Fibroblast Growth Factor 23 and Exercise Capacity in Heart Failure with Preserved Ejection Fraction

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Abstract

Background: Heart failure with preserved ejection fraction (HFpEF) is characterized by left ventricular hypertrophy (LVH) and reduced exercise capacity. Fibroblast growth factor 23 (FGF23), a hormone involved in phosphate, vitamin D and iron homeostasis, is linked to LVH and HF. We measured c-terminal FGF23 (cFGF23) and intact FGF23 (iFGF23) levels and examined their associations with exercise capacity in patients with HFpEF.

Methods: Using multivariable linear regression and linear mixed models, we studied the associations of cFGF23 and iFGF23 with baseline and mean weekly change over 24 weeks in peak oxygen consumption (VO_2) and 6-minute walk distance (6MWD) in individuals enrolled in the Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in HFpEF trial.

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Our study population comprised of 172 individuals with available plasma for cFGF23 and iFGF23 measurements.

Results: Median (25th–75th percentile) baseline cFGF23 and iFGF23 levels were 208.7 (132.1–379.5) RU/ml and 90.3 (68.6–128.5) pg/ml, respectively. After adjustment for cardiovascular disease, hematologic and kidney parameters, higher cFGF23 was independently associated with lower peak VO_2 at baseline. Higher iFGF23 was independently associated with shorter 6MWD at baseline. No significant associations were appreciated with the longitudinal outcomes.

Conclusion: In patients with HFpEF, higher FGF23 levels are independently associated with reduced exercise capacity at baseline.

Graphical Abstract

Iron deficiency, inflammation, and reduced kidney function are potential factors that result in elevated c-terminal fibroblast growth factor 23 (cFGF23) and intact fibroblast growth factor 23 levels (iFGF23) in patients with heart failure with preserved ejection fraction (HFpEF). Both cFGF23 and iFGF23 were associated with at least one measure of reduced exercise capacity in patients with established HFpEF: cFGF23 was independently associated with reduced oxygen consumption and iFGF23 was associated with reduced 6 minute walk distance in patients with HFpEF.
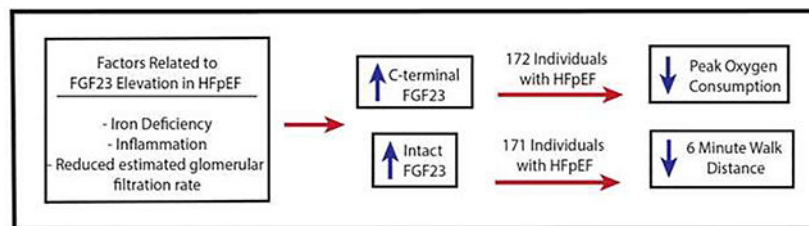
Keywords

Heart failure with preserved ejection fraction; fibroblast growth factor 23; exercise capacity; chronic kidney disease

Introduction

Heart failure with preserved ejection fraction (HFpEF) is an emerging public health epidemic with over 3.3 million adults diagnosed with HFpEF in the United States.(1) Of the total incident cases of heart failure, at least half are HFpEF.(1,2) HFpEF is characterized by elevation in left sided filling pressures, and is commonly manifested by concentric left ventricular remodeling or left ventricular hypertrophy on echocardiography and reduced exercise capacity.(3,4) Given that exercise intolerance is a cardinal manifestation of HFpEF that significantly contributes to morbidity, exercise capacity is commonly used as an endpoint for clinical trials in HFpEF.(5) Systemic and microvascular inflammation, endothelial dysfunction and alterations in myocardial bioenergetics are thought to contribute to exercise intolerance in patients with HFpEF; however, the exact pathophysiologic mechanisms of HFpEF remain under investigation.(6,7)

Fibroblast growth factor 23 (FGF23) is a bone-derived hormone involved in phosphate, vitamin D and iron homeostasis.(8) The biologically active intact FGF23 hormone has target effects in the kidney and parathyroid glands. Circulating FGF23 levels are determined by a balance between production of the full FGF23 protein and its post-translational modification through cleavage into C-terminal and N-terminal fragments within the osteocytes and bone marrow stromal cells.(8,9)Traditional regulators of FGF23 production include phosphate, calcium, parathyroid hormone and 1,25dihydroxyvitamin D. Other non-traditional regulators of FGF23 that increase FGF23 transcription include iron deficiency, inflammation and hypoxia.(9) Effects of these factors on FGF23 production and cleavage may be discerned by measuring FGF23 levels with two available assays. The c-terminal FGF23 (cFGF23) assay measures both the biologically active intact FGF23 (iFGF23) hormone and its C-terminal fragments, whereas the iFGF23 assay measures levels of only the intact FGF23 hormone.(8) In health, increased transcription due to iron deficiency and/or inflammation are matched to increased post-translational cleavage, resulting in elevated levels of circulating cFGF23, which measures both fragments and the intact hormone, but normal levels of iFGF23, which only measures the intact hormone.(8) In advanced chronic kidney disease (CKD), post-translational cleavage is impaired and increased transcription secondary to iron deficiency and/or inflammation result in elevation in both cFGF23 and iFGF23 levels.(8,10)

