

Serum Fibroblast Growth Factor-23 Is Associated with Incident Kidney Disease

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ABSTRACT

Fibroblast growth factor-23 is a bone-derived hormone that increases urinary phosphate excretion and inhibits hydroxylation of 25-hydroxyvitamin D. Recent studies suggest that fibroblast growth factor-23 may be an early biomarker of CKD progression. However, its role in kidney function decline in the general population is unknown. We assessed the relationship between baseline (1990–1992) serum levels of intact fibroblast growth factor-23 and incident ESRD in 13,448 Atherosclerosis Risk in Communities study participants (56.1% women, 74.7% white) followed until December 31, 2010. At baseline, the mean age of participants was 56.9 years and the mean eGFR was 97 ml/min per 1.73 m². During a median follow-up of 19 years, 267 participants (2.0%) developed ESRD. After adjustment for demographic characteristics, baseline eGFR, traditional CKD risk factors, and markers of mineral metabolism, the highest fibroblast growth factor-23 quintile (>54.6 pg/ml) compared with the lowest quintile (<32.0 pg/ml) was associated with risk of developing ESRD (hazard ratio, 2.10; 95% confidence interval, 1.31 to 3.36; trend $P < 0.001$). In a large, community-based study comprising a broad range of kidney function, higher baseline fibroblast growth factor-23 levels were associated with increased risk of incident ESRD independent of the baseline level of kidney function and a number of other risk factors.

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CKD is a global public health problem due to its high prevalence and strong association with cardiovascular disease, ESRD, and mortality.^{1–4} Exploring novel biomarkers for risk of future CKD, particularly among those with relatively preserved kidney function, may contribute to understanding of CKD pathogenesis and new targets for therapy. Fibroblast growth factor-23 (FGF-23) was recently proposed as an early indicator of kidney injury.^{5,6}

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FGF-23 is a 32-kD bone-derived hormone with several endocrine functions in the kidney, including the promotion of urinary phosphate excretion and the inhibition of the hydroxylation of 25-hydroxyvitamin D, to reduce the production of the active 1,25-dihydroxyvitamin D.⁷ Recent studies suggest that FGF-23 is a risk factor for cardiovascular disease as well as all-cause mortality, and that these associations may be stronger among persons with CKD.^{8–15} Furthermore, higher levels of FGF-23 are linked to kidney disease progression in persons with established CKD, although this association may not be independent of baseline eGFR and albuminuria.^{11,16,17} There is limited information about the association between FGF-23 and the development of CKD in the general population.

The objective of this study was to test the hypothesis that higher baseline serum levels of intact FGF-23 are associated with an elevated risk of incident kidney disease, independent of baseline kidney function and a large number of risk factors among participants of the community-based Atherosclerosis Risk in Communities (ARIC) study.

RESULTS

Participant Baseline Characteristics

There were 13,448 ARIC study participants. Of the participants, 72.7% had a creatinine-based eGFR ($eGFR_{Cr}$) ≥ 90 ml/min per 1.73 m^2 based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation, 25.3% had $eGFR_{Cr}$ of 60–89 ml/min per 1.73 m^2 , and 2.0% had $eGFR_{Cr} < 60$ ml/min per 1.73 m^2 . Overall, 56.1% of participants were women and 74.7% were white. The mean age was 56.9 years and the mean baseline $eGFR_{Cr}$ was 97 ml/min per 1.73 m^2 . At baseline, participants with higher FGF-23 levels were more likely to have $eGFR_{Cr} < 60$ ml/min per 1.73 m^2 , diabetes, or a history of coronary heart disease and were more likely to use antihypertensive medications (Table 1). Participants with higher FGF-23 levels also had higher blood concentrations of parathyroid hormone, phosphate, calcium, and β_2 -microglobulin, as well as lower $eGFR_{Cr}$ ($r = -0.21$), cystatin C–based eGFR ($eGFR_{Cys}$) ($r = -0.27$), and creatinine-based and cystatin C–based eGFR ($eGFR_{Cr-Cys}$) ($r = -0.27$). FGF-23 was weakly, positively correlated with calcium only among those with $eGFR \geq 60$ ml/min per 1.73 m^2 ($eGFR \geq 60$ ml/min per 1.73 m^2 , $r = 0.12$; $eGFR < 60$ ml/min per 1.73 m^2 , $r = 0.02$).

FGF-23 Level Associated with Risk of ESRD

During a median follow-up of 19 years, 267 participants (2.0%) had developed ESRD. Those participants with baseline levels of FGF-23 in higher quintiles had a higher cumulative incidence of ESRD (log-rank $P < 0.001$; Figure 1). For example, participants with baseline levels of FGF-23 in the highest quintile (corresponding to > 54.6 pg/ml) developed ESRD at nearly five times the rate of those with FGF-23 levels in

the lowest quintile (corresponding to < 32.0 pg/ml; hazard ratio [HR], 4.88; 95% confidence interval [95% CI], 3.16 to 7.52; P for trend across all quintiles, $P < 0.001$; Table 2, model 1).

