

10. Isakova T, Gutierrez OM, Chang Y *et al.* Phosphorus binders and survival on hemodialysis. *J Am Soc Nephrol* 2009; 20: 388–396
11. Benner D, Nissenson AR, Van Wyck D. Focused clinical campaign improves mineral and bone disorder outcomes. *J Ren Care* 2012; 38: 2–8
12. Port FK, Pisoni RL, Bommer J *et al.* Improving outcomes for dialysis patients in the International Dialysis Outcomes and Practice Patterns Study. *Clin J Am Soc Nephrol* 2006; 1: 246–255
13. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease–mineral and bone disorder (CKD–MBD). *Kidney Int Suppl* 2009; 113: S1–S130
14. Toussaint ND, Pedagogos E, Beavis J *et al.* Improving CKD–MBD management in haemodialysis patients: barrier analysis for implementing better practice. *Nephrol Dial Transplant* 2011; 26: 1319–1326
15. Weed-Collins M, Hogan R. Knowledge and health beliefs regarding phosphate-binding medication in predicting compliance. *ANNA J* 1989; 16: 278–282, 285, discussion 286
16. Milas NC, Nowalk MP, Akpele L *et al.* Factors associated with adherence to the dietary protein intervention in the Modification of Diet in Renal Disease Study. *J Am Diet Assoc* 1995; 95: 1295–1300
17. Cianciaruso B, Capuano A, D'Amaro E *et al.* Dietary compliance to a low protein and phosphate diet in patients with chronic renal failure. *Kidney Int Suppl* 1989; 27: S173–S176
18. Bame SI, Petersen N, Wray NP. Variation in hemodialysis patient compliance according to demographic characteristics. *Soc Sci Med* 1993; 37: 1035–1043
19. Morduchowicz G, Sulkes J, Aizic S *et al.* Compliance in hemodialysis patients: a multivariate regression analysis. *Nephron* 1993; 64: 365–368
20. Arenas MD, Malek T, Alvarez-Ude F *et al.* Phosphorus binders: preferences of patients on haemodialysis and its impact on treatment compliance and phosphorus control. *Nefrologia* 2010; 30: 522–530
21. Arenas MD, Malek T, Gil MT *et al.* Challenge of phosphorus control in hemodialysis patients: a problem of adherence?. *J Nephrol* 2010; 23: 525–534
22. Chiu YW, Teitelbaum I, Misra M *et al.* Pill burden, adherence, hyperphosphatemia, and quality of life in maintenance dialysis patients. *Clin J Am Soc Nephrol* 2009; 4: 1089–1096
23. Peterson AM, Nau DP, Cramer JA *et al.* A checklist for medication compliance and persistence studies using retrospective databases. *Value Health* 2007; 10: 3–12
24. Leslie R. Calculating Medication Compliance, Adherence and Persistence in Administrative Pharmacy Claims Databases. In Proceedings of the Western Users of SAS Software Meeting. 5–7 November 2008, Universal City, CA, USA.
25. Mehrotra R, Martin KJ, Fishbane S *et al.* Higher strength lanthanum carbonate provides serum phosphorus control with a low tablet burden and is preferred by patients and physicians: a multicenter study. *Clin J Am Soc Nephrol* 2008; 3: 1437–1445
26. Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare and Medicaid programs; Conditions for coverage for ESRD facilities, Final Rule. *Fed Regist* 2008; 73: 20370–20484
27. Lindberg M, Lindberg P. Overcoming obstacles for adherence to phosphate binding medication in dialysis patients: a qualitative study. *Pharm World Sci* 2008; 30: 571–576
28. Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. *Clin Ther* 2001; 23: 1296–1310

Received for publication: 20.12.2012; Accepted in revised form: 11.5.2013

Nephrol Dial Transplant (2014) 29: 2099–2105

doi: 10.1093/ndt/gfu101

Advance Access publication 29 April 2014

Association of fibroblast growth factor-23 with arterial stiffness in the Multi-Ethnic Study of Atherosclerosis

Jeffrey J. Hsu¹, Ronit Katz³, Joachim H. Ix², Ian H. de Boer⁴, Bryan Kestenbaum⁴ and Michael G. Shlipak¹

¹Division of General Internal Medicine, San Francisco VA Medical Center, University of California, San Francisco, CA, USA, ²Division of Nephrology, Department of Medicine, University of California, San Diego, La Jolla, CA, USA, ³Department of Biostatistics, University of Washington, Seattle, WA, USA and ⁴Division of Nephrology, Department of Medicine, University of Washington, Seattle, WA, USA

Correspondence and offprint requests to: Michael G. Shlipak; E-mail: michael.shlipak@ucsf.edu

ABSTRACT

Background. Serum fibroblast growth factor-23 (FGF-23) is associated with cardiovascular disease (CVD), yet the mechanisms remain uncertain. Our objective was to determine whether higher FGF-23 concentrations are associated with arterial stiffness.

