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ORIGINAL RESEARCH

Fibroblast Growth Factor 23 and Risk of New Onset Heart Failure With Preserved or Reduced Ejection Fraction: The PREVEND Study

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BACKGROUND: The role of fibroblast growth factor 23 (FGF23) in the development of new-onset heart failure (HF) with reduced (HFrEF) or preserved ejection fraction (HFpEF) in the general population is unknown. Therefore, we set out to investigate associations of C-terminal FGF23 with development of new-onset HF and, more specifically, with HFrEF or HFpEF in a large, prospective, population-based cohort.

METHODS AND RESULTS: We studied 6830 participants (aged 53.8±12.1 years; 49.7% men; estimated glomerular filtration rate, 93.1±15.7 mL/min per 1.73 m^2) in the community-based PREVEND (Prevention of Renal and Vascular End-Stage Disease) study who were free of HF at baseline. Cross-sectional multivariable linear regression analysis showed that ferritin (standardized β, −0.24; P<0.001) and estimated glomerular filtration rate (standardized β, −0.13; P<0.001) were the strongest independent correlates of FGF23. Multivariable Cox proportional hazard regression was used to study the association between baseline FGF23 and incident HF, HFrEF (ejection fraction ≤40%) or HFpEF (ejection fraction ≥50%). After median follow-up of 7.4 [IQR 6.9–7.9] years, 227 individuals (3.3%) developed new-onset HF, of whom 132 had HFrEF and 88 had HFpEF. A higher FGF23 level was associated with an increased risk of incident HF (fully adjusted hazard ratio, 1.29 [95% CI, 1.06–1.57]) and with an increased risk of incident HFrEF (fully adjusted hazard ratio, 1.31 [95% CI, 1.01–1.69]). The association between FGF23 and incident HFpEF lost statistical significance after multivariable adjustment (hazard ratio, 1.22 [95% CI, 0.87–1.71]).

CONCLUSIONS: Higher FGF23 is independently associated with new-onset HFrEF in analyses fully adjusted for cardiovascular risk factors and other potential confounders. The association between FGF23 and incident HFpEF lost statistical significance upon multivariable adjustment.

Key Words: fibroblast growth factor 23 ■ general population ■ heart failure with preserved ejection fraction ■ heart failure with reduced ejection fraction

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that stimulates urinary phosphate excretion and suppresses activation of 25-hydroxy-vitamin D in the kidney. In addition to these effects on phosphate and mineral metabolism in the kidney, elevated FGF23 levels can trigger off-target effects, including the induction of left ventricular hypertrophy.[1,2] In patients with chronic kidney disease (CKD), higher FGF23 has been consistently linked with adverse cardiovascular outcomes including incident heart failure (HF).[3] Moreover, our group and others have demonstrated that in patients with established HF, a higher FGF23 level predisposes to

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CLINICAL PERSPECTIVE

What Is New?

- Higher fibroblast growth factor 23 levels in the general population are significantly associated with an increased risk of developing heart failure with reduced ejection fraction, independent of potential confounders.

What Are the Clinical Implications?

- Fibroblast growth factor 23 may be a promising biomarker to identify individuals at risk for the development of heart failure with reduced ejection fraction.
- The currently observed relationship between fibroblast growth factor 23 and heart failure with reduced ejection fraction sets the stage for prospective studies investigating fibroblast growth factor 23 as a potential therapeutic target in heart failure with reduced ejection fraction prevention.

Nonstandard Abbreviations and Acronyms

FGF23	fibroblast growth factor 23
HFrEF	heart failure with reduced ejection fraction
HFpEF	heart failure with preserved ejection fraction
PREVEND	Prevention of Renal and Vascular End-Stage Disease
PTH	parathyroid hormone
TSAT	transferrin saturation

adverse outcomes including (re)hospitalization and premature mortality.[4]

While initial studies focused on the role of FGF23 in populations with impaired baseline kidney function or heart failure, less is known about the potential relationship between higher FGF23 levels and the risk of new-onset HF in the general population. Although some studies have shown associations between higher baseline concentration of FGF23 and increased risk of HF in the general population,[5–9] little is known about the role of FGF23 in the development of new-onset HF with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF) as individual outcomes. Recent mechanistic studies indicate that FGF23 can affect functional properties of cardiomyocytes without changing contractility, suggesting a role in diastolic dysfunction.[10] Moreover, FGF23 has been associated with endothelial dysfunction.[11] At the same time, FGF23 has been linked with regulation of renal sodium reabsorption[12] and renin-angiotensin-aldosterone system activation.[13] Thus, although FGF23 has been linked with processes driving both HFpEF and HFrEF, it is currently unclear whether it is associated with predominantly incident HFpEF, HFrEF, or both. Therefore, in the current study, we aimed to investigate the association between FGF23 and HF and focus on HFpEF and HFrEF individually, in the general population-based PREVEND (Prevention of Renal and Vascular End-Stage Disease) cohort.

METHODS

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Population

This study was conducted within the PREVEND cohort, which is designed to prospectively investigate the natural course of increased levels of urinary albumin excretion and its relation to renal and cardiovascular disease in a large cohort of Dutch men and women aged 28 to 75 years drawn from the general population. In total, 8592 subjects constituted the PREVEND study at baseline in 1997 to 1998. Details of this cohort have been described elsewhere.[14,15]

A flowchart indicating participant distribution is provided in Figure 1. For the current analysis, we used data from the second survey in 2002 to 2003 (n=6894) and defined this survey as baseline. Since we were interested in new-onset HF, we excluded participants with prevalent HF at the second survey. The PREVEND study was approved by the medical ethics committee of the University Medical Center Groningen and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Data Collection

