FGF-23 Associates with Death, Cardiovascular Events, and Initiation of Chronic Dialysis

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ABSTRACT

Concentrations of the phosphate-regulating hormone fibroblast growth factor-23 (FGF-23) are elevated in patients with chronic kidney disease (CKD), but whether higher plasma FGF-23 concentrations associate with all-cause mortality, cardiovascular events, or initiation of chronic dialysis is not completely understood. Here, we measured FGF-23 concentration in stored plasma samples from 1099 patients with advanced CKD who participated in The Homocysteine in Kidney and End Stage Renal Disease study. Mean serum phosphorus concentration was 4.3 mg/dl, median FGF-23 concentration was 392 RU/ml, and mean GFR was 18 ml/min/1.73 m^2. During a median follow-up of 2.9 yr, 453 (41%) patients died from any cause, 215 (20%) had a cardiovascular event, and 615 (56%) initiated chronic dialysis. Compared with the lowest quartile of FGF-23, each subsequent quartile associated with a progressively higher risk for death, adjusted for confounders (HR [95% CI] of 1.24 [0.91 to 1.69], 1.76 [1.28 to 2.44], and 2.17 [1.56 to 3.08] for the second through fourth quartiles, respectively). In addition, compared with the lowest quartile, the two highest quartiles of FGF-23 also associated with a significantly elevated risk for cardiovascular events and initiation of chronic dialysis. In conclusion, in advanced CKD, FGF-23 strongly and independently associates with all-cause mortality, cardiovascular events, and initiation of chronic dialysis.

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Chronic kidney disease (CKD) often confers poor clinical outcomes, which are mainly driven by extraordinarily high rates of cardiovascular events and progression to end-stage renal disease (ESRD) with its attendant complications.[1–3] Although populations with advanced CKD have a high prevalence of traditional cardiovascular risk factors, the severity and extent of their cardiovascular disease (CVD) appear to be disproportionate to these risk factor profiles.[4] In addition, risk-factor modification strategies proven to attenuate risk in the general population are often shown to be nonefficacious in advanced CKD patients.[5] Hence, identification of other risk factors and biomarkers that identify pa-tients at risk for CVD and kidney disease progression in this patient population is critical.

Small absolute increases in serum phosphorus concentrations, even within the normal range, are independently associated with all-cause mortality

and cardiovascular events in epidemiologic studies, which have included both populations with normal kidney function and CKD.[6–10] Similarly, observational studies have suggested that higher serum phosphorus concentrations predict more rapid decline of kidney function.[11,12] The mechanisms by which the small increases in serum phosphorus are associated with worse clinical outcomes are unclear. Fibroblast growth factor-23 (FGF-23) is a 251-amino-acid protein secreted by osteocytes in adults.[13] It is a key regulator that maintains serum phosphorus within the normal range in CKD patients.[13] FGF-23 increases urinary phosphorus excretion by decreasing phosphorus reabsorption in the proximal tubule and inhibits 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) synthesis, resulting in decreased dietary phosphorus absorption from the gastrointestinal tract.[13,14] FGF-23 concentrations increase early in the course of CKD, long before the development of overt hyperphosphatemia,[13] suggesting that elevated FGF-23 concentrations are an early abnormality of disordered phosphorus metabolism in CKD patients.

Recently, elevated FGF-23 concentrations were found to be associated with increased mortality in chronic dialysis patients and progression of kidney disease in a small cohort of patients with mainly mild CKD.[15,16] No studies have examined the relationship between FGF-23 concentrations with all-cause mortality, cardiovascular events, and initiation of chronic dialysis in patients with advanced CKD not requiring renal replacement therapy. Therefore, we conducted a longitudinal analysis to test the hypothesis that elevated plasma FGF-23 concentrations are a predictor for death, cardiovascular events, and chronic dialysis initiation in patients with advanced kidney disease not requiring dialysis who participated in The Homocysteine in Kidney and End Stage Renal Disease (HOST) study.[17]

RESULTS

Of the 1099 participants included in this analysis, the mean (SD) age of the participants was 69 ± 11 yr, 98% were male, and 26% were African American. The mean estimated Modified Diet Renal Disease-GFR (MDRD-GFR) was 18 ± 6 ml/min/1.73m^2, and most participants (n = 718; 65%) had an estimated MDRD-GFR of 15 to 29 ml/min/1.73m.^{2} The distribution of plasma FGF-23 concentration was skewed to the right, with a median of 392 RU/ml (IQR 216 to 945 RU/ml). The mean serum phosphorus concentration was 4.3 ± 1.3 mg/dl; 36% and 12% had a serum phosphorus concentration greater than 4.5 and 5.5 mg/dl, respectively.

Higher FGF-23 concentrations were significantly associated with younger age, the Caucasian race, higher prevalence of diabetes and cardiovascular disease, greater body mass index (BMI), greater systolic blood pressure (BP), and higher serum phosphorus and intact parathyroid hormone (iPTH) concentrations. GFR, hemoglobin concentrations, serum albumin, and plasma $1,25(OH)_2D$ concentrations were lower in the higher FGF-23 groups (Table 1). Plasma FGF-23 concentra-

tions correlated with serum phosphorus ($r = 0.46$) and plasma iPTH ($r = 0.34$) concentrations and inversely with plasma $1,25(OH)_2D$ concentrations ($r = -0.39$); there was no correlation with serum calcium or plasma 25-hydroxyvitamin D (25(OH)D) concentrations.