Methods. In this cross-sectional study, serum FGF-23 concentrations were measured in 5977 participants without known CVD in the Multi-Ethnic Study of Atherosclerosis. The primary outcomes of interest were large (LAE) and small artery elasticity (SAE), pulse pressure and ankle-brachial index (ABI) > 1.30. LAE and SAE were measured by pulse contour analysis of the radial artery. Pulse pressure was measured with an automated sphygmomanometer using the average of two

resting blood pressure measurements. ABI was calculated as the ratio of the ankle and brachial systolic blood pressures.

Results. Serum FGF-23 concentrations were not significantly associated with LAE [relative difference (RD) per doubling: 0%; 95% confidence interval (CI): -2 – 1 %], SAE (RD per doubling: 0%; 95% CI: -3 – 2 %), pulse pressure (β per doubling: 0.44; 95% CI: -0.31 – 1.19), or a high ABI (odds ratio per doubling: 1.14; 95% CI: 0.84–1.55). Findings were similar irrespective of chronic kidney disease status.

Conclusions. Higher serum FGF-23 concentrations are not associated with arterial stiffness, as measured by pulse pressure, LAE, SAE or high ABI, in a community-based population without CVD.

Keywords: ABI, arterial elasticity, arterial stiffness, FGF-23, pulse pressure

INTRODUCTION

Fibroblast growth factor-23 (FGF-23) is a circulating osteocyte-derived hormone that plays a key role in regulating serum phosphorous metabolism. FGF-23 responds to increased serum phosphorous concentrations by promoting phosphorous excretion and by inhibiting 1- α -hydroxylase in the renal proximal tubule [1, 2]. Markedly elevated serum concentrations of FGF-23 are often seen in patients with advanced chronic kidney disease (CKD) [3], likely as a compensatory response to the hyperphosphatemia prevalent in this population.

Previous studies have shown an association of FGF-23 concentrations with left ventricular mass and left ventricular hypertrophy, even after adjustment for kidney function, and this finding appears to be causal in animal studies [4, 5]. Additionally, multiple studies have demonstrated an association of FGF-23 with mortality in patients with end-stage renal disease, advanced CKD [6, 7] and coronary artery disease [8]. A recent study demonstrated that FGF-23 is independently associated with mortality and incident heart failure in a community-based elderly population, and this association is most pronounced in patients with CKD [9].

While these findings linking higher FGF-23 with poor cardiovascular outcomes are very strong, particularly in patients with CKD, the mechanisms remain unclear. One possible contributor is direct cardiotoxicity of FGF-23, as high FGF-23 has been shown, experimentally, to promote left ventricular hypertrophy [5]. Nonetheless, the role of FGF-23 in ventricular remodeling has not been definitively delineated, as another group found that neutralizing antibodies to FGF-23 did not have any effect on cardiac hypertrophy in rats with CKD [10]. Aberrant calcium and phosphorous homeostasis has consistently been linked with arterial calcification and stiffness. Because FGF-23 is integral in regulating phosphorous and vitamin D metabolism, it may also induce arterial calcification and stiffness. However, there is considerable controversy because some [11, 12], but not all, prior studies have observed associations of FGF-23 with arterial calcification [13]. Moreover, FGF-23 typically requires its co-receptor, Klotho, to bind to target tissues and exert its biological effects. Most studies

show no expression of Klotho in human vascular cells [13, 14], although one recent study has suggested that its expression may be induced by vitamin D receptor activators in human aortic smooth muscle cells [15].

In addition, FGF-23 concentrations are strongly related to kidney function, and arterial stiffness has strong associations with estimated glomerular filtration rate (eGFR) in both cross-sectional and longitudinal studies. Multiple community-based studies have demonstrated associations between measures of increased arterial stiffness [such as lower large artery elasticity (LAE) and small artery elasticity (SAE) [16–18], higher pulse pressure [16, 19] and high ankle-brachial index (ABI) [20–23]] with increased cardiovascular morbidity and mortality, as well as incident hypertension, coronary heart disease, stroke, heart failure and kidney function decline.

The objective of this study was to determine whether there is an independent association between FGF-23 and arterial stiffness, as measured by LAE, SAE, pulse pressure and high ABI.

MATERIALS AND METHODS

Participants

The study was a cross-sectional analysis from the baseline examination of the Multi-Ethnic Study of Atherosclerosis (MESA), conducted in 2000–2002 [24]. Briefly, MESA was designed to understand subclinical cardiovascular disease (CVD) and its progression in a multi-ethnic cohort. The study recruited 6814 men and women aged 45–84 years who were free of clinically apparent CVD from six cities in the USA: Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; Northern Manhattan and the Bronx, NY; and St. Paul, MN. Participants identified themselves as white, black, Hispanic or Chinese.