In demographic-adjusted analyses, the relationship between higher FGF-23 and increased risk of ESRD remained (Figure 2A). For example, participants with levels of FGF-23 in the highest quintile had a risk of ESRD that was 4.47 times higher than those in the lowest quintile (95% CI, 2.89 to 6.90; P for trend across all quintiles, $P < 0.001$; Table 2, model 2).

After adjustment for baseline $eGFR_{Cr-Cys}$, risk estimates for ESRD associated with FGF-23 quintile were substantially attenuated but remained statistically significant (HR, 1.92; 95% CI, 1.23 to 3.02; Table 2, model 3). Results were qualitatively similar after adjusting for other estimates of GFR (quintile 5 versus quintile 1: HR adjusted for age, sex, race, and $eGFR_{Cr}$, 2.60; 95% CI, 1.67 to 4.05; HR adjusted for age, sex, race, and $eGFR_{Cys}$, 2.01; 95% CI, 1.29 to 3.14).

In fully adjusted models, higher FGF-23 levels were associated with a higher risk of ESRD (Figure 2B). For each 10-pg/ml increase of FGF-23, the fully adjusted risk of ESRD was 12% higher (model 5: HR, 1.12; 95% CI, 1.07 to 1.17; $P < 0.001$). There was a 2.10-fold higher risk of ESRD for those in the highest FGF-23 quintile compared with those in the lowest quintile, after adjusting for demographics, $eGFR_{Cr-Cys}$, kidney disease risk factors, and markers of mineral metabolism (95% CI, 1.31 to 3.36; P for trend across all quintiles, $P < 0.001$; Table 2, model 5).

There was no observed effect modification between FGF-23 and age, sex, or race. Baseline eGFR category was not a statistically significant effect modifier of the association between FGF-23 quintile and ESRD (P for interaction, $P > 0.20$). However, given inherent interest, stratified analyses by baseline eGFR category were conducted. The association between FGF-23 and ESRD risk persisted when the sample was restricted to those with baseline $eGFR \geq 90$ ml/min per 1.73 m^2 (HR per 10-pg/ml FGF-23 increment, 1.11; 95% CI, 1.01 to 1.22; $P = 0.03$; Table 3, model 5).

FGF-23 Level Associated with Risk of CKD

There were 1818 cases (13.8%) of incident CKD ($n = 13,180$). A higher FGF-23 level at baseline was associated with incident CKD in the unadjusted analysis (quintile 5 versus quintile 1: HR, 1.74; 95% CI, 1.51 to 2.02; P for trend across all quintiles, $P < 0.001$; Table 4, model 1). This association was attenuated with adjustment for baseline eGFR, and was not statistically significant in the fully adjusted model (model 5, quintile 5 versus 1: HR, 1.10; 95% CI, 0.94 to 1.29; P for trend across all quintiles, $P = 0.07$). In all models with FGF-23 as a continuous variable, FGF-23 was statistically significantly associated with incident CKD, independent of demographics, $eGFR_{Cr-Cys}$, kidney disease risk factors, and markers of mineral metabolism (model 1: HR per 10-pg/ml increment, 1.09; 95% CI, 1.07 to 1.10; $P < 0.001$; model 5: HR per 10-pg/ml increment, 1.03; 95% CI, 1.00 to 1.06; $P = 0.03$).

Table 1. Baseline characteristics by quintile of serum level of FGF-23 in the ARIC study (1990–2010; n=13,448)

Characteristic	Quintile of Serum Level of FGF-23 (pg/ml)					P Value ^a
	1 (<32.0)	2 (32.0–38.5)	3 (38.6–45.3)	4 (45.4–54.6)	5 (>54.6)	
Participants (n)	2690	2690	2689	2690	2689	
Median FGF-23 (pg/ml)	27.5	35.6	41.9	49.5	63.5	<0.001
Age (yr)	56.3 (5.7)	56.6 (5.7)	56.7 (5.7)	57.2 (5.7)	57.6 (5.7)	<0.001
Women	60.0	56.8	54.1	53.0	56.4	<0.001
Caucasian	74.1	76.6	76.7	74.2	72.1	<0.001
Body mass index (kg/m ²)	27.0 (5.4)	27.7 (5.3)	28.0 (5.2)	28.3 (5.4)	29.1 (5.7)	<0.001
HDL (mg/dl)	53.0 (17.6)	50.6 (16.6)	49.4 (16.5)	48.0 (16.1)	47.5 (16.6)	<0.001
Diabetes status	14.3	13.3	13.1	15.2	18.8	<0.001
Systolic BP (mmHg)	120.0 (18.3)	120.7 (18.4)	121.0 (18.3)	122.0 (19.1)	123.7 (19.6)	<0.001
Antihypertensive medication	25.9	28.0	30.5	34.9	45.6	<0.001
History of coronary heart disease	4.3	5.4	5.6	6.1	7.7	<0.001
eGFR (ml/min per 1.73 m ²)						
eGFR _{Cr} category						
<60	0.5	0.6	0.8	1.5	6.5	<0.001
60–89	18.7	22.0	24.9	29.3	31.6	<0.001
≥90	80.9	77.4	74.3	69.1	61.9	<0.001
eGFR _{Cr}	100.0 (13.9)	98.1 (13.7)	97.3 (14.2)	95.6 (15.1)	91.7 (19.1)	<0.001
eGFR _{Cys}	96.3 (16.2)	93.8 (16.3)	92.4 (16.6)	89.1 (17.8)	82.5 (20.8)	<0.001
eGFR _{Cr-Cys}	100.5 (15.3)	97.7 (14.9)	96.4 (15.1)	93.6 (16.3)	87.9 (19.8)	<0.001
C-reactive protein (mg/dl)	4.41 (8.87)	3.98 (5.99)	4.06 (5.96)	4.43 (6.86)	5.07 (7.81)	<0.001
Parathyroid hormone (pg/ml)	40.5 (20.4)	41.3 (15.6)	41.7 (15.6)	42.8 (16.6)	45.1 (24.1)	<0.001
Phosphate (mg/dl)	3.47 (0.47)	3.51 (0.47)	3.51 (0.47)	3.56 (0.47)	3.63 (0.52)	<0.001
Calcium (mg/dl)	9.28 (0.41)	9.31 (0.44)	9.33 (0.40)	9.39 (0.42)	9.45 (0.46)	<0.001
β ₂ -Microglobulin (mg/L)	1.80 (0.41)	1.84 (0.43)	1.88 (0.40)	1.95 (0.47)	2.19 (1.07)	<0.001

