CLINICAL STUDY

Relationship of serum fibroblast growth factor 23 with cardiovascular disease in older community-dwelling women

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Abstract

Objective: Although fibroblast growth factor 23 (FGF23) has been implicated in the pathogenesis of cardiovascular disease, the relationship between FGF23 and cardiovascular disease has not been well characterized in the general population. The aim of this study was to determine whether serum FGF23 is independently associated with cardiovascular disease in older community-dwelling women.

Design and methods: A cross-sectional design was used to examine the relationship between serum FGF23 and cardiovascular disease. The subjects consisted of a population-based sample of 659 women, aged 70–79 years, who participated in the Women's Health and Aging Studies in Baltimore, Maryland. Prevalent cardiovascular disease (coronary heart disease, stroke, congestive heart failure, and peripheral artery disease) was assessed through diagnostic algorithms and physician adjudication.

Results: Of the 659 women, 185 (28.1%) had cardiovascular disease. Median (25th, 75th percentile) intact serum FGF23 was 34.6 (25.2, 46.2) pg/ml. The prevalence of cardiovascular disease in the lowest, middle, and highest tertile of serum FGF23 was 22.6, 24.9, and 36.7% respectively ($P=0.002$). Serum log FGF23 was associated with cardiovascular disease (odds ratio per 1 s.d. increase = 1.23, 95% confidence interval 1.17, 1.30; $P<0.0001$) in a multivariable logistic regression model, adjusting for age, race, smoking, education, body mass index, cognition, diabetes, hypertension, physical activity, total cholesterol, high-density lipoprotein cholesterol, and renal function.

Conclusion: Elevated serum FGF23 concentrations are independently associated with prevalent cardiovascular disease in older community-dwelling women. Further studies are needed to elucidate the potential biological mechanisms by which FGF23 may be involved in the pathogenesis of cardiovascular disease.

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Introduction

Abnormalities in phosphate and calcium metabolism have recently been implicated as risk factors for cardiovascular disease and all-cause mortality, particularly in patients with chronic kidney disease (1–3). Fibroblast growth factor 23 (FGF23) is a recently discovered 30 kDa secreted hormone glycoprotein that plays an important role in the complex and tightly regulated mechanism of mineral metabolism (4). In healthy individuals, FGF23 is secreted from bone osteocytes in response to an increase in dietary phosphate (5). FGF23 acts through one of the FGF-receptors, with klotho as a co-receptor, to inhibit renal phosphate reabsorption and decrease circulating levels of $1,25(\text{OH})_2\text{D}$, and possibly inhibit PTH secretion by the parathyroid glands (6–8). Thus, its net effect is a reduction in serum phosphate and $1,25(\text{OH})_2\text{D}$ levels, which may result in hypocalcemia.

Increased serum FGF23 concentrations were an independent predictor of coronary artery disease in patients with mild chronic kidney disease and mortality in patients undergoing hemodialysis (9, 10). Recently, FGF23 has been found to be associated with total body atherosclerosis and vascular dysfunction (11, 12). However, another study found that circulating FGF23 did not correlate with coronary artery calcification in patients with normal renal function (13). In men and women with cardiovascular disease, increased levels of FGF23 were an independent predictor of cardiac events (14). Elevated FGF23 levels have been associated with left ventricular hypertrophy (15, 16).

The relationship between FGF23 and cardiovascular disease in the general population has not been characterized. The specific aim of this study was to determine whether elevated serum FGF23 concentrations are associated with cardiovascular disease.

To address this aim, we examined the relationship between serum FGF23 and prevalent cardiovascular disease in older women living in the community.

Materials and methods

Participants

A cross-sectional study was conducted among 659 women, from the Women's Health and Aging Studies (WHAS) I and II, two population-based cohorts of community-dwelling women living in Baltimore, Maryland. Both cohorts were designed to investigate factors associated with the progression of physical disability. WHAS I, which consisted of 1002 women aged 65 and older, represented one-third of the most disabled women living in Baltimore. WHAS II was composed of 436 women aged 70–79 recruited from among the two-thirds least disabled in the same community. This study involved 299 women, age 70–79 years, from WHAS I, and 360 women from WHAS II.