We measured FGF-23 in stored baseline fasting serum samples for all MESA participants with available samples ($n = 6552$; 96.2%). For these analyses, we excluded persons who lacked measurements of serum ($n = 20$) or urine phosphorous ($n = 52$), serum ($n = 5$) or urine creatinine ($n = 3$), small and large artery elasticities ($n = 448$), ABI ($n = 45$) or systolic and diastolic blood pressures ($n = 2$). After exclusions, the total sample size was 5977 persons.

Primary predictor variable

FGF-23. Serum intact FGF-23 was measured using a commercially available immunoassay that measures the full-length peptide by recognizing both mid-molecule and distal epitopes (Kainos Laboratories, Japan), with a lower limit of detection of 3 pg/mL. Notably, a recent study showed poor agreement between the commercially available assays and found the Kainos assay to have the most reproducible performance characteristics [25]. Standardized high- and low-value FGF-23 controls were used within each run to monitor quality control. The coefficient of variation for high and low control samples were 6.7 and 12.4%, respectively. Serum and urine phosphorous were measured using the timed-rate colorimetry reaction on a Beckman Coulter DxC automated analyzer. Urine

samples were acidified prior to measurement to reduce calcium-phosphorous precipitation.

Primary outcome variables

SAE and LAE. The methods for measuring LAE and SAE were as previously described [16, 17]. Radial artery pulse waveforms were measured with the HDI PulseWave CR-2000 Research Cardiovascular Profiling Instrument (Hypertension Diagnostics, Inc., Eagan, Minnesota). With the patient supine, the pulse pressure sensor was placed on the supported wrist of the dominant arm, and a blood pressure cuff was placed on the contralateral arm over the brachial artery. Measurements were taken for 30 s.

Arterial capacitive (C1) and oscillatory (C2) indices were obtained using a third-order, six-element Windkessel model that was derived from the diastolic pulse contour, as previously described [17]. The pressure at the radial artery at time (t) at the beginning of diastole was modeled as a decaying exponential function plus a sinusoidal function dampened by a decaying exponential. Systemic vascular resistance (SVR) was calculated as mean arterial pressure divided by cardiac output (CO), and CO was estimated using heart rate, ejection time and body surface area. Estimates combining information from the waveform and SVR variables were used to determine C1 and C2. The terms LAE and SAE were used to describe C1 and C2, respectively, as recommended by Hypertension Diagnostics, Inc. and as done in previous studies [16, 17]. One previous MESA study described the reproducibility of these elasticity measures and found the between-measure correlations for LAE and SAE to be 0.74 and 0.84, respectively [26].

Pulse pressure. Seated, resting blood pressure was measured three times in each participant using an automated oscillometric sphygmomanometer, and the average of the last two measurements were used in the analyses. Pulse pressure was calculated as the difference between the systolic and diastolic blood pressures.

ABI. ABI was measured as previously described [27]. Briefly, the systolic blood pressure (SBP) measurements in the bilateral brachial, dorsalis pedis and posterior tibial arteries were obtained in the supine position using a hand-held Doppler instrument with a 5-mHz probe. The higher of the brachial artery pressures was used as the denominator. For each lower extremity, the ABI numerator used was the highest pressure (dorsalis pedis or posterior tibial) from that leg. The leg cuff was inflated to a maximum of 300 mmHg, and if a pulse was still detected at this level, the ABI was classified as 'incompressible.' For each patient, the higher of the two leg-specific ABI measurements was used. ABI was categorized as low (<0.9), normal (0.9–1.3) or high (>1.3 or 'incompressible'). High ABI was considered to be a marker of increased arterial stiffness, as it has been strongly linked to medial arterial calcification [28] and has been associated with mortality [20]. Low ABI was considered to be a marker of atherosclerosis.

Other measurements. Age, gender, race/ethnicity, medical history and smoking history were obtained using standardized

questionnaires. Smoking history was categorized as ever versus never. Diabetes was defined as a fasting glucose of ≥ 126 mg/dL or use of hypoglycemic medications. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Total and high-density lipoprotein (HDL) cholesterol and triglyceride concentrations were measured from venous samples using standard methods. Urine albumin and creatinine were measured in a single morning urine sample by nephelometry and the rate Jaffé equation, respectively, and eGFR was calculated from the serum creatinine and cystatin C concentrations using the Chronic Kidney Disease Epidemiology Collaboration combined creatinine-cystatin C equation [29].