FGF-23 and the Risk of Death from All Causes
The median follow-up for survival analyses was 2.9 yr (interquartile range, 2.1 to 3.7 yr), and there were 453 deaths from all causes. There were 82, 104, 132, and 135 deaths across increasing FGF-23 quartiles, corresponding to crude death rates of 10.2, 13.4, 18.1, and 19 per 100 person-years, respectively. Higher plasma FGF-23 concentrations were associated with greater risk of all-cause mortality in crude analysis (Figure 1; $P<0.0001$ by the log-rank test for the overall comparison among the subgroups). The adjusted associations between FGF-23 concentrations and death by quartiles are shown in Figure 2. The first and second quartile of FGF-23 concentrations had a similar mortality rate, the third quartile was associated with significantly increased risk of death, and the fourth quartile was associated with an approximate tripling of mortality in models adjusted for age, sex, and race. Additional adjustment for traditional cardiovascular risk factors, baseline kidney function, and other components of the mineral metabolism axis attenuated the association, yet the highest quartile remained associated with an approximately twofold greater risk for death. Further adjustment for cardioprotective medications had no additional effect. Similarly, when plasma FGF-23 concentrations were evaluated as a continuous variable, higher concentrations of FGF-23 were associated with an increased risk of all-cause mortality, with a hazard ratio (HR) of 1.63 (95% CI 1.29 to 2.07; $P<0.0001$) per SD increase in log FGF-23 concentrations in the fully adjusted model (Model 3). Similarly, the relationship of FGF-23 concentration with all-cause mortality, in which the time before chronic dialysis onset was left-truncated, showed an increase in HR of 1.47 (95% CI 1.04 to 2.10; $P = 0.03$) for each SD increase in the log FGF-23 concentration. FGF-23 was the strongest risk factor for death. Other variables independently associated with death were older age, history of CVD, and lower serum concentrations of albumin (Supplemental Table 1 and Table 2). The model was repeated with serum phosphorus concentration instead of FGF-23 as an independent variable. After multivariate adjustment (Model 3), the serum phosphorus concentration was not associated with all-cause mortality (HR 1.02, 95% CI 0.92 −1.13 per mg/dl increase; $P = 0.71$).

FGF-23 and Risk of Cardiovascular Events
A total of 215 cardiovascular disease events occurred over a median follow-up of 2.9 yr. The crude rates for cardiovascular events were 5.0, 7.0, 9.7, and 10.1 per 100 person-years in the respective increasing FGF-23 quartiles. In unadjusted analysis, participants with high and moderate plasma FGF-23 concentrations were much more likely to have any of the cardiovascular composite events than those with lower FGF-23 concen-

Table 1. Baseline characteristics of study participants by quartiles of baseline plasma FGF-23 concentrations

FGF-23 concentrations	Q1 ≤216 RU/ml	Q2 217–380 RU/ml	Q3 381–945 RU/ml	Q4 >946 RU/ml	P Value for Trend
Age (years)	72 ± 11	70 ± 10	68 ± 11	65 ± 11	<0.0001
Sex (% Male)	98.5	98.9	97.8	98.2	0.52
Race (% African American)	34.2	25.0	27.4	19.0	0.0003
Diabetes (%)	45.1	54.7	62.8	57.3	0.0009
Hypertension (%)	96.7	94.6	96.4	98.2	0.22
CVD (%)	49.1	59.1	62.8	57.0	0.01
Charlson score	4.5 ± 2.2	5.1 ± 2.2	5.4 ± 2.2	5.2 ± 2.1	<0.0001
Current smoking (%)	10.5	18.8	17.0	27.0	<0.0001
Medications (%)					
ACEI/ARB	44.7	40.6	41.6	37.6	0.13
CCB	60.0	67.4	68.2	62.8	0.48
Beta-blocker	53.1	62.3	61.7	60.6	0.09
Lipid-lowering	54.2	57.6	59.9	55.1	0.71
Sevelamer	2.5	1.8	3.3	6.2	0.01
BMI (kg/m^2)	26.8 ± 4.2	27.6 ± 4.5	28.6 ± 5.2	28.7 ± 5.1	<0.0001
SBP (mmHg)	140 ± 22	143 ± 23	146 ± 23	144 ± 24	0.007
DBP (mmHg)	72 ± 13	73 ± 13	73 ± 12	74 ± 13	0.11
MDRD-GFR (ml/min/1.73m^2)	23 ± 6	20 ± 6	16 ± 5	15 ± 5	<0.0001
Hemoglobin (g/dl)	12.3 ± 1.6	12.1 ± 1.6	11.6 ± 1.6	11.5 ± 1.8	<0.0001
Albumin (mg/dl)	4.2 ± 0.5	4.2 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	0.0001
Calcium (mg/dl)	9.0 ± 0.5	8.9 ± 0.7	8.9 ± 0.7	8.9 ± 0.9	0.21
Phosphorus (mg/dl)	3.8 ± 0.8	4.1 ± 0.9	4.5 ± 1.6	5.1 ± 1.4	<0.0001
LDL-C (mg/dl)	97 ± 33	95 ± 30	93 ± 34	94 ± 34	0.27
HDL-C (mg/dl)	45 ± 17	43 ± 15	42 ± 14	40 ± 13	<0.0001
25(OH)D (ng/ml)	21 ± 11	21 ± 10	20 ± 10	21 ± 11	0.70
1,25(OH)$_2$D (pg/ml)	25 ± 11	22 ± 10	18 ± 10	19 ± 9	<0.0001
iPTH (pg/ml)	122 ± 77	170 ± 109	223 ± 160	269 ± 224	<0.0001