Participants were recruited from an age-stratified random sample of women aged 65 years and older selected from Medicare enrollees residing in 12 contiguous zip code areas in eastern Baltimore city and Baltimore County. Baseline assessment occurred from November 1992 to February 1995 in WHAS I and from August 1994 to February 1996 in WHAS II. Standardized questionnaires, a physical examination, and a physical performance testing were administered by trained interviewers the participant's home in WHAS I and in the Johns Hopkins Functional Laboratory in WHAS II. Body mass index was calculated as weight/height² (kg/m²). Mini-Mental State Examination (MMSE) was recorded (17). Chronic kidney disease was defined as estimated glomerular filtration rate of <60 ml/min per 1.73 m² using the four-variable Modification of Diet in Renal Disease Study (MDRD) equation of Levey *et al.* (18). Race was assessed in a questionnaire as black, white, or other, current smoking as yes or no, and education as 0–8, 9–11, 12 years or more than 12 years as the highest level of formal education achieved. Chronic diseases were adjudicated by WHAS co-investigators based on standardized questionnaires, physical examination, physician contact, and diagnostic algorithms. Further details on the methods and sampling design of the WHAS studies are published elsewhere (19, 20). Cardiovascular disease was defined as a combination of any of these adjudicated conditions: coronary artery disease, congestive heart failure, stroke, and peripheral artery disease. Written informed consent was obtained from all participants. The study protocol complied with the Declaration of Helsinki. The Johns Hopkins Medical Institutions Institutional Review Board approved the research protocols.

Laboratory measurements

Non-fasting blood specimens were collected at the participants' homes in WHAS I and at the Johns Hopkins General Clinical Research Center in WHAS II. Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by enzymatic methods at Quest Diagnostics. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (21). Active intact full-length FGF23 was measured in previously unfrozen serum samples collected at baseline using a commercial sandwich ELISA according to the manufacturer's protocol (Kainos Laboratories, Inc., Tokyo, Japan). This ELISA utilizes two murine MABs for two separate sites (22). In this study, the intra- and inter-assay coefficients of variation were 2.8 and 4.9% respectively.

Statistical analysis

Variables are reported as medians (25th, 75th percentiles) or as percentages. Characteristics of subjects were compared across tertiles of serum FGF23 and according to cardiovascular disease by Kruskal–Wallis rank sum tests for continuous variables and χ^2 tests for categorical variables. Bivariate and multivariable logistic regression models were used to examine the relationship between serum FGF23 and cardiovascular disease. Variables that were significantly associated with cardiovascular disease or tertiles of FGF23 in the bivariate analyses and total and HDL cholesterol were entered into the multivariable analyses. All analyses were performed by SAS (v. 9.1.3, SAS Institute, Inc., Cary, NC, USA). The level of significance used in this study was $P < 0.05$.

Results

Serum FGF23 concentrations were skewed in distribution. Overall, the mean (s.d.) of log serum FGF23 concentration (pg/ml) was 3.52 (0.52) and median (25th, 75th percentile) serum FGF23 was 34.6 (25.2, 46.2) pg/ml. The health and demographic characteristics of the 659 women across tertiles of serum FGF23 concentrations are shown in Table 1. Higher serum FGF23 concentrations were associated with older age, higher body mass index, greater education, lower physical activity, and lower estimated glomerular filtration rate; hypertension, coronary artery disease, peripheral artery disease, and cardiovascular disease. There were no significant differences across the tertiles of serum FGF23 concentrations by race, alcohol intake, current smoking, cognitive impairment, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, or by the prevalence of congestive heart failure, stroke, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease, depression, and cancer. The proportion of participants who had cardiovascular

Table 1 Characteristics of 659 women, aged 70–79 years, in the WHAS, by tertiles of serum FGF23.