Statistical methods. Serum FGF-23 concentrations were log-transformed and analyzed as a continuous linear variable and also categorized by quintiles. Baseline characteristics of the MESA participants were compared by FGF-23 quintile, and differences in these characteristics across quintiles were evaluated using ANOVA or the Kruskal–Wallis test for continuous variables, and the χ^2 test for categorical variables, as appropriate. In addition, because the range of FGF-23 concentrations is lower in the general population than in persons with CKD, the highest decile of FGF-23 concentrations was also evaluated as a potential cut point.

The associations of FGF-23 concentrations and the ABI categories were evaluated using multinomial logistic regression, with the intermediate ABI category serving as the reference group. LAE, SAE and pulse pressure were analyzed as continuous variables using linear regression. In these models, LAE and SAE were log-transformed due to their skewed distributions. To improve interpretation of FGF-23's associations with LAE and SAE, the β coefficients were back-transformed to represent a relative difference (RD) in LAE or SAE. For example, an RD of -5% for LAE signifies that a higher increment of FGF-23 is associated with 5% lower LAE.

The initial models were adjusted for demographics (age, gender, race and clinic site), and the fully adjusted models were adjusted for demographics, diabetes (DM), smoking status, BMI, SBP, total cholesterol, HDL cholesterol, eGFR, serum calcium, intact parathyroid hormone (PTH), serum phosphorus, 25-hydroxy (25-OH) vitamin D, antihypertensive medication use and statin use. However, SBP was left out of the fully adjusted models for the pulse pressure and ABI analyses, as SBP is used in the calculations for both outcomes. In addition, all models were repeated after stratification by CKD status, and interaction testing between FGF-23 and CKD status was performed.

All statistical analyses were conducted with S-Plus (version 8.0, Tibco, Seattle, WA, USA) and SPSS statistical software (version 15.0.1.1, SPSS, Inc., Chicago, IL, USA).

RESULTS

The mean age of the 5977 study participants was 62 ± 10 years, and 53% were female. Thirty-eight percent were white, 13% were Chinese, 27% were black and 22% were Hispanic. The mean serum FGF-23 concentration was 40 ± 18 pg/mL

Table 1. Characteristics of study participants by quintiles of FGF-23: the MESA

Range	FGF-23 quintiles (pg/mL)					P-value
	1 <29	2 29–34	3 35–40	4 41–49	5 >49	
N	1201	1182	1203	1208	1183	
Demographics						
Age (y)	60 (10)	62 (10)	62 (10)	62 (10)	64 (10)	<0.001
Male	494 (41%)	549 (46%)	597 (50%)	616 (51%)	580 (49%)	<0.001
Race/ethnicity						
White	378 (32%)	433 (37%)	447 (37%)	506 (42%)	530 (45%)	<0.001
Chinese	140 (12%)	152 (13%)	169 (14%)	137 (11%)	154 (13%)	
Black	355 (30%)	305 (26%)	332 (28%)	297 (25%)	299 (25%)	
Hispanic	328 (27%)	292 (25%)	255 (21%)	268 (22%)	200 (17%)	
Medical history						
Hypertension	477 (40%)	475 (40%)	501 (42%)	551 (46%)	625 (53%)	<0.001
Antihypertensive meds	366 (30%)	372 (32%)	403 (34%)	466 (39%)	571 (48%)	<0.001
Diabetes	145 (12%)	158 (13%)	134 (11%)	127 (11%)	179 (15%)	0.281
Smoking status						
Never	589 (49%)	603 (51%)	612 (51%)	601 (50%)	643 (54%)	<0.001
Former	400 (33%)	421 (36%)	435 (36%)	462 (38%)	440 (37%)	
Current	207 (17%)	157 (13%)	155 (13%)	140 (12%)	98 (8%)	
Statin use	149 (12%)	167 (14%)	159 (13%)	191 (16%)	209 (18%)	0.002
Measurements						
BMI (kg/m ²)	27.7 (5.4)	27.8 (5.2)	27.9 (5.3)	28.8 (5.3)	28.9 (5.7)	<0.001
SBP (mmHg)	125 (22)	125 (21)	125 (21)	127 (21)	128 (22)	<0.001
Total cholesterol (mg/dL)	193 (34)	194 (35)	193 (34)	195 (36)	195 (37)	0.101
HDL cholesterol (mg/dL)	54 (15)	51 (14)	51 (14)	49 (14)	50 (14)	<0.001
Triglycerides (mg/dL)	105 [73, 152]	107 [77, 158]	107 [74, 156]	118 [84, 171]	122 [84, 177]	<0.001
Serum calcium (mg/dL)	9.61 (0.38)	9.62 (0.37)	9.63 (0.38)	9.68 (0.39)	9.71 (0.44)	<0.001
eGFR (mL/min/1.73 m ²)	90 (15)	87 (16)	85 (16)	82 (16)	76 (19)	<0.001
25-OH vitamin D (ng/mL)	23 (11)	25 (11)	25 (11)	25 (11)	28 (12)	<0.001
Intact PTH (pg/mL)	40 [31, 53]	40 [31, 51]	41 [31, 53]	42 [32, 54]	41 [32, 55]	0.064
Serum phosphorous (mg/dL)	3.61 (0.49)	3.65 (0.51)	3.64 (0.50)	3.68 (0.51)	3.75 (0.57)	<0.001
eGFR < 60 mL/min/1.73 m ²	39 (3%)	48 (4%)	72 (6%)	113 (9%)	218 (19%)	<0.001
ACR ≥ 30 mg/g	86 (7%)	86 (7%)	102 (9%)	104 (9%)	163 (14%)	<0.001

