Fibroblast Growth Factor–23 and Cardiac Structure and Function

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Background—Fibroblast growth factor–23 (FGF-23) is a phosphaturic factor previously associated with left ventricular hypertrophy and systolic dysfunction among individuals with chronic kidney disease. Whether FGF-23 acts directly to induce left ventricular hypertrophy, potentially independent of its klotho coreceptor, remains uncertain. We investigated associations of FGF-23 with cardiac structural abnormalities among individuals with a broad range of kidney function and explored potential biological mechanisms using cardiac magnetic resonance imaging and histology in klotho-null mice, an established model of constitutively elevated FGF-23.

Methods and Results—Among 887 participants with coronary artery disease in the Heart and Soul Study, FGF-23 was modestly associated with worse left ventricular ejection fraction (−1.0% per standard deviation increase in lnFGF-23; standard error, 0.4%), but was not associated with the overall prevalence of concentric hypertrophy (odds ratio, 1.5; CI, 0.9 to 2.4) or eccentric hypertrophy (odds ratio, 1.1; CI, 0.9 to 1.3). FGF-23 was only associated with concentric hypertrophy among individuals with diminished kidney function (eGFR <60 mL/min per 1.73 m²; odds ratio, 2.3; CI, 1.0 to 5.3; P-interaction=0.28). Comparing klotho-null with wild-type mice, null mice did not have greater left ventricular mass (P=0.37) or a lower ejection fraction (P=0.94).

Conclusions—Together, our results suggest that FGF-23 is unlikely to have major effects on cardiovascular structure and function among patients free of substantial chronic kidney disease, and these effects may not be independent of the klotho coreceptor. (J Am Heart Assoc. 2014;3:000584 doi: 10.1161/JAHA.113.000584)

Key Words: chronic kidney disease • hypertrophy • structure

Fibroblast growth factor–23 (FGF-23) is a circulating hormone secreted primarily by osteocytes. In the renal tubules, FGF-23 binds to the FGF receptor 1c and the klotho coreceptor to promote phosphaturia and lower circulating levels of 1,25-dihydroxyvitamin D. Circulating levels of FGF-23 can rise 100- to 1000-fold in patients with a diminished capacity for phosphorous excretion, such as those with chronic kidney disease (CKD). In patients with CKD, supraphysiologic levels of FGF-23 are strongly associated with left ventricular mass index (LVMI), left ventricular hypertrophy (LVH), cardiovascular events, and mortality.3–5 Recently, these findings have also been variably extended to individuals with intact renal function,6–8 but associations have not been consistent throughout the population.6,9

Given these inconsistencies, one of the key open questions that remains in the field is whether the association between FGF-23 and LVH is likely to be causal.10 Several studies have failed to replicate associations of FGF-23 and LVMI,11 and others have suggested that associations with cardiovascular disease may be context dependent, manifesting primarily among individuals with renal tubular dysfunction.9 If the latter is true, FGF-23 could simply be a biomarker of underlying kidney damage among individuals with CKD, rather than being directly toxic to the cardiovascular system.10

To address this question, several research groups have investigated whether FGF-23 has direct effects on cardiac myocytes in experimental models, but again results have been inconsistent.12–14 In 1 study, a 5/6-nephrectomized rat model of CKD developed LVH that was significantly attenuated with administration of the FGF receptor antagonist PD-173074.12 However, an FGF-23-neutralizing antibody did not reduce the hypertrophy in another investigation using the same animal

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model. In a third study, a Col4a3−/− mouse model of CKD with elevated FGF-23 demonstrated increased gene expression of markers of pathological hypertrophy but no increase in cardiomyocyte size or ventricular wall thickness. The possibility of a klotho-independent direct effect of FGF-23 on cardiomyocytes has been raised by the observation that klotho-deficient mice, an established animal model of constitutively elevated FGF-23, develop echocardiographic LVH. This latter finding has not yet been widely replicated or tested in the context of other imaging modalities.

We investigated the relationships between FGF-23, left ventricular mass, structure, and systolic function among patients in the Heart and Soul Study, a well-characterized cohort of patients with stable coronary heart disease and a broad range of kidney function. We also explored the putative effects of FGF-23 activity in homozygous and heterozygous klotho-deficient mice using cardiac magnetic resonance imaging (MRI), a potentially more accurate method to quantify LVH than the echocardiography used previously. Together, our population and mouse results raise questions about the generalizability of the effect of FGF-23 on cardiac structure.