The procedures at each examination in the PREVEND study have been described in detail previously.[16] In brief, each examination included 2 visits to an outpatient clinic separated by 3 weeks. Before the first visit, all participants completed a self-administered questionnaire regarding demographics, cardiovascular and renal disease history, smoking habits, alcohol consumption, and medication use. During the first visit, participants' height and weight were assessed. Height was measured to the nearest 0.5 cm. Weight was measured to the nearest 0.5 kg after removing shoes and heavy clothing with a Seca balance scale (Vogel and Halke, Hamburg, Germany). During each

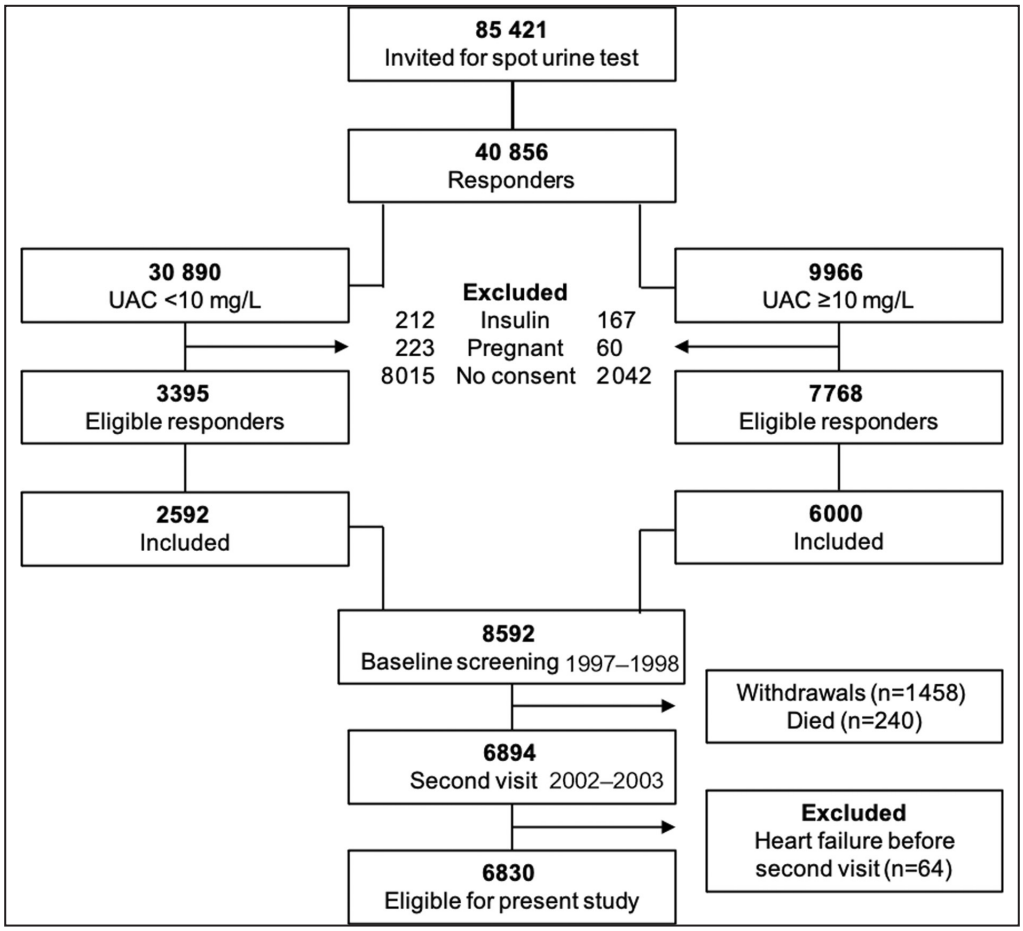


Figure 1. Flowchart of 6830 PREVEND participants selected for final analysis.
UAC indicates urinary albumin concentration

visit, blood pressure was measured on the right arm, every minute for 10 and 8 minutes, respectively, by an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical, Tampa, FL). The mean of the last 2 recordings from each of the 2 visits was used. In the last week before the second visit, subjects had to collect 2 consecutive 24-hour specimens after thorough oral and written instruction. After handing in the urine collections, the urine specimens were stored at −20 °C. Furthermore, fasting blood samples were provided and stored at −80 °C.

Measurement of Plasma FGF23 Concentration

FGF23 was measured in EDTA plasma using a commercially available ELISA kit (Quidel, San Diego, CA). This ELISA, which detects both the intact protein and C-terminal fragments, has intra- and interassay coefficients of variation of <5% and<16% in blinded replicated samples, respectively.[17]

Measurement of Other Variables

Body mass index was calculated as weight (kg) divided by height squared (m^2). Smoking status was categorized as ever (current and former) and never. Alcohol consumption was categorized as no/almost never (<1 standardized drink per month) and yes (≥1 standardized drink per month). Type 2 diabetes was defined as a fasting glucose ≥7.0 mmol/L (126.0 mg/dL), nonfasting glucose >11.1 mmol/L (199.8 mg/dL), or use of glucose-lowering drugs. Hypercholesterolemia was defined as total serum cholesterol ≥0.21 mmol/L (≥240 mg/dL) or use of lipid-lowering treatment. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg or use of blood pressure–lowering treatment. Measurement of plasma creatinine, phosphate, and albumin was performed by an isotope dilution mass spectrometry traceable enzymatic method on a Roche Modular analyzer using reagents and calibrators from Roche (Roche Diagnostics, Mannheim, Germany). Estimated glomerular filtration rate (eGFR)

was calculated with the Chronic Kidney Disease Epidemiology Collaboration formula.[18] Concentrations of 25-hydroxy-vitamin D were measured using liquid chromatography–tandem mass spectrometry as previously described.[19] An automated 2-site immunoassay (Roche Diagnostics, Indianapolis, IN) was used to measure plasma intact parathyroid hormone (PTH). Plasma NT-proBNP (N-terminal pro-B-type natriuretic peptide) was measured on the Roche Modular E170 (Roche Diagnostics) with commercially available kits as previously described.[20] Serum iron was measured using a colorimetric assay, ferritin using immunoassay, and transferrin using an immunoturbidimetric assay (all Roche Diagnostics). Transferrin saturation (TSAT, %) was calculated as 100 × serum iron (μmol/L) ÷ 25 x transferrin (g/L).[21] Urinary albumin excretion was calculated as the average value from 2 consecutive 24-hour urine collections. Other variables were measured using routine laboratory tests in venous blood and the collected urine.