Data are *n* (%) for categorical variables, mean (SD) or median [range] for continuous variables, as appropriate. BMI, body mass index; SBP, systolic BP; DBP, diastolic BP; ACR, urine albumin-to-creatinine ratio; eGFR, estimated GFR.

(median, 38 pg/mL; interquartile range, 30–46 pg/mL); mean eGFR was 84 ± 17 mL/min per 1.73 m², and 490 participants (8%) had eGFR < 60 mL/min per 1.73 m². Compared with participants with lower FGF-23 concentrations, those in the highest quintile were older, more likely to be white, to have hypertension, diabetes and to use statins (Table 1). Other notable differences for the highest FGF-23 quintile were higher SBP, triglycerides, 25-OH vitamin D, serum phosphorous concentrations, urine albumin-to-creatinine ratio (ACR) and lower eGFR.

In both the continuous and categorical analyses, no significant association was observed between FGF-23 concentrations and either LAE or SAE (Table 2). Threshold analyses, comparing participants with FGF-23 > 57 pg/mL (top decile) to the rest of the cohort, also revealed no significant association of FGF-23 with either LAE or SAE. The fully adjusted analyses were repeated without adjusting for SBP, and the results were generally similar (data not shown).

In the demographically adjusted continuous models for pulse pressure, there was a significant association between higher FGF-23 concentrations and larger pulse pressure, but this association was substantially attenuated in the fully

adjusted model. In the categorical models, the highest quintile of FGF-23 had a significantly higher pulse pressure compared with the lowest quintile (referent group) in the demographically adjusted analysis, but this association was also substantially attenuated in the fully adjusted model. The highest decile of FGF-23 was associated with a large pulse pressure difference, and while this association was attenuated in the fully adjusted model, it remained statistically significant.

In the analyses of the association of FGF-23 with ABI, no significant association was apparent between FGF-23 and either high or low ABI in any of the models (Table 3).

In subgroup analyses of MESA participants with CKD (*n* = 905), defined by either eGFR < 60 mL/min/1.73 m² or ACR > 30 mg/g, the findings were generally similar (Supplementary data Table S1 and S2). The median values [with interquartile ranges] for eGFR, ACR and FGF-23 in this group were 54 [47, 57] mL/min/1.73 m², 9.0 [4.3, 31.3] mg/g and 47.2 [38.1, 58.6] pg/mL, respectively. Additionally, no significant interaction was observed between FGF-23 and CKD status (P interactions all >0.05; Table 4).

Table 2. Association of fibroblast growth factor-23 (FGF-23) with large artery elasticity (LAE), small artery elasticity (SAE) and pulse pressure by linear regression analyses in the MESA cohort

FGF-23	LAE (log)		SAE (log)		Pulse pressure (mmHg)	
	Demographically adjusted ^a	Fully adjusted ^b	Demographically adjusted	Fully adjusted	Demographically adjusted	Fully adjusted
	RD ^c , % change (95% CI)	RD, % change (95% CI)	RD, % change (95% CI)	RD, % change (95% CI)	β (95% CI)	β (95% CI)
Continuous models						
Per doubling	0 (−2, 1)	0 (−2, 1)	1 (−2, 4)	0 (−3, 2)	1.35 (0.62, 2.08)*	0.44 (−0.32, 1.19)
Multicategory models						
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0 (−2, 3)	0 (−3, 3)	0 (−4, 5)	0 (−5, 3)	−0.36 (−1.53, 0.81)	−0.47 (−1.62, 0.67)
Quintile 3	2 (−1, 5)	2 (−1, 4)	3 (−1, 7)	1 (−3, 5)	−0.14 (−1.31, 1.02)	−0.33 (−1.48, 0.82)
Quintile 4	2 (−1, 5)	1 (−1, 4)	2 (−2, 6)	0 (−4, 4)	0.92 (−0.24, 2.09)	0.18 (−0.99, 1.34)
Quintile 5	0 (−3, 3)	0 (−3, 3)	3 (−1, 7)	1 (−4, 5)	1.74 (0.56, 2.91)**	0.44 (−0.78, 1.65)
Threshold models						
FGF-23 ≤ 57 pg/mL	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
FGF-23 > 57 pg/mL	−3 (−6, 0)	−1 (−5, 2)	−2 (−7, 2)	−3 (−7, 1)	2.60 (1.37, 3.83)*	1.35 (0.10, 2.61)**

^aAdjusted for demographics (age, gender, race and clinic site).

