Plasma FGF23 and Calcified Atherosclerotic Plaque in African Americans with Type 2 Diabetes Mellitus

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Key Words

African American · Albuminuria · Calcified atherosclerotic plaque · FGF23 · Kidney disease · Type 2 diabetes mellitus

Abstract

Background: Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone implicated in disorders of serum phosphorus concentration and vitamin D. The role of FGF23 in vascular calcification remains controversial. **Methods:** Relationships between FGF23 and coronary artery calcified atherosclerotic plaque (CAC), aortoiliac calcified plaque (CP), carotid artery CP, volumetric bone mineral density (vBMD), albuminuria, and estimated glomerular filtration rate (eGFR) were determined in 545 African Americans with type 2 diabetes (T2D) and preserved kidney function in African American-Diabetes Heart Study participants. Generalized linear models were fitted to test associations between FGF23

and cardiovascular, bone, and renal phenotypes, and change in measurements over time, adjusting for age, gender, African ancestry proportion, body mass index, diabetes duration, hemoglobin A1c, blood pressure, renin-angiotensin-system inhibitors, statins, calcium supplements, serum calcium, and serum phosphate. **Results:** The sample was 56.7% female with a mean (SD) age of 55.6 (9.6) years, diabetes duration of 10.3 (8.2) years, eGFR 90.9 (22.1) ml/min/1.73 m², urine albumin:creatinine ratio (UACR) 151 (588) (median 13) mg/g, plasma FGF23 161 (157) RU/ml, and CAC 637 (1,179) mg. In fully adjusted models, FGF23 was negatively associated with eGFR ($p < 0.0001$) and positively associated with UACR ($p < 0.0001$) and CAC ($p = 0.0006$), but not with carotid CP or aortic CP. Baseline FGF23 concentration did not associate with changes in vBMD or CAC after a mean of 5.1 years follow-up. **Conclusions:** Plasma FGF23 concentrations were independently associated with subclinical coronary artery disease, albuminuria, and kidney function in the understud-

ied African American population with T2D. Findings support relationships between FGF23 and vascular calcification, but not between FGF23 and bone mineral density, in African Americans lacking advanced nephropathy.

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Introduction

Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone produced primarily by osteocytes and osteoblasts and implicated in hypo- and hyper-phosphatemia, vitamin D metabolism, and ectopic mineralization [1]. Reduced FGF23 activity may lead to hyperphosphatemia, likely accounting for the association with ectopic mineralization [2, 3]. FGF23 inhibits renal tubular reabsorption of phosphate, and reduces circulating 1,25 dihydroxyvitamin D through the inhibition of formation through Cyp27b1 and by stimulation of degradation through the actions of Cyp24 [4]. Expression of FGF is induced by serum calcium and phosphate, 1,25 dihydroxyvitamin D, metabolic acidosis, leptin, as well as parathyroid hormone (PTH) [2, 5]. Increased FGF23 levels are early manifestations of chronic kidney disease-mineral bone disorder (CKD-MBD) in patients with estimated glomerular filtration rates (eGFR) below 90 ml/min/1.73 m² [6–8]. FGF23 levels appear to be relevant in populations with type 2 diabetes mellitus (T2D), including those with preserved kidney function [9].

CKD-MBD increases the propensity to develop subclinical cardiovascular disease (CVD) manifesting as calcified atherosclerotic plaque (CP), with enhanced risk for myocardial infarction, stroke and CVD-related death [10, 11]; reducing FGF23 may lower rates of CVD events and death [12]. Hyperphosphatemia, circulating Wingless/Integration 1 (Wnt) inhibitor family member Dickkopf-1, sclerostin, FGF23, and Klotho all play roles in CKD-MBD [13–17]. Circulating FGF23 levels are associated with mortality and left ventricular hypertrophy in these patients [18–26].

The role of FGF23 in vascular calcification remains controversial. Although reports in patients with CKD-MBD demonstrated an association between FGF23 and coronary artery CP (CAC) and thoracic or abdominal aorta CP [27–32], FGF23 levels were not associated with prevalence or severity of CAC in the Chronic Renal Insufficiency Cohort (CRIC) [33]. Another report in Turkish patients failed to detect the association between FGF23 with CAC or aorta CP in patients with CKD [34], and the Multi-Ethnic Study of Atherosclerosis (MESA) did not

detect relationships between FGF23 and arterial stiffness measured by pulse pressure, small artery elasticity, or ankle-brachial indices [35]. Relationships between bone and vascular health differ markedly between populations with recent African vs. European descent [36] and there is a paucity of data on associations between plasma FGF23 and vascular calcification in African Americans. To address this concern, African American-Diabetes Heart Study (AA-DHS) participants with T2D and preserved kidney function were assessed for associations between FGF23 and CP in the coronary arteries, carotid arteries, and aorta, vertebral volumetric bone mineral density (vBMD), and kidney disease phenotypes. The study contained both cross-sectional (baseline relationships) and longitudinal components (baseline measures of FGF23 vs. change in CP, kidney phenotypes, and vBMD).