Follow-Up and Ascertainment of HF Events

HF was identified using criteria described in the Heart Failure Guidelines of the European Society of Cardiology, and an end point adjudication committee ascertained the diagnosis of HF as described elsewhere.[22] In addition, HF was subclassified as HFrEF or HFpEF based on the left ventricular ejection fraction (LVEF) at the time of diagnosis (LVEF fraction ≤40% or ≥50%, respectively). Individuals with an LVEF 41% to 49% (n=7) were excluded from the cause-specific analyses of HFpEF and HFrEF to prevent blending and dilution of differential epidemiologic profiles. The etiology and the date of onset of HF were also derived from clinical charts. Data on LVEF were available in 98.4% of cases with new-onset HF. In 6 cases, diagnosis was confirmed through joint decision, because of insufficient data on LVEF.

Follow-up for the present investigation was defined as the time between the second survey and the date of new-onset HF, death, or January 2010. Information on dates of death for every participant was obtained from Statistics Netherlands.

Statistical Analysis

We performed multiple imputations using multivariate imputation by chained equations of any of the covariates with missing values to lessen the loss of precision and power resulting from exclusion of individuals with incomplete variables. The variable with the largest percentage of missingness was CRP (C-reactive protein) (6.3%). Five percent of FGF23 data was missing. There were no missing data on the outcome variables HF, HFpEF, and HFrEF. Missingness was dependent on observed variables in our data (data not shown). Before imputing the missing values, skewed variables were transformed to approximate a normal distribution. Transformed values were converted back to the original scale after multiple imputations. In addition to all baseline variables that predicted missingness, the multiple imputations model included the outcome variables and all covariates that were in the final Cox regression models. We imputed 10 data sets to avoid producing a large Monte Carlo error. Estimates of the 10 data sets were pooled using Rubin's rules to account for variability in the imputation procedure. As pooled estimates from imputed data are considered superior to those from complete case analysis, the estimates in this article are those of the imputed data.[23,24]

Baseline characteristics are presented according to tertiles of plasma FGF23 concentration. Continuous data are presented as mean with SD or as median and interquartile range in case of skewed distribution. Categorical data are presented as percentiles.

Linear regression analyses adjusted for age and sex were used to identify correlates of FGF23 in PREVEND. Variables with a *P* value <0.2 in age- and sex-adjusted analysis were subsequently added to multivariable linear regression models. To study the association of FGF23 with HFpEF and HFrEF risk, we considered HFpEF and HFrEF as competing risks and therefore performed multivariate cause-specific Cox proportional hazards regression analyses (eg, censoring for the opposing HF type). FGF23 concentration was analyzed as a continuous term per 2-log increment (ie, per doubling) and in tertiles. We included variables in the multivariable models that in previous studies were associated with the risk of incident HF. The variables that were independently associated with FGF23 were also included in the multivariable models. The following Cox regression models were composed: model 1, adjusted for age and sex; model 2, additionally adjusted for body mass index, smoking (ever versus never), alcohol use (yes versus no/almost never), hypercholesterolemia (yes versus no), hypertension (yes versus no), diabetes type 2 (yes versus no), myocardial infarction (yes versus no), atrial fibrillation (yes versus no), and eGFR and urinary albumin excretion; model 3, as model 2 and additionally adjusted for CRP, hemoglobin, ferritin and TSAT; model 4, as model 3 and additionally adjusted for NT-proBNP (fully adjusted model). Then, the bone mineral biomarkers plasma 25-hydroxy-vitamin D, plasma calcium, plasma phosphate and PTH were separately added to the final model, to study the potential influence of each bone mineral biomarker (eg, potential mediators). Finally, cubic splines were generated for fully adjusted models to illustrate the hazard ratios of HF, HFpEF, and HFrEF outcomes according to FGF23 concentrations.

Effect modification by age, sex, or eGFR was analyzed using multiplicative interaction terms in fully adjusted Cox regression models with HF, HFpEF, or HFrEF as outcomes. In sensitivity analysis, we compared the estimates derived from a complete case analysis with the estimates derived from the multiple imputations data set.

The proportional hazard assumption was assessed for every predictor variable using graphical approaches, and we found no evidence of departure from this assumption.

All statistical analyses were performed with SPSS software version 23.0 for Windows (IBM, Armonk, NY), and R version 3.4.2 (R Foundation for Statistical Analysis, Vienna, Austria) (http://cran.r-project.org/). A 2-sided *P* value of <0.05 was considered statistically significant for all analyses except for analyses including interaction terms for which a 2-sided *P* value of <0.1 was considered statistically significant.

RESULTS

In the 6830 PREVEND participants eligible for analysis (Figure 1), age at baseline was 53.8±12.1 years, 49.7% were men, and plasma FGF23 level was 69.5 [IQR 56.4–87.7] RU/mL. Baseline characteristics according to tertiles of FGF23 are presented in Table 1.

Associations of FGF23 With Clinical Variables

Individuals with higher FGF23 level were more often older, women, more likely to have cardiovascular risk factors, and had higher body mass index, plasma CRP, glucose, PTH, calcium, phosphate, NT-proBNP, and urinary albumin excretion. Participants with a higher FGF23 level had a lower eGFR, plasma albumin, hemoglobin, ferritin, TSAT, 25-hydroxy-vitamin D and high-density lipoprotein cholesterol.