FGF23 is elevated in rare diseases of hypophosphatemia and in patients with CKD, in whom elevated FGF23 levels preserve normophosphatemia as kidney disease advances.(11) Chronic FGF23 elevation may be maladaptive. Observational data demonstrate significant associations between FGF23 and cardiovascular disease, specifically congestive heart failure, left ventricular hypertrophy, atrial fibrillation and hypertension, independent of kidney function.(12–15) Although most studies do not differentiate between heart failure subtypes, direct links between FGF23 and HFpEF pathogenesis are supported by mouse models that establish that FGF23 binding to fibroblast growth factor receptor 4 (FGFR4) on the myocardium results in cardiac myocyte hypertrophy.(16) Understanding the relationships between FGF23 and exercise impairment in patients with HFpEF may provide insights into the pathophysiology of HFpEF.

We performed an ancillary study in patients enrolled in the Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Heart Failure with Preserved Ejection Fraction (RELAX) trial (17) to: 1) investigate the degree of cFGF23 and iFGF23 elevation in a HFpEF population with and without CKD, 2) study the relationship between FGF23 levels and known regulators of FGF23 homeostasis in the RELAX trial, and 3) investigate the associations of FGF23 with baseline, and 24-week exercise capacity in patients with HFpEF.

Methods

Study Population

The RELAX trial (Clinicaltrials.gov identifier: NCT00763867 2013) was a double-blind, placebo-controlled randomized clinical trial conducted by the Heart Failure Clinical Research Network to investigate whether phosphodiesterase-5 inhibition with sildenafil would improve exercise capacity in 216 individuals with diagnosed HFpEF.(17)

Cardiopulmonary exercise testing (CPET) and 6-minute walk test (6MWT) were used to determine peak oxygen consumption and exercise capacity. For inclusion into the RELAX trial, individuals were required to have a normal ejection fraction (50%) and New York Heart Association (NYHA) class II through IV heart failure.(17) They were also required to have a peak oxygen consumption (peak VO_2) of 60% of age- and sex- specific normal values and a respiratory exchange ratio of 1.0 on CPET screening. Additional requirements included either an N-terminal pro-brain-type natriuretic peptide (NT-pro BNP) 400 pg/mL or elevated left ventricular filling pressures on cardiac catheterization.(17) Individuals with an estimated glomerular filtration rate (eGFR) of < 20 ml/min/1.73m^2 were excluded from the study.(17) All participants provided written and informed consent and the study was approved by institutional review boards of each of the participating sites.

In an ancillary study to the RELAX trial, we used stored samples to measure baseline plasma cFGF23 and iFGF23, and baseline serum hematologic markers associated with FGF23 regulation.(8) We aimed to investigate the degree of FGF23 elevation at baseline in an HFpEF population and its associations with baseline and 24-week oxygen consumption and exercise capacity. Our study population comprised of 172 individuals with samples available for baseline cFGF23 and iFGF23 measurement. CPET was available for 171 of these individuals, 6MWT was available for all 172 individuals at baseline. For our longitudinal analyses, 24-week CPET was available in 153 individuals and 24-week 6MWT was available in 152 individuals.

Primary Exposure

The primary exposures were plasma cFGF23 and iFGF23 concentrations. Frozen plasma samples were shipped from the University of Vermont Core Laboratory to the Immunoassay and Biomarker Laboratory at the Diabetes Research Institute at the University of Miami, where FGF23 measurements were performed. All samples had previously undergone a single thaw. Plasma cFGF23 and iFGF23 were measured by ELISA testing using kits from Immutopics, Inc. (San Clemente, CA). Estimates of intra-assay coefficients of variation (CV) were 3.9% and 4.5% for cFGF23 and iFGF23, respectively. The inter-assay CV was 4.2% for cFGF23 and 3.3% for iFGF23. Reference ranges for cFGF23 and iFGF23 are not standardized, but have been reported as 21–80 RU/ml for cFGF23 and 3.8–18.7 pg/ml for iFGF23 in elderly well-nourished adults >65 years of age with an eGFR 90 mL/min/1.73 m^2.(18)

Outcomes

The primary outcomes were baseline peak VO_2 measured by CPET and 6-minute walk test distance (6MWD). Both measurements have previously been described.(17) The CPET protocol in the RELAX trial utilized either cycle or treadmill ergometry. The protocol involved a 5-minute rest period where gas exchange data was collected, 3-minutes of low-level exercise and a symptom-limited incremental ramp of 10 watts per minute. Breath-by-breath data was captured using metabolic carts and securely transferred to the RELAX CPET Core Laboratory for VO_2 measurements (Massachusetts General Hospital, Boston, MA). The highest 30 second median breath-by-breath VO_2 during the final minute of

incremental exercise was determined to be the peak VO_2.(19) Longitudinal outcomes were 24-week peak VO_2 and 6MWD.

