Serum FGF23 and Risk of Cardiovascular Events in Relation to Mineral Metabolism and Cardiovascular Pathology

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Summary

Background and objectives Circulating fibroblast growth factor-23 is associated with adverse cardiovascular outcomes in CKD and non-CKD individuals, but the underlying mechanism remains unclear. This study tested whether this association is independent of mineral metabolism and indices of subclinical cardiovascular pathology.

Design, setting, participants, & measurements The prospective association between fibroblast growth factor-23 and major cardiovascular events (a composite of hospital-treated myocardial infarction, hospital-treated stroke, or all-cause mortality) was investigated in the community-based Prospective Investigation of the Vasculature in Uppsala Seniors (n=973; mean age=70 years, 50% women) using multivariate logistic regression. Subjects were recruited between January of 2001 and June of 2004.

Results During follow-up (median=5.1 years), 112 participants suffered a major cardiovascular event. In logistic regression models adjusted for age, sex, and estimated GFR, higher fibroblast growth factor-23 was associated with increased risk for major cardiovascular events (odds ratio for tertiles 2 and 3 versus tertile 1=1.92, 95% confidence interval=1.19–3.09, $P<0.01$). After additional adjustments in the model, adding established cardiovascular risk factors, confounders of mineral metabolism (calcium, phosphate, parathyroid hormone, and 25 (OH)-vitamin D), and indices of subclinical pathology (flow-mediated vasodilation, endothelial-dependent and -independent vasodilation, arterial stiffness, and atherosclerosis and left ventricular mass) attenuated this relationship, but it remained significant (odds ratio for tertiles 2 and 3 versus tertile 1=1.69, 95% confidence interval=1.01–2.82, $P<0.05$).

Conclusions Fibroblast growth factor-23 is an independent predictor of cardiovascular events in the community, even after accounting for mineral metabolism abnormalities and subclinical cardiovascular damage. Circulating fibroblast growth factor-23 may reflect novel and important aspects of cardiovascular risk yet to be unraveled.

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Introduction

Fibroblast growth factor-23 (FGF23) is an endocrine factor that regulates mineral metabolism in health and disease (1–4). Its circulatory levels are increased in patients with CKD (5) and predict adverse cardiovascular outcomes and CKD progression across all strata of impaired kidney function (6–10). Recent studies further reported that even modest increments in FGF23 levels are associated with cardiovascular mortality as well in the community-based setting (11,12). In these previous studies of the normal population, a reduced kidney function was put forward as the most important mediator of these associations. Still, the underlying mechanisms linking FGF23 to cardiovascular disease remain largely unknown.

Several models explaining the link between FGF23 and cardiovascular disease have been proposed: FGF23 may directly influence cardiac remodeling, leading to left ventricular hypertrophy (13) or possibly, vascular function through the presence of its coreceptor Klotho in the vascular endothelium from human arterial specimens (14); FGF23 could also indirectly modify cardiovascular risk through regulation of essential parameters of mineral metabolism, such as serum phosphorous, calcium, parathyroid hormone (PTH), and vitamin D (15). To date, these hypotheses have not been fully explored in longitudinal studies, because critical confounding parameters of mineral metabolism and assessments of subclinical cardiovascular pathology were not available in either our recently reported study of elderly Swedish men (12) or a study by Ix *et al.* (11).

Accordingly, our goal was to examine the relation between FGF23 and cardiovascular events in a community-based cohort, with the specific aim to test whether this association is independent of mineral

metabolism abnormalities and indices of subclinical cardiovascular pathology.

Materials and Methods

All 70-year-old individuals living in Uppsala, Sweden, between 2001 and 2004 were eligible for the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (16) (described in detail on http://www.medsci.uu.se/pivus/pivus.htm). Of 2025 invited individuals, 1014 agreed to participate. Of these individuals, 11 participants were excluded because of missing data on FGF23. All participants gave written informed consent, and the Ethics Committee of Uppsala University approved the study protocols.