Data are presented as the mean (SD) or percentage unless otherwise specified.
^aANOVA for continuous variables and chi-squared test for categorical variables.

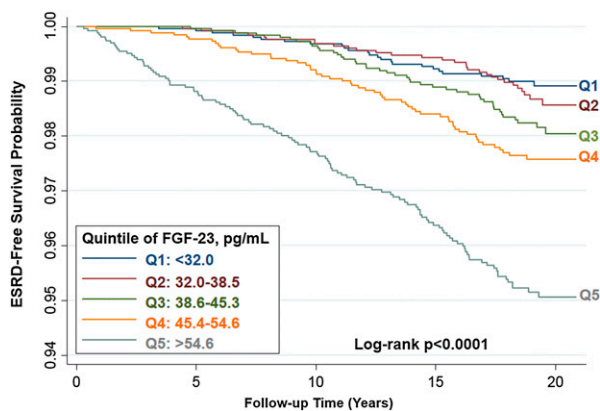


Figure 1. Probability of survival without incident ESRD is lower among those with higher quintiles of baseline serum level of FGF-23.

Phosphate Level Associated with Risk of ESRD

We used multivariable regression models to evaluate whether FGF-23 was a mediator of the relationship between phosphate and ESRD. When FGF-23 was excluded from the covariates, phosphate levels >3.5 mg/dl were significantly associated with higher risk of ESRD in models 1–3, but not in models 4 and 5 after accounting for β₂-microglobulin. In models 3–5, when log₁₀-transformed FGF-23 was added to the model, the association between phosphate level and ESRD was substantially attenuated and no longer statistically significant.

Sensitivity Analyses

To evaluate whether albuminuria confounded the association between FGF-23 and ESRD, a sensitivity analysis was performed with adjustment for the available data on the urine albumin/creatinine ratio (measured at study visit 4, 1996–1998, in 79.1% of participants) and restricting the analysis to incident ESRD after the measurement of urine albumin/creatinine ratio. Adjusting for the urine albumin/creatinine ratio did not materially affect the results. For example, in the full model (model 5), after adjusting for demographics, eGFR_{Cr-Cys}, kidney disease risk factors, markers of mineral metabolism, and urine albumin/creatinine ratio, there was a 1.85-times higher risk of ESRD for those in the highest FGF-23 quintile relative to the lowest quintile (95% CI, 1.02 to 3.37).

To assess the effect of misclassification of ESRD status as a result of death before RRT, analyses were repeated after accounting for the competing risk of death. The results of the competing risk analyses were consistent with the main findings.

DISCUSSION

In a large, community-based study of middle-aged, healthy at enrollment, black and white men and women with up to 21 years of follow-up, higher baseline levels of FGF-23 were associated with an increased risk ESRD after adjustment for

Table 2. Frequency, incidence rates, HRs, and 95% CIs for incident ESRD by quintile of serum levels of FGF-23 in the ARIC study (1990–2010; n=13,448)

Rate	Quintile of Serum Level of FGF-23 (pg/ml)					P Value for Trend ^a
	1 (<32.0)	2 (32.0–38.5)	3 (38.6–45.3)	4 (45.4–54.6)	5 (>54.6)	
Participants (n)	2690	2690	2689	2690	2689	
Events	25 (0.9)	30 (1.1)	43 (1.6)	56 (2.1)	113 (4.2)	<0.001
Incidence rate ^b	0.53 (0.36 to 0.78)	0.63 (0.44 to 0.90)	0.91 (0.67 to 1.22)	1.21 (0.93 to 1.57)	2.55 (2.12 to 3.06)	<0.001
Model ^c						
1	1 [Ref]	1.19 (0.70 to 2.03)	1.72 (1.05 to 2.81)	2.30 (1.43 to 3.68)	4.88 (3.16 to 7.52)	<0.001
2	1 [Ref]	1.20 (0.70 to 2.04)	1.72 (1.05 to 2.82)	2.18 (1.36 to 3.50)	4.47 (2.89 to 6.90)	<0.001
3	1 [Ref]	1.10 (0.65 to 1.87)	1.41 (0.86 to 2.32)	1.42 (0.88 to 2.28)	1.92 (1.23 to 3.02)	<0.001
4	1 [Ref]	1.08 (0.63 to 1.85)	1.48 (0.90 to 2.45)	1.33 (0.82 to 2.17)	1.97 (1.25 to 3.11)	<0.001
5	1 [Ref]	1.10 (0.64 to 1.91)	1.58 (0.95 to 2.64)	1.37 (0.83 to 2.25)	2.10 (1.31 to 3.36)	<0.001