In multivariable linear regression analysis, ferritin and eGFR were most strongly associated with FGF23 (standardized β, −0.24 and −0.13, respectively; *P* for both variables <0.001). Furthermore, in decreasing correlation strength, plasma calcium, plasma phosphate, sex, body mass index, smoking, high-sensitivity CRP, PTH, urinary albumin excretion, atrial fibrillation, age, hypercholesterolemia, and type 2 diabetes were independently positively associated with FGF23. Hemoglobin, TSAT, and 25-hydroxy-vitamin D were independently inversely associated with FGF23 (Table 2).

FGF23 and New-Onset HF

During a median follow-up 7.4 [IQR 6.9–7.9] years, 227 individuals (3.3%) were diagnosed with new-onset HF. In age- and sex-adjusted Cox proportional hazard regression analysis, each doubling of FGF23 was associated with an increased risk of HF (hazard ratio [HR], 1.48 [95% CI, 1.26–1.73]; *P*<0.001) (Table 3). After adjustment for known risk factors for HF, this association remained independent (HR, 1.29 [95% CI, 1.06–1.57]; *P*=0.01).

The estimates derived from the fully adjusted model did not change essentially when bone mineral biomarkers 25-hydroxy-vitamin D, plasma phosphate, calcium, and PTH were added separately (Tables S1 and S2). We found no evidence for effect modification by sex, age, or eGFR ($P_{interaction}$=0.3, 0.3, and 0.5, respectively). Complete case analysis (n=5187) yielded 124 individuals with new-onset HF. In the fully adjusted complete case analysis, the association of FGF23 with HF lost statistical significance (HR, 1.12 [95% CI, 0.84–1.50]; *P*=0.4).

FGF23 and HF Subtypes

Of the 227 individuals that were diagnosed with new-onset HF, 88 (38.8%) were classified as HFpEF and 132 (58.1%) as HFrEF. The other 7 individuals with new-onset HF were classified as HFwith midrange ejection fraction and were left out of the analyses. Cause-specific Cox proportional hazard regression analysis showed that the association between FGF23 and HF subtype was statistically significant for both types of HF in the analysis adjusted for age and sex (HR, 1.54 [95% CI, 1.27–1.88]; *P*<0.001 for HFrEF; and HR, 1.39 [95% CI, 1.07–1.82]; *P*=0.01 for HFpEF) (Table 3). After full adjustment, each doubling of FGF23 was associated with an estimated 31% greater risk of incident HFrEF (Table 3 and Figure 2; final model: HR, 1.31 [95% CI, 1.01–1.68]; *P*=0.04). The association between FGF23 and the risk of HFpEF lost statistical significance in fully adjusted analyses (final model: HR, 1.22 [95% CI, 0.87–1.71]; *P*=0.26). The results of the fully adjusted models did not materially change when plasma 25-hydroxy-vitamin D, phosphate, calcium, and PTH were added separately (Tables S1 and S2). We found no evidence for effect modification by sex, age, and eGFR for HFrEF nor for HFpEF ($P_{interaction}$ all>0.1). The estimates derived from complete case analysis (Table S2) with 88 cases of new-onset HFrEF lost statistical significance (final model: HR, 1.19 [95% CI, 0.84–1.68]; *P*=0.3).

DISCUSSION

Our study confirms previous findings linking FGF23 to incident HF and extended these findings by focusing on the specific associations with incident HFpEF or HFrEF. Only the association with HFrEF persisted upon multivariable adjustment.

Table 1. Baseline Characteristics According to Tertiles of C-Terminal FGF23 in 6830 PREVEND Participants