Covariates

Information on participant demographics, medication use and clinical data including self-reported history of diabetes and smoking were collected at the baseline RELAX trial visit. Given the relationship between iron deficiency and FGF23,(9) and that iron deficiency is associated with poor outcomes in heart failure with reduced and preserved ejection fraction, (20,21) we measured iron parameters in the RELAX trial as potential confounders. Serum iron, ferritin, and iron binding capacity were measured on a Roche Cobas 6000 analyzer using manufacturer's reagent and protocols for instrument set up, operation and analyte analyses (Roche Diagnostics, Indianapolis, IN) at the University of Miami using stored frozen serum. Iron deficiency was defined as ferritin <100 ng/mL or ferritin 100–299 ng/mL and TSAT < 20%. Transferrin saturation (TSAT) was calculated by the formula *iron / (iron + iron binding capacity)*. Serum high-sensitivity C-reactive protein (CRP), phosphate, hemoglobin, and NT-proBNP were measured via standard assays. Chronotropic index was computed using baseline CPET data with the formula *(peak exercise heart rate [HR] – resting HR) / (age-predicted maximal HR – resting HR)*, as previously done.(22) Estimated glomerular filtration rate (eGFR) was calculated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.(23) CKD was defined as an eGFR $\leq$ 60 ml/min/1.73m^2.

Statistical Analysis

We first investigated the degree of FGF23 elevation in the HFpEF population by CKD status using cFGF23 and iFGF23 quartiles. Spearman correlation coefficients were then estimated between cFGF23 and iFGF23 in the whole population, and with each of our two primary outcomes, peak VO_2 and 6MWD. We used linear regression models to investigate predictors of cFGF23 and iFGF23. We used linear regression models to examine the associations between baseline plasma cFGF23 and iFGF23, expressed as continuous variables, and peak VO_2 and 6MWD. We hierarchically adjusted for demographics (age, gender, race), cardiovascular disease risk factors (diabetes, systolic blood pressure, CRP, smoking status, body mass index, NT-proBNP, eGFR, chronotropic index). Given biologic relationships between hematologic parameters of iron deficiency and anemia with FGF23, in our final model, we adjusted for TSAT, ferritin and hemoglobin. CPET exercise modality, cycle or treadmill, was also included as a covariate in our models with peak VO_2 as the primary outcome. cFGF23 and iFGF23 were log transformed for all analyses.

Given relationships between sex and exercise capacity,(24) we assessed whether the association of cFGF23 and iFGF23 with peak VO_2 and 6MWD was modified by sex. We also tested if our associations were modified by eGFR and diabetes by testing the significance of the interaction terms of each with cFGF23 and iFGF23.

For longitudinal analyses that investigated the associations of baseline cFGF23 and iFGF23 with 24-week peak VO_2 and 6MWD, we performed linear mixed models for repeated measures with an unstructured covariance structure using baseline, 12-week and 24-week

measurements. Time was considered a fixed effect. We used hierarchical adjustment approach in the linear mixed models similar to our primary analyses and additionally adjusted the models for baseline measurements. Given the RELAX Trial was a randomized interventional trial, we also adjusted our final models for randomization arm.

All analyses were performed using SAS version 9.4 (Cary, NC, USA). Two-sided P values < 0.05 were considered statistically significant.

Results

Baseline Descriptive Characteristics

Baseline characteristics of the study population are presented in Table 1, and were similar to the overall RELAX trial participants.(17) The mean age of the study population was 68.4 ± 10.0 years, 50.6% were female and 3.5% were black. The mean eGFR in the overall study population was 63.9 ± 22.0 ml/min/1.73m^2 and median CRP in the study population was 3.7 (1.7 – 8.5) mg/L. More than half of the study population (58.5%) had iron deficiency (Table 1). Baseline exercise capacity was reduced in our study population (mean peak VO$_2$ 12.3 ± 3.0 ml/min/kg, mean 6MWD 315.3 ± 105.4 m). Median baseline cFGF23 level was 208.7 (132.1 – 379.5) RU/ml and median baseline iFGF23 level was 90.3 (68.6 – 128.5) pg/ml.

Correlates of baseline cFGF23 and iFGF23

Within the HFpEF population with available eGFR data, 79 individuals had an eGFR value 60 ml/min/1.73m^2 and were defined as having CKD. Ninety-one individuals did not meet criteria for CKD. Within the HFpEF population, cFGF23 and iFGF23 levels were higher in individuals with CKD than those without CKD (p values 0.003 and < 0.001, respectively). Median cFGF23 and iFGF23 levels were 247.9 (164.5, 513.4) RU/ml and 111.7 (80, 137.8) pg/ml in individuals with CKD and 184.9 (116.2, 290) RU/ml and 77 (63.1, 99.9) pg/ml in individuals without CKD. Both populations had higher FGF23 levels than previously reported normal ranges (Figure 1). cFGF23 was modestly correlated with iFGF23 (r = 0.32, p <0.0001). Systolic blood pressure, NT-pro-BNP and hematologic parameters of ferritin and hemoglobin were all significantly associated with cFGF23 in multivariable linear regression models (p value for all < 0.05). NT-pro-BNP was also significantly associated with iFGF23 (p value 0.04). Lower eGFR was independently associated with higher iFGF23 (p value 0.0001; Table 2), but the association between eGFR and cFGF23 was not significant in multivariable models.