Data are presented as n (%) or HR (95% CI) unless otherwise specified. Ref, reference group.

^aLinear trend across the quintiles using the median of each quintile.

^bUnadjusted incidence rate (95% CI) per 1000 person-years.

^cModels are as follows: model 1 is unadjusted (no covariates included in the model); model 2 is adjusted for age, sex, and race; model 3 is adjusted for the variables in model 2 plus eGFR_{Cr}, eGFR_{Cys}, and eGFR_{Cr-Cys}; model 4 is adjusted for the variables in model 3 plus diabetes, systolic BP, antihypertensive medication, HDL cholesterol, body mass index, C-reactive protein, and β_2 -microglobulin; and model 5 is adjusted for variables in model 4 plus phosphate, calcium, and parathyroid hormone.

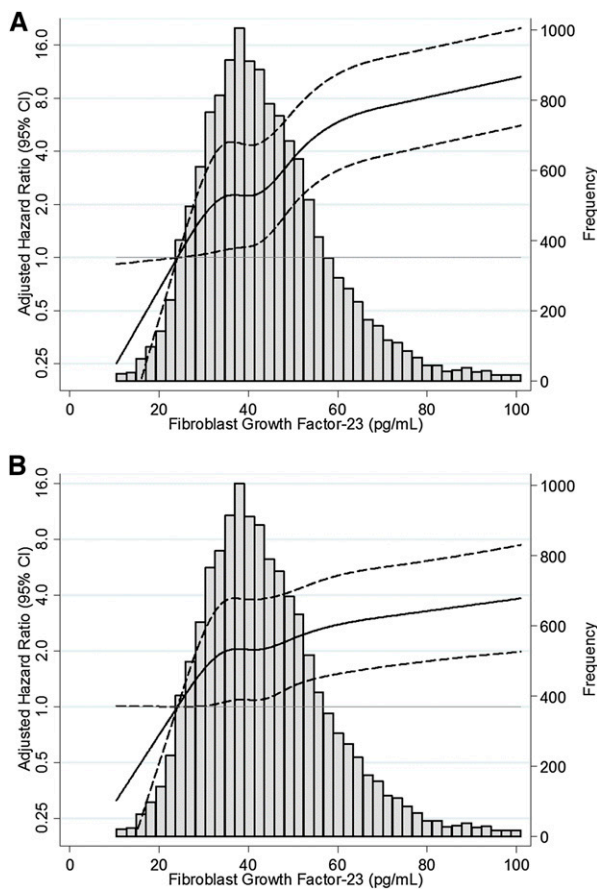


Figure 2. Relative hazard of ESRD is higher at higher baseline levels of FGF-23. (A) Adjusted for age, sex, and race. (B) Adjusted for age, sex, race, eGFR_{Cr-Cys}, diabetes, systolic BP, antihypertensive medication, HDL cholesterol, body mass index, C-reactive protein, β_2 -microglobulin, phosphate, calcium, and parathyroid hormone. Restricted cubic spline with knots at 24.1, 34.8, 41.9, 50.6, and 72.3 pg/ml. The shaded area represents the 95% CI.

demographic characteristics, kidney function, other risk factors, and mineral metabolism markers. These findings were robust to sensitivity analyses accounting for the strong competing risk of death as well as in analyses adjusting for a measure of albuminuria.

Three studies have investigated the association between FGF-23 and kidney disease in CKD cohorts.^{11,16,17} In the Mild to Moderate Kidney Disease (MMKD) cohort study of 177 participants with CKD (mean GFR 63.9 ml/min per 1.73 m²) followed for a median of 53 months, intact FGF-23 levels above the median value (35 pg/ml) were associated with kidney disease progression, defined as a doubling of serum creatinine concentration or need for RRT.¹⁶ This association was independent of age, sex, eGFR_{Cr}, proteinuria, and serum levels of calcium, phosphate, and parathyroid hormone (HR per 10-pg/ml increment, 1.06; 95% CI, 1.02 to 1.11; *P*=0.01). In the African-American Study of Kidney Disease and Hypertension (AASK), among 809 participants with CKD (mean GFR 45.2 ml/min per 1.73 m²) followed for a median of 7.9 years, the highest (≥ 64.4 pg/ml) versus lowest (≤ 30.7 pg/ml) quartile of intact FGF-23 level was associated with higher risk of ESRD or death, independent of demographics, kidney disease risk factors, kidney function (GFR, urine protein/creatinine ratio), and markers of mineral metabolism (HR, 2.26; 95% CI, 1.34 to 3.83; *P* for trend, *P*<0.01).¹⁷ In the Chronic Renal Insufficiency Cohort (CRIC) study of 3879 participants with CKD (mean GFR 42.8 ml/min per 1.73 m²) followed for a median of 42 months, higher quartiles of C-terminal FGF-23 were associated with ESRD risk, but not after adjustment for eGFR.¹¹ However, in a subgroup analysis, FGF-23 was independently associated with ESRD among participants with an eGFR of 30–45 ml/min per 1.73 m² and ≥ 45 ml/min per 1.73 m², but not among those with eGFR<30 ml/min per 1.73 m².