	All n=6830	Tertiles of C-terminal FGF23			*P* Value*
		I n=2277	II n=2277	III n=2276	
FGF23, RU/mL	69.5 [56.4–87.7]	52.0 [46.3–56.3]	69.5 [65.0–74.4]	100.2 [87.7–127.3]	<0.001
Age, y	53.8±12.1	51.9±12.0	53.6±12.1	55.8±12.5	…
Men	3394 (49.7)	1250 (54.9)	1195 (52.5)	949 (41.7)	…
White, n (%)	6551 (95.9)	2159 (94.8)	2193 (96.3)	2199 (96.6)	0.02
BMI, kg/m^2	26.7±4.4	26.2±3.9	26.6±4.4	27.4±4.9	<0.001
≥30	1318 (19.3)	331 (14.5)	406 (17.8)	581 (25.5)	<0.001
Systolic blood pressure, mm Hg	126.5±18.9	125.5±18.5	125.9±18.9	128.1±20.2	0.05
Diastolic blood pressure, mm Hg	73.4±9.1	73.5±9.5	73.3±9.3	73.5±9.4	0.8
Smoking status, ever	4868 (71.3)	1518 (66.7)	1637 (71.9)	1713 (75.3)	<0.001
Alcohol use, yes vs no/almost never	5100 (74.7)	1787 (78.5)	1740 (76.4)	1573 (69.1)	<0.001
Hypertension, yes	2320 (34.0)	626 (27.5)	743 (32.6)	951 (41.8)	<0.001
Myocardial infarction, yes	172 (2.5)	43 (1.9)	46 (2.0)	83 (3.6)	<0.001
Atrial fibrillation	58 (0.8)	8 (0.4)	11 (0.5)	39 (1.7)	<0.001
Diabetes type 2, yes	432 (6.3)	107 (4.7)	122 (5.4)	203 (8.9)	<0.001
Hypercholesterolemia, yes	2122 (31.0)	628 (27.6)	672 (29.5)	822 (36.1)	0.001
Hemoglobin, mmol/L	8.5±0.8	8.6±0.7	8.6±0.7	8.4±0.9	0.1
Ferritin, µg/L	96.0 [48.0–172.0]	109.5.1 [61.1–188.1]	100.4 [53.4–177.6]	76.0 [30.1–150.2]	<0.001
Transferrin saturation	25.0±9.7	26.6±9.9	25.8±9.4	22.7±10.0	<0.001
High-sensitivity CRP, mg/L	1.4 [0.6–3.1]	1.2 [0.6–2.6]	1.3 [0.6–2.9]	1.7 [0.8–3.8]	<0.001
Plasma creatinine, µmol/L	73.0±20.8	70.8±14.2	72.7±14.5	75.3±30.5	<0.001
eGFR, mL/min*1.73 m^2	93.1±15.7	96.8±13.9	93.4±14.8	89.1±18.1	<0.001
<60	219 (3.2)	17 (0.7)	49 (2.2)	153 (6.7)	<0.001
Plasma glucose, mmol/L	5.1±1.2	5.0±1.2	5.0±1.2	5.2±1.4	0.002
Total cholesterol, mmol/L	5.4±1.1	5.4±1.1	5.4±1.1	5.5±1.1	0.5
HDL cholesterol, mg/dL	48.5±12.2	48.8±12.3	48.6±12.1	48.1±12.8	<0.001
Plasma 25-OH-vitamin D, nmol/L	59.0±26.3	60.3±27.0	60.3±26.7	56.5±25.8	<0.001
Plasma parathyroid hormone, pmol/L	4.9 [4.0–5.9]	4.8 [4.0–5.7]	4.8 [4.0–5.8]	5.0 [4.1–6.1]	<0.001
Plasma albumin, g/L	43.9±4.7	44.1±5.4	44.0±4.3	43.6±4.7	0.2
Plasma phosphate, mmol/L	1.02±0.36	1.00±0.35	1.01±0.31	1.05±0.44	0.002
Plasma calcium, mmol/L	2.30±0.12	2.29±0.11	2.31±0.12	2.31±0.13	<0.001
NT-proBNP, ng/L	41.3 [21.0–80.6]	37.5 [19.1–72.6]	39.9 [19.8–75.6]	47.3 [24.7–94.2]	0.04
Urinary creatinin excretion, mmol/24 h	12.4±3.4	12.8±3.5	12.5±3.5	11.9±3.4	0.2
Urinary albumin excretion, mg/24 h	8.8 [6.1–16.3]	8.5 [6.0–15.1]	8.6 [6.1–14.9]	9.5 [6.2–19.9]	<0.001
Albuminuria	950 (13.9)	266 (11.7)	274 (12.0)	410 (18.0)	<0.001
Urinary phosphate excretion, mmol/24 h	26.5±13.6	26.9±13.6	27.1±15.3	25.4±12.01	0.8
Medication					
ACEi/ARB	799 (11.7)	232 (10.2)	259 (11.4)	308 (13.5)	0.007
Statins	541 (7.9)	119 (5.2)	153 (6.7)	269 (11.8)	<0.001
Antidiabetics	248 (3.6)	63 (2.8)	71 (3.1)	114 (5.0)	<0.001
Calcium	84 (1.2)	30 (1.3)	24 (1.1)	30 (1.3)	0.6
Vitamin D	48 (0.7)	16 (0.7)	11 (0.5)	21 (0.9)	0.2

Data are presented as n (%), mean±SD, or median [interquartile range] for nominal, normally distributed, and nonnormally distributed data, respectively. 25-OH-vitamin D indicates 25-hydroxycholecalciferol; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

*The *P* value represents the *P* for trend in multinominal linear regression analysis adjusted for age and sex.

Table 2. Multivariable Associations of Log2-Transformed C-Terminal FGF23 With Clinical Variables in 6830 PREVEND Participants

Clinical parameter	Log_2FGF23	
	Std. β	*P* Value*
Ferritin, µg/L	−0.24	<0.001
eGFR, mL/min per 1.73 m^2	−0.13	<0.001
Hemoglobin, mmol/L	−0.08	<0.001
Plasma calcium, mmol/L	0.08	<0.001
Plasma phosphate, mmol/L	0.07	<0.001
Sex, male vs female	0.07	<0.001
BMI, kg/m^2	0.06	<0.001
Smoking status, ever vs never	0.05	<0.001
Transferrin saturation, %	−0.05	<0.001
High-sensitivity CRP, mg/L	0.04	<0.001
Plasma parathyroid hormone, pmol/L	0.04	<0.001
Urinary albumin excretion, mg/24 h	0.03	<0.001
Plasma 25-OH vitamin D, nmol/L	−0.03	<0.001
Atrial fibrillation	0.03	<0.001
Age, y	0.03	0.01
Hypercholesterolemia, yes vs no	0.02	0.02
Diabetes type 2, yes vs no	0.02	0.04
Hypertension, yes vs no	0.02	0.07
Alcohol use, yes vs no/almost never	−0.01	0.08
Myocardial infarction, yes vs no	0.01	0.19
Caucasian, yes vs no	−0.01	0.22
NT-proBNP, ng/L	0.004	0.69

25-OH-vitamin D indicates 25-hydroxycholecalciferol; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

*The *P* value is obtained with multivariable linear regression analyses with forward selection method. Total R^2=0.27.

Associations of FGF23 With Clinical Variables

In line with its physiological functions, FGF23 was independently associated with eGFR and urinary albumin excretion, and with bone mineral biomarkers 25-OH-vitamin D, plasma phosphate, calcium, and PTH. Of interest, ferritin was the strongest independent determinant of FGF23, showing an inverse relationship. Our groups and others have shown an inverse relationship between C-terminal FGF23 levels and parameters of iron status.[25–28] In addition, longitudinal data in patients with CKD showed an independent association of baseline FGF23 with change in hemoglobin over time and risk of incident anemia in patients with CKD stages 2 to 4. We added ferritin, TSAT, and hemoglobin as a covariate in Cox regression analyses, since iron and anemia levels have also been associated with new-onset HF,[29–31] but this did not materially change the associations between FGF23 and HF outcomes.

Furthermore, FGF23 was independently positively associated with CRP. Other data have linked FGF23 to the immune response both directly[32] and indirectly through inflammation[33] and vitamin D metabolism.[34] CRP was also added as a potential confounder in the Cox regression analyses.