Methods

Participants

The Heart and Soul Study is a longitudinal cohort study designed to investigate the relationship of psychosocial stressors and cardiovascular disease progression. Study methods have been described in detail elsewhere. Briefly, inclusion required ≥1 of the following: history of myocardial infarction, angiographic evidence of >50% stenosis in ≥1 coronary vessel, evidence of exercise-induced ischemia by treadmill or nuclear testing, history of coronary revascularization, or documented diagnosis of coronary artery disease. Individuals were excluded if they were unable to walk 1 block, survived a myocardial infarction within the past 6 months, or planned to move out of the area within 3 years. The institutional review boards of all participating institutions approved the study protocol, and all study participants provided written informed consent.

Between September 2000 and December 2002, 1024 participants from 12 outpatient clinics in the San Francisco Bay area were enrolled. In primary analyses, we excluded 11 individuals (1.1%) with missing values of echocardiographic data, 34 individuals (3.3%) with missing values of FGF-23, and 92 individuals (9.0%) with missing values of other demographic or clinical measurements, resulting in a final sample size of 887 participants. We performed sensitivity analyses using multiple imputation with the Markov Chain Monte Carlo method and 10 imputations to include all individuals, which produced essentially identical results as the complete case approach.

Exposure and Outcome Assessment

We obtained serum and plasma samples from all participants at their baseline visit after a 12-hour fast. FGF-23 was measured in duplicate (and averaged) from frozen samples in 2007 using a carboxy-terminal human enzyme-linked immunosorbent assay (Immutopics). The intra-assay coefficient of variation was 5.0%; the interassay coefficient of variation was 9.9% at a concentration of 36.4 RU/mL and 12.6% at a concentration of 379 RU/mL. Participants underwent a complete 2-dimensional echocardiogram at baseline. We obtained standard 2-dimensional parasternal short axis and apical 2- and 4-chamber views during suspended inspiration. A cardiologist blinded to all other clinical information interpreted the echocardiograms.

We determined left ventricular mass on the basis of wall thickness measurements using a truncated ellipsoid technique and indexed results to body surface area to generate LVMI. We evaluated whether associations with LVMI were sensitive to further adjustment by body mass index (BMI). We considered participants who had LVMI >90 g/m² to have LVH. We calculated relative wall thickness as: (posterior wall thickness−septal wall thickness)/end-diastolic cavity dimension. We further categorized LVH into mutually exclusive categories of eccentric and concentric LVH. We classified LVH as eccentric if relative wall thickness was ≤0.43 and concentric if relative wall thickness was >0.43. Because concentric and eccentric LVH result from different modes of cardiomyocyte growth, differences in their respective associations with FGF-23 may provide insight about the underlying mechanism of FGF-23 activity in the heart. We determined LVEF by the American Society of Echocardiography recommended biplane method of disks.

Ascertainment of Demographic and Clinical Data

At baseline, participants reported age, sex, ethnicity, prevalent hypertension and diabetes, current smoking status, smoking history (used to calculate pack-years of smoking), and level of physical activity (relative to others of the same age and sex) by questionnaire. Trained study personnel measured weight and height (used to calculate BMI) and measured blood pressure after 5 minutes of rest in the supine position. We measured total cholesterol, high-density lipoprotein cholesterol, triglycerides, serum phosphorus, and serum calcium using standard clinical chemistry analyzers on fasting serum samples taken at baseline. We measured urine creatinine using the rate Jaffé method. We measured urine albumin using nephelometry, and indexed urine albumin to

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Statistical Analysis
We  examined  the  distribution  of  the  above-listed  covariates  by  quartile  of  FGF-23  and  examined  Spearman  correlation  coefficients.  We  evaluated  dose-response relationships  of  FGF-23  with  LVMI  and  LVEF  and  used  multivariable-adjusted linear  regression  with  least-square  means  to  estimate  adjusted  LVMI  and  resting  LVEF  by quartile  of  FGF-23.  After  ln-transforming  FGF-23  because  of  its  skewed  distribution, we  used  multivariable-adjusted  linear  regression  to  examine  continuous  relationships  per  standard  deviation  (SD)  of  lnFGF-23.  Finally,  we  used  multinomial  logistic  regression  to  evaluate  the  simultaneous  odds  ratios  of  concentric  or  eccentric  hypertrophy  compared  with  no  hypertrophy.  We  used  multinomial  logistic  regression  for  this  analysis,  as  these  forms  of  LVH  were,  by  definition,  mutually  exclusive.

