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ORIGINAL ARTICLE

Relationship of fibroblast growth factor 21 with kidney function and albuminuria: multi-ethnic study of atherosclerosis

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ABSTRACT

Background. Fibroblast growth factor 21 (FGF21) may play a role in the development of chronic kidney disease (CKD). We therefore investigated the relationship of plasma FGF21 levels with kidney function and albuminuria in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods. The analysis included 5724 MESA participants ages 45–84 years between 2000 and 2002, free of clinically apparent cardiovascular disease (CVD). Participants were followed up in person at four additional clinic visits over 10 years. Plasma FGF21 levels were measured at baseline examination by enzyme-linked immunosorbent assay. Kidney function was assessed by estimated glomerular filtration rate (eGFR). Outcomes were urinary albumin:creatinine ratio (UACR) progression, incident CKD by eGFR (reaching eGFR <60 mL/min/1.73 m^2 with eGFR loss rate ≥ 1 mL/min/1.73 m^2 per year) and rapid kidney function decline (eGFR decline $>5\%$/year).

Results. At baseline, higher FGF21 levels, assessed as both continuous and categorical quartile variables, were significantly associated with lower eGFR and higher UACR, after adjusting for demographic, socioeconomic and other confounding factors [adjusted mean differences of -2.63 mL/min/1.73 m^2 in eGFR and 0.134 in log normally transformed UACR (mg/g) for the highest FGF21 quartile compared with the lowest quartile, all $P < 0.001$]. However, in longitudinal analyses, baseline FGF21 levels did not predict incident CKD by eGFR, rapid kidney function decline or UACR progression. No significant interaction with sex and race/ethnicity was found (all $P > 0.05$).

Conclusions. Our study does not support a role of FGF21 as a biomarker for predicting kidney function decline or albuminuria in adults free of clinically apparent CVD at baseline.

Keywords: albuminuria, biomarkers, chronic kidney disease, fibroblast growth factor 21, glomerular filtration rate

INTRODUCTION

Chronic kidney disease (CKD) affects 8–16% of the adult population worldwide [1]. Recently, several new biomarkers with the potential to predict and detect the progression of kidney function decline have been identified. One of the most promising newly identified biomarkers is fibroblast growth factor 21 (FGF21) [2].

FGF21 is a member of the FGF family [3]. In animal studies, FGF21 has anti-inflammatory, antidiabetic and hypolipidemic effects [4–7]. However, its circulating levels are often elevated in different cardiometabolic disorders [8–12], probably due to the compensatory responses to the underlying metabolic stress or the presence of FGF21 resistance [13] as a result of impaired FGF21 signaling. A few studies have demonstrated elevated FGF21 levels in adults with non-dialysis-dependent CKD or atherosclerosis [2, 4, 14]. In addition, existing data suggest that FGF21 plays a role in the development or prediction of kidney function decline [2]. Although limited data have shown that increased FGF21 levels are associated with kidney function decline, this conclusion is limited by a small sample size with limited racial/ethnic diversity and a cross-sectional study design [2].

We previously reported that elevated FGF21 levels at baseline were associated with the presence of microvascular disease, especially nephropathy at baseline and a higher risk of new on-study total microvascular disease in patients with type 2 diabetes [14]. Elevated FGF21 levels were also reported to be predictive of estimated glomerular filtration rate (eGFR) decline in another longitudinal study of patients with type 2 diabetes [15]. Beyond this, information remains limited on how FGF21 levels are related to the progression of renal function in healthy people. For example, it is not known whether the relationship between FGF21 levels and kidney function decline differs by

gender and race/ethnicity. This is particularly important since African Americans have been reported to be at higher risk of CKD incidence and albuminuria [16]. Given this, we examined the relationship of plasma FGF21 levels with kidney function decline and albuminuria among participants from the Multi-Ethnic Study of Atherosclerosis (MESA), a cohort of four racial/ethnic groups without baseline cardiovascular disease (CVD).

MATERIALS AND METHODS

Study participants

The MESA is a multicenter, community-based cohort study on the prevalence, correlates and progression of subclinical CVD and the present study was a secondary analysis of data from the MESA. The MESA cohort consists of 6814 men and women ages 45–84 years, who were free of clinically apparent CVD at baseline. Participants from four major ethnic groups (Caucasian, African American, Hispanic American and Chinese American) were recruited from six communities in the USA. After the initial baseline examination in 2000–02, participants attended up to four additional clinic visits over a 10-year period. Details regarding the study objectives, design and protocol have been published [17]. The study was approved by the institutional review boards at all participating centers and informed written consent was obtained from all participants. The study was performed in compliance with the principles of the Declaration of Helsinki.