Values are expressed as means ± standard deviation unless otherwise specified. CVD, cardiovascular disease; BMI, body mass index; ACE Inhibitor, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; GFR, glomerular filtration rate; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; iPTH, intact parathyroid hormone.
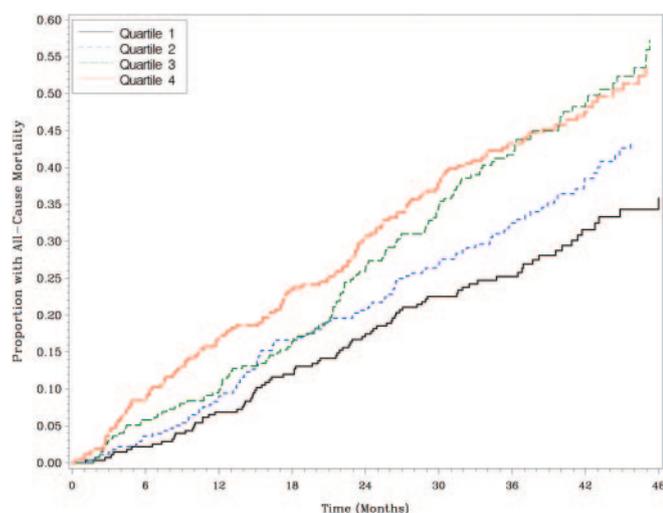

Figure 1. Kaplan-Meier curve of all-cause mortality according to quartiles of baseline plasma fibroblast growth factor-23 (FGF-23) concentrations.

trations (Figure 3; $P = 0.001$ by the log-rank test for the overall comparison among the groups). The association of FGF-23 concentrations with the composite of cardiovascular events

was similar to its association with death from any causes (Figure 4). When FGF-23 was log-transformed and evaluated as a continuous predictor, each SD increase in log FGF-23 was associated with a 54% higher cardiovascular event risk in fully adjusted models (Model 3; HR 1.54, 95% CI 1.07 to 2.21; $P = 0.02$). Serum phosphorus as a continuous variable was related to the composite of cardiovascular event in multivariate analyses (Model 3; adjusted HR 1.15, 95% CI 1.05 to 1.27 per mg/dl of serum phosphorus increase; $P = 0.002$). FGF-23 was also the strongest risk factor for the composite of cardiovascular events (Supplemental Table 1 and Table 2). Other independent risk factors associated with the composite of cardiovascular events are shown in Supplemental Table 2.

Estimates of the association of FGF-23 concentrations with specific cardiovascular events were based on few events and had limited precision (Table 2); nonetheless, trends were evident for two outcomes. The associations of FGF-23 concentrations with myocardial infarction (n = 143 events) were strong (adjusted HR 2.44, 95% CI 1.25 to 4.76; $P = 0.009$) for the highest FGF-23 quartile. Those participants in the highest FGF-23 quartile had a markedly increased adjusted HR for amputations (n = 44) of 7.00 (95% CI 1.54 to 31.83; $P = 0.01$). In contrast, the adjusted HR for stroke (n = 43) was not significant.
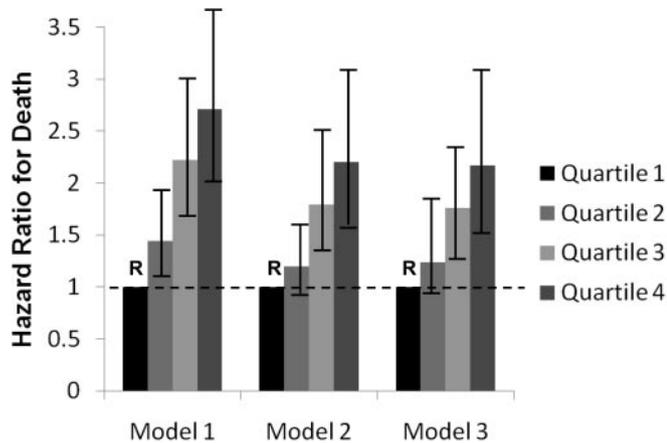
Figure 2. Hazard ratio (95% CI) for all-cause mortality according to quartiles of baseline plasma fibroblast growth factor-23 (FGF-23) concentrations. Model 1: adjusted for age, gender, race Model 2: adjusted for covariates in Model 1 plus smoking, alcohol intake, diabetes, hypertension, prevalent cardiovascular disease, Charlson score, body mass index, systolic and diastolic BP, homocysteine, folate, vitamin B_{12}, treatment arm, hemoglobin, estimated GFR, albumin, calcium, phosphorus, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, intact parathyroid hormone, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides Model 3: adjusted for covariates in Model 2 plus use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs, and sevelamer Quartile 1 is the reference group in all models. R denotes reference and bars represent 95% confidence intervals.
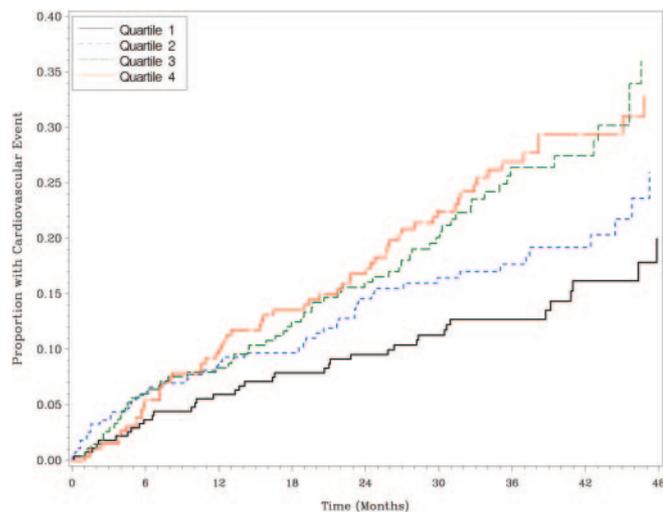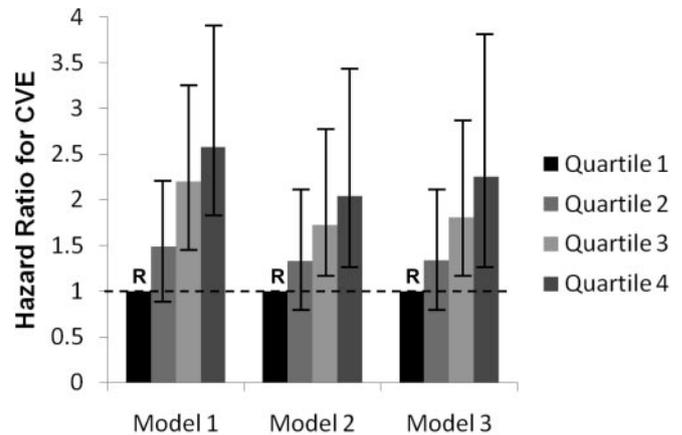