Anthropometrical measurements, BP, blood sampling, and questionnaires regarding socioeconomic status, medical history, smoking habits, medication, and physical activity level were performed as previously described (16). Serum FGF23 was measured using an intact FGF23 ELISA (Kainos Laboratories, Japan). The intra-assay coefficient of variation was <4%. The LIAISON 25(OH)D_3 assay (DiaSorin Inc., Saluggia, Italy) was performed on the LIAISON analyzer according to the manufacturer's instructions. PTH levels were analyzed using the Immulite 2000 Intact PTH Assay (Diagnostic Products Corporation, Los Angeles, CA). Estimated GFR (eGFR) was derived from the CKD Epidemiology Collaboration Equation (17). Mineral metabolism variables (calcium, phosphate, PTH, and 25-hydroxy vitamin D), left ventricular mass and ejection fraction assessed by echocardiography, endothelial-dependent and -independent vasodilation by the invasive forearm model, flow-mediated vasodilation, intima media thickness of the carotid arteries, and pulse-wave analysis were measured as previously described (16,18,19). For endothelial function measurements, the forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin; Kullavik). Evaluations of FBF were made by calculations of the mean of at least five consecutive recordings. For evaluation of endothelial function, an arterial cannula was placed in the brachial artery. After evaluation of resting FBF, local intra-arterial drug infusions were given during 5 minutes for each dose, with a 20-minute washout period between the drugs. The infused dosages were 25 and 50 μg/min for acetylcholine (Clin-Alpha) to evaluate endothelium-dependent vasodilation and 5 and 10 μg/min for sodium nitroprusside (Nitropress; Abbot) to evaluate endothelium-independent vasodilation. The dosages of these drugs have been chosen to result in FBFs on the steep part of the dose–response curve without giving systemic effects, and calculations of endothelial function are described elsewhere (16,18,19).

Follow-Up and End Point Definitions

The cohort was invited to a re-examination 1 month after the 75th birthday, to which 827 individuals (81%) attended. Participants were asked if they had been treated in the hospital for a myocardial infarction or stroke. Their medical records were evaluated by an experienced physician (L.L.) to validate the cases. L.L. was also blinded to the results of FGF23 measurements. In this chart review, myocardial infarction was considered to be present if the subject presented with acute chest pain and showed raised troponin levels and electrocardiogram changes. Stroke was considered to be present if the subject presented with acute onset neurologic deficits, such as hemiparesis, aphasia, ataxia, etc., and other disorders than stroke could be excluded. There was excellent agreement between the self-reported cardiovascular events and the chart review. The Swedish cause of death register was used to identify all those individuals who died between examinations and during follow-up. All nonparticipants of the follow-up examination that were still alive were contacted by telephone, and their medical records were screened to obtain their medical history at 75 years of age. By this action, only two nonparticipants were lost to follow-up. The primary outcome, major adverse cardiovascular events (MACEs), was defined as all-cause mortality, hospital-treated myocardial infarction, or hospital-treated stroke during follow-up as previously described (20). All-cause mortality was used in the definition of MACE, because data on cause-specific mortality was not available.

Statistical Analyses

The prospective association between serum FGF23 and MACE was analyzed. Because we did not have data on time to event, we had to use logistic regression rather than Cox regression in our analyses using the following multivariable models:

Model A adjusted for age and sex.
Model B adjusted for age, sex, and eGFR.
Model C adjusted for covariates in model B and variables reflecting mineral metabolism (PTH, vitamin D, calcium, and phosphate).
Model D adjusted for covariates in model B and markers of cardiovascular pathology (left ventricular mass, left ventricular ejection fraction, endothelial-dependent and -independent vasodilation, flow-mediated dilation, arterial stiffness, and intima media thickness).

We also performed secondary analyses, where we combined models A–D and also, separate models, where each of the covariates in models C and D was added individually to multivariable model B.