Among 6814 participants at baseline, data on plasma FGF21 levels were available on 5792 of them. After further excluding 68 participants with missing data on urinary albumin:creatinine ratio (UACR) and eGFR at baseline, 5724 participants were included in the analysis.

Plasma FGF21 measurement

Venous blood samples were collected after a 12-h fast by certified technicians using standardized venepuncture procedures. FGF21 levels were measured from stored plasma samples in singlicate obtained at the baseline examination by enzyme-linked immunosorbent assay kits from the Antibody and Immunoassay Services, University of Hong Kong, Hong Kong (www.antibody.hku.hk) as described previously [14, 18, 19]. These samples were stored at -80°C for $\sim$15 years with one freeze–thaw cycle only before FGF21 measurement. In a previous pilot study, serum FGF21 levels were demonstrated to be stable after one to six freeze–thaw cycles, with the coefficients of variation (CVs) being 8.1% [18]. Two in-house controls were run in duplicate in each assay. The intra-assay and interassay CVs were $<$10%.

eGFR and UACR measurement

We calculated eGFR from serum creatinine and cystatin C using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine–cystatin C equation [20]. Serum creatinine was measured via rate reflectance spectrophotometry and calibrated to the standardized creatinine measurements obtained at the Cleveland Clinic Research Laboratory (Cleveland, OH, USA). Cystatin C was measured via a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, IL, USA). Data on eGFR were available in Examinations 1, 3, 4 and 5.

Urinary creatinine was measured using a similar method to serum creatinine. Urinary albumin was measured using the Array 360 CE Protein Analyzer (Beckman Instruments, Brea, CA, USA). Data on UACR were available in Examinations 1, 2, 3 and 5.

Outcomes

Several outcomes were analyzed. In the cross-sectional analysis at baseline, reduced eGFR was defined as eGFR $<$60 mL/min/1.73 m^2. Different stages of albuminuria were classified using sex-specific UACR cut-points into normal, mildly increased, moderately increased and severely increased UACR as described previously [21]. Supplementary data, Table S1, shows the number of participants with different stages of albuminuria at each examination.

For longitudinal analyses, incident CKD by eGFR was defined as an eGFR $<$60 mL/min/1.73 m^2 with a minimum rate of eGFR loss of 1 mL/min/1.73 m^2/year among participants with eGFR $\geq$60 mL/min/1.73 m^2 at baseline [22, 23]. Incident rapid kidney function decline was defined as a decline in eGFR $>$5%/year as described in previous MESA reports [22, 23]. Supplementary data, Table S2, shows the number of participants with CKD by eGFR and rapid kidney function decline at each follow-up examination. For UACR progression, the following three categories were used: no UACR progression, definite UACR progression and intermediate UACR progression as described previously [21]. Participants with no UACR progression stayed in the same UACR category (normal or high normal) across all examinations or improved to a lower UACR category. Participants with definite UACR progression progressed to a higher UACR category at Examination 2 or 3 (e.g. from normal UACR at baseline to mildly, moderately or severely increased UACR at Examination 2 or 3 or from mildly increased UACR at baseline to moderately or severely increased UACR at Examination 2 or 3) and either stayed in the same category or progressed to a higher category at Examination 5. Participants who did not fit any of these categories were placed into the intermediate UACR progression category.

Other covariates of interest

Information on age, gender, race, education, smoking, alcohol use and physical activity was obtained from the questionnaires. Resting seated brachial artery blood pressure was measured three times and the results of the last two readings were averaged. Antihypertensive medications were classified into those targeting the renin–angiotensin–aldosterone system (RAAS) (including angiotensin-converting enzyme inhibitors and angiotensin II antagonists) and those not targeting the RAAS system (including beta-blockers, diuretics, calcium channel blockers and other vasodilators). Participants with fasting blood glucose $\geq$126 mg/dL or using glucose-lowering medications were classified as diabetic. Physical activity was measured as the total number of hours of moderate and vigorous activity

per week multiplied by the metabolic equivalent level [24]. Insulin resistance was measured using the homeostasis model assessment index of insulin resistance (HOMA2-IR) according to the updated computer model [25]. Interleukin-6, high-sensitivity C-reactive protein and fibrinogen were measured as described previously [26].

Statistical analysis

Data were presented as mean ± standard deviation (SD) or number (%). For variables with a skewed distribution, data were presented as median [interquartile range (IQR)] and were log transformed before analysis. Distributions of demographic data, cardiovascular risk factors and measures of renal function at baseline were compared across FGF21 quartiles among all the participants at baseline examination. Age, sex, body mass index (BMI), race/ethnicity and other variables that showed an increasing or decreasing trend with FGF21 levels after adjusting for age, sex and race/ethnicity ($P < 0.1$) were used as covariates in subsequent multiple regression analyses. In all analyses, data were adjusted for age, sex and race/ethnicity in Model 1. In Model 2, data were further adjusted for all other covariates at baseline. In Model 3, data were further adjusted for baseline eGFR and UACR, where appropriate.