FGF23 and New-Onset HF

Our study confirms previous findings linking FGF23 to incident HF after adjustment for cardiovascular risk factors and other potential confounders. Our group and others have shown that the strength of FGF23 as a cardiovascular risk predictor can be attenuated by adjustment for NT-proBNP.[22,35,36] We demonstrated in this study that FGF23 is significantly associated with incident HF independent of NT-proBNP levels, underlining an independent role for FGF23 as a risk factor for new-onset HF. In line, previous intervention studies showed that FGF23 reduction with calcimimetics resulted in lower left ventricular mass index[37] and incident HF in patients on hemodialysis.[38]

FGF23 and New-Onset HFrEF

Although the observational design of our study precludes a definite mechanistic explanation for the positive association between FGF23 and new-onset HFrEF, we can speculate on potential factors involved. First, FGF23 correlates with the presence of left ventricular hypertrophy (LVH). Although LVH is associated with both types of HF, in the Cardiovascular Health Study, LVH was especially associated with HFrEF.[39] Nehgme et al[40] showed that children with X-linked hypophosphatemic rickets, an X-linked dominant disease with FGF23 overexpression and consecutive hypophosphatemia, have signs of LVH. In a study by Reindl et al,[41] FGF23 was significantly higher in patients who developed left ventricular remodeling following ST-segment–elevation myocardial infarction even after adjustment for biomarkers of myocardial necrosis, myocardial stress, and inflammation. Moreover, some experimental data labeled FGF23 as a direct and causal stimulator for LVH via specific myocardial FGF23-receptor activation.[1,2,42–44] On the contrary, there are also studies in patients on hemodialysis that have found no association between FGF23 and ventricular mass,[45,46] and children with X-linked hypophosphatemic rickets do not universally develop LVH.[40,47]

In addition to the potential role of FGF23 in cardiac remodeling, its association with reduced renal function may represent a potential mechanism for the observed association with HF and HFrEF. Chronic kidney disease is a major risk factor for development of new-onset HF.[48] In our study, FGF23 strongly

Table 3. Hazard Ratios of Incident HF, HFpEF, and HFrEF By Tertiles of FGF23 Concentration and per Doubling of FGF23 Concentration in 6830 PREVEND Participants

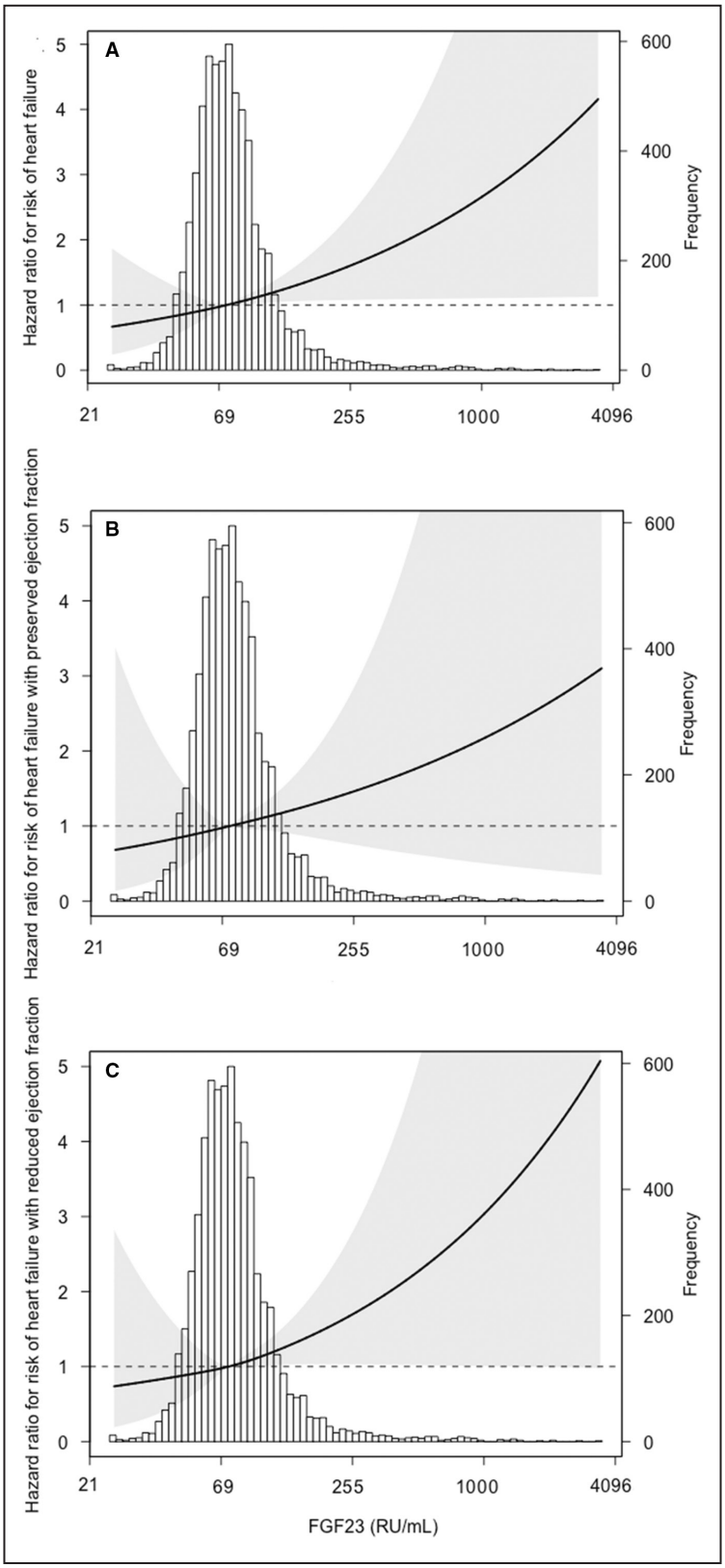
	Hazard Ratio (95% CI)			
	FGF23 concentration			
	Tertile 1 (21–61)	Tertile 2 (61–81)	Tertile 3 (81–3495)	Per doubling of FGF23 concentration
Heart failure				
Events, No.	45	61	121	227
Model 1	1	1.18 (0.79–1.76)	1.92 (1.34–2.76)	1.48 (1.26–1.73)
Model 2	1	1.14 (0.76–1.71)	1.42 (0.97–2.07)	1.29 (1.08–1.55)
Model 3	1	1.12 (0.75–1.68)	1.35 (0.92–1.98)	1.32 (1.08–1.62)
Model 4	1	1.13 (0.75–1.70)	1.36 (0.93–2.00)	1.29 (1.06–1.57)
HFpEF				
Events, No.	15	26	47	88
Model 1	1	1.39 (0.73–2.67)	1.94 (1.06–3.56)	1.39 (1.07–1.82)
Model 2	1	1.31 (0.68–2.53)	1.50 (0.80–2.82)	1.25 (0.91–1.70)
Model 3	1	1.30 (0.67–2.50)	1.43 (0.75–2.73)	1.21 (0.86–1.69)
Model 4	1	1.29 (0.67–2.50)	1.42 (0.74–2.72)	1.22 (0.87–1.71)
HFrEF				
Events, No.	29	33	70	132
Model 1	1	1.04 (0.62–1.76)	1.90 (1.20–3.00)	1.54 (1.27–1.88)
Model 2	1	1.01 (0.60–1.72)	1.34 (0.82–2.18)	1.33 (1.06–1.68)
Model 3	1	0.99 (0.58–1.69)	1.28 (0.78–2.09)	1.41 (1.09–1.84)
Model 4	1	0.99 (0.58–1.69)	1.28 (0.78–2.09)	1.31 (1.01–1.69)