We  derived  3 sequential  models  for  multivariable  linear  and  logistic  regression  analyses.  Model 1 adjusted  for  age,  sex,  ethnicity,  and  BMI.  Model 2  further  adjusted  for  systolic  blood  pressure,  prevalent  diabetes,  prevalent  hypertension,  physical  activity  score,  current  smoking  status,  pack-years,  total  cholesterol  (quintiles),  high-density  lipoprotein  cholesterol  (log),  and  triglycerides  (log).  Model 3  in addition  adjusted  for  serum  phosphorous  and  calcium,  albumin-to-creatinine  ratio  (ACR; log),  and  eGFR.  Covariate  forms  were  chosen  after  graphical  examination  and  comparison  of  model  likelihood  statistics.

Given  the  strong  prior  hypothesis  of  effect  modification  by  underlying  kidney  disease,25  we  performed  analyses  stratified  by  the  presence  or  absence  of  CKD,  defined  as estimated GFR <60 versus ≥60 mL/min per 1.73 m².  We  subsequently  tested  the  multiplicative  interaction  of  FGF-23  with  CKD.  We  also  conducted  sensitivity  analyses  restricting  the  range  of  FGF-23  to  levels  observed  among  patients  with  normal  kidney  function (<350 RU/mL).  We  tested  multiplicative  interaction  by  sex  and  race,  but  no  interactions  were  observed.

We performed analyses using SAS software, version 9.2, and Stata Statistical Software, version 10.1.  P values <0.05 were  considered  statistically  significant.

Murine Experiments
We  crossbred  heterozygous  klotho-null  mice  to  obtain  homozygous  klotho(−/−)  and  heterozygous  klotho(−/+)+  mutants.  We  identified  genotypes  by  polymerase  chain  reaction  using genomic  DNA  extracted  from  tail  clips.26 Klotho-deficient  mice  lack  the  klotho  coreceptor  required  for  FGF-23  to  promote  phosphaturia  in  the  kidney;  as  a  result,  they  display  significantly  elevated  circulating  levels  of  FGF-23  accompanied  by  soft-tissue  and  vascular  calcifications  by  3–6  weeks  of  age.26

We  performed  MRI  on  wild-type  (n=4),  klotho(−/+)+  (n=5),  and  klotho(−/−)  (n=5)  mice  at  4  weeks  of  age.  As  positive  controls  for  LVH,27  we  also  imaged  2  homozygous  and  1  heterozygous  1-α  hydroxylase–null  mice.

We  conducted  MRI  with  a  Bruker  9.4  Tesla  Biospec  94/20  system  (Bruker  Instruments,  Billerica,  MA)  using  a  quadrature  40-mm  mouse  body  volume  coil.  We  anesthetized  animals  with  0.5%  to  3%  vaporized  isoflurane  and  monitored  ECG  and  respiratory  signals  during  imaging.  We  used  a  cardiac- and  respiratory-gated  fast  gradient  echo  sequence  (FLASH-cine)  with  the  following  parameters:  30-degree  flip  angle;  time  to  echo,  2.8  ms;  time  to  repeat,  20  ms;  matrix  size,  192×192;  field  of  view,  25.6×25.6  mm;  and  4  averages.  We  acquired  contiguous  image  slices  (1  mm  thick)  of  the  heart’s  short  axis  from  the  apex  covering  the  heart’s  full  volume.

For  each  image  slice,  we  manually  traced  the  left  ventricular  wall  region  and  internal  chamber  region  using  Image  J  software.  We  used  the  pixel-to-area  conversion  factor  1  pixel/0.018  mm²  to  estimate  the  left  ventricular  wall  area,  left  ventricular  chamber  area  at  systole,  and  left  ventricular  chamber  area  at  diastole.  We  multiplied  the  area  measurements  by  slice  thickness  to  obtain  volume  measurements  for  each  slice  and  summed  the  volume  measurements  to  determine  total  volume.28 We  determined  LVEF  on  the  basis  of  the  estimated  left  ventricular  chamber  volume  at  systole  and  diastole.  After  ln-transformation  to  achieve  normality  and  similar  variances  across  genotypes,  we  compared  left  ventricular  wall  volumes  and  ejection  fractions  between  genotypes  using  ANOVA.  For  left  ventricular  wall  volume,  we  adjusted  our  results  for  left  ventricular  internal  chamber  volume  to  account  for  differences  in  mouse  size.