Figure 4. Hazard ratio (95% CI) for the composite of cardiovascular events according to quartiles of baseline plasma fibroblast growth factor-23 (FGF-23) concentrations Model 1: adjusted for age, gender, race Model 2: adjusted for covariates in Model 1 plus smoking, alcohol intake, diabetes, hypertension, prevalent cardiovascular disease, Charlson score, body mass index, systolic and diastolic BP, homocysteine, folate, vitamin B_{12}, treatment arm, hemoglobin, estimated GFR, albumin, calcium, phosphorus, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, intact parathyroid hormone, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides Model 3: adjusted for covariates in Model 2 plus use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs, and sevelamer Quartile 1 is the reference group in all models. R denotes reference and bars represent 95% confidence intervals.

Figure 3. Kaplan-Meier curve of cardiovascular events according to quartiles of baseline plasma fibroblast growth factor-23 (FGF-23) concentrations.

We examined the association of FGF-23 concentrations with the composite of all-cause mortality and cardiovascular events within subgroups defined by age, race, diabetes status, and baseline kidney function as well as plasma 1,25(OH)$_2$D and phosphorus concentrations. The point estimates in multi-

variate model (Model 3) were similar within each subgroup, and no significant interactions between FGF-23 concentrations and age, race, diabetes status, baseline kidney function, 1,25(OH)$_2$D, or phosphorus concentrations were observed (Figure 5).

FGF-23 and Risk of Chronic Dialysis Initiation

During the follow-up period, 56% (n = 615) of participants initiated chronic dialysis. The primary causes of kidney disease in those who initiated dialysis were diabetes (46%), hypertension (29%), glomerulonephritis (4%), polycystic kidney disease (3%), obstructive nephropathy (2%), atherosclerotic renovascular disease (2%), and tubulo-interstitial disease (1%). The remaining 13% either had unknown causes of kidney disease or causes other than those listed. The crude chronic dialysis rates were 13%, 25%, 46%, and 68% in FGF-23 quartiles 1, 2, 3, and 4, respectively. Cumulative incidence curves depicted in Supplemental Figure 1 show that there was a higher incidence of dialysis initiation across increasing FGF-23 quartiles (P<0.0001). After adjustments for demographics, traditional cardiovascular risk factors, baseline kidney function, and other variables of mineral metabolism, the two highest quartiles (381 to 945 RU/ml and >946 RU/ml) of FGF-23 concentrations remained significantly associated with incident chronic dialy-

Table 2. Associations between quartiles of baseline plasma FGF-23 concentrations with specific cardiovascular events

FGF-23 concentrations	Q1 ≤216 RU/ml	Q2 217–380 RU/ml	Q3 381–945 RU/ml	Q4 >946 RU/ml
Myocardial Infarction				
No. Myocardial Infarction	25	35	43	40
Hazard Ratio (95% CI)				
Model 1	1.00	1.61 (0.96–2.70)	2.31 (1.40–3.81)	2.52 (1.49–4.26)
Model 2	1.00	1.52 (0.88–2.63)	1.95 (1.09–3.50)	2.17 (1.13–4.15)
Model 3	1.00	1.58 (0.91–2.76)	2.10 (1.15–3.81)	2.44 (1.25–4.76)
Amputation				
No. Amputation	3	9	14	18
Hazard Ratio (95% CI)				
Model 1	1.00	3.46 (0.93–12.84)	5.73 (1.63–20.12)	8.15 (2.33–28.47)
Model 2	1.00	3.01 (0.75–12.13)	4.01 (0.98–16.50)	6.01 (1.38–26.12)
Model 3	1.00	3.35 (0.81–13.9)	4.75 (1.12–20.14)	7.00 (1.54–31.83)
Stroke				
No. Strokes	11	10	9	13
Hazard Ratio (95% CI)				
Model 1	1.00	0.94 (0.40–2.23)	0.92 (0.37–2.25)	1.40 (0.60–3.29)
Model 2	1.00	0.63 (0.26–1.55)	0.44 (0.15–1.27)	0.76 (0.26–2.27)
Model 3	1.00	0.59 (0.23–1.46)	0.41 (0.14–1.19)	0.78 (0.26–2.35)