Because a restricted cubic spline analyses suggested a nonlinear association between FGF23 and MACE risk (data not shown), we modeled FGF23 in multicategory and threshold models. Missing values for covariates were estimated by multiple imputation. Missing serum biochemistries were $n \leq 5$ for all variables except PTH (n=31). Missing cardiovascular variables were systolic and diastolic BP (n=4), eGFR (n=4), intima-media thickness (n=61), left ventricular hypertrophy (n=92), endothelium-independent vasodilation (n=130), endothelium-dependent vasodilation (n=147), pulse-wave reflection index (n=138), left ventricular ejection fraction (n=178), and flow-mediated vasodilation (n=182).

Results

Baseline characteristics of the PIVUS study participants are shown in Table 1. There was a significant

Table 1. Baseline characteristics of participants in the Prospective Investigation of the Vasculature in Uppsala Seniors study

Variable	
Number of subjects	1003
Female no. (%)	497 (50)
Age (yr)	70.2±0.2
Body mass index (kg/m^2)	27±4
Systolic BP (mmHg)	150±23
Diastolic BP (mmHg)	79±10
Serum total cholesterol (mg/dl)	5.4±1.0
Serum HDL cholesterol (mg/dl)	1.5±0.4
Fasting plasma glucose (mg/dl)	5.3±1.6
Diabetes	117 (12)
Smoking no. (%)	109 (11)
Previous cardiovascular disease no. (%)	164 (16)
Lipid-lowering treatment no. (%)	159 (16)
Antihypertensive treatment no. (%)	314 (31)
Estimated GFR (ml/min per 1.73 m^2)	80±14
Serum calcium (mg/dl)	9.44±0.52
Serum phosphate (mg/dl)	3.40±0.53
Serum 25(OH)D_3 (nmol/L)	58±20
Serum intact PTH (pmol/L)	47±21
Serum FGF23 (pg/ml)	47±24
Left ventricular mass (g)	225±75
Left ventricular ejection fraction (%)	66±8
Endothelium-dependent vasodilatation (%)	529±316
Endothelium-independent vasodilatation (%)	369±213
Flow-mediated dilatation (%)	4.8±3.6
Pulse-wave reflection index (%)	−31±14
Intima media thickness (mm)	0.92±0.16

Data are mean ± SD for continuous variables and *n* (%) for categorical variables. 25(OH)D_3, 25-hydroxyvitamin D_3; PTH, parathyroid hormone; FGF23, fibroblast growth factor-23.

cross-sectional association between FGF23 and eGFR (Spearman rank correlation coefficient=−0.02, $P<0.001$). During follow-up (median=5.1 years, range=4.8–5.8 years), 52 participants died, 25 participants had a myocardial infarction, 33 participants had a stroke, and 2 participants suffered from both diseases, contributing to 112 instances of MACEs (overall risk=11.0%).

In multicategory and threshold models adjusted for age, sex, and eGFR (model B), participants in tertiles 2 and 3 had up to a twofold increased risk compared with those participants in the lowest tertile (Table 2). These associations were attenuated after additional adjustment for mineral metabolism factors (model C) and parameters reflecting subclinical cardiovascular damage (model D). The association remained significant in a combined model with all covariates in models A–D (odds ratio for tertiles 2 and 3 versus tertile 1=1.75, 95% confidence interval=1.07–2.86, $P=0.03$) and after addition of established cardiovascular risk factors (body mass index, diabetes, systolic BP, antihypertensive medication, total cholesterol, HDL cholesterol, lipid-lowering medication, and smoking; odds ratio for tertiles 2 and 3 versus tertile 1=1.69, 95% confidence interval=1.01–2.82, $P<0.05$).

In analyses with separate addition of the mineral metabolism parameters and cardiovascular covariates to multivariable model B, left ventricular mass, endothelium-dependent vasodilation, serum phosphate, and calcium seemed to attenuate the associations the most (Table 3), although the associations remained statistically significant in all analyses.