Materials and Methods

Study Participants

Self-reported and unrelated African Americans with T2D were recruited from medicine clinics and community advertising in the AA-DHS [37, 38]. Participant examinations were conducted in the Clinical Research Unit of the Wake Forest School of Medicine (WFSM), including interviews for medical history, anthropometric measures, blood pressure, fasting blood sampling, and urine collection for urine albumin:creatinine ratio (UACR). The CKD-EPI equation was used to compute eGFR [39]. T2D was clinically defined in participants diagnosed after age 30 years and receiving blood sugar lowering medication in the absence of diabetic ketoacidosis or insulin-only treatment since diagnosis. Exclusion criteria included prior serum creatinine concentrations ≥ 2 mg/dl or physician-diagnosed kidney disease. Hypertension was based on physician diagnosis, use of anti-hypertensive medications, or a study visit blood pressure $>140/90$ mm Hg. The study was approved by the WFSM Institutional Review Board and participants provided written informed consent. Fifty-two percent ($n = 282$) of 545 participants returned in the AA-DHS longitudinal follow-up assessment, where full examinations were repeated. Baseline recruitment was performed from September 5, 2007 to August 30, 2010; follow-up recruitment was performed from August 30, 2012 to June 10, 2014.

Plasma Vitamin D and Intact PTH Assays

The vitamin D assays were developed and performance characteristics determined by Quest Diagnostics Nichols Institute (San Juan Capistrano, Calif., USA). The 25-hydroxyvitamin D assay was performed using liquid chromatography tandem mass spectroscopy, a radio-receptor assay was used for 1,25 dihydroxyvitamin D as described [40]. iPTH was measured at LabCorp using an electrochemiluminescence immunoassay [40].

Plasma FGF23 Assay

FGF23 (intact and C-terminal) was measured in ethylene diamine tetraacetic acid plasma samples stored continuously at -80°C since collection at baseline visits. Frozen plasma was thawed

in a 37°C water bath for 15 min, placed on ice, and then centrifuged at 1,700 g (2,800 rpm) for 30 min at 4°C. FGF23 was determined using a second generation Human FGF-23 (C-terminal) ELISA Kit (Cat. #60-6100; Immutopics, Inc., San Clemente, Calif., USA) according to manufacturer's instructions. Intra- and inter-assay coefficients of variation were <6% at 32 and <2.5% at 279 RU/ml. Assays were performed using a single lot of kits over a 10 day period.

CT Imaging of Arterial CP

CAC and CP in the carotid arteries and abdominal aortoiliac bed were determined using 4 or 16 channel multidetector CT (LightSpeed Qxi and 16 Pro, GE Healthcare, Waukesha, Wis., USA). Techniques for AA-DHS coronary, carotid and aorta scans have been described in detail (online suppl. methods; for all online suppl. material, see www.karger.com/doi/10.1159/000443241) [37, 38, 41].

Bone Imaging

Single and multidetector CT systems incorporating a standardized scanning protocol based on the National Heart Lung and Blood Institute's MESA were performed as reported (online suppl. methods) [42].