Heart Weight and Histology
We  obtained  murine  data  on  heart  weight  and  histology  in  a  distinct  set  of  mice  to  confirm  the  results  we  obtained  via  cardiac  MRI.  We  anesthetized  mice  at  6  weeks  of  age  and  perfused  with  0.1  mol/L  phosphate-buffered  saline  and  fixed  in  10%  buffered  formalin.  We  measured  wet  heart  weight  and
calculated the ratio of wet heart weight to body weight to determine the index of cardiac hypertrophy. After embedding hearts in paraffin blocks, we cut 5-μm sections and stained them with hematoxylin/eosin solution. Cross-sectional images were documented using a DP controller and Manager (Olympus Soft Imaging Solutions).

We determined heart weight/body weight ratio in wild-type (n=6), klotho(+/−) (n=5), and klotho(−/−) (n=12) mice. After checking normality and similar variances, we compared heart weight/body weight ratio between genotypes using ANOVA.

We obtained 1 to 3 histological cross-sections at the midventricular level of the heart from wild-type (n=4) and klotho(+/−) (n=4) mice. Using Image J software, we manually traced the total area of the LV in each section and compared LV area in wild-type (n=4) and klotho(+/−) (n=4) mice using generalized estimating equations with a Gaussian link and a compound symmetry covariance structure to account for the repeated sections within individual mice.

Table 1. Characteristics of Heart and Soul Study Subjects by Quartile of FGF-23 (RU/mL)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Quartile 1 (≤27.7, n=222)</th>
<th>Quartile 2 (27.8 to 43.0, n=221)</th>
<th>Quartile 3 (43.1 to 71.4, n=222)</th>
<th>Quartile 4 (≥71.5, n=222)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66 (10)</td>
<td>67 (11)</td>
<td>67 (11)</td>
<td>67 (12)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>193 (87)</td>
<td>187 (85)</td>
<td>182 (82)</td>
<td>167 (75)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>133 (60)</td>
<td>124 (56)</td>
<td>137 (62)</td>
<td>149 (67)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129 (19)</td>
<td>134 (22)</td>
<td>133 (20)</td>
<td>135 (23)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>47 (21)</td>
<td>58 (26)</td>
<td>62 (28)</td>
<td>64 (29)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>140 (63)</td>
<td>160 (72)</td>
<td>160 (72)</td>
<td>163 (73)</td>
</tr>
<tr>
<td>Lifestyle factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.9 (4.6)</td>
<td>28.2 (4.8)</td>
<td>28.9 (5.4)</td>
<td>28.4 (6.0)</td>
</tr>
<tr>
<td>Above average physical activity, n (%)</td>
<td>83 (37)</td>
<td>89 (40)</td>
<td>78 (35)</td>
<td>52 (23)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>36 (16)</td>
<td>35 (16)</td>
<td>38 (17)</td>
<td>68 (31)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>20 (21)</td>
<td>19 (21)</td>
<td>20 (22)</td>
<td>24 (22)</td>
</tr>
<tr>
<td>Laboratory values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>175 (40)</td>
<td>176 (38)</td>
<td>181 (43)</td>
<td>181 (49)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>46 (13)</td>
<td>47 (13)</td>
<td>45 (14)</td>
<td>46 (16)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>96 (66)</td>
<td>106 (92)</td>
<td>122 (90)</td>
<td>119 (109)</td>
</tr>
<tr>
<td>Serum phosphorous, mg/dL</td>
<td>3.5 (0.5)</td>
<td>3.6 (0.5)</td>
<td>3.7 (0.6)</td>
<td>3.8 (0.7)</td>
</tr>
<tr>
<td>Serum calcium, mg/dL</td>
<td>9.5 (0.5)</td>
<td>9.5 (0.5)</td>
<td>9.5 (0.5)</td>
<td>9.5 (0.6)</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin-to-creatinine ratio ([ACR]×1000)</td>
<td>30 (118)</td>
<td>33 (118)</td>
<td>53 (210)</td>
<td>146 (497)</td>
</tr>
<tr>
<td>eGFR, mL/min per 1.73 m²</td>
<td>80 (18)</td>
<td>75 (19)</td>
<td>71 (24)</td>
<td>58 (21)</td>
</tr>
</tbody>
</table>

Results

Participant Characteristics

Compared with participants in the lowest quartile of FGF-23, participants in the higher quartiles were more frequently female, hypertensive, diabetic, and current smokers (Table 1). Those in higher quartiles of FGF-23 were also less physically active, had higher triglycerides, higher serum phosphorous, higher ACR, and lower eGFR.