Characteristics	Tertile of serum FGF23 (pg/ml)			P
	<28.1 (n=221)	28.1–42.4 (n=217)	>42.4 (n=221)	
Age (years)	73 (71, 76)	74 (72, 77)	74 (71, 76)	0.009
Race, white (%)	77.4	73.7	77.0	0.62
Education <12 years (%)	77.0	68.1	61.0	0.001
Uses alcohol (%)	25.8	23.0	26.0	0.74
Smoking (%)				0.55
Never	47.5	54.0	46.4	
Former	39.8	35.0	41.4	
Current	12.7	11.5	12.3	
BMI (kg/m ²)	26.1 (22.1, 30.6)	26.6 (23.3, 30.1)	28.1 (24.6, 31.0)	0.002
Physical activity (%)				<0.0001
Low	41.9	54.7	64.2	
Medium	27.2	15.6	15.6	
High	30.9	29.8	20.3	
MMSE score <24 (%)	5.9	7.4	7.3	0.79
Cholesterol (mg/dl)				
Total	228 (202, 256)	230 (207, 257)	226 (203, 254)	0.61
HDL	55 (45, 66)	54 (44, 66)	51 (43, 63)	0.16
LDL (mg/dl)	140 (118, 163)	141 (117, 166)	138 (116, 164)	0.98
Triglycerides (mg/dl)	132 (89, 207)	141 (98, 199)	140 (96, 203)	0.67
SBP (mmHg)	145.0 (131.5, 163.0)	144.0 (130.0, 159.0)	145.5 (130.0, 160.0)	0.75
DBP (mmHg)	70.5 (63.0, 80.0)	70.0 (61.0, 80.0)	69.0 (62.0, 79.0)	0.49
Estimated GFR (ml/min per 1.73 m ²)	62.9 (51.7, 74.3)	61.9 (51.7, 70.3)	57.6 (51.1, 65.8)	0.05
Hypertension (%)	44.3	51.2	61.4	0.001
Coronary artery disease (%)	12.2	13.4	21.3	0.02
Congestive heart failure (%)	5.0	4.6	8.6	0.15
Peripheral artery disease (%)	8.1	3.8	17.2	0.02
Stroke (%)	2.7	2.3	4.5	0.37
Cardiovascular disease (%) ^a	22.6	24.9	36.7	0.002
Diabetes mellitus (%)	13.1	9.7	16.3	0.12
COPD (%)	26.7	28.1	24.4	0.68
Chronic kidney disease (%)	49.1	49.3	56.9	0.20
Depression (%)	18.8	19.8	14.4	0.51
Cancer (%)	9.1	9.7	10.9	0.81

HDL, high-density lipoprotein; LDL, low-density lipoprotein; GFR, glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; COPD, chronic obstructive pulmonary disease.

^aCoronary heart disease, congestive heart failure, peripheral artery disease, and stroke.

disease was highest in the highest tertile and lowest in the lowest tertile of serum FGF23 ($P=0.002$).

Of the 659 women, 185 (28.1%) had cardiovascular disease. The demographic and health characteristics of women according to the diagnosis of cardiovascular disease are shown in Table 2. Serum FGF23 was significantly higher in adults with than those without cardiovascular disease ($P=0.0001$). Participants who had cardiovascular disease were more likely to be white, current smokers, less active, with cognitive impairment, and with hypertension; coronary artery disease, congestive heart failure, peripheral artery disease, stroke, and diabetes mellitus. There were no significant differences between participants who had cardiovascular disease by age, education level, alcohol use, body mass index, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, estimated glomerular filtration rate, chronic obstructive pulmonary disease, chronic kidney disease, depression, and cancer.

The relationship between serum FGF23 and cardiovascular disease was examined using separate

multivariable logistic regression models for all participants (Table 3). Higher serum FGF23 was associated with increased odds of cardiovascular disease after adjusting for age, race, smoking, education, body mass index, cognition, diabetes mellitus, hypertension, and physical activity ($P<0.0001$). In another model with additional adjustment for total cholesterol, HDL cholesterol, and renal function, the association between serum FGF23 and cardiovascular disease was nearly unchanged ($P<0.0001$).

The relationship between serum FGF23 and cardiovascular disease was examined in additional separate multivariable logistic regression models that were stratified by the presence or absence of chronic kidney disease at baseline. In women without chronic kidney disease, higher serum FGF23 was associated with increased odds of cardiovascular disease in multivariable models after adjusting for age, race, smoking, education, body mass index, cognition, diabetes mellitus, hypertension, physical activity, total cholesterol, and HDL cholesterol (Table 3). The magnitude of the

Table 2 Characteristics of 659 women, aged 70–79 years, in the WHAS, with or without CVD. Numbers in parentheses indicate 25th, 75th percentile.