Our findings are similar to some aspects of these three prior studies of FGF-23 and CKD progression. The cross-sectional

Table 3. HRs and 95% CIs for ESRD per 10-pg/ml FGF-23 increment according to category of baseline kidney function in the ARIC study (1990–2010; *n*=13,448)

eGFR Model (ml/min per 1.73 m ²) ^a	Participants (<i>n</i>)	HR (95% CI)	P Value	P Value for Interaction ^b
eGFR _{Cr} ≥90				
Model 1	9779	1.09 (1.04 to 1.15)	<0.001	—
Model 5		1.11 (1.01 to 1.22)	0.03	—
eGFR _{Cr} 60–89	3404			
Model 1		1.18 (1.07 to 1.30)	0.001	0.78
Model 5		1.07 (0.95 to 1.21)	0.25	0.56
eGFR _{Cr} <60	265			
Model 1		1.10 (1.07 to 1.13)	<0.001	0.60
Model 5		1.11 (1.01 to 1.23)	0.03	0.21

^aModels are as follows: model 1 is unadjusted (no covariates included in the model); and model 5 is adjusted for age, sex, race, diabetes, systolic BP, antihypertensive medication, eGFR_{Cr}, eGFR_{Cys}, eGFR_{Cr-Cys}, HDL cholesterol, body mass index, C-reactive protein, β₂-microglobulin, phosphate, calcium, and parathyroid hormone.

^bInteraction of GFR category using eGFR_{Cr}≥90 ml/min per 1.73 m² as the reference group (all *P*>0.20). Dash represents reference group for test of interaction.

Table 4. Frequency, incidence rates, HRs, and 95% CIs for incident CKD (hospitalizations and deaths) by quintile of serum levels of FGF-23 in the ARIC study (1990–2010; *n*=13,180)

Rate	Quintile of Serum Level of FGF-23 (pg/ml)					P Value for Trend ^a
	1 (<32.0)	2 (32.0–38.5)	3 (38.6–45.3)	4 (45.4–54.6)	5 (>54.6)	
Participants (<i>n</i>)	2678	2674	2665	2649	2514	
Events ^b	303 (11.3)	307 (11.5)	338 (12.7)	410 (15.5)	460 (18.3)	<0.001
Incidence rate ^c	6.76 (6.04 to 7.57)	6.81 (6.09 to 7.61)	7.55 (6.79 to 8.40)	9.49 (8.62 to 10.5)	11.6 (10.6 to 12.7)	<0.001
Model ^d						
1	1 [Ref]	1.01 (0.86 to 1.18)	1.12 (0.96 to 1.31)	1.42 (1.22 to 1.65)	1.74 (1.51 to 2.02)	<0.001
2	1 [Ref]	0.98 (0.84 to 1.15)	1.08 (0.92 to 1.26)	1.30 (1.12 to 1.51)	1.59 (1.37 to 1.84)	<0.001
3	1 [Ref]	0.92 (0.78 to 1.08)	0.96 (0.82 to 1.12)	1.07 (0.92 to 1.25)	1.20 (1.03 to 1.39)	0.001
4	1 [Ref]	0.94 (0.80 to 1.11)	1.00 (0.86 to 1.18)	1.06 (0.91 to 1.24)	1.11 (0.95 to 1.29)	0.05
5	1 [Ref]	0.95 (0.80 to 1.12)	1.00 (0.85 to 1.18)	1.07 (0.92 to 1.25)	1.10 (0.94 to 1.29)	0.06

Data are presented as *n* (%) or HR (95% CI) unless otherwise specified. Ref, reference group.

^aLinear trend across the quintiles using the median of each quintile.

^bAmong all incident CKD patients, 20.5% (*n*=373) developed a eGFR_{Cr}<60 ml/min per 1.73 m² and at least a 25% decline in eGFR_{Cr}, 7.9% (*n*=143) developed ESRD identified by the USRDS registry, and 71.6% (*n*=1302) had a CKD-related hospitalization or death.

^cUnadjusted incidence rate (95% CIs) per 1000 person-years.