Associations of Baseline FGF23 and Baseline Exercise Capacity

cFGF23 and iFGF23 were each negatively correlated with peak VO$_2$ (cFGF23: r = − 0.37, p <0.0001; iFGF23: r = −0.26, p=0.0007) and 6MWD (cFGF23: r = −0.22, p=0.004; iFGF23: r = −0.18, p=0.02). In unadjusted linear regression models, higher cFGF23 was associated with lower peak VO$_2$ (Table 3) and shorter 6MWD (Table 3). After adjusting for demographics (age, black race, sex, exercise modality), cardiovascular disease risk factors (diabetes, systolic blood pressure, CRP, smoking status, body mass index, NT-proBNP, eGFR, chronotropic index), and hematologic parameters (hemoglobin, TSAT, ferritin), cFGF23 remained significantly associated with lower peak VO$_2$, but not with 6MWD (Table

3, Figure 2). In our fully adjusted multivariable linear regression models, iFGF23 was only significantly associated with 6MWD (Table 3, Figure 2).

The associations of higher cFGF23 and iFGF23 with peak VO$_2$ or 6MWD were not modified by sex, eGFR, nor diabetes (P for interactions all > 0.18).

Associations of Baseline FGF23 and 24-Week Exercise Capacity

Among the153 individuals with 24-week VO$_2$ data, mean (standard deviation) 24-week peak VO$_2$ was 12.2 (3.2) ml/min/kg. Among the 152 individuals with 24-week 6MWD data, mean 24 week (standard deviation) 6MWD was 331.8 (108.0) meters. In fully adjusted linear mixed models that included randomization arm and baseline measures of exercise capacity, neither cFGF23 nor iFGF23 was associated with mean weekly change in exercise capacity over time (Table 4).

Discussion

In participants enrolled in the RELAX trial, we performed an ancillary study to investigate the associations of baseline cFGF23 and iFGF23 with baseline and 24-week and oxygen consumption and 6MWD in patients with diagnosed HFpEF. Compared to previously reported normal reference ranges, levels of both cFGF23 and iFGF23 were elevated at baseline in patients with HFpEF with and without kidney disease enrolled in RELAX trial. (18) Higher levels of both cFGF23 and iFGF23 were associated with at least one marker of reduced exercise capacity. Higher baseline cFGF23 was independently associated with lower baseline peak VO$_2$ in patients with HFpEF. Higher baseline iFGF23 was significantly associated with reduced 6MWD at baseline in patients with HFpEF. Neither was associated with longitudinal outcomes of exercise capacity.

Exact mechanisms of FGF23 elevation in HFpEF are unknown. Inflammation is one key regulator of FGF23 transcription.(9) Based on the American Heart Association and U.S. Centers for Disease Control and Prevention risk categories for high sensitivity CRP, the median CRP value in our patient population was categorized in the high risk group for cardiovascular disease.(25) The inflammatory milieu of HFpEF may represent one mechanism of increased cFGF23 levels in patients with HFpEF. Iron deficiency also upregulates FGF23 transcription,(9) and more than half of our study population had iron deficiency at baseline. Hematologic parameters were independently associated with cFGF23 at baseline, suggesting that iron deficiency may contribute to elevation in cFGF23 levels in patients with HFpEF. Kidney disease modifies FGF23 regulation, likely by increasing *FGF23* transcription and reducing FGF23 cleavage.(26) Prior studies suggest that in advanced CKD, cFGF23 and iFGF23 are strongly correlated and most circulating FGF23 is the intact FGF23 hormone.(10) Our study supports this hypothesis as eGFR was the main determinant of iFGF23 in our study population. Underlying kidney dysfunction is one possible explanation for iFGF23 elevation in our patient population. Finally, it is also possible that cFGF23 and iFGF23 rise as a result of HFpEF pathology. Experimental data suggest a potential reverse causal relationship in which the altered myocardium is a source of circulating FGF23.(27) Together, increased production and reduced cleavage of FGF23

may establish HFpEF as a state of secondary cFGF23 and iFGF23 elevation, higher levels of which lead to or are a consequence of worse clinical parameters in HFpEF.

Studies investigating the associations of FGF23 with heart failure subtypes or in patients with HFpEF are limited. In the Multiethnic Studies of Atherosclerosis (MESA), iFGF23 was significantly associated with incident HFpEF.(28) iFGF23 was not associated with mortality in patients with existing HFpEF in the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study.(29) In a recent study of 143 individuals with HFpEF, cFGF23 was significantly associated with cardiac fibrosis and mortality.(30) None of these studies included measurements of both cFGF23 and iFGF23.(28–30) A recent ancillary study to the Trial of Intensified verses Standard Medical Therapy in Elderly Patients with Congestive Heart Failure (TIME-CHF) measured both cFGF23 and iFGF23 and found levels of both were elevated, but neither was independently associated with mortality or heart failure hospitalizations.(31) Although TIME-CHF included individuals with preserved ejection fraction, the primary intent was not to study an HFpEF population and HFpEF constituted only one fifth of the total TIME-CHF study population.(31) Neither the MESA, LURIC nor TIME-CHF studies incorporated assessments of exercise capacity, an important endpoint in HFpEF. In our ancillary study that conducted simultaneous measurements of cFGF23 and iFGF23, we demonstrate that both cFGF23 and iFGF23 were associated with at least one measure of reduced exercise capacity at baseline in a population with established HFpEF.