Characteristics	With CVD (n=185)		Without CVD (n=474)		P
	n	% or median	n	% or median	
Age (years)	185	74 (71, 77)	474	74 (72, 76)	0.44
Race, white (%)	126	68.1	375	79.1	0.003
Education < 12 years (%)	118	63.8	333	70.6	0.09
Uses alcohol (%)	38	20.5	126	26.6	0.10
Smoking (%)					0.007
Never	73	39.5	251	53.1	
Former	84	45.4	170	36.0	
Current	28	15.1	52	11.0	
BMI (kg/m ²)	171	27.6 (23.6, 31.4)	458	26.8 (23.1, 30.5)	0.20
Physical activity					0.0002
Low	114	65.1	229	49.1	
Medium	33	18.9	92	19.7	
High	28	16.0	145	31.1	
MMSE score < 24 (%)	22	11.9	23	4.9	0.001
Cholesterol (mg/dl)					
Total	169	228 (199, 256)	448	229 (206, 254)	0.39
HDL	172	52.0 (43.0, 62.0)	451	54.0 (44.0, 66.0)	0.17
LDL	169	138 (115, 162)	448	139 (118, 166)	0.54
Triglycerides (mg/dl)	169	141 (100, 202)	448	137 (92, 203)	0.68
Log serum FGF23 (pg/ml)	185	3.7 (3.3, 4.0)	474	3.5 (3.2, 3.8)	0.0001
SBP (mmHg)	185	143.0 (129.0, 160.0)	474	146.0 (131.0, 160.0)	0.25
DBP (mmHg)	185	67.0 (60.0, 74.0)	474	72.0 (64.0, 81.0)	<0.0001
Estimated GFR (ml/min per 1.73 m ²)	169	57.7 (47.2, 69.9)	448	58.3 (51.7, 70.4)	0.18
Hypertension (%)	119	64.3	225	47.6	0.0001
Diabetes mellitus (%)	45	24.3	41	8.7	<0.0001
COPD (%)	53	28.7	121	25.5	0.41
Chronic kidney disease (%)	93	55.0	226	50.5	0.31
Depression (%)	24	18.3	27	16.1	0.61
Cancer (%)	18	9.7	47	9.9	0.94

HDL, high-density lipoprotein; LDL, low-density lipoprotein; GFR, glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; COPD, chronic obstructive pulmonary disease.

odds ratio (OR) was higher in the final model for women with chronic kidney disease compared with those without chronic kidney disease (OR 1.44 vs 1.10 respectively).

Alternative multivariable models were used to examine the relationship between tertiles of serum FGF23 and cardiovascular disease. Women in the highest and middle tertiles of FGF23, respectively, had a high odds of cardiovascular disease compared with those in the lowest tertile after adjusting for age, race, smoking, education, body mass index, cognition, diabetes mellitus, and hypertension (OR 1.57, 95% confidence interval (CI) 1.38, 1.78; OR 0.88, 95% CI 0.77, 1.01), additionally for physical activity (OR 1.52, 95% CI 1.33, 1.73; OR 0.89, 95% CI 0.78, 1.02), and finally with addition of total cholesterol and HDL cholesterol to the model (OR 1.49, 95% CI 1.30, 1.70; OR 0.91, 95% CI 0.79, 1.05).

Discussion

This study demonstrates that older community-dwelling women, aged 70–79 years, with high serum FGF23 concentrations have higher odds of cardiovascular

disease. To our knowledge, this is the first study to show that high serum FGF23 concentrations are independently associated with cardiovascular disease in the general population. This finding is consistent with previous reports that have shown increased levels of FGF23 are an independent risk factor for cardiovascular disease and adverse cardiovascular events (11–13). Currently, there are two types of assays for measuring circulating FGF23. In this study, we measured intact FGF23, in contrast to assays that measure the inactive C-terminal fragment. Many studies have shown a high correlation between the two assays (23).

The potential role of FGF23 in the pathogenesis of cardiovascular disease may be partly explained through its involvement in the complex process of vascular calcification. Vascular calcification involves complex interplay between calcification stimulators and inhibitors, key players being hyperphosphatemia and increased levels of parathyroid hormone and vitamin D (24). Increased phosphate levels, parathyroid hormone, and 1,25(OH)₂D₃ are all independently associated with cardiovascular-related morbidity and mortality (25–27). Higher levels of FGF23 are also associated with the development of coronary artery

Table 3 Multivariate logistic regression models of the relationship between serum FGF23 and cardiovascular disease in 659 women, aged 70–79 years, in the WHAS, for all participants and stratified by chronic kidney disease at baseline^a.