^dModels are as follows: model 1 is unadjusted (no covariates included in the model); model 2 is adjusted for age, sex, and race; model 3 is adjusted for the variables in model 2 plus eGFR_{Cr}, eGFR_{Cys}, and eGFR_{Cr-Cys}; model 4 is adjusted for the variables in model 3 plus diabetes, systolic BP, antihypertensive medication, HDL cholesterol, body mass index, C-reactive protein, and β₂-microglobulin; and model 5 is adjusted for variables in model 4 plus phosphate, calcium, and parathyroid hormone.

relationships between FGF-23, eGFR, phosphate, and parathyroid hormone were similar in direction and magnitude in the ARIC, MMKD, AASK, and CRIC studies.^{11,16,17} Our fully adjusted risk estimates for ESRD, when expressed per 10-pg/ml increment of FGF-23, were very similar to those from the MMKD study (MMKD: HR per 10-pg/ml increment, 1.06; 95% CI, 1.02 to 1.11; *P*=0.01; ARIC: HR per 10-pg/ml increment, 1.12; 95% CI, 1.07 to 1.17; *P*<0.01).¹⁶ Our fully adjusted risk estimates for ESRD were also similar in magnitude to those from the AASK trial, with a 2-fold increased risk of ESRD for the highest versus lowest quintile.¹⁷

However, differences between this study and the CRIC study were noted in the relationship between FGF-23 and ESRD risk, which may be the because of adjustment factors, FGF-23 measurement, or the study populations. First, in the CRIC study, fully adjusted models included baseline albuminuria, a key risk factor that was not measured at baseline in the ARIC

study. However, sensitivity analyses adjusting for albuminuria at ARIC study visit 4 did not substantially alter the observed relationship. Second, C-terminal FGF-23 was measured in the CRIC study, whereas intact FGF-23 was measured in the MMKD, AASK, and current ARIC studies. Whereas C-terminal FGF-23 levels appear to be correlated with FGF-23 bioactivity, intact FGF-23 is thought to be the biologically active moiety and the predominant circulating form of FGF-23 in the setting of renal impairment.¹⁸ Third, the study populations differ in that ARIC participants represent the range of kidney function seen in the general population. By contrast, the CRIC study specifically selected participants on the basis of having CKD.

In addition to analyses of ESRD, we report in this study that higher baseline levels of FGF-23 are associated with incident CKD after accounting for demographic characteristics and GFR. This relationship remained significant in some, but not all, models after adjusting for kidney disease risk factors and

other markers of mineral metabolism. Our findings are generally consistent with the Women's Health and Aging Study I that investigated the relationship between FGF-23 and incident CKD.¹⁹ Among 307 older, disabled women (median eGFR 70.3 ml/min per 1.73 m²) followed for a maximum of 2 years, a 1-SD increase (0.45 pg/ml) in log-transformed intact FGF-23 was associated with incident stage 3 CKD defined as eGFR < 60 ml/min per 1.73 m², after adjusting for baseline eGFR, demographics, kidney disease risk factors, other markers of mineral metabolism, and history of cardiovascular disease (HR, 1.51; 95% CI, 1.06 to 2.16; *P*=0.02). The stronger estimates of incident CKD risk in the Women's Health and Aging Study I may be attributed to the older study population (mean age of 74 years versus 57 years in this study), a shorter follow-up period (2 years versus 21 years in this study), or differences in FGF-23 increments used for estimating risk. The observation that FGF-23 is more strongly related to incident ESRD than incident CKD in this study suggests that FGF-23 may be a marker of faster kidney disease progression.

There are several potential mechanisms to explain the association between FGF-23 and ESRD. First, FGF-23 may be a proxy for abnormalities of mineral metabolism, such as elevated phosphate. Higher circulating levels of phosphate may promote vascular calcification and lead to kidney injury due to calcium phosphate deposition in the kidney.^{20–22} However, our findings suggest that the association between FGF-23 and ESRD is statistically independent of serum phosphate and other markers of mineral metabolism, namely, calcium and parathyroid hormone. Another major role of FGF-23 is to inhibit 1 α -hydroxylase activity in the kidney, reducing the conversion of 25-hydroxyvitamin D into the active 1,25-dihydroxyvitamin D, that may in turn contribute to kidney disease progression.^{7,23} Animal studies have shown that elevated levels of active vitamin D contribute to factors that are hypothesized to be associated with a reduction of kidney disease progression, including reduction in proteinuria, decrease in TGF- β , inhibition of mesangial cell proliferation, and preservation of the structural integrity of glomerular podocytes.^{24–27} Finally, FGF-23 might directly contribute to kidney injury, similar to the direct effect of FGF-23 on cardiac myocytes.⁹ Previous studies suggest that FGF-23 elevation precedes abnormalities in other mineral metabolism markers, which supports the potential role of FGF-23 as an early indicator of renal pathology.^{28–30}

This study has several strengths. The ARIC study is a large, well characterized cohort. The representation of men and women, African Americans and Caucasians, and four United States communities allows for relatively broad generalizability of the study results. Many important factors, including kidney function measured by multiple filtration markers as well as calcium, phosphate, and parathyroid hormone, were measured and included in adjusted regression models. Participants were followed for an extended period (up to 21 years) and we identified cases of ESRD with linkage to the US Renal Data System (USRDS) registry.