Statistical Analyses

Generalized linear models were fitted to test for cross-sectional associations between plasma FGF23 concentration and eGFR, UACR, thoracic vertebral and lumbar vertebral vBMD, CAC, carotid artery CP, and aortoiliac CP, and between FGF23 and change in these measurements. The Box-Cox method was applied to identify the appropriate transformation best approximating the distributional assumptions of conditional normality and homogeneity of variance of the residuals [43]. This suggested taking the natural logarithm (log) of (CAC + 1), (carotid artery CP + 1), (aortoiliac CP + 1), (UACR + 1) and the square root of lumbar and thoracic vBMD. FGF23, the main predictor in these association analyses, was also modeled on the log scale. For UACR and eGFR, an initial unadjusted analysis was performed, followed by a model adjusting for age, gender, African ancestry proportion, body mass index (BMI), T2D duration, hemoglobin A1c (HbA1c), systolic blood pressure (SBP), and angiotensin converting enzyme inhibitor/angiotensin receptor blocker (ACEi/ARB) medications. For UACR, the fully adjusted model also included eGFR. For CP, an initial unadjusted model was followed by a full-model, adjusting for age, gender, African ancestry proportion, BMI, T2D duration, HbA1c, SBP, smoking, statins, calcium supplements, and eGFR. For vBMD, an unadjusted model was followed by a full-model adjusting for age, gender, African ancestry proportion, BMI, T2D duration, HbA1c, SBP, smoking, use of hormone replacement therapy, steroids, bisphosphonates, calcium supplements, and eGFR. Additional final adjustments were made for serum phosphate, calcium, and the serum calcium × phosphate ion product. Prior to modeling the data, the CAC, carotid artery CP, and aortoiliac CP values were winsorized at their observed mean ± 2 SD. To assess change in the 282 participants who completed a second visit, models were constructed initially with time between readings as a covariate, then a second model adding the level of the predictor observed at the first visit was fitted. The final model adjusted for all the covariates included in the fully adjusted model described earlier. All participants who had undergone coronary artery angio-

plasty, stent placement, or bypass grafting were excluded from the baseline and longitudinal analyses for CAC (but were included in analyses of aorta and carotid artery CP). A total of 235 participants with 2 measures of CAC and without prior coronary artery interventions were longitudinally evaluated. SAS software was used.

Results

The study sample consisted of 545 African Americans with T2D (236 male, 309 female), with a mean (SD) age of 55.6 (9.6) years, diabetes duration of 10.3 (8.2) years, BMI 35.3 (8.6) kg/m²; 82.9% had hypertension and 51.4% were receiving lipid lowering medications. Table 1 contains demographic information; laboratory and imaging results are displayed in table 2. At the first visit, participants had mean (SD) HbA1c concentration of 8.1 (2.0%), and generally preserved kidney function based on eGFR 90.9 (22.1) ml/min/1.73 m² and UACR 151 (588) mg/g (median UACR was 13 mg/g). Plasma FGF23 concentrations differed by gender 125 (120) RU/ml in males and 188 (176) RU/ml in females ($p < 0.0001$). CP was detectable in the aorta, coronary arteries, and carotid arteries in 84.7, 74.5, and 60.6% of participants, respectively. Analyses for the effect of baseline FGF23 concentrations on change in CP, BMD, eGFR and UACR were performed in 282 participants who returned for a follow-up visit after mean (SD) median follow-up of 5.1 (0.9) years. Demographic, laboratory and imaging results at follow-up are displayed in the lower portions of tables 1 and 2. Of participants with longitudinal data, 34.7% (104) had a baseline UACR >30 mg/g and 5.8% (17) had a baseline UACR >300 mg/g. At follow-up, 37.2% (111/298) had a UACR >30 mg/g and 11.4% (34/298) a UACR >300 mg/g. In addition, an eGFR <60 ml/min/1.73 m² was present in 6.7% (20/294) at baseline and 16.0% (48/298) at follow-up. At baseline and follow-up, respectively, 5.3 and 32.8% of participants were taking over the counter (OTC) vitamin D. At baseline, 13% were taking OTC calcium supplements, 7.9% steroids (oral glucocorticoids and/or inhaled steroids), and 0.4% bisphosphonates. At follow-up, 19.9% were taking calcium, 8.9% steroids, and 0% bisphosphonates. No participant reported having osteoporosis.

Cross-sectional analyses were performed to test for the association between the natural log of FGF23 concentration with eGFR and UACR at visit 1 (table 3). Adjusting for age, gender, African ancestry proportion, BMI, T2D duration, HbA1c, smoking, SBP, and ACEi/ARB, log (FGF23), and therefore, FGF23 exhibited the expected negative association with eGFR (estimate (β) -8.50, SE

Table 1. Demographic data in the AA-DHS study sample, baseline and 5.1 year follow-up