The cross-sectional relationship of baseline FGF21 levels with continuous UACR and eGFR at baseline was assessed by multivariable linear regression. The regression coefficient (*B*) and 95% confidence interval (CI) were estimated. No multicollinearity was detected. In a separate analysis we assessed the relationship of the FGF21 quartile with reduced eGFR using multivariable logistic regression and with different stages of albuminuria using multinomial logistic regression. The odds ratio (OR) and 95% CI were estimated.

In longitudinal analysis, the associations of baseline FGF21 levels with incident CKD by eGFR and rapid kidney function decline over the follow-up period were assessed using Cox proportional hazards regression analysis. The hazard ratio (HR) and 95% CI were estimated. For each participant who developed incident CKD by eGFR or rapid kidney function decline, the time to events was considered as the time interval between the date of the visit at which the events were ascertained and the date of baseline Examination 1. For participants who remained event-free, the follow-up time was censored at the last examination. The time was left truncated to the Examination 3 date because no one could be observed to have an event before Examination 3. That is, follow-up time accrued from baseline, but the participants only entered the risk set at Examination 3. The proportional hazards assumption was checked using Schoenfeld residuals and no significant violation was found. For UACR progression over the follow-up period, analysis was performed among participants with valid data on UACR at Examinations 1–3 and 5 who did not have severely increased UACR at baseline. Multinomial logistic regression was then used to assess the relationship of baseline FGF21 levels with UACR progression. In a separate analysis we assessed the relationship of baseline FGF21 levels with longitudinal change in eGFR using a linear mixed-effects model among participants with eGFR data available at baseline and at least one follow-up examination. Similarly, we assessed the relationship of baseline FGF21 levels with longitudinal change in log normally (ln) transformed UACR using a linear mixed-effects model among participants with eGFR data available at baseline and at least one follow-up examination.

In all the above cross-sectional and longitudinal analyses, FGF21 levels were highly skewed and therefore ln-transformed in the regression models. This can prevent unstable estimates of effects since extreme values may have an undue influence on the estimate of the regression coefficient. To investigate the interaction with sex and race/ethnicity, the P-value for interaction was estimated by including each interaction term in the regression models in the full sample after adjusting for the main effects of the covariates. In a separate analysis, we also investigated the interaction with diabetes status at baseline, as previous studies suggested an association of FGF21 levels with kidney function and albuminuria in people with type 2 diabetes [14, 15]. In all the analyses, participants with missing data were excluded. A two-tailed P-value < 0.05 was considered statistically significant. SPSS 24 (IBM, Armonk, NY, USA) and STATA 14.0 (StataCorp, College Station, TX, USA) were used for statistical analysis.

RESULTS

Baseline characteristics

Table 1 shows baseline characteristics of all the 5724 participants according to FGF21 quartiles. Participants with higher FGF21 levels were more likely to be older, women, Caucasian or Hispanic American and have a poorer cardiorenal risk profile at baseline compared with those with lower FGF21 levels (all $P < 0.01$). Participants with higher FGF21 levels were also likely to have diabetes, hypertension and a lower high-density lipoprotein cholesterol level and use lipid-lowering medication at baseline (all $P < 0.001$).

Association of FGF21 levels with eGFR and UACR at baseline

As shown in Table 1, participants with higher FGF21 levels had lower eGFR and higher UACR at baseline (both $P < 0.001$). The association of FGF21 levels with eGFR and UACR remained significant even after adjusting for confounding factors ($P = 0.003$ and 0.011, respectively; Table 2). As shown in Supplementary data, Table S3, the relationship of FGF21 quartiles with reduced eGFR and albuminuria was assessed in a separate analysis in which a higher FGF21 quartile was associated with higher odds of reduced eGFR ($P < 0.001$), moderately increased UACR ($P = 0.016$) and severely increased UACR ($P = 0.021$). No significant interaction with sex and race/ethnicity was found in any of the above analyses ($P > 0.05$). A significant interaction with diabetes was found for eGFR, which was robust, as the association of both ln-transformed FGF21 levels and FGF21 quartiles was significantly stronger in those with diabetes than in those without diabetes (regression coefficient −2.00 versus −0.47, respectively, per SD increase in ln-transformed FGF21 levels, P for sex interaction = 0.009 and regression coefficients 0.40, 0.45 and −3.91 versus −0.14, −0.82