The participants included in these analyses had a broad range of kidney function. In the entire study population, 167 participants had normal kidney function (eGFR ≥90 mL/min per 1.73 m²), 449 participants had eGFR between 60 and 90 mL/min per 1.73 m², and 271 participants had eGFR <60 mL/min per 1.73 m².

FGF-23 concentrations varied markedly among patients with different levels of kidney function. Median FGF-23 levels were 32.3 RU/mL (interquartile range 26.7) in participants...
with normal kidney function, 39.1 RU/mL (interquartile range 30.8) among those with eGFR between 60 and 90 mL/min per 1.73 m², and 68.5 RU/mL (interquartile range 90.2) in participants with eGFR <60 mL/min per 1.73 m².

FGF-23, LVMI, and LVEF
In our crude model, FGF-23 was positively associated with LVMI (Table 2). These results were attenuated and no longer statistically significant after adjustment for cardiovascular risk factors, including serum phosphorous, serum calcium, ACR, and eGFR. FGF-23 was inversely associated with LVEF in both crude and multivariable-adjusted models, but with only a modest decrease in LVEF across quartiles of FGF-23 (Table 2).

FGF-23 and Concentric and Eccentric Hypertrophy
In multinomial logistic regression, FGF-23 was not statistically significantly associated with the prevalence of concentric hypertrophy (odds ratio, 1.5; CI, 0.9 to 2.4 per SD increase in lnFGF-23) or eccentric hypertrophy (odds ratio, 1.1; CI, 0.9 to 1.3) compared with no hypertrophy. The differential associations of FGF-23 with concentric versus eccentric hypertrophy (with no hypertrophy as the referent) were not statistically significant (P=0.27).

Interactions With Kidney Function
In stratified analyses adjusting for eGFR as a continuous variable (Table 3), FGF-23 was strongly related to concentric hypertrophy in patients with eGFR <60 mL/min per 1.73 m² but not among patients with eGFR ≥60 mL/min per 1.73 m². To determine if the greater association among participants with CKD reflected their greater range in FGF-23 levels, we performed analyses restricted to individuals with FGF-23 <350 RU/mL. Associations in this restricted population were similar to our previous results. In the total population, kidney function did not appear to modify the relationship between FGF-23 and LVMI (P-interaction=0.87) or LVEF (P-interaction=0.31).

Cardiac MRI in Mice
As expected, 1-α hydroxylase-deficient mice used as positive controls had significantly greater wall volumes than did wild-type mice (P=0.04). In contrast, klotho-deficient mice did not differ significantly from wild-type mice (−0.28 SD smaller intranformed wall volume in klotho-deficient mice; CI, −0.87 to 0.32 SD; P=0.37). Similar to wild-type mice, klotho-deficient mice had significantly lower wall volumes than 1-α hydroxylase-deficient mice (−1.14 SD; CI, −2.13 to −0.14 SD; P=0.02). Left ventricular function among klotho-deficient mice also did not differ significantly from wild-type mice (0.04 SD higher In-transformed LVEF in klotho-deficient mice; CI, −0.99 to 1.06 SD; P=0.94).

Heart Weight and Histology in Mice
No statistically significant differences could be observed in the heart weight/body weight ratio comparing wild-type, klotho(−/−), and klotho(−/−) mice (P=0.97; Figure) or in left ventricular wall area measurements comparing wild-type and klotho(−/−) mice (P=0.94).