There are multiple pathways that could explain the significant associations of FGF23 and exercise capacity. Circulating FGF23 may directly exert toxicity on cardiac myocytes through FGFR4 activation.(16) Blocking FGFR4 ameliorates this phenotype, suggesting a causal role for FGF23 in the pathogenesis of both heart failure with reduced and preserved ejection fraction.(32) FGF23 may activate the renin-angiotensin-aldosterone system via suppression of angiotensin converting enzyme-2, which may promote cardiac remodeling and fibrosis, features indicative of HFpEF.(33,34) FGF23 may indirectly contribute to HFpEF pathophysiology through inhibition of *CYP27B1* and stimulation of *CYP24A1* expression, resulting in decreased production and increased degradation of $1,25(OH)_2D$ levels.(35) Low levels of vitamin D are associated with reduced functional capacity and poor clinical outcomes in patients with HFpEF.(36) FGF23 may also indirectly impact HFpEF through reduced bone marrow erythropoiesis and development of anemia, (37) which is associated with worse outcomes in patients with HFpEF.(22) FGF23 may also simply be a fidelity marker of more severe illness and inflammation. Most of these studies do not discriminate between the effects of cFGF23 and iFGF23 as investigators measured FGF23 levels with one assay. We find that both cFGF23 and iFGF23 were each associated with one marker of exercise capacity in patients with HFpEF. Regarding the discrepant results between our cross-sectional and longitudinal analyses, the absence of a substantial change in peak VO_2 and 6MWD over the 24 week period may have limited our ability to detect significant associations.

Although we were able to investigate the cross-sectional and longitudinal associations between cFGF23 and iFGF23 and oxygen consumption and exercise capacity in a well-defined population with clear objective endpoints, we acknowledge additional limitations to our study. While significant associations were found between baseline FGF23 levels and

baseline HFpEF parameters, cross-sectional analyses cannot prove causation. Our study is limited by single FGF23 measurements which also restricts our ability to make causal inferences. Although we have measurements of eGFR, we do not have measurements of proteinuria or 1,25(OH)$_2$D in our study population. Finally, although we detected no significant interactions by sex, eGFR or diabetes status, the small sample size of our study did not allow us to conduct subgroup analyses or definitively exclude the possibility of effect modification by subgroups.

Whether FGF23 is a modifiable target that could result in improvements of cardiovascular disease is yet to be determined. Phosphate binders and dietary phosphate reduction may lower FGF23 levels in more advanced kidney disease,(38) but have not been studied in patients with HFpEF. Several studies demonstrate that treatment of iron deficiency can lower cFGF23 levels and improve functional status in heart failure with reduced ejection fraction, and may be one innovative approach to FGF23 lowering in HFpEF populations.(39,40) However, prior to implementation of FGF23 reducing strategies, further mechanistic studies investigating whether direct FGF23 toxicity contributes to HFpEF pathogenesis should be undertaken. Given the lack of guideline directed medical therapy for patients with HFpEF, identifying risk factors and pathophysiologic mechanisms of disease pathogenesis and severity remains an important area in need for further investigation.

Acknowledgments

Abbreviations

6MWD	6-minute walk distance

6MWT	6 minute walk test
Ace/ARB	Angiotensin converting enzyme inhibitor/angiotensin receptor blocker
BMI	Body mass index
cFGF23	c-terminal FGF23
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CPET	Cardiopulmonary exercise testing
CRP	C-reactive protein
C-terminal	Carboxy terminal
CV	Coefficients of variation
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
FGF23	Fibroblast growth factor 23
FGFR4	Fibroblast growth factor receptor 4
HFpEF	Heart failure with preserved ejection fraction
HR	Heart rate
iFGF23	intact FGF23
IQR	interquartile range
LURIC	Ludwigshafen Risk and Cardiovascular Health
MESA	Multiethnic Studies of Atherosclerosis
N-terminal	Amino terminal
NT-pro BNP	N-terminal pro-brain-type natriuretic peptide
NYHA	New York Heart Association
RELAX	Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Heart Failure with Preserved Ejection Fraction
RU	Relative units
SAS	Statistical analysis software

SBP	Systolic blood pressure
SD	Standard deviation
TIME-CHF	Trial of Intensified verses Standard Medical Therapy in Elderly Patients with Congestive Heart Failure
TSAT	Transferrin saturation
Peak VO$_2$	Peak oxygen consumption