^bAdjusted for demographics, DM, smoking (ever), BMI, SBP (only for LAE and SAE), total cholesterol, HDL, eGFR, serum calcium, intact PTH, serum phosphorus, statin and antihypertensive medication use, and 25-OH vitamin D.

^cRelative difference in LAE or SAE per increment in FGF-23 concentration from a linear regression with ln(LAE) or ln(SAE) as the dependent variable, respectively. For example, an RD of −3% represents a 3% decrease compared with the referent group.

*P < 0.001,

**P < 0.05

Table 3. Association of FGF-23 with high and low ankle-brachial index (ABI) using multinomial logistic regression in the MESA cohort

FGF-23	High ABI (>1.3), n = 208		Low ABI (<0.9), n = 202	
	Demographically adjusted ^a	Fully adjusted ^b	Demographically adjusted	Fully adjusted
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Continuous models				
Per doubling	1.21 (0.91, 1.60)	1.13 (0.83, 1.54)	1.21 (0.92, 1.61)	1.04 (0.76, 1.42)
Multicategory models				
Quintile 1	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.03 (0.65, 1.63)	1.00 (0.61, 1.63)	1.16 (0.71, 1.88)	1.09 (0.65, 1.79)
Quintile 3	1.00 (0.63, 1.58)	1.09 (0.68, 1.74)	1.03 (0.63, 1.67)	1.08 (0.65, 1.81)
Quintile 4	1.11 (0.71, 1.74)	0.97 (0.60, 1.56)	1.07 (0.66, 1.73)	1.01 (0.61, 1.68)
Quintile 5	1.09 (0.69, 1.72)	1.08 (0.68, 1.73)	1.35 (0.85, 2.13)	1.15 (0.69, 1.90)
Threshold models				
FGF-23 ≤ 57 pg/mL	Ref.	Ref.	Ref.	Ref.
FGF-23 > 57 pg/mL	1.27 (0.82, 1.97)	1.13 (0.72, 1.79)	1.15 (0.74, 1.78)	0.85 (0.53, 1.38)

No values in this table achieved statistical significance (P < 0.05).

^aAdjusted for demographics (age, gender, race and clinic site).

^bAdjusted for demographics, DM, smoking (ever), BMI, total cholesterol, HDL, eGFR, serum calcium, intact PTH, serum phosphorus, statin and antihypertensive medication use, and 25-OH vitamin D.

Table 4. Interaction testing between FGF-23 and CKD status

Outcome	P-value for interaction
LAE	0.712
SAE	0.253
Pulse pressure	0.935
ABI < 0.9	0.256
ABI > 1.3	0.997

FGF-23 was analyzed as a continuous variable. CKD was defined as eGFR < 60 mL/min/1.73 m² or ACR > 30 mg/g.

DISCUSSION

In this study, we explored whether higher FGF-23 concentrations were associated with markers of arterial stiffness. We chose a cohort without prevalent CVD to understand the potential associations with incident CVD risk and minimize reverse causality.

Smaller prior studies have evaluated the association of FGF-23 with arterial stiffness measures. In a cohort of 142

patients with CKD, Desjardins *et al.* found no association between FGF-23 and pulse wave velocity (PWV) [12]. Similarly, in a study of 200 patients with CKD stage III–IV, Ford *et al.* also found no association between FGF-23 and PWV in their fully adjusted model [30]. Though different measures of arterial stiffness were used, and though we evaluated a community-living cohort predominantly without CKD, the results of these studies are consistent with those of the present study. In contrast, Mirza *et al.* analyzed a community-based cohort of 967 persons aged 70 and found that higher serum FGF-23 concentrations were associated with arterial stiffness, as measured by pulse wave analysis, and this association was stronger in patients with impaired renal function (eGFR < 60 mL/min/1.73 m²) [31]. Notably, this study analyzed an older population from a single community in Sweden and used a measure of arterial stiffness (reflection index) not used in our study, potentially explaining the difference in our findings. Given the larger size and demographic diversity of MESA and the multiple measures of arterial stiffness used in our study, our analysis strongly suggests that there is minimal, if any, association between FGF-23 and arterial stiffness in the general adult population in the USA.