Certain limitations are important to consider in interpreting these results. There was a single measurement of FGF-23 obtained from stored samples. Intact FGF-23 has high intra-individual biologic variability and preanalytical instability.^{31,32} However, the assay used in this study demonstrated the best analytical performance characteristics compared with other available commercial assays of intact FGF-23.³³ Given that ARIC participants were selected to be representative of community-dwelling adults regardless of disease status, there were relatively few participants with a baseline eGFR < 60 ml/min per 1.73 m². As such, there was limited statistical power to make definitive conclusions in this subgroup. We did not have baseline measurements of albuminuria, which is a strong risk factor for kidney failure.³⁴ However, results were robust to adjustment for a measure of albuminuria at an intermediate time point. The incident CKD outcome in this study identifies stage 3 and worse, but not patients with CKD with proteinuria and preserved kidney filtration (CKD stages 1 and 2) due to the lack of albuminuria measurements. Furthermore, the incident CKD outcome is a composite of eGFR < 60 ml/min per 1.73 m², 25% eGFR decline, USRDS-identified ESRD, hospitalizations, and deaths, with the majority of patients being identified through CKD-related hospitalizations and deaths. Given the long span of time between study visits and creatinine measurements, this composite variable was created to mitigate selection bias caused by potential differential loss to follow-up based on kidney disease status.

In conclusion, this study demonstrates that high serum levels of intact FGF-23 are associated with an elevated risk of incident ESRD in a community-based population, independent of demographic characteristics, kidney disease risk factors, baseline eGFR, and mineral metabolism markers. Future research should investigate whether FGF-23 plays a direct role in kidney disease pathogenesis, separate from or in combination with other markers of mineral metabolism.

CONCISE METHODS

Study Design and Study Participants

The ARIC study is a community-based cohort originally designed to study risk factors for cardiovascular disease and atherosclerosis.³⁵ The participants at enrollment (1987–1989) included 15,792 predominantly black and white men and women aged 45–64 years, randomly selected and recruited from four United States communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Of these, 14,348 participants (90.9%) attended ARIC study visit 2 during 1990–1992 (baseline for this study). Participants who attended ARIC study visit 2 (1990–1992), had stored serum samples, did not have ESRD at baseline, and were either black or white were eligible for inclusion in this study (*n*=13,448, 93.7%).

The institutional review board at each participating study center approved the study protocol, and all study participants provided written documentation of informed consent. Procedures were followed

in accordance with the ethical standards of the institutional review boards and the Declaration of Helsinki.

Measurements

Study participants provided 12-hour fasting blood samples and spot urine samples at baseline (study visit 2, 1990–1992). Blood samples were centrifuged within 30 minutes of venipuncture at $3000 \times g$ for 10 minutes at 4°C , aliquoted into multiple vials, frozen at -70°C , shipped to the ARIC central laboratory, and stored at -70°C until analysis.

Several biochemical analyses were performed on the specimens as part of the original ARIC study visit 2 protocol (1990–1992). Fasting glucose was measured by the modified hexokinase/glucose-6-phosphate dehydrogenase method. HDL cholesterol levels were measured enzymatically after precipitation with dextran sulfate-magnesium.³⁶ Creatinine was measured by the modified kinetic Jaffé method, and values were calibrated to the National Institute of Standards and Technology standard.³⁷ GFR was estimated using standardized eGFR_{Cr} , eGFR_{Cys} , and $\text{eGFR}_{\text{Cr-Cys}}$ by CKD-EPI estimating equations.^{38,39}

Additional biochemical analyses were performed on these ARIC visit 2 stored blood samples in 2012–2013 at the University of Minnesota Advanced Research and Diagnostic Laboratory and were quantified using the Roche Modular P800 Chemistry Analyzer unless otherwise specified (Roche Diagnostics, Indianapolis, IN). Serum levels of intact FGF-23 were measured using a commercially available, two-site sandwich ELISA according to the manufacturer's protocol (Kainos Laboratories, Inc., Kyoto, Japan). The coefficient of variation (CV) for FGF-23 based on ARIC blind duplicate samples was 16.6%, and the CV for internal laboratory quality control samples was 8.8% at 41.4 pg/ml. Serum β_2 -microglobulin was measured turbidimetrically after agglutination of antigens from the sample to latex-bound anti- β_2 -microglobulin antibodies (CV=5.9%–7.3%). Calcium and phosphate were measured using colorimetric methods (CV=2.3% and 2.2%, respectively). Intact parathyroid hormone was measured using a sandwich immunoassay and quantified using a Roche Elecsys 2010 Analyzer (CV=2.9%–5.1%). C-reactive protein was measured using a high-sensitivity latex-particle-enhanced immunoturbidimetric assay kit (CV=4.5%). Cystatin C was measured using a particle-enhanced turbidimetric assay (Gentian, Moss, Norway; CV=2.1%–3.5%).

Baseline information on demographic characteristics, lifestyle habits, medical history, and medication use was obtained using a questionnaire administered in person by trained interviewers at the same study visit 2 (1990–1992). Participants were classified as having diabetes if they had fasting glucose ≥ 126 mg/dl, had nonfasting glucose ≥ 200 mg/dl, reported a history of diabetes, or were taking diabetes medication in the past 2 weeks. Three seated measurements of BP were taken by a certified technician using a random-zero sphygmomanometer after 5 minutes of rest, and the mean of the second and third readings was used for analysis. Body mass index was calculated as weight (in kilograms)/height (in meters squared) using measurements taken while participants wore light clothing without shoes.