Discussion

We confirm that FGF23 is associated with incident cardiovascular events in the community-based setting independently of eGFR; this association was only weakly modified by adjustment for components of mineral metabolism (vitamin D, PTH, calcium, and phosphate) or assessments of vascular function (flow-mediated vasodilation and endothelial-dependent and -independent vasodilation), arterial stiffness (pulse-wave reflection index), atherosclerosis (intima media thickness), or left ventricular mass and function. Of the cardiovascular variables, adjustment for left ventricular mass and endothelial function seemed to attenuate the associations the most, which is in accordance with experimental studies suggesting a direct effect of FGF23 in myocardium (13) and vascular wall (14). Of the mineral metabolism variables, phosphate and calcium seemed to attenuate the association between FGF23 and MACE the most, whereas no such effect was seen after adjustment for PTH or vitamin D. This observation corroborates with the fact that FGF23 expression is regulated by phosphate and calcium (21,22), which in turn, are vital components for vascular calcification and additional cardiovascular complications, such as endothelial dysfunction and cellular senescence (23). However, recent studies in patients with moderate impairments in kidney function support that FGF23 is essentially unaltered or only weakly lowered by treatment with phosphate binders, whereas no parallel changes in serum phosphate were observed (24–26). This finding shows the difficulties in manipulating serum phosphate level when kidney function is relatively well preserved and substantiates our observation that adjustment for serum phosphate does not completely disrupt the relationship between FGF23 and MACE.

Our main finding that the association between FGF23 and MACE remained significant in all multivariable models indicates that the underlying mechanism linking FGF23 to cardiovascular outcomes still is incompletely understood. We speculate that a possible mechanism could be an impaired renal response to hormonal action (*i.e.*, FGF23 resistance). Indeed, this phenomenon is a cardinal feature of CKD and is mediated by reduced expression of the FGF23 coreceptor Klotho (27), which has been shown to preserve and protect vascular integrity through multiple independent mechanisms (14,28–31). It is further possible that other aspects of tubular dysfunction modulate cardiovascular risk independently of baseline eGFR; indeed, tubular injury could cause long-term interstitial fibrosis and secondary glomerular scarring, which translates into a more rapid loss of GFR and increased cardiovascular risk. Another causal pathway could be that FGF23 directly stimulates cardiomyocyte growth and development of left

Table 2. The association between serum fibroblast growth factor-23 and major cardiovascular events in the Prospective Investigation of the Vasculature in Uppsala Seniors cohort

	No. of events/ no. at risk	Model A	Model B	Model C	Model D
Multicategory models					
Tertile 1 (<36 pg/ml)	24/335	Referent	Referent	Referent	Referent
Tertile 2 (36–50 pg/ml)	45/334	2.05 (1.22–3.47)[a]	2.05 (1.22–3.46)[a]	1.95 (1.15–3.32)[b]	2.00 (1.18–3.39)[b]
Tertile 3 (≥50 pg/ml)	43/334	1.83 (1.08–3.11)[b]	1.79 (1.05–3.06)[b]	1.70 (0.98–2.94)[c]	1.68 (0.97–2.90)[c]
Threshold models					
Tertile 1 (<36 pg/ml)	24/335	Referent	Referent	Referent	Referent
Tertile 2 and 3 (≥36 pg/ml)	88/668	1.94 (1.21–3.12)[a]	1.92 (1.19–3.09)[a]	1.83 (1.12–2.98)[b]	1.84 (1.14–2.98)[b]

Model A is adjusted for age and sex. Model B is adjusted for covariates in model A and estimated GFR. Model C is adjusted for covariates in model B and variables reflecting mineral metabolism (parathyroid hormone, vitamin D, calcium, and phosphate). Model D is adjusted for covariates in model B and markers of cardiovascular pathology (left ventricular mass, left ventricular ejection fraction, endothelial-dependent and -independent vasodilation, flow-mediated dilation, arterial stiffness, and intima media thickness). Model data are given as odds ratio (95% confidence interval).
[a]$P<0.01$.
[b]$P<0.05$.
[c]$P=0.06$.