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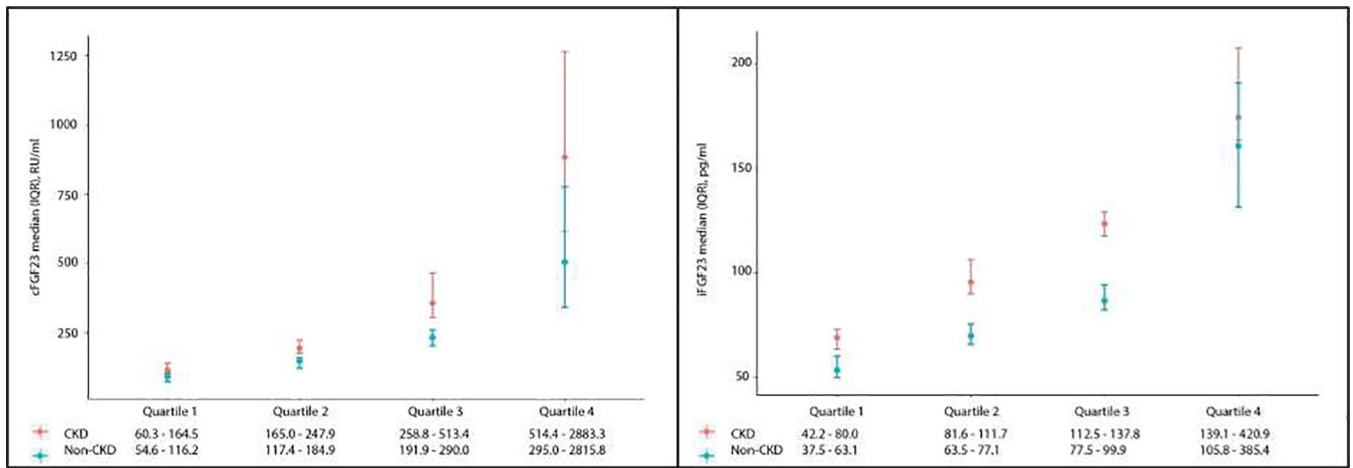
Figure 1. cFGF23 and iFGF23 quartiles by CKD status in patients with HFpEF.
Quartiles of cFGF23 and iFGF23 in 79 individuals with CKD and 91 individuals without CKD. Results reported as medians with interquartile ranges. Abbreviations: cFGF23, c-terminal fibroblast growth factor 23; iFGF23, intact fibroblast growth factor 23; CKD, chronic kidney disease; HFpEF, heart failure with preserved ejection fraction; IQR, interquartile range
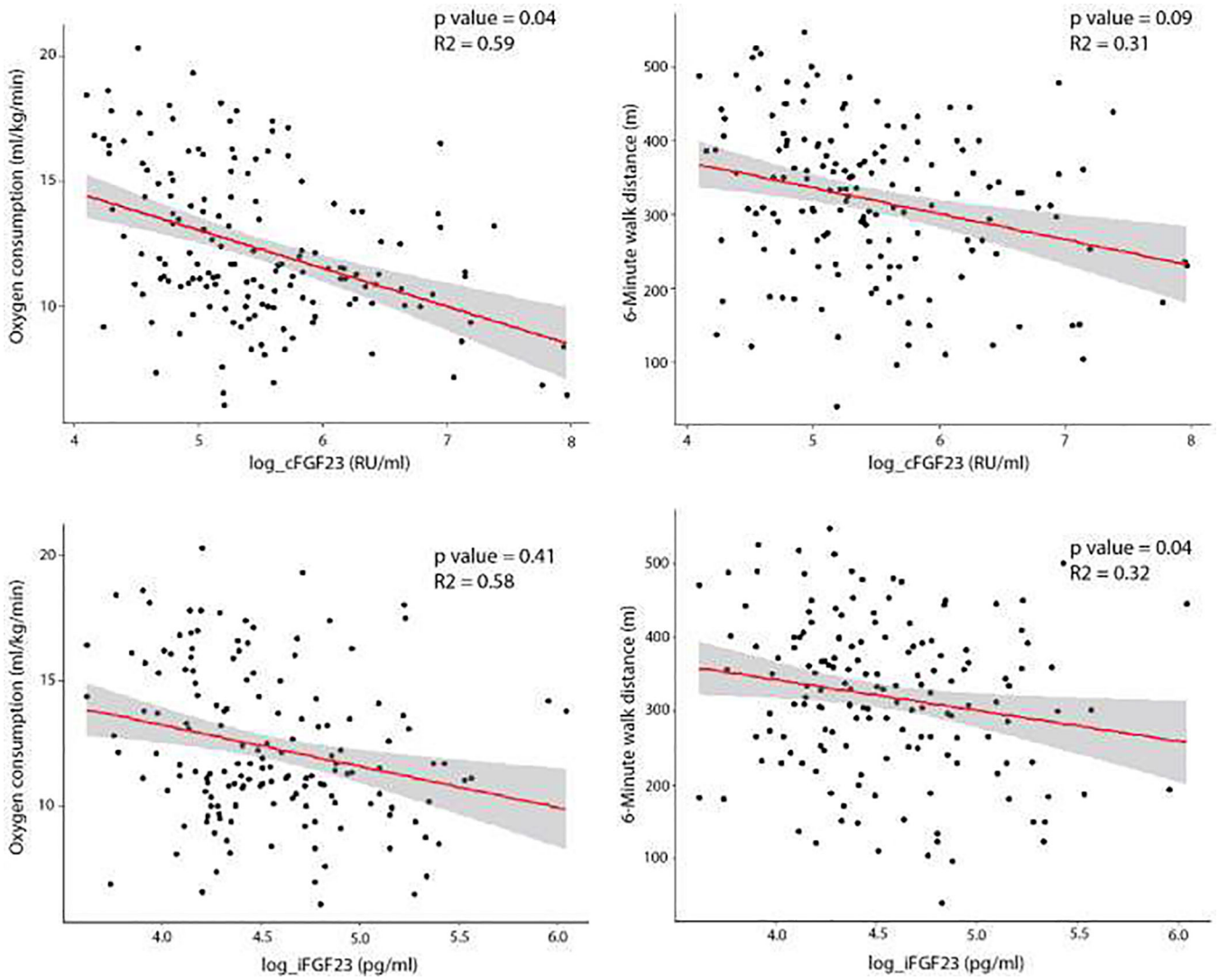
Figure 2. Associations of cFGF23 and iFGF23 with peak VO2 and 6MWD.*
Scatter plot with regression line adjusted for age, sex, Black race, diabetes, exercise modality, systolic blood pressure, C-reactive protein, smoking status, body mass index, NTpro-BNP, chronotropic index, eGFR, transferrin saturation, ferritin, and hemoglobin. *No exercise modality in the model with 6MWD. Abbreviations: cFGF23, c-terminal fibroblast growth factor 23; iFGF23, intact fibroblast growth factor 23; 6MWD, 6-minute walk distance; NT-proBNP, N-terminal pro-brain natriuretic peptide; eGFR, estimated glomerular filtration rate.