Model 1 adjusted for age, gender, race. Model 2 adjusted for covariates in Model 1 plus smoking, alcohol intake, diabetes, hypertension, prevalent cardiovascular disease, Charlson score, body mass index, systolic and diastolic blood pressures, homocysteine, folate, vitamin B_{12}, treatment arm, hemoglobin, estimated glomerular filtration rate, albumin, calcium, phosphorus, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, intact parathyroid hormone, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides. Model 3 adjusted for covariates in Model 2 plus use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs, and sevelamer. Quartile 1 is the reference group in all models.
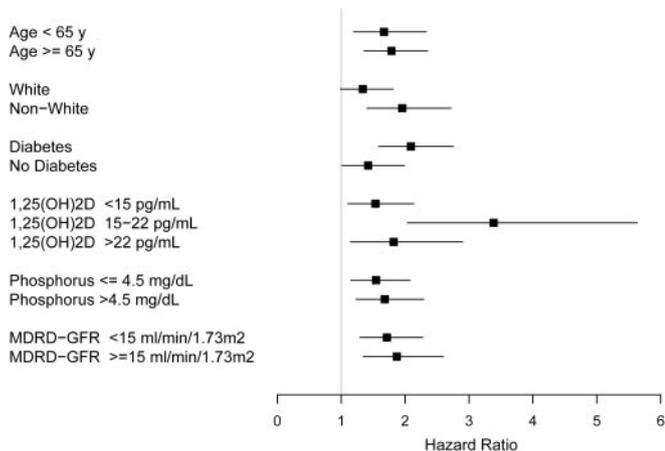

Figure 5. Association of baseline plasma fibroblast growth factor-23 with the composite of all-cause mortality and cardiovascular events within subgroups. Error bars represent 95% confidence intervals. eGFR = estimated GFR; 1,25D = 1,25-dihydroxyvitamin D.

sis (Figure 6). Further adjustment for reno-protective and cardio-protective medications did not change this association appreciably. The analysis was repeated using FGF-23 concentrations as a continuous variable instead of quartiles. After multivariable adjustment (Model 3), the HR of chronic dialysis initiation increased by 1.68 (95% CI 1.38 to 2.05; $P < 0.0001$) per SD increase in log FGF-23 concentrations. Serum phosphorus concentration was independently associated with initiation of chronic dialysis in multivariate analyses (Model 3; HR 1.09, 95% CI 1.02 to 1.17; $P = 0.01$ per mg/dl increase); however, the magnitude of the association was modest.
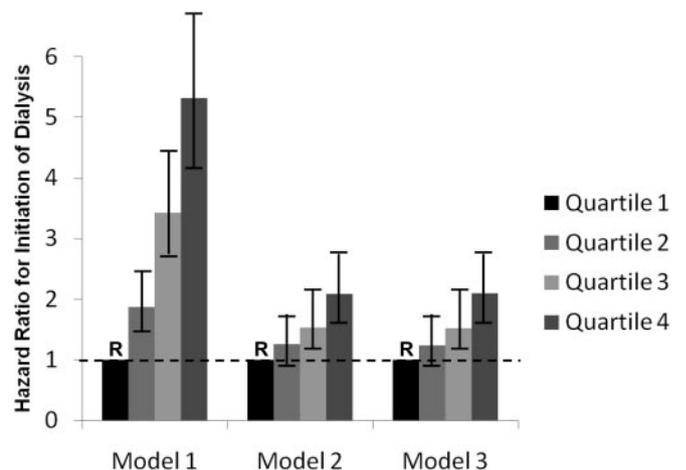

Figure 6. Hazard ratio (95% CI) for initiation of chronic dialysis according to quartiles of baseline plasma fibroblast growth factor-23 (FGF-23) concentrations Model 1: adjusted for age, gender, race Model 2: adjusted for covariates in Model 1 plus smoking, alcohol intake, diabetes, hypertension, prevalent cardiovascular disease, Charlson score, body mass index, systolic and diastolic BP, homocysteine, folate, vitamin B_{12}, treatment arm, hemoglobin, estimated GFR, albumin, calcium, phosphorus, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, intact parathyroid hormone, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides Model 3: adjusted for covariates in Model 2 plus the use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs and sevelamer Quartile 1 is the reference group in all models. R denotes reference and bars represent 95% confidence intervals.

Table 3. Hazard ratio (95% CI) for the composite outcome of initiation of chronic dialysis or death according to quartiles of baseline plasma FGF-23 concentrations

Models	Q1 ≤ 216 RU/ml	Q2 217–380 RU/ml	Q3 381–945 RU/ml	Q4 >946 RU/ml
Model 1	1.00	1.70 (1.37–2.10)	2.92 (2.36–3.61)	4.43 (3.57–5.49)
Model 2	1.00	1.26 (1.01–1.57)	1.66 (1.31–2.11)	2.32 (1.80–2.98)
Model 3	1.00	1.26 (1.01–1.58)	1.65 (1.30–2.10)	2.30 (1.78–2.96)

Model 1 included adjustment for age, gender, race. Model 2 included adjustment for covariates in Model 1 plus smoking, alcohol intake, diabetes, hypertension, cardiovascular disease, Carlson score, body mass index, systolic and diastolic BP, homocysteine, folate, vitamin B_{12}, treatment assignment, hemoglobin, MDRD-GFR, as well as concentrations of serum albumin, calcium, phosphorus, 25-hydroxyvitamin D, 1,25 dihydroxyvitamin D, intact parathyroid hormone, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides. Model 3 included adjustment for covariates in Model 2 plus the use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs and sevelamer. Quartile 1 is the reference group in all models.

Because of the potential risk of informative censoring of incident dialysis by the competing risk of death, the association of plasma FGF-23 concentrations with the composite outcome of incident chronic dialysis or death was examined. A total of 839 participants reached the composite end point of initiating chronic dialysis (n = 615) or dying without the initiation of dialysis (n = 224). Almost identical associations were observed when the composite outcome of dialysis initiation or death was examined (Table 3).

Sensitivity Analyses

To minimize potential confounding, all analyses were repeated including only participants with an MDRD-GFR of 15 to 29 ml/min/1.73m^2 and normal serum phosphorus (<4.5 mg/dl) at baseline (n = 718). In these analyses, there was a significant and strong association between quartiles of FGF-23 concentrations with death, cardiovascular events, and initiation of chronic dialysis. In addition, to better understand the relationship between FGF-23 concentrations and kidney function, the cross-sectional relationship between baseline FGF-23 concentrations as a continuous variable and baseline MDRD-GFR was further explored and was found to be approximately linear. In addition, the results of the SIMEX adjustment to MDRD-GFR did not have any appreciable effect on the FGF-23 coefficients for all outcomes when examined as a continuous variable in statistical Model 1 and Model 2.