Table 2. Least-Squares Mean Estimates of Left Ventricular Mass Index (g/m²) and Left Ventricular Ejection Fraction (%) by Quartile of FGF-23 (RU/mL)

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Left ventricular mass index, g/m²</th>
<th>Left ventricular ejection fraction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;27.7)</td>
<td>93.6 (90.2 to 96.9)</td>
<td>62.9 (61.7 to 64.2)</td>
</tr>
<tr>
<td>2 (27.8 to 43.0)</td>
<td>96.1 (92.7 to 99.4)</td>
<td>62.5 (61.2 to 63.7)</td>
</tr>
<tr>
<td>3 (43.1 to 71.4)</td>
<td>96.9 (93.8 to 100.3)</td>
<td>62.2 (60.9 to 63.5)</td>
</tr>
<tr>
<td>4 (≥71.5)</td>
<td>105.3 (101.9 to 108.7)</td>
<td>62.2 (60.9 to 63.5)</td>
</tr>
</tbody>
</table>

Increase per SD lnFGF-23 | P Value |<0.001 |<0.001 |<0.001 |<0.001 |0.01

We provide least-squares mean estimates and confidence intervals (by quartiles) and regression coefficients and standard errors per SD InFGF-23 (in the overall population). P values were estimated by linear regression to test for trend. ACR indicates albumin-to-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor–23; HDL, high-density lipoprotein; SBP, systolic blood pressure.

*Adjusted for age, sex, ethnicity, BMI.
†Adjusted for age, sex, ethnicity, BMI, SBP, diabetes, hypertension, physical activity score, smoking history, total cholesterol, HDL cholesterol, triglycerides.
‡Adjusted for age, sex, ethnicity, BMI, SBP, diabetes, hypertension, physical activity score, smoking history, total cholesterol, HDL cholesterol, triglycerides, serum phosphorous, serum calcium, ACR, and eGFR.
Table 3. Estimated Odds Ratios and Confidence Intervals Per Standard Deviation (SD) Increase in lnFGF-23, Stratified by Chronic Kidney Disease (eGFR <60 mL/min per 1.73 m²)

<table>
<thead>
<tr>
<th></th>
<th>eGFR &lt;60 mL/min per 1.73 m²</th>
<th>eGFR ≥60 mL/min per 1.73 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=271 ODDS RATIO P VALUE</td>
<td>n=616 ODDS RATIO P VALUE</td>
</tr>
<tr>
<td>No hypertrophy</td>
<td>87 1.0 (ref) —</td>
<td>305 1.0 (ref) —</td>
</tr>
<tr>
<td>Eccentric hypertrophy</td>
<td>171 1.0 (0.7 to 1.4) 0.93</td>
<td>300 1.1 (0.9 to 1.4) 0.37</td>
</tr>
<tr>
<td>Concentric hypertrophy</td>
<td>13 2.3 (1.0 to 5.3) 0.04</td>
<td>11 1.0 (0.4 to 2.5) 0.94</td>
</tr>
</tbody>
</table>

ACR indicates albumin-to-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; HDL, high-density lipoprotein; SBP, systolic blood pressure. Adjusted for age, sex, ethnicity, BMI, SBP, diabetes, hypertension, physical activity score, smoking history, total cholesterol, HDL cholesterol, triglycerides, serum phosphorous, serum calcium, ACR, and eGFR. P-interaction=0.28.

Discussion

Our study in a well-characterized population with stable coronary artery disease and a broad range of kidney function extends previously published evidence on the association between circulating levels of FGF-23 and cardiac hypertrophy. In 887 individuals enrolled in the Heart and Soul Study, FGF-23 was not associated with left ventricular mass or odds of concentric or eccentric hypertrophy. We observed a strong association between FGF-23 and concentric hypertrophy only among individuals with CKD. In our murine experiments, we were unable to confirm previous findings of LVH in klotho-deficient mice, an established animal model of elevated FGF-23. Together, our results raise questions about the generalizability of the effect of FGF-23 on cardiac structure and suggest that further research is needed to prove a direct effect of FGF-23 on the cardiovascular system in humans.

Although FGF-23 has been variably associated with abnormal cardiac structure and function and adverse cardiovascular outcomes in individuals with and without CKD, findings have been inconsistent, and when present, associations have generally been stronger among individuals with impaired renal function. At present, it remains controversial whether FGF-23 has a direct effect on the cardiovascular system.

There are several possible mechanisms by which FGF-23 may affect cardiac structure and function indirectly. It is possible that FGF23 provides an alternative measure of renal impairment (imperfectly captured by eGFR or ACR) that can indirectly lead to cardiovascular remodeling and adverse events via hemodynamic overload and pressure and volume expansion. Alternatively, FGF-23 may be a marker of time-averaged serum phosphorous and, by extension, vascular calcification—a known risk factor for cardiovascular remodeling. In this study, associations of FGF-23 with LVEF were not significantly attenuated by adjustment for serum phosphorous levels measured at a single time. Nonetheless, a single serum phosphorous measurement may not accurately represent cumulative phosphorous burden, given the high intrindividual biological variability in serum phosphorous levels when measured repeatedly over time. Finally, FGF-23 may affect the cardiovascular system indirectly by inducing hypovitaminosis D via inhibition of 1a-hydroxylase, given the large body of evidence for vitamin D deficiency as a cardiovascular risk factor.