Table 1.

Baseline Characteristics of the Total Study Population

Baseline characteristics	Number	
Age, years	172	68.4 ± 10.0
Female, N (%)	172	87 (50.6)
Black, N (%)	172	6 (3.5)
Current Smoking, N (%)	172	26 (15.1)
Hypertension, N (%)	172	145 (84.3)
Diabetes, N (%)	172	69 (40.1)
NYHA Functional Class	172	
NYHA Functional Class 2, N (%)		85 (49.4)
NYHA Functional Class 3, N (%)		87 (50.6)
>1 heart failure hospitalization in prior year, N (%)	172	78 (45.4)
Ace/ARB use, N (%)	172	114 (66.3)
BMI, kg/m^2	172	33.9 ± 7.2
SBP, mmHg	172	127.2 ± 17.0
eGFR, ml/min/1.73m^2	170	63.9 ± 22.0
High sensitivity CRP, mg/L	172	3.7 (1.7 – 8.5)
Phosphate, mg/dL	164	3.5 ± 0.6
CPET Hemoglobin, mg/dl	166	13.1 ± 1.5
Core lab NT-proBNP, pg/ml	171	648.1 (220.8 – 1388.0)
Intact FGF23, pg/ml	172	90.3 (68.6 – 128.5)
C-terminal FGF23, RU/ml	172	208.7 (132.1 – 379.5)
Iron, ug/dL	164	79.8 ± 32.2
Ferritin, ng/mL	164	112.4 (58.3 – 209.8)
TSAT, %	164	23.9 ± 10.5
Iron Deficiency, N (%)	164	96 (58.5)
Exercise Mode	171	
Exercise Mode, Treadmill %		57 (33.3)
Exercise Mode, Bicycle %		114 (66.7)
Chronotropic Index	169	0.5 ± 0.3
Ejection fraction, %	171	60.9 ± 7.2
Left ventricular mass, g	127	171.6 ± 69.6
Left ventricular end systolic volume, ml	94	43.4 ± 20.2
Left ventricular end diastolic volume, ml	94	118.6 ± 34.4

Results are reported as means ± standard deviation, proportions or medians with interquartile ranges

Abbreviations: cFGF23, c-terminal fibroblast growth factor 23; N, number; NYHA, New York Heart Association; Ace/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BMI, body mass index; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; CPET, cardiopulmonary exercise testing; NT-proBNP, N-terminal pro-brain natriuretic peptide, TSAT, transferrin saturation

Table 2.

Determinants of cFGF23 and iFGF23 in multivariable linear regression models

Covariates	cFGF23		iFGF23	
	F-value	***p*-value**	**F-value**	***p*-value**
Age	1.26	0.26	3.68	0.06
Female	0.82	0.37	2.65	0.11
Black	0.05	0.83	1.39	0.24
Diabetes	0.09	0.76	0.18	0.67
Systolic blood Pressure	5.72	0.02	0.14	0.71
C-reactive protein	0.08	0.78	0.06	0.81
Smoking	0.53	0.47	0.27	0.60
Body mass index	0.01	0.93	0.01	0.90
NT-proBNP	22.2	<.0001	4.44	0.04
Chronotropic Index	2.31	0.13	0.03	0.85
eGFR	0.02	0.90	15.77	0.0001
Transferrin Saturation	1.00	0.32	0.44	0.51
Ferritin	4.38	0.04	0.84	0.36
Hemoglobin	6.44	0.01	1.01	0.32

Model adjusted for age, gender, black race, diabetes, systolic blood pressure, C-reactive protein, smoking, body mass index, NT-proBNP, chronotropic index, estimated glomerular filtration rate, transferrin saturation, ferritin and hemoglobin

Abbreviations: NT-proBNP, N-terminal pro-brain natriuretic peptide; eGFR, estimated glomerular filtration rate

Table 3.

