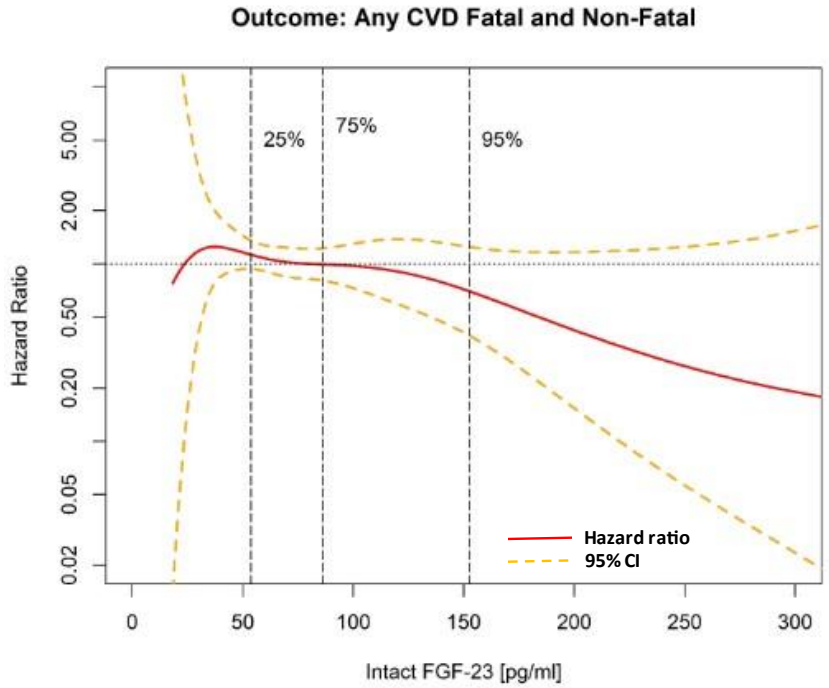
Model 1 is adjusted for age, sex, race, and educational attainment

Model 2 is adjusted for variables in Model 1 plus smoking status (former, current, never), physical activity, body mass index, diabetes, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein-cholesterol, and statin use.

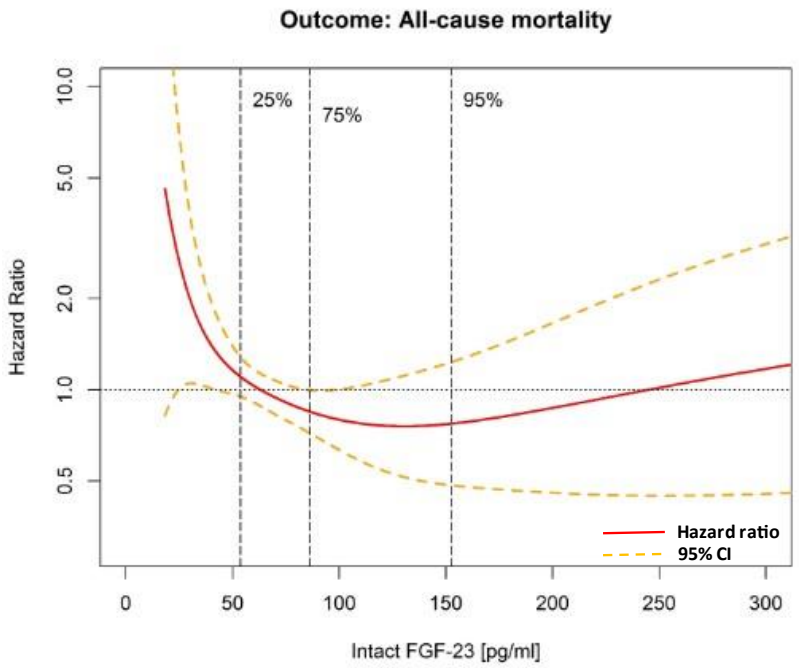
Model 3 is adjusted for variables in Model 2 plus estimated glomerular filtration rate and urine albumin to creatinine ratio.

Figure S1. Hazard ratios (95% confidence interval) of incident cardiovascular disease (CVD) events (panel A) and death (panel B) as a function of intact fibroblast growth factor 23 (expressed as picograms per milliliter [pg/ml]).

A



B



The models are using restricted quadratic splines adjusted for age, sex, race, and educational attainment, smoking status (former, current, never), physical activity, body mass index, diabetes, systolic blood pressure, antihypertensive drug use, total cholesterol, high-density lipoprotein-cholesterol, statin use, estimated glomerular filtration rate and urine albumin to creatinine ratio. Vertical dashed lines represent 25^{th}, 75^{th}, and 95^{th} percentile of cFGF23 distribution.