DISCUSSION

In this prospective cohort of patients with advanced CKD not yet requiring dialysis, we found higher plasma FGF-23 concentrations to be strongly associated with all-cause mortality, cardiovascular events, and progression to chronic dialysis initiation independently of established risk factors for cardiovascular disease, including age, hypertension, diabetes, smoking, kidney function, and other abnormalities of mineral metabolism. The association was especially strong for all-cause mortality, acute myocardial infarction, and lower extremity amputations. These significant associations were stronger than those with the traditional CVD risk factors, persisted in participants who had serum phosphorus concen-

trations within the reference interval, and were not altered by adjustment for vitamin D analytes.

FGF-23 concentrations increase early in the course of kidney disease. By the time patients reach ESRD, FGF-23 concentrations are often 100 times above the normal range, whereas serum phosphorus concentrations are usually only mildly increased or normal.[15,18] The increase in FGF-23 concentrations appears to be a compensatory mechanism to maintain serum phosphorus levels in the normal range. Indeed, in our patient population, FGF-23 concentrations were significantly increased but serum phosphorus concentrations remained within the normal range in over 50% of the population. These increases in FGF-23 concentrations, although an appropriate adaptive mechanism to maintain normal serum phosphorus concentrations in the presence of kidney disease,[19] may also be detrimental as suggested by our data, in which increased FGF-23 concentrations had a strong relationship with death, cardiovascular events, and initiation of dialysis.

Previous observational studies have found a similar relationship of FGF-23 and adverse outcomes. In a prospective cohort of incident dialysis patients, the risk of all-cause mortality increased across ascending FGF-23 concentrations, with those in the highest quartile having a sixfold increased risk of death compared with subjects in the lowest quartile.[15] Recently, increased FGF-23 concentrations have also been shown to be associated with all-cause mortality in patients with stable coronary artery disease[20] and with left ventricular hypertrophy in predialysis subjects.[21] Furthermore, higher FGF-23 concentrations were independently associated with vasoreactivity and increased arterial stiffness in a recent community-based cohort.[22] Our findings also support the association of higher FGF-23 concentrations with vascular stiffness, as we found that FGF-23 was associated with lower extremity amputations in patients with advanced CKD.

The role of FGF-23 in kidney disease progression is largely unknown. A recent study of 177 nondiabetic patients with mild-to-moderate CKD found that higher FGF-23 concentrations, but not serum calcium, phosphorus, or iPTH, were independently associated with more rapid progression of CKD.[16] The main limitations of this study were the small sample size and the inability to adjust for potentially important confounders, such as vitamins 25(OH)D

and 1,25(OH)$_2$D and the availability of adjudicated end points. Thus, to our knowledge, this is the first study that thoroughly examines the relationship between plasma FGF-23 concentrations with the most important complications of CKD, including death, cardiovascular events, and initiation of chronic dialysis. In addition, the uniqueness of this analyses is derived from having 1,25(OH)$_2$D measurements in all study participants, which is a key potential confounder that has not been examined in previous analyses.

The mechanisms that underlie the association between plasma FGF-23 concentration with death, cardiovascular events, and CKD progression are unknown. It is possible that FGF-23 has direct cardiac and vascular toxicity.[23] Alternatively, it is conceivable that this association is mediated or confounded by other factors. Further studies are needed to elucidate these potential mechanisms.

The results of the present study suggest that plasma FGF-23 concentrations can potentially be used to guide therapies in patients with CKD and normal serum phosphorus concentrations who are not yet requiring chronic dialysis. FGF-23 has many characteristics of a clinically applicable therapeutic target, including the facts that it appears early in the course of kidney function decline and it is strongly associated with disordered phosphorus metabolism.[24] Alternatively, plasma FGF-23 concentration may also be useful to identify subjects with CKD that may benefit the most from lowering serum phosphorus concentrations even if they lie in the nominally normal range. A recent study of incident dialysis patients found that treatment with phosphorus binders within the first 90 d of dialysis was associated with a significant reduction in mortality.[25] Interestingly, the survival benefit was independent of phosphorus concentrations. The observations that phosphorus loading increases serum FGF-23 concentrations in healthy subjects and that the use of phosphate binders decreases serum FGF-23 concentrations in chronic dialysis patients provide a plausible mechanistic explanation for the association between the early use of phosphate binders and improved clinical outcomes.[25,26,27] Further interventional trials are necessary to determine if the reduction of plasma FGF-23 concentrations indeed provides cardio- and renoprotective effects in CKD patients.

Our study has several limitations. First, as an observational study, the causal relationship between FGF-23 concentrations and clinical outcomes cannot be established. Second, data on the presence or degree of proteinuria are not available in the parent HOST study. Nonetheless, we were able to adjust for BP control, a significant modulator of proteinuria, and for the use of renoprotective medications. Third, we did not have information on the use of active vitamin D analogues or nutritional vitamin D supplements. However, given the time period in which the HOST study was performed, vitamin D analogues and vitamin D supplementation were not prevalent in the studied patient population. Fourth, the definition of kidney disease was based

upon estimated MDRD-GFR rather than more precise measures of kidney function, such as iothalamate clearance. Finally, this study was comprised of mainly male subjects with advanced kidney disease and elevated plasma homocysteine concentrations; therefore, caution should be used when extrapolating these results to other populations.