Definition of Incident ESRD

The ARIC cohort was linked with the USRDS registry to obtain cases of incident ESRD between baseline (ARIC study visit 2, 1990–1992) through December 31, 2010, as reported to the US Centers for

Medicare and Medicaid Services on the Medical Evidence Form 2728 within 45 days of RRT initiation. Participants with USRDS-identified ESRD at baseline were excluded from analyses of incident ESRD ($n=12$).

Definition of Incident CKD

As a secondary outcome, CKD was defined by CKD-related International Classification of Disease diagnostic codes (revisions 9 and 10) for hospitalizations and deaths that occurred from baseline through December 31, 2010, USRDS-identified ESRD, or eGFR decline (defined as $\text{eGFR}_{\text{Cr}} < 60$ ml/min per 1.73 m^2 and at least 25% decline in eGFR_{Cr} from baseline). This outcome definition uses both study visit data and intervening hospitalization data in an attempt to mitigate selection bias caused by potential differential loss to follow-up based on kidney disease status. This definition is based on Kidney Disease Improving Global Outcomes criteria and was previously validated in the ARIC study.^{2,40} Compared with an outpatient $\text{eGFR} < 60$ ml/min per 1.73 m^2 , the sensitivity was 34.7% and specificity was 96.2% for this diagnostic code CKD definition. Participants with CKD at baseline were excluded from analyses of incident CKD ($n=268$).

Statistical Analyses

For descriptive purposes, we examined the associations of baseline levels of serum FGF-23 quintiles with demographic and clinical characteristics of study participants using means, medians, SDs, and proportions. We tested for differences in demographic and clinical characteristics across FGF-23 quintiles using ANOVA and chi-squared tests for continuous and categorical variables, respectively. In a cross-sectional analysis, pairwise Pearson correlation coefficients were calculated for FGF-23, other mineral metabolism biomarkers, and eGFR. Because of the non-normal distribution of FGF-23 levels, we used logarithmic transformation and categorized values by quintiles.

Cox proportional regression models were used to assess the association between baseline levels of serum FGF-23 and incident ESRD during follow-up, incorporating time until event. Restricted cubic splines were used to present age, sex, and race-adjusted as well as fully adjusted HRs for ESRD by continuous FGF-23 level with five knots at 24.1, 34.8, 41.9, 50.6, and 72.3 pg/ml corresponding to the 5th, 27.5th, 50th, 72.5th, and 95th percentiles, respectively, as suggested by Harrell.⁴¹ For the splines, FGF-23 levels were truncated at the 1st and 99th percentiles. Kaplan–Meier survival curves were used to visually depict ESRD-free survival probabilities over follow-up time according to FGF-23 quintiles.

To examine the independent association of FGF-23 with incident ESRD, multivariable models were adjusted for several baseline covariates. Model 1 did not include any covariates. Model 2 included basic demographic characteristics (age, sex, and race). Model 3 included all variables in model 2 plus $\text{eGFR}_{\text{Cr-Cys}}$. Model 4 included kidney disease risk factors (diabetes status, systolic BP, antihypertensive medication use, HDL cholesterol, body mass index, C-reactive protein, β_2 -microglobulin) in addition to all the variables in model 3. Finally, model 5 included all variables in model 4 plus markers of mineral metabolism (phosphate, parathyroid hormone, calcium). The quintile with the lowest baseline FGF-23 level was used as the reference group. To test for linear trend, the median FGF-23 value in

each quintile was treated as a continuous variable in regression models. In a continuous analysis, ESRD risk was expressed per 10-pg/ml FGF-23 increment. To determine whether serum phosphate was independently associated with ESRD, the same modeling approach described above was utilized, except FGF-23 was the last covariate added to the multivariable models. Phosphate was modeled as a linear spline with one knot at 3.5 mg/dl. The linear spline was used to better characterize the shape of the association since ESRD risk changed at phosphate level of 3.5 mg/dl.

In a sensitivity analysis, the available data on the urine albumin/creatinine ratio (measured at study visit 4, 1996–1998) was included as a covariate in regression models, and the observation period was limited to follow-up time after measurement of the urine albumin/creatinine ratio among participants without ESRD at study visit 4 ($n=10,642$; 79.1%). The proportional hazards assumption was tested using Schoenfeld residuals and by assessing the statistical significance of FGF-23 quintiles and $\ln(\text{follow-up time})$. Interaction terms were tested in all of the main regression models to assess effect modification by age, sex, race, and eGFR on the association between FGF-23 and kidney disease outcomes. Competing risk regression models were used to examine the association between FGF-23 and kidney disease outcomes, accounting for the competing risk of death before kidney events.⁴² All tests were two-sided and $P<0.05$ was considered statistically significant. Analyses were conducted using Stata software (version 12; StataCorp., College Station, TX).

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DISCLOSURES

None.

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See related editorial, “The Biomarker Niche for Fibroblast Growth Factor 23 Testing in CKD,” on pages 7–9.