Baseline	Male (n = 236)	Female (n = 309)	All (n = 545)	p value
Age, years	56.0 (9.8)	55.4 (9.5)	55.6 (9.6)	0.46
Age at diabetes onset, years	45.5 (10.9)	45.2 (10.2)	45.3 (10.5)	0.70
Diabetes duration, years	10.5 (8.7)	10.2 (7.8)	10.3 (8.2)	0.71
BMI, kg/m ²	32.5 (7.4)	37.4 (8.9)	35.3 (8.6)	<0.0001
SBP, mm Hg	132.0 (18.1)	133.5 (19.9)	132.8 (19.2)	0.36
Diastolic blood pressure, mm Hg	78.8 (10.9)	76.5 (11.4)	77.5 (11.2)	0.017
Hypertension, %	84.8	80.5	82.9	0.19
Lipid meds, %	52.1	50.8	51.4	0.76
Smokers, %				
Current	28.0	19.7	23.3	0.0003
Past	40.3	31.4	35.2	
Insulin use, %	40.7	39.2	39.8	0.72
Hormone replacement therapy, %	NA	25.6	NA	NA
Follow-up (mean 5.1 years)	Male (n = 139)	Female (n = 143)	All (n = 282)	p value
Age, years	59.7 (9.3)	60.4 (8.5)	60.1 (8.9)	0.52
Age at diabetes onset, years	44.7 (9.3)	44.8 (9.6)	44.8 (9.5)	0.92
Diabetes duration, years	15.1 (8.1)	15.6 (6.6)	15.3 (7.4)	0.52
BMI, kg/m ²	32.6 (6.8)	37.2 (8.0)	34.9 (7.8)	<0.0001
SBP, mm Hg	129.7 (18.0)	132.6 (17.4)	131.2 (17.7)	0.17
Diastolic blood pressure, mm Hg	76.7 (11.5)	77.1 (11.0)	76.9 (11.2)	0.78
Hypertension, %	69.1	67.1	68.1	0.73
Lipid meds, %	51.8	54.5	53.2	0.64
Smokers, %				
Current	23.7	14.1	18.9	0.006
Past	40.3	31.7	35.9	
Hormone replacement therapy, %	NA	24.1	24.1	NA

1.30; $p < 0.0001$). With additional adjustment for eGFR, FGF23 was positively associated with UACR (β 0.49, SE 0.11; $p < 0.0001$). Adjusting for age, gender, African ancestry proportion, BMI, T2D duration, smoking, HbA1c, SBP, statins, calcium supplements, and eGFR, FGF23 was positively associated with CAC (β 0.50, SE 0.18; $p = 0.005$), but not carotid artery CP nor aortic CP. These associations were not significantly influenced by the inclusion of serum phosphate, calcium, or the (phosphate $\times$ calcium) ion product as additional covariates in the model (data not shown). The association between FGF23 and CAC was similar in the subset of participants who had CKD (eGFR < 60 ml/min/1.73 m² and/or UACR > 30 mg/g; β 0.5, SE 0.27; $p = 0.063$ full-model) and in those without CKD (β 0.45, SE 0.24; $p = 0.069$ full-model). No significant relationships were detected between lumbar or thoracic vBMD and FGF23 at visit 1 (table 3).

Online supplementary table S1 displays the relationships between the log (FGF23), serum calcium, serum phosphate, intact PTH and 1,25 dihydroxyvitamin D.

FGF23 concentration was positively correlated with intact PTH and serum phosphorus concentrations. Serum calcium was positively correlated with serum phosphate and 1,25 dihydroxyvitamin D. Serum phosphate was negatively correlated with intact PTH and 1,25 dihydroxyvitamin D. In addition, 1,25 dihydroxyvitamin D was positively correlated with intact PTH. These relationships are as expected in individuals lacking advanced nephropathy and support findings in other ethnic groups. Serum phosphate was also associated with carotid artery CP in fully adjusted models, and trended toward association with aorta CP ($p = 0.05$ – 0.10), but not CAC ($p > 0.7$) or BMD ($p > 0.15$) (online suppl. table S2). These associations were not altered by adjustment for FGF23.

Longitudinal analyses were performed to assess relationships between FGF23 at the first visit with changes in eGFR, UACR, CAC, aorta CP, carotid artery CP, and vBMD over a mean of 5.1 years. First visit FGF23 levels were available in 282 returning AA-DHS participants, with mean (SD) values 143 (127) RU/ml; differences were

Table 2. Results of initial and 5.1 year follow-up lab and imaging results in AA-DHS, mean (SD)