In conclusion, plasma FGF-23 concentrations were strongly and independently associated with death, cardiovascular events, or progression to chronic dialysis in patients with advanced CKD not requiring dialysis. Further studies are needed to determine the mechanisms by which higher plasma FGF-23 concentrations are associated with clinical outcomes and whether therapeutic interventions to lower FGF-23 concentrations are clinical beneficial in CKD patients.

CONCISE METHODS

Homocysteinemia in Kidney and End Stage Renal Disease

The HOST study was a multicenter, prospective, randomized, double-blind, placebo-controlled trial examining the effects of folate, pyridoxine hydrochloride (vitamin B$_6$), and cyanocobalamin (vitamin B$_{12}$) replacement on all-cause mortality and cardiovascular events in patients with advanced kidney disease with elevated plasma total homocysteine concentrations.[17] A prespecified plan was to examine the time to initiation of chronic dialysis in the subgroup with advanced CKD but not requiring maintenance dialysis at the initiation of the study. The trial enrolled 2056 participants aged 21 yr of age or older, with ESRD receiving either maintenance hemodialysis or peritoneal dialysis (n = 751), or with an estimated creatinine clearance (calculated by the Cockcroft-Gault formula) of less than 30 ml/min (n = 1305) not requiring chronic dialysis. A plasma homocysteine concentration of 15 μmol/L or higher was also an inclusion criterion. Participants were excluded if they were pregnant; had a life expectancy of less than 6 mo; had end-stage liver disease or metastatic cancer; were taking methotrexate, antifolate medication, or anticonvulsants; expected to receive a living-related kidney donation in the next 6 mo; were noncompliant with medications; or were unable to give informed consent. The patients were enrolled from 36 Veterans Affairs medical centers after providing informed consent. Each center's institutional review board approved the study. The participants were randomly assigned to receive a once-daily capsule containing 40 mg of folic acid, 100 mg of vitamin B$_6$, and 2 mg of vitamin B$_{12}$ or a daily placebo capsule. Participant enrollment began in September 2001 and ended in October 2003.

Blood samples were collected from the participants in ethylenediaminetetraacetate (EDTA) at 3 mo after randomization in HOST. Plasma was separated and stored in a central repository at −80 °C until assays were performed. The HOST executive committee and the Cooperative Studies Program (CSP) of the Department of Veterans Affairs authorized the use of these plasma samples for the measurement of 25(OH)D, 1,25(OH)$_2$D, iPTH, and FGF-23. All participants with advanced CKD not requiring maintenance dialysis at the 3-mo

follow-up visit, and from whom plasma samples were available, were included in the present analysis, resulting in a final cohort of 1099 participants. The mean follow-up duration of this cohort from the 3-mo visit until the end of the study was 2.8 ± 1.1 yr (median 2.9 yr).

Measurement of Demographic, Biochemical, and Clinical Data

Information collected at the time of randomization included a complete history and physical examination; demographics; smoking status; history of alcohol intake; etiology of kidney disease; history of hypertension, diabetes, and cardiovascular disease; and use of medications, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), β-blockers, and aspirin. Systolic and diastolic BP was measured in the sitting position.[17] Serum creatinine was measured by a modified kinetic Jaffé method at each local site.[28] Serum total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), albumin, calcium, and serum phosphorus was also measured at local sites using standard techniques. Although estimated creatinine clearance (calculated by the Cockcroft-Gault formula) was used for eligibility of the HOST study, for the purpose of this analysis, GFR was estimated using the four-variable MDRD prediction equation.[29]

Fibroblast Growth Factor-23 Measurements

The plasma FGF-23 concentration measured 3 mo after randomization was the primary exposure variable in this analysis. C-terminal FGF-23 concentrations were measured in stored EDTA-plasma samples using a two-site second-generation ELISA kit (Immutopics, San Clemente, CA), with antibodies directed against two epitopes within the C-terminal region of the FGF-23 molecule. In addition to detecting intact FGF-23, this assay also detects its catabolic C-terminal fragments.[30] All laboratory measurements were performed at Associated Regional University Pathologists (ARUP) Laboratories at the University of Utah. The analytical measurement range for the FGF-23 assay is 3.0–2300 RU/ml. The intra-assay coefficients of variations (CVs) were 2.6% and 1.4% at 32.1 and 299.2 RU/ml, respectively. The interassay CVs were 3.4% and 4.4% at 32.1 and 299.2 ng/ml, respectively.

Outcomes

All fatal events were reviewed and classified by the HOST Endpoints Committee using information obtained from hospital discharge summaries, autopsy reports, Medicare End Stage Renal Disease Death Notification form, or death certificate. Deaths were also tracked with the Beneficiary Identification and Records Locator Subsystem, a VA file used to record death and dates.[31] The endpoints of cardiovascular events and initiation of chronic dialysis were obtained through self-reporting by participants during the follow-up period, verified by clinic and hospital records at the local site, and adjudicated by the HOST Central Endpoints Committee. Myocardial infarction was diagnosed when two of the following three criteria were present: (1) typical cardiac symptoms, (2) elevated serum cardiac biomarker concentrations, and (3) diagnostic electrocardiographic changes.[32] Thrombotic stroke was defined as an acute onset of persistent neurologic deficits compatible with an obstruction in the arterial system in the brain.[33] Amputations of all or part of a lower extremity were

ascertained through self-reporting. These diagnoses were verified using information provided by discharge summaries as well as neurologic examinations, imaging, cardiac biomarker, and electrocardiogram results in medical records.

The individual outcomes for this analysis were (1) time to death from any cause, (2) time to any cardiovascular event (composite of myocardial infarction, amputation, and stroke), (3) time to each specific cardiovascular event, and (4) time to initiation of chronic maintenance dialysis that occurred at least three months after randomization.