Model 1: adjusted for age and sex. Model 2: model 1 additionally adjusted for White race (yes vs no), body mass index, smoking (ever vs never), alcohol use (yes vs no/almost never), hypercholesterolemia (yes vs no), hypertension (yes vs no), diabetes type 2 (yes vs no), myocardial infarction (yes vs no), atrial fibrillation (yes vs no), estimated glomerular filtration rate, and urinary albumin excretion. Model 3: model 2 additionally adjusted for high-sensitivity C-reactive protein, hemoglobin, ferritin, and transferrin saturation. Model 4: model 3 additionally adjusted for N-terminal pro-B-type natriuretic peptide. FGF23 indicates fibroblast growth factor 23; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

correlated with renal function measured with eGFR, a relationship consistently observed in a vast number of studies.[49] However, in our study, the predictive value of FGF23 proved to be statistically significant after adjustment for eGFR and remained virtually unchanged comparing patients with eGFR of ≥90 (62% of participants) and<90 (38% of participants) mL/min per 1.73 m^2. Only 3% of participants had an eGFR <60 mL/min per 1.73 m^2 and therefore further studies in patients with CKD with a clear discrimination of HF entities are warranted.

Other mechanisms by which FGF23 could lead to pathological pathways involved in both HFrEF and HFpEF include stimulation of distal tubular sodium absorption[12]; suppression of angiotensin-converting enzyme 2 transcription in the kidney, leading to a direct stimulatory effect on the renin-angiotensin-aldosterone system[13]; and disturbed phosphate and vitamin D homeostasis. Hyperphosphatemia has been shown to be associated with incident HF and an elevated risk for cardiovascular events in the general population and patients with stable coronary artery disease.[50–52] Similarly, low levels of vitamin D are linked with increased mortality in HF.[53] In our study, FGF23 revealed a moderate correlation with phosphate and a stronger negative correlation with 25-hydroxy-vitamin D. Nevertheless, multivariable adjustment for both variables did not statistically significantly affect the association of FGF23 with HF and HFrEF and may therefore be of minor importance in this context.

Figure 2. Prospective associations between fibroblast growth factor 23 (FGF23) levels and development of heart failure (A), heart failure with preserved ejection fraction (B), and heart failure with reduced ejection fraction (C).
Adjusted for age, sex, White race, body mass index, smoking, alcohol use, hypercholesterolemia, hypertension, diabetes type 2, myocardial infarction, atrial fibrillation, estimated glomerular filtration rate, urinary albumin excretion, high-sensitivity C-reactive protein, hemoglobin, ferritin, transferrin saturation, and N-terminal pro-B-type natriuretic peptide, according to model 4. Knots have been placed at 10th, 50th, and 90th percentiles of $\log_2$ FGF23 levels. Line represents hazard ratio, and gray area represent the 95% CI.



In our study, patients in the highest tertile of FGF23 were more likely to have cardiovascular risk factors for ischemic cardiomyopathy (eg, smoking, coronary artery disease, diabetes type 2, hypertension, hypercholesterolemia). Although multivariable adjustment for these cardiovascular risk factors did not significantly change the association of FGF23 with HFrEF, residual confounding cannot be excluded.

Finally, recent studies show evidence of myocardial production and release of FGF23.[54,55] This challenges the concept of skeletal FGF23 leading to cardiotoxic effects. Richter el al found cultured cardiomyocytes to express FGF23.[54] In subsequent clinical studies, FGF23 was shown to be present in the explanted hearts of patients with ischemic or dilated cardiomyopathy undergoing heart transplantation but not in healthy hearts. In another set of experiments, Andrukhova et al found that, in the setting of experimental myocardial infarction in mice, circulating FGF23 is increased in the circulation.[55] In addition to increased FGF23 production in the bone, myocardial FGF23 was also increased at both protein and mRNA levels, suggesting that increased circulating FGF23 following myocardial infarction is at least partly derived from the myocardium itself.[55] In the light of these observations, we cannot exclude the possibility that patients in our study may have had higher circulating FGF23 levels originating at least partly from the myocardium, which may have influenced the association with new-onset HF.