Baseline: mean (SD) or median (P25, P75)	Male (n = 236)	Female (n = 309)	All (n = 545)	p value
Plasma FGF23, RU/ml	94 (66, 138)	125 (87, 209)	111 (79, 168)	<0.0001
Aorta CP mass, mg Ca ⁺	1,243 (85, 6,814)	817 (10, 5,810)	1,049 (28, 6,368)	0.53
Aorta CP mass = 0, %	35 (15.3)	68 (22.2)	103 (19.3)	0.044
Carotid artery CP mass, mg Ca ⁺	10 (0, 169)	2 (0, 85)	4 (0, 104)	0.023
Carotid artery CP mass = 0, %	91 (39.4)	146 (47.7)	237 (44.1)	0.055
Coronary artery CP mass*, mg Ca ⁺	55 (0, 702)	18 (0, 196)	27 (0, 348)	0.015
Coronary artery CP mass* = 0, %	51 (25.5)	72 (25.2)	123 (25.3)	>0.99
Lumbar vertebrae vBMD, mg/cm ³	178 (147, 211)	177 (143, 209)	177 (144, 210)	0.73
Thoracic vertebrae vBMD, mg/cm ³	196 (170, 233)	205 (169, 239)	202 (169, 235)	0.15
Serum creatinine, mg/dl	1.09 (0.28)	0.88 (0.26)	0.97 (0.28)	<0.0001
High sensitivity CRP, mg/dl	0.70 (1.06)	1.32 (2.13)	1.05 (1.77)	<0.0001
UACR, mg/g	130 (451)	168 (675)	151 (588)	0.43
eGFR, ml/min/1.73 m ²	92.0 (20.3)	90.1 (23.5)	90.2 (22.1)	0.31
Fasting glucose, mg/dl	158 (72)	145 (62)	150 (67)	0.028
HbA1c, %	8.24 (1.9)	8.02 (2.0)	8.10 (2.0)	0.20
Total cholesterol, mg/dl	177 (49)	186 (44)	182 (46)	0.023
HDL-cholesterol, mg/dl	44.6 (11.9)	50.2 (14.1)	47.8 (13.4)	<0.0001
LDL-cholesterol, mg/dl	105 (38)	111 (37)	108 (38)	0.06
Serum calcium, mg/dl	9.49 (0.44)	9.59 (0.44)	9.54 (0.44)	0.007
Serum phosphorus, mg/dl	3.43 (0.58)	3.68 (0.53)	3.57 (0.57)	<0.0001
Intact parathyroid hormone, pg/ml	49.9 (25.8)	58.4 (32.0)	54.7 (29.8)	0.001
Follow-up (mean 5.1 years)	Male (n = 139)	Female (n = 143)	All (n = 282)	p value
Aorta CP mass, mg Ca ⁺	2,922 (342, 10,026)	2,295 (408, 10,344)	2,599 (371, 10,026)	0.89
Aorta CP mass = 0, %	0	2 (1.4)	2 (0.7)	0.50
Carotid artery CP mass, mg Ca ⁺	39 (0, 304)	17 (0, 166)	26 (0, 243)	0.51
Carotid artery CP mass = 0, %	41 (29.9)	41 (28.7)	82 (29.3)	0.078
Coronary artery CP mass**, mg Ca ⁺	116 (16, 993)	74 (4, 468)	89 (9, 688)	0.44
Coronary artery CP mass** = 0, %	13 (11.4)	25 (19.7)	38 (15.8)	0.11
Lumbar vertebrae vBMD, mg/cm ³	163 (136, 191)	166 (133, 203)	163 (133, 194)	0.5
Thoracic vertebrae vBMD, mg/cm ³	174 (149, 203)	193 (158, 225)	184 (155, 218)	0.004
Serum creatinine, mg/dl	1.19 (0.44)	0.93 (0.34)	1.06 (0.42)	<0.0001
High sensitivity CRP, mg/dl	0.74 (0.98)	1.24 (1.66)	0.99 (1.39)	0.0021
UACR, mg/g	14.1 (3.7, 88.2)	11.4 (4.3, 61.5)	13.5 (4.2, 68.2)	0.87
eGFR, ml/min/1.73 m ²	84.5 (22.0)	83.9 (23.2)	83.7 (22.7)	0.87
Fasting glucose, mg/dl	153 (58)	148 (58)	151 (58)	0.51
HbA1c, %	8.04 (1.77)	8.19 (1.84)	8.1 (1.8)	0.48
Total cholesterol, mg/dl	176 (51)	189 (46)	182 (49)	0.02
HDL-cholesterol, mg/dl	47.6 (12.9)	55.7 (17.7)	51.7 (16.0)	<0.0001
LDL-cholesterol, mg/dl	103 (43)	109 (38)	106 (41)	0.21
Serum calcium, mg/dl	9.47 (0.44)	9.59 (0.46)	9.52 (0.45)	0.022
Serum phosphorus, mg/dl	3.48 (0.53)	3.62 (0.54)	3.55 (0.54)	0.032
Intact parathyroid hormone, pg/ml	49.4 (27.4)	53.5 (29.2)	51.5 (28.3)	0.22