Association of c-terminal FGF23 and intact FGF23 with baseline exercise capacity

Baseline	Peak VO$_2$ (ml/kg/min)			6-Minute Walk Distance (meters)		
cFGF23 Models *	Parameter Estimate 95% CI	*p* - value	R^2	Parameter Estimate 95% CI	*p* - value	R^2
Unadjusted	−1.16 (−1.59 – −0.74)	<.001	0.15	−22.79 (−38.37 – −7.22)	0.004	0.05
Model 1	−1.08 (−1.46 – −0.70)	<.001	0.34	−20.48 (−35.92 – −5.05)	0.01	0.09
Model 2	−0.49 (−0.87 – −0.11)	0.01	0.58	−18.57 (−35.59 – −1.55)	0.03	0.27
Model 3	−0.44 (−0.85 – −0.02)	0.04	0.59	−15.88 (−34.08 – 2.32)	0.09	0.31
iFGF23 Models *	Parameter Estimate 95% CI	*p* - value		Parameter Estimate 95% CI	*p* - value	
Unadjusted	−0.75 (−1.19 – −0.31)	0.001	0.06	−20.66 (−36.31 – −5.02)	0.01	0.04
Model 1	−0.63 (−1.03 – −0.23)	0.002	0.26	−19.29 (−34.86 – −3.71)	0.02	0.09
Model 2	−0.19 (−0.55 – 0.18)	0.31	0.56	−18.52 (−34.68 – −2.37)	0.02	0.27
Model 3	−0.16 (−0.54 – 0.22)	0.41	0.58	−17.22 (−33.73 – −0.71)	0.04	0.32

Results presented as β-estimate per 1 SD increase in ln_cFGF23 and ln_iFGF23 in ml/kg/min for peak VO$_2$ and meters for 6-minute walk distance

Model 1 adjusts for age, sex, black race, and exercise modality

Model 2 adjusts Model 1 variables plus diabetes, systolic blood pressure, C-reactive protein, smoking status, body mass index, NTpro-BNP, chronotropic index, and eGFR (CKD-EPI equation)

Model 3 adjusts for Model 2 plus transferrin saturation, ferritin, hemoglobin

*Exercise modality was only included in the model when peak VO$_2$ was the outcome

Abbreviations: FGF23, fibroblast growth factor 23; HFpEF, heart failure with preserved ejection fraction; CI, confidence intervals; NT-proBNP, N-terminal pro-brain natriuretic peptide; eGFR, estimated glomerular filtration rate

Table 4.

Association of c-terminal FGF23 and intact FGF23 with mean weekly change in exercise capacity over 24 weeks

	Mean weekly change in peak VO$_2$ over 24 weeks (ml/kg/min)	P value	Mean weekly change in 6-Minute walk distance over 24 weeks (meters)	P value
cFGF23 Models [*]				
Unadjusted	−0.084 (−0.200 – 0.031)	0.08	0.19 (−4.32 – 4.69)	0.86
Model 1	−0.083 (−0.199 – 0.033)	0.08	0.22 (−4.28 – 4.73)	0.88
Model 2	−0.086 (−0.203 – 0.032)	0.07	−0.09 (−4.66 – 4.48)	0.73
Model 3	−0.069 (−0.191 – 0.053)	0.17	0.97 (−3.70 – 5.64)	0.83
Model 4	−0.071 (−0.192 – 0.051)	0.16	1.40 (−3.37 – 6.16)	0.63
iFGF23 Models [*]				
Unadjusted	−0.135 (−0.289 – 0.018	0.05	−2.40 (−8.51 – 3.70)	0.25
Model 1	−0.133 (−0.286 – 0.021)	0.05	−2.34 (−8.45 – 3.76)	0.26
Model 2	−0.137 (−0.293 – 0.019)	0.04	−2.50 (−8.70 – 3.70)	0.24
Model 3	−0.128 (−0.290 – 0.033)	0.07	−2.32 (−8.59 – 3.95)	0.26
Model 4	−0.125 (−0.287 – 0.037)	0.08	−2.23 (−8.65 – 4.19)	0.30

Results presented as mean weekly change over 24 weeks in ml/kg/min for peak VO$_2$ and meters for 6-minute walk distance with 95% confidence intervals

Model 1 adjusts for age, sex, black race, and exercise modality

Model 2 adjusts Model 1 variables plus diabetes, systolic blood pressure, C-reactive protein, smoking status, body mass index, NTpro-BNP, chronotropic index, and eGFR (CKD-EPI equation)

Model 3 adjusts for Model 2 plus transferrin saturation, ferritin, hemoglobin

Model 4 adjusts for Model 3 plus randomization arm and baseline exercise capacity

[*] Exercise modality was only included in the model when peak VO$_2$ was the outcome

Abbreviations: FGF23, fibroblast growth factor 23; HFpEF, heart failure with preserved ejection fraction; CI, confidence intervals; NT-proBNP, B-natriuretic peptide; eGFR, estimated glomerular filtration rate