Although a direct effect of FGF-23 on the heart has been hypothesized, evidence to support this hypothesis is limited. In 1 murine experiment, intravenous or direct injection of recombinant FGF-23 protein into the myocardium resulted in LVH within 5 or 14 days, respectively. However, confirmatory studies of LVH reduction by FGF-23 neutralization have produced mixed results. Faul et al reported that klotho-deficient mice, an established murine model of elevated FGF-23, develop echocardiographic LVH—and interpreted this as evidence that FGF-23 action on cardiac tissue is independent of the klotho coreceptor. Our study using cardiac MRI, a potentially more accurate 3-dimensional imaging modality than echocardiography, did not provide evidence of LVH or diminished ejection fraction in klotho-deficient mice. Our MRI findings were substantiated by heart weight and histological data, which also showed no significant differences between wild-type and klotho-deficient mice.

Figure. Heart weight/body weight ratio in wild-type (WT) (n=6), klotho(+/-) (n=5), and klotho(-/-) (n=12) mice at 6 weeks of age. Values are expressed as mean ± standard error (SE) for each group.
Several caveats are necessary when comparing our results with those reported previously. First, mice in our study were 4 to 6 weeks old, whereas klotho-deficient mice found to have echocardiographic LVH were 8 to 12 weeks old at the time that cardiac size was assessed. It is possible that differences in left ventricular size and systolic function do not manifest at an early age, although klotho-deficient mice do display significantly elevated circulating levels of FGF-23 accompanied by soft-tissue and vascular calcifications as early as 3–6 weeks of age.26 Another possible explanation for our failure to detect LVH in klotho-deficient mice is our small sample sizes (n=5 to 6 mice/genotype), although our sample sizes were similar to those in previous studies that observed LVH and were sufficient to observe LVH in our positive controls. A final possible explanation for our findings is that the use of cardiac MRI, a potentially more accurate imaging modality than echocardiography, reduced measurement error in assessing cardiac size and produced more accurate results. To validate our findings, further in vitro and in vivo experimentation with direct injection of FGF-23 and inhibition of the klotho coreceptor, ideally in murine models with and without CKD at earlier and later ages, is needed.

This study has several strengths. Few previous studies have had the necessary data to examine whether FGF-23 is associated with left ventricular mass and systolic dysfunction in patients without CKD. With 887 participants, we were able to study the association of FGF-23 and cardiac structure and function with precision. We adjusted for important potential confounders not available in most previous studies, including serum phosphorous and calcium, ACR, and cystatin-based eGFR, though we cannot exclude the possibility of residual confounding or confounding by other unknown characteristics. FGF-23 was measured in duplicate, with low intra- and interassay coefficients of variation. Echocardiograms were examined by a blinded cardiologist before the FGF23 measurements were conducted, reducing the likelihood of measurement error and misclassification bias.

Our study also has important limitations. As a result of our cross-sectional study design, we are unable to determine temporality. Because the participants in our study were mostly white men with stable coronary heart disease, caution must be taken in generalizing our results to other populations. Furthermore, we did not have information on variables such as erythropoietin levels or vasomotor responses in this cohort study, which could have shed light on the observed association among individuals with CKD.

Despite recent pharmacological advances, long-term outcomes in patients with CKD remain poor. If the observed relationships between elevated FGF-23 and LVH among individuals with CKD are causal, it is tempting to speculate that FGF-23 may become a therapeutic target in these patients, and clinical trials are currently ongoing. However, our results suggest that FGF-23 is unlikely to be a major target among patients without underlying CKD.

In conclusion, we observed a modest association of FGF-23 with worse left ventricular systolic function in the entire population and an association of FGF-23 with LVMI and concentric LVH only among individuals with CKD. Our murine experiments found no evidence of LVH in klotho-deficient mice, using both cardiac MRI and histological analysis. Our results suggest that FGF-23 is unlikely to have major direct effects on cardiovascular structure and function among patients free of substantial CKD; furthermore, if present, any direct effect on the myocardium may not be independent of the klotho coreceptor.

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Disclosures
None.

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