In our fully adjusted threshold model, the highest decile of FGF-23 was associated with larger pulse pressure. Although this finding was statistically significant, the strength of the association was relatively weak. Given the null findings when we evaluated continuous and categorical models and the large sample size of this study, we conclude that there is not a robust association between FGF-23 and pulse pressure.

In our subgroup analysis of participants with CKD, we did not find associations between FGF-23 concentrations and any of the measures of arterial stiffness, although power was lower for this analysis ($n = 905$). This finding is interesting given that the association of FGF-23 concentrations with all-cause mortality and incident heart failure is stronger in patients with CKD [9, 32]. Thus, while the presence of CKD has been found to augment the risk of CVD outcomes in patients with higher FGF-23 concentrations, we found that it did not appear to modify the association of FGF-23 with arterial stiffness.

A recent experimental study found that protein levels of Klotho, the obligate co-receptor for FGF-23 in kidney and parathyroid tissues, were undetectable in arteries harvested from mice, and FGF-23 treatment did not affect arterial function in *ex vivo* studies of murine mesenteric arteries [14]. Similarly, Scialla *et al.* recently found no association between serum FGF-23 concentrations and either coronary artery or thoracic aorta calcification in patients with mild-to-moderate CKD [13]. The same study also demonstrated that *in vitro* exposure of human vascular smooth muscle cells to FGF-23 did not have any effect on phosphate-induced calcification, and these cells do not express mRNA for FGF-23 or Klotho. It should be noted, however, that FGF-23 may have indirect effects on the vessel wall, as its effects on the myocardium were found to be Klotho-independent [5]. Further, a recent study found that higher FGF-23 concentrations were associated with an increased risk of major cardiovascular events in an older community-based population, and this association was attenuated, yet still significant, after adjusting for measures

of arterial stiffness [33]. These findings, combined with the results of our current study, suggest that the association of FGF-23 with risk of CVD outcomes is unlikely to be mediated through a pathway inducing arterial stiffness.

The strengths of our study include its large sample size, its inclusion of multiple different ethnicities and its evaluation of multiple measures of arterial stiffness. The study also has important limitations. The first is the lack of PWV data in the MESA cohort, which is one of the more widely used measures of arterial stiffness. However, the measures used in our study are also widely regarded as surrogate measures of arterial stiffness. Prior studies in MESA and other studies have demonstrated that these measures are strongly associated with incident CVD and kidney outcomes [16–23]. Additionally, our study focused on persons without CVD and has a limited representation of persons with CKD. We still found a wide range of FGF-23 concentrations in our cohort, so we should have been able to detect meaningful associations in our analysis. Yet we acknowledge that this range is relatively low compared with values seen in CKD populations. Further, this study is cross-sectional, and as such, does not assess longitudinal outcomes. We do not exclude the possibility that baseline FGF-23 concentrations may influence changes in arterial stiffness over time.

In summary, higher FGF-23 concentrations are known to be strongly associated with poor cardiovascular outcomes, especially in the setting of advanced CKD. We provide insights that this effect is unlikely to be mediated through increased arterial stiffness.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://ndt.oxfordjournals.org>.

ACKNOWLEDGEMENTS

The authors thank the other investigators, the staff and the participants of MESA for their valuable contributions. A full list of participating MESA investigators and institutions can be found at <http://www.mesa-nhlbi.org>. This research was supported by grants 5R01-AG-027002–06 from the National Institute on Aging (NIA) and R01-HL-096875 from the National Heart, Lung and Blood Institute, and the UCSF Department of Medicine.

CONFLICT OF INTEREST STATEMENT

Ix has received honoraria from Shire Pharmaceuticals and Keryx Biopharmaceuticals. All of the other authors have declared that they have no other relevant financial interest.

(See related article by Ketteler and Biggar. FGF23: more a matter of the heart than of the vessels? *Nephrol Dial Transplant* 2014; 29: 1987–1988.)