* Coronary data at baseline based on 486 subjects without coronary stent or CABG; ** coronary data at follow-up based on 235 subjects without coronary stent or CABG at follow-up visit.

observed by gender (115 (100) RU/ml in 139 males; 171 (144) RU/ml in 143 females; $p = 0.0002$). As a significant proportion of the study sample lacked CP in a vascular bed at the initial visit, longitudinal analyses assessed quantitative changes in CP in each bed (data not shown)

or limited to participants with detectable CP in a given arterial site at visit 2 (table 4); results were comparable. Unadjusted and fully adjusted models failed to reveal relationships between baseline FGF23 and change in kidney function, CP in any vascular bed, or vBMD. Although

Table 3. Cross-sectional associations between plasma FGF23 and clinical variables

Outcome + sequentially-adjusted models	Estimate	SE	p value
<i>Estimated GFR</i>			
Unadjusted	-8.36	1.34	<0.0001
Age, sex, ancestry, BMI, T2D duration, smoking	-8.27	1.28	<0.0001
Above + HbA1c, SBP, ACEi/ARB medicines	-8.50	1.30	<0.0001
<i>log (UACR + 1)</i>			
Unadjusted	0.38	0.11	0.0007
Age, sex, ancestry, BMI, T2D duration, smoking	0.45	0.11	0.0001
Above + HbA1c, SBP, ACEi/ARB medicines	0.52	0.11	<0.0001
Above + eGFR	0.49	0.11	<0.0001
<i>log (aorta CP + 1)</i>			
Unadjusted	0.49	0.22	0.027
Age, sex, ancestry, BMI, T2D duration, smoking	0.24	0.18	0.17
Above + HbA1c, SBP, statin medicines	0.28	0.18	0.12
Above + calcium supplements, eGFR	0.20	0.19	0.29
<i>log (coronary artery CP + 1)</i>			
Unadjusted	0.27	0.16	0.089
Age, sex, ancestry, BMI, T2D duration, smoking	0.23	0.15	0.12
Above + HbA1c, SBP, statin medicines	0.25	0.15	0.091
Above + calcium supplements, eGFR	0.13	0.15	0.38
<i>log (CAC + 1)</i>			
Unadjusted	0.45	0.19	0.015
Age, sex, ancestry, BMI, T2D duration, smoking	0.42	0.17	0.015
Above + HbA1c, SBP, statin medicines	0.48	0.17	0.006
Above + calcium supplements, eGFR	0.50	0.18	0.005
<i>Square root (lumbar vertebral vBMD)</i>			
Unadjusted	-0.035	0.11	0.75
Age, sex, ancestry, BMI, T2D duration, smoking	0.011	0.10	0.91
Above + HbA1c, SBP, HRT, steroids, bisphosphonates, calcium supplements	0.035	0.10	0.73
Above + eGFR	0.019	0.11	0.86
<i>Square root (thoracic vertebral vBMD)</i>			
Unadjusted	-0.0009	0.11	0.99
Age, sex, ancestry, BMI, T2D duration, smoking	0.017	0.11	0.88
Above + HbA1c, SBP, HRT, steroids, bisphosphonates, calcium supplements	0.055	0.11	0.62
Above + eGFR	0.031	0.12	0.79

change in albuminuria had an association p value of 0.01 with baseline FGF23 in the fully adjusted model (table 4), this was not considered significant, accounting for multiple comparisons.

Discussion

This unique cohort consists of unrelated and extensively phenotyped African Americans with T2D lacking advanced nephropathy. The AA-DHS, with its partner study DHS in European Americans, were performed to better understand complex relationships between renal,

bone, and vascular health between populations of recent African and European descent [36]. The present analyses detected significant cross-sectional associations between plasma FGF23 concentration with eGFR and UACR, confirming that FGF23 is a powerful biomarker of incident kidney disease [44]. Significant positive cross-sectional associations were also detected between plasma FGF23 concentration and CAC in diabetic African Americans with mean (SD) eGFR 90.9 (22.1) ml/min/1.73 m², but not with carotid artery or aorta CP. This is consistent with increased atherosclerotic plaque calcification in diabetic subjects with preserved kidney function, but not with increased medial calcification stimulated by

Table 4. Longitudinal associations between initial plasma FGF23 and change in clinical variables