FGF23 and New-Onset HFpEF

In this study, the association between FGF23 and incident HFpEF lost statistical significance upon multivariable adjustment. Our study may have been underpowered to detect a statistically significant association between FGF23 and HFpEF in our fully adjusted model. Probably because of the relatively young age at baseline in the current study (mean age, 53.8 years), the number of individuals with new-onset HFpEF is relatively low, compared with other community-based studies.[22] However, theoretically, the involvement of FGF23 in HFpEF is plausible. FGF23 may promote myocardial fibrosis through upregulation of B-catenin, transforming growth factor-β, procollagen I, and procollagen III, which may exacerbate diastolic dysfunction.[56] Moreover, some of the aforementioned pathways potentially linking FGF23 with HFrEF, including sodium retention and renin-angiotensin-aldosterone system activation, have also been implicated in the development of HFpEF.[12,57]

To our knowledge, our study is the first to study the association between C-terminal FGF23 and HFpEF or HFrEF as specific outcomes in the general population. There is 1 previous study reporting on the association of FGF23 and HFpEF and HFrEF separately, but in this study, the full-length intact FGF23 molecule was measured.[57] In contrast with our results, the authors found higher FGF23 levels to be associated with HFpEF events but not HFrEF events. Subjects in this multiethnic cohort (Multiethnic Study of Atherosclerosis) were older, had a higher prevalence of type 2 diabetes, and were free of coronary heart disease at baseline. Sample size and length of follow-up were comparable with our study; however, more HFrEF as well as HFpEF cases were identified, most likely as a result of the higher average age at baseline. These differences, as well as the different type of FGF23 assay used, may explain the difference in the observed associations. Two recent systematic reviews both demonstrated marked heterogeneity in the strengths of associations with outcomes based on the FGF23 assay used for measurement in the underlying individual studies.[58,59] In addition, previous studies examined the association between FGF23 with ejection fraction as a continuous variable. FGF23 was associated with reduced ejection fraction in participants with CKD,[3] and in a population undergoing elective coronary angiography,[60] which supports a specific association of FGF23 with impaired left ventricular systolic function as observed in our study. With respect to diastolic function, Okamoto et al[61] did not find an association of FGF23 with diastolic impairment in a cross-sectional study comprising 269 patients with HFpEF.

Our study has several strengths and limitations. The large and well-characterized cohort with long-term follow-up, and the thorough validation of incident HFpEF and HFrEF diagnoses by an adjudication committee, with little loss to follow-up, are strengths of our study. Our study is limited by the fact that the PREVEND study subjects are predominantly White, and our results can therefore not be extrapolated to subjects from other races or ethnicities. Because of the observational nature of our study, residual confounding cannot be excluded. Further potential limitations are possible recall bias (since surveys were used to collect information regarding cardiovascular history, smoking habits, alcohol consumption, and medication use), the absence of detailed echocardiographic parameters, and the fact that we analyzed only a single baseline measurement of FGF23. Future population studies with a clear discrimination of HF entities and experimental studies investigating the exact pathophysiological mechanisms will be necessary to further elucidate the differential role of FGF23 in HFrEF and HFpEF. This will help determine whether therapies targeting FGF23 may positively influence the risk of incident HF.

In conclusion, we found that in the general population, a higher plasma C-terminal FGF23 level is independently associated with new-onset HFrEF in analyses fully adjusted for cardiovascular risk factors and other potential confounders. The association between FGF23 and incident HfpEF lost statistical significance upon multivariable adjustment.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Hazard ratios of incident HF, HFpEF and HFrEF per doubling of FGF23 concentration of the fully adjusted model supplemented with bone mineral markers (e.g. potential mediators) in 6830 participants of PREVEND.

	Hazard Ratio (95% CI)		
	Heart failure	**HFpEF**	**HFrEF**
Model 4	1.35 (1.10-1.64)	1.33 (0.97-1.83)	1.32 (1.01-1.79)
+ Plasma 25-OH vitamin D	1.29 (1.06-1.58)	1.22 (0.87-1.72)	1.31 (1.02-1.70)
+ Plasma phosphate	1.29 (1.05-1.59)	1.22 (0.87-1.72)	1.31 (1.01-1.70)
+ Plasma calcium	1.26 (1.03-1.54)	1.15 (0.82-1.62)	1.30 (1.00-1.68)
+ Plasma parathyroid hormone	1.29 (1.06-1.57)	1.23 (0.88-1.72)	1.31 (1.01-1.69)

Abbreviations: HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; FGF23, fibroblast growth factor 23; PREVEND Prevention of Renal and Vascular End-Stage Disease; 25-OH-vitamin D, 25-hydroxycholecalciferol.

Table S2. Hazard ratios of incident HF, HFpEF and HFrEF per doubling of FGF23 concentration in complete case analysis (n=5491)

	Hazard Ratio (95% CI)		
	Heart failure	**HFpEF**	**HFrEF**
Events, No.	126	38	98
Model 1	1.43 (1.15-1.77)	1.37 (0.88-2.13)	1.45 (1.13-1.86)
Model 2	1.16 (0.90-1.51)	1.10 (0.64-1.89)	1.16 (0.86-1.57)
Model 3	1.13 (0.84-1.52)	0.89 (0.49-1.62)	1.22 (0.86-1.73)
Model 4	1.12 (0.84-1.50)	0.93 (0.52-1.67)	1.19 (0.84-1.68)

Model 1	Adjusted for age and sex
Model 2	Model 1 + additionally adjusted for Caucasian (yes vs. no), body mass index, smoking (ever versus never), alcohol use (yes versus no/almost never), hypercholesterolemia (yes versus no), hypertension (yes versus no), diabetes mellitus type 2 (yes versus no), myocardial infarction (yes versus no), atrial fibrillation (yes versus no), estimated glomerular filtration rate and urinary albumin excretion
Model 3	Model 2 + additionally adjusted for High sensitive C-Reactive Protein, hemoglobin, ferritin and transferrin saturation
Model 4	Model 3 + additionally adjusted for n-terminal pro-brain natriuretic peptide

Abbreviations: HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; FGF23, fibroblast growth factor 23.