REFERENCES

- Gutierrez O, Isakova T, Rhee E *et al.* Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol* 2005; 16: 2205–2215
- Burnett SM, Gunawardene SC, Bringham FR *et al.* Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. *J Bone Miner Res* 2006; 21: 1187–1196
- Shigematsu T, Kazama JJ, Yamashita T *et al.* Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. *Am J Kidney Dis* 2004; 44: 250–256
- Mirza MA, Larsson A, Melhus H *et al.* Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. *Atherosclerosis* 2009; 207: 546–551
- Faul C, Amaral AP, Oskouei B *et al.* FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011; 121: 4393–4408
- Gutierrez OM, Mannstadt M, Isakova T *et al.* Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008; 359: 584–592
- Isakova T, Xie H, Yang W *et al.* Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011; 305: 2432–2439
- Parker BD, Schurgers LJ, Brandenburg VM *et al.* The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. *Ann Intern Med* 2010; 152: 640–648
- Ix JH, Katz R, Kestenbaum BR *et al.* Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: CHS (Cardiovascular Health Study). *J Am Coll Cardiol* 2012; 60: 200–207.
- Shalhoub V, Shatzen EM, Ward SC *et al.* FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J Clin Invest* 2012; 122: 2543–2553
- Nasrallah MM, El-Shehaby AR, Salem MM *et al.* Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. *Nephrol Dial Transplant* 2010; 25: 2679–2685
- Desjardins L, Liabeuf S, Renard C *et al.* FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int* 2012; 23: 2017–2025
- Scialla JJ, Lau WL, Reilly MP *et al.* Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney Int* 2013; 83: 1159–1168
- Lindberg K, Olauson H, Amin R *et al.* Arterial Klotho expression and FGF23 effects on vascular calcification and function. *PLoS One* 2013; 8: e60658
- Lim K, Lu TS, Molostvov G *et al.* Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* 2012; 125: 2243–2255
- Peralta CA, Jacobs DR, Jr, Katz R *et al.* Association of pulse pressure, arterial elasticity, and endothelial function with kidney function decline among adults with estimated GFR >60 mL/min/1.73 m²: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis* 2012; 59: 41–49
- Duprez DA, Jacobs DR, Jr, Lutsey PL *et al.* Association of small artery elasticity with incident cardiovascular disease in older adults: the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2011; 174: 528–536
- Peralta CA, Adeney KL, Shlipak MG *et al.* Structural and functional vascular alterations and incident hypertension in normotensive adults: the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2010; 171: 63–71
- Bangalore S, Messerli FH, Franklin SS *et al.* Pulse pressure and risk of cardiovascular outcomes in patients with hypertension and coronary artery disease: an International Verapamil SR-trandolapril Study (INVEST) analysis. *Eur Heart J* 2009; 30: 1395–1401
- O'Hare AM, Katz R, Shlipak MG *et al.* Mortality and cardiovascular risk across the ankle-arm index spectrum: results from the Cardiovascular Health Study. *Circulation* 2006; 113: 388–393
- Resnick HE, Lindsay RS, McDermott MM *et al.* Relationship of high and low ankle brachial index to all-cause and cardiovascular disease mortality: the Strong Heart Study. *Circulation* 2004; 109: 733–739
- Criqui MH, McClelland RL, McDermott MM *et al.* The ankle-brachial index and incident cardiovascular events in the MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2010; 56: 1506–1512
- Ix JH, Katz R, Peralta CA *et al.* A high ankle brachial index is associated with greater left ventricular mass MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2010; 55: 342–349
- Bild DE, Bluemke DA, Burke GL *et al.* Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol* 2002; 156: 871–881
- Smith ER, McMahon LP, Holt SG. Method-specific differences in plasma fibroblast growth factor 23 measurement using four commercial ELISAs. *Clin Chem Lab Med* 2013; 51: 1971–1981
- Brumback LC, Jacobs DR Jr, Dermond N *et al.* Reproducibility of arterial elasticity parameters derived from radial artery diastolic pulse contour analysis: the multi-ethnic study of atherosclerosis. *Blood Press Monit* 2010; 15: 312–315
- Ix JH, De Boer IH, Peralta CA *et al.* Serum phosphorus concentrations and arterial stiffness among individuals with normal kidney function to moderate kidney disease in MESA. *Clin J Am Soc Nephrol* 2009; 4: 609–615
- Ix JH, Miller RG, Criqui MH *et al.* Test characteristics of the ankle-brachial index and ankle-brachial difference for medial arterial calcification on X-ray in type 1 diabetes. *J Vasc Surg* 2012; 56: 721–727
- Inker LA, Schmid CH, Tighiouart H *et al.* Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; 367: 20–29
- Ford ML, Smith ER, Tomlinson LA *et al.* FGF-23 and osteoprotegerin are independently associated with myocardial damage in chronic kidney disease stages 3 and 4. Another link between chronic kidney disease-mineral bone disorder and the heart. *Nephrol Dial Transplant* 2012; 27: 727–733
- Mirza MA, Larsson A, Lind L *et al.* Circulating fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis* 2009; 205: 385–390
- Arnlov J, Carlsson AC, Sundstrom J *et al.* Higher fibroblast growth factor-23 increases the risk of all-cause and cardiovascular mortality in the community. *Kidney Int* 2013; 83: 160–166
- Arnlov J, Carlsson AC, Sundstrom J *et al.* Serum FGF23 and risk of cardiovascular events in relation to mineral metabolism and cardiovascular pathology. *Clin J Am Soc Nephrol* 2013; 8: 781–786

Received for publication: 3.1.2014; Accepted in revised form: 30.3.2014