Other Measurements

Plasma 25(OH)D, 1,25(OH)$_2$D, and iPTH measurements were also performed at the ARUP laboratories. Plasma 25(OH)D concentrations were measured by a commercial competitive chemiluminescent immunoassay (DiaSorin, Stillwater, MN) on a Liaison analyzer and 1,25(OH)$_2$D was measured by a commercial competitive RIA (DiaSorin). The analytical measurement range for the 25(OH)D assay was 7 to 150 ng/ml. The intra-assay CVs were 5.6% and 4.5% at 11 and 28 ng/ml, respectively, while the interassay CVs were 9.1% and 5.6% at 16 and 51 ng/ml, respectively. For 1,25(OH)$_2$D, the range of the assay was 5 to 200 pg/ml. The intra-assay CVs were 12.6% and 9.7% at 13 and 45 pg/ml, respectively, while the interassay CVs were 21.4% and 14.7% at 25 pg/ml and 56 pg/ml, respectively. Plasma iPTH was measured using an electrochemiluminescent immunoassay, with a reference interval of 15 to 65 pg/ml. The intra and interassay CVs were both less than 5%.

Statistical Analyses

Subjects with CKD who had initiated chronic hemodialysis within 3 mo after randomization (n = 34; 2.6%) or for whom a plasma sample was not available for FGF-23 measurement (n = 172, 13.2%) were excluded, resulting in a final sample of 1099 subjects for the present study. Compared with those excluded, demographics, death, and cardiovascular event rates were similar to those in the subjects who were retained in this analysis (not shown). Demographic, cardiovascular risk factors, and risk factors for kidney disease progression were compared across quartiles of FGF-23 concentrations through the use of the chi-squared test for categorical data, ANOVA for continuous variables that are approximately normally distributed, and the Kruskall-Wallis test for skewed continuous variables. Graphical methods and measures of skewness and kurtosis were used to determine the distribution of FGF-23 concentrations in the plasma samples. Given the positively skewed distribution of FGF-23 concentrations, the values were transformed to the log base of 10. Spearman correlations were used to investigate the correlation of calcium, phosphorus, 25(OH)D, 1,25(OH)$_2$D, iPTH, and FGF-23 concentrations with one another.

Quartiles of FGF-23 concentrations were chosen as the primary predictor variable, with the lowest quartile serving as the reference category. Subsequently, the associations between plasma FGF-23 concentrations and all-cause mortality, cardiovascular events, or initiation of chronic dialysis were analyzed using Cox proportional hazard models. All-cause mortality was censored for analyses of cardiovascular events and initiation of dialysis.

The covariates for adjustment in the final models were primarily

identified by using models in which FGF-23, as a continuous predictor of each outcome and each candidate variable, was entered separately. Variables that changed the parameter estimate (beta coefficient) of FGF-23 by 5% or more were retained in the final models. In addition, candidate variables that were significantly associated with the outcome and had biologic plausibility for such associations were also retained in the final model. Three sequential sets of covariates were considered. In Model 1, the covariates included age, gender, and race. In Model 2, the covariates included those used in Model 1 plus comorbidity and biochemical measures, which we viewed as potential confounders of the relationship between FGF-23 concentration and death, cardiovascular events, or progression to chronic dialysis; these were smoking status, alcohol intake, diabetes, hypertension, history of cardiovascular disease, Charlson comorbidity score, BMI, systolic and diastolic BP, plasma homocysteine, serum folate and serum vitamin B_{12} concentrations at baseline, HOST treatment assignment, hemoglobin, MDRD-GFR, serum albumin, serum calcium, serum phosphorus, plasma 25(OH)D, plasma 1,25(OH)$_2$D, plasma iPTH, as well as total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. In Model 3, the covariates included those used in the first two models plus additional medication use, which are also possible confounders, but their inclusion in Cox regression models incurs a risk of bias by indication. These medications included ACEI, ARB, calcium channel blockers, β-blockers, aldosterone antagonists, lipid-lowering agents, aspirin, anticoagulants, anti-arrhythmic drugs, and sevelamer.

Proportional hazards assumptions were tested by using log-minus-log survival plots and plots of Schoenfeld residuals *versus* survival time. Analyses were repeated using log-transformed values for FGF-23 as a continuous variable. To further test the robustness of our results, a Cox model was performed using SAS PHREG with an ENTRY option on the model statement in which time before chronic dialysis onset was left-truncated, and a fully adjusted model was run using log-transformed values for FGF-23 concentration as the predictor. This analysis was performed with the objective of examining the association between plasma FGF-23 concentrations and death following the initiation of chronic dialysis in the participants who experienced this event during the follow-up period in the HOST study.

In addition, it is possible that including estimated MDRD-GFR as a covariate may not have fully controlled for confounding between MDRD-GFR and FGF-23 concentrations due to measurement error associated with short-term longitudinal variability in the estimated MDRD-GFR. To address this issue, we used statistical simulation/extrapolation (SIMEX) model[34] in secondary analyses to determine if the FGF-23's hazard ratio was overstated due to attenuation of the correlation between FGF-23 and MDRD-GFR in a Cox regression including Model 1 and Model 2 covariates. The SIMEX procedure was carried out assuming a measurement error variance in log MDRD-GFR of 0.015 based on repeat estimated GFR measurements during the baseline phases of the MDRD[35] and the African American Study of Kidney Disease (AASK)[36] studies. Application of the delta method yielded as estimated SD for the measurement error of 2.23 ml/min/1.73m^2 in the HOST data set, which corresponds to a reliability coefficient of 0.88.

Two-tailed values of $P<0.05$ were considered statistically signifi-

cant. All statistical analyses were performed with SAS software, version 9.13 (SAS Institute, Cary, NC).

DISCLOSURES
None.

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