	Model 1 ^b			Model 2 ^c			Model 3 ^d		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
All participants	1.26	1.20, 1.33	<0.0001	1.23	1.16, 1.30	<0.0001	1.24	1.18, 1.31	<0.0001
Chronic kidney disease ^e									
Absent	1.14	1.05, 1.24	0.001	1.10	1.01, 1.19	0.04	1.10	1.01, 1.20	0.03
Present	1.41	1.30, 1.53	<0.0001	1.42	1.31, 1.55	<0.0001	1.44	1.32, 1.57	<0.0001

^aOdds ratio (OR) expressed per 1 s.d. of log serum FGF23 (1 s.d. = 0.52 pg/ml).^bModel 1 – adjusted for age, race, smoking, education, body mass index, MMSE, diabetes, and hypertension.^cModel 2 – adjusted for age, race, smoking, education, body mass index, MMSE, diabetes, hypertension, and physical activity.^dModel 3 – adjusted for age, race, smoking, education, body mass index, cognition, diabetes, hypertension, physical activity, total cholesterol, HDL cholesterol, and renal function.^eFor analyses stratified by chronic kidney disease, Model 3 includes all covariates above except renal function.

calcification, especially in the presence of chronic kidney disease (28). Since FGF23 is a known regulator of all three factors, it is plausible that FGF23 could be involved in the mechanism of vascular calcification. FGF23 has also been implicated in the development of left ventricular hypertrophy in older community-dwelling adults (15) and in patients with chronic kidney disease (16), which may help explain the association between elevated FGF23 and prevalent cardiovascular disease.

The observed role of FGF23 in vascular calcification may be explained by the role of *klotho* as an important co-factor for FGF23, without which FGF23 bioactivity declines (29). Inactivating mutations in the *klotho* gene in mice show premature aging phenotypes including hyperphosphatemia, extensive vascular calcification, atherosclerosis, and increased levels of vitamin D, which are also shared by FGF23-null mice (4, 30). In humans, previous studies have shown that decreased plasma α -*klotho* concentrations are associated with vascular calcification and independently predict longevity and mortality (31, 32). Consequently, excess FGF23 levels with declining *klotho* levels may be a result of unresolved hyperphosphatemia and abnormal vitamin D metabolism, which are also important factors in vascular calcification (23). Coronary artery calcification is a strong predictor of cardiac events and all-cause mortality in healthy older adults (33, 34). Thus, FGF23 may be an important biomarker for the development and progression of vascular calcification and the pathogenesis of cardiovascular disease.

There are several limitations of the study including the use of the MDRD Study equation, which has not yet been validated in adults > 70 years of age. The study was conducted in only community-dwelling women, 70 to 79 years old, and the present findings cannot therefore be generalized to older men. Residual confounding by other factors such as serum phosphate and vitamin D levels could be a potential limitation. Further prospective studies are needed in other populations to determine if elevated FGF23 levels are predictive of incident cardiovascular disease

and to further elucidate the potential role of FGF23 in the pathogenesis of cardiovascular disease. A recent nested case-control study showed that in the Health Professionals Follow-Up Study, a prospective cohort involving male health professionals, aged 40–75 years, with normal renal function, plasma FGF23 was not an independent predictor of incident coronary heart disease (35). This cohort involved men with normal kidney function without cardiovascular disease at baseline. It may be possible that FGF23 is associated with severity but not the incidence of cardiovascular disease or that elevated FGF23 is primarily a risk factor for cardiovascular disease in adults with impaired renal function. In addition, the 10-year follow-up interval between baseline and incident cardiovascular disease may have attenuated the risk magnitude associated with a single measure of FGF23 (35).

In conclusion, this study demonstrates that FGF23 is associated with prevalent cardiovascular disease in older, community-dwelling women. Further studies are needed to elucidate the potential biological mechanisms by which FGF23 may be involved in the pathogenesis of cardiovascular disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

We confirm that all of the named authors contributed substantially to the article and agreed to take public responsibility for the validity of its content.

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