Outcome + sequentially adjusted models	p value	Estimate (SE)
<i>Δ in log aorta CP*</i>		
Adjusted for time between measures	0.48	−0.11 (0.16)
Above + log (aorta CP + 1 at visit 1)	0.08	0.15 (0.08)
Above + age, sex, % African ancestry, diabetes duration, statin use, smoking, and change in eGFR, BMI, HbA1c and SBP	0.35	0.09 (0.09)
<i>Δ in log carotid CP*</i>		
Adjusted for time between measures	0.21	0.14 (0.11)
Above + log (carotid CP + 1 at visit 1)	0.16	0.16 (0.11)
Above + age, sex, % African ancestry, diabetes duration, statin use, smoking, and change in eGFR, BMI, HbA1c and SBP	0.28	0.13 (0.12)
<i>Δ in log coronary artery CP*</i>		
Adjusted for time between measures	0.17	0.10 (0.07)
Above + log (coronary CP + 1 at visit 1)	0.24	0.08 (0.07)
Above + age, sex, % African ancestry, diabetes duration, statin use, smoking, and change in eGFR, BMI, HbA1c and SBP	0.27	0.10 (0.08)
<i>Δ square root lumbar vBMD</i>		
Adjusted for time between measures	0.82	−0.35 (1.60)
Above + eGFR at visit 1	0.62	−0.78 (1.60)
Above + age, sex, % African ancestry, diabetes duration, use of HRT, steroids, bisphosphonates, and calcium supplements, smoking, and change in eGFR, BMI, HbA1c and SBP	0.39	−1.44 (1.69)
<i>Δ square root thoracic vBMD</i>		
Adjusted for time between measures	0.78	0.62 (2.20)
Above + eGFR at visit 1	0.92	0.21 (2.20)
Above + age, sex, % African ancestry, diabetes duration, use of HRT, steroids, bisphosphonates, and calcium supplements, smoking, and change in eGFR, BMI, HbA1c and SBP	0.42	−1.80 (2.25)
<i>Δ in log UACR</i>		
Adjusted for time between measures	0.02	−0.12 (0.05)
Above + eGFR at visit 1	0.05	−0.10 (0.05)
Above + age, sex, % African ancestry, diabetes duration, statin use, ACE/ARB use, smoking, and change in eGFR, BMI, HbA1c and SBP	0.01	−0.15 (0.06)
<i>Δ in eGFR</i>		
Adjusted for time between measures	0.07	3.10 (1.70)
Above + eGFR at visit 1	0.81	0.42 (1.70)
Above + age, sex, % African ancestry, diabetes duration, statin use, ACE/ARB use, smoking, and change in UACR, BMI, HbA1c and SBP	0.23	−2.10 (1.70)
* Values were winsorized.		

CKD. Additional adjustment for serum phosphorus, calcium, or (calcium × phosphorus) concentrations in this population without advanced nephropathy did not impact findings. Although there has been controversy surrounding the relationship between FGF23 and subclinical atherosclerosis, our results support findings by Kestenbaum et al. [32] in population-based MESA participants, a sample not enriched for T2D.

After accounting for eGFR, an independent association was found to be present between albuminuria and

FGF23 in this African American cohort, replicating findings in Europeans [45]. After mean 5.1 year follow-up, changes in eGFR, albuminuria, BMD, or level of CP in major vascular beds were not associated with baseline FGF23 (p value 0.01 for change in albuminuria (full model) was not considered significant based upon multiple comparisons). In order to focus on atherosclerosis and bone health, AA-DHS excluded participants with severe nephropathy. As such, no participant required phosphate binders or active 1,25 di-hydroxyvitamin D therapy at the

first visit. The strongest associations with FGF23 in AA-DHS participants were with eGFR and UACR. This suggests that increased FGF23 associates with early kidney disease, in support of CRIC data that the CKD-MBD manifests earlier in patients with T2D [33] and data that mineral metabolites associate with nephropathy progression more strongly in African Americans than European Americans [46]. In general, AA-DHS participants appeared to have had good access to healthcare based on achieved blood pressures and high rates of receiving statins/antihypertensives.