Table 3. The association between fibroblast growth factor-23 and major cardiovascular events in the Prospective Investigation of the Vasculature in Uppsala Seniors cohort: separate models for different mineral metabolism variables and indices of subclinical cardiovascular patholog

	Tertile 1 (FGF23≤36 pg/ml)	Tertiles 2 and 3 (FGF23>36 pg/ml)
Model B (age, sex, and estimated GFR)	Referent	1.92 (1.19–3.09)[a]
Mineral metabolism variables		
Model B + serum parathyroid hormone	Referent	1.92 (1.19–3.10)[a]
Model B + serum vitamin D	Referent	1.99 (1.23–3.21)[a]
Model B + serum calcium	Referent	1.87 (1.15–3.02)[b]
Model B + serum phosphate	Referent	1.77 (1.09–2.86)[b]
Indices of subclinical cardiovascular pathology		
Model B + left ventricular mass	Referent	1.84 (1.14–2.98)[b]
Model B + left ventricular ejection fraction	Referent	1.92 (1.19–3.09)[a]
Model B + endothelium-dependent vasodilation	Referent	1.88 (1.17–3.03)[a]
Model B + endothelium-independent vasodilation	Referent	1.88 (1.17–3.03)[a]
Model B + flow-mediated dilatation	Referent	1.92 (1.19–3.10)[a]
Model B + pulse-wave reflection index	Referent	1.94 (1.20–3.12)[a]
Model B + intima media thickness	Referent	1.92 (1.19–3.10)[a]

Data are odds ratios for tertiles 2 and 3 versus tertile 1 of FGF23. Tertile data are given as odds ratio (95% confidence interval). FGF23, fibroblast growth factor-23.
[a]$P<0.01$.
[b]$P<0.05$.

ventricular hypertrophy (13) and that our adjustments for left ventricular mass did not fully capture all aspects of this pathophysiological pathway. Finally, we cannot exclude the possibility that FGF23 exerts off-target effects in other tissues than the myocardium, which translate into end organ toxicity and increased cardiovascular risk. Indeed, FGF23 was shown to be an independent predictor of a more rapid loss of GFR and independently associated with albuminuria (6,9,32). Regardless, our present findings support that FGF23 is an independent risk factor that portrays dimensions of cardiovascular risk beyond traditional cardiovascular risk factors, alterations in mineral metabolism, and subclinical cardiovascular pathology.

The major strengths are the longitudinal study design and detailed characterization of the study participants with regard to mineral metabolism and state-of-the art static and dynamic assessments of cardiovascular function and geometry. Study limitations are inclusion of elderly

Caucasian individuals only, and therefore, extrapolations of our findings to other ethnicities and age groups should be done with caution. Data on cause-specific mortality were not available in PIVUS; hence, a composite end point that included nonfatal cardiovascular events and all-cause mortality was used that may have diluted the strength of the associations. However, the results were similar when excluding total mortality from the composite end point (data not shown). Because of the complexity of FGF23 biology, residual confounding cannot be excluded. For example, we were unable to adjust for 24-hour urinary phosphate excretion, which may be an important modifier of FGF23 and/or portray an integrative value of circulating FGF23. Another potential limitation is the adjustment for a single baseline measurement of confounding variables of mineral metabolism that can vary considerably over time. It is particularly true for vitamin D status, which has a substantial seasonal variation in Scandinavian countries. However, because vitamin D and FGF23 were measured from the same samples, it is unlikely that such seasonal variations substantially could modify the relationship between FGF23 and MACE. Finally, because the influence of a biomarker not only is determined by the underlying pathophysiology but also by its specificity and sensitivity, no firm conclusions regarding the relative causal effect of specific variables on the association between FGF23 and cardiovascular events should be made.

In conclusion, FGF23 is a predictor of cardiovascular events in the community, and its predictive value is independent of confounders of mineral metabolism and measurements portraying multiple dimensions of cardiovascular pathology. Circulating FGF23 may, therefore, reflect novel and important aspects of cardiovascular risk yet to be unraveled.

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Disclosures

The study was investigator-initiated and -driven. The authors report no conflicts of interests in connection with this study. T.E.L. is a part-time employee of Astellas.

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