Our results support that FGF23 associates with CAC in patients without advanced CKD, most existing reports of FGF23 associations with CAC are in non-African populations with CKD [27–31, 47]. The present results differ from those in CRIC and a report from Turkey [33, 34]. CRIC analyzed baseline FGF23 levels in 1,501 participants with advanced stage CKD (mean (SD) eGFR 47 (17) ml/min/1.73 m²), approximately 50% had diabetes [33]. Unlike AA-DHS, CRIC measures of CP were not strictly coincident with biomarker measures, performed an average of 376 days after baseline visits for plasma collection. Although 32% of CRIC participants were African American, statistical analyses adjusted for the effects of ethnicity but did not stratify. This adjustment may be insufficient to capture substantial ethnic differences in biologic behaviors and propensities to CP and kidney disease known to be present between African Americans and European Americans, based in part on genetic variation [36]. Analyses performed separately in each CRIC racial/ethnic group might be informative. Failure to detect FGF23 receptor expression in vascular smooth muscle cells (SMCs) also did not consider the ethnicity/genetic composition of human cell donors, and co-existing rodent studies may not generalize to different human populations, although this is a controversial area with recent studies suggesting FGF23 and FGFR expression in calcified coronary arteries of explants from heart transplant recipients [48]. Differences have been observed in relationships between FGF23 and CP based on gender [49]. Finally, higher serum phosphate concentrations and prescription of vitamin D analogues and calcium-containing phosphate binders to CRIC participants with advanced CKD may contribute to the development of CP and confound relationships between FGF23 and CP.

Although FGF23 levels are associated with CAC and aorta CP in several reports including this study, there is limited evidence demonstrating that FGF23 directly influences arterial wall biology. Expression of FGF receptors (R1 and R3) is detectable in vascular SMCs and in

human aorta [33, 50, 51], although FGF23 mRNA expression was not detectable in these tissues. There is immunohistochemical evidence for the presence of FGF23 protein in human coronary arteries and rodent aorta [52], although the source of this protein is unclear and could be derived from osteoblastic/osteocytic transition in coronary artery plaques or from deposition of circulating FGF23. Recent studies suggest that Klotho, the FGF23 co-receptor, is expressed at the mRNA level in human aorta, although Klotho does not appear to be expressed by human, mouse, or bovine aortic SMCs in culture [33, 50]. Likewise, cultured arterial SMCs do not appear to respond to treatment with exogenous FGF23 [33]. However, there is evidence that FGF23 can inhibit endothelium-dependent vasodilatory responses to acetylcholine in mouse aorta through reduced nitric oxide availability via increases in superoxide [53], although the mechanisms underlying such an effect are unclear. The potential for direct effects of FGF23 on arterial biology was also suggested by in vitro studies of recombinant FGF23 effects on aortic rings derived from uremic (but not healthy) rats, which led to increases in phosphate-induced calcium deposition [54]. Native aorta SMCs did not express Klotho and did not respond to FGF23 treatment, but SMCs overexpressing Klotho due to transfection with a Klotho expression vector responded to FGF23 with increases in ERK1/2 phosphorylation and transcripts for factors involved in osteoblastic differentiation.

Taken together, studies support that FGF23 is not likely to directly induce de novo vascular calcification. There are other potential explanations for association between circulating FGF23 and CAC in the AA-DHS, which do not require direct actions of FGF23 on vascular cells. FGF23 is a powerful biomarker of early CKD, and its correlation with CP may reflect the effects of incident kidney disease in this diabetic sample. Additional studies are required to verify these findings and define the exact mechanisms involved. Elevations in FGF23 may be due in part to bone cell responses to subclinical alterations in circulating Klotho, calcium, phosphate, and PTH, which may have their own effects on promotion of calcium accumulation and accretion in atherosclerotic plaques. Serum phosphate was associated with carotid artery CP and to a lesser extent aorta CP, but not CAC as shown in this study.

Strengths of this study include the focus on the understudied African American population, extensive phenotyping, and that 282 (52%) of participants returned for a second exam after an average of 5.1 years. Despite higher

CP and lower BMD over time, associations were not detected between baseline FGF23 concentrations and changes in CP or BMD. Limitations were the relatively small number of participants involved in the collection of data during the second visit; 300 participants were targeted for return visits based on grant support, and the lack of measures of urine phosphorus or fractional excretion of phosphorus [55, 56]. The small number of participants during the second visit may have reduced the power to detect relationships between FGF23 and changes in atherosclerosis or BMD.

This cross-sectional analysis in African Americans with T2D and preserved kidney function revealed that FGF23 concentrations were significantly and positively associated with albuminuria and CAC, while being negatively associated with eGFR. Results confirm and extend recent MESA findings in a non-T2D enriched population-based sample [32], clarifying the controversial relationships between FGF23 and CP in patients with diabetes who lack advanced nephropathy. In this report, significant associations were not observed between baseline FGF23 and progression of CP or fall in BMD in African Americans; FGF23 was positively associated with intact PTH and phosphate concentrations. In contrast to some studies in European Americans and African Americans

with advanced nephropathy, FGF23 concentrations were significantly associated with severity of CAC, perhaps as a biomarker of subclinical incident nephropathy, but absent advanced nephropathy.

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Disclosure Statement

The authors have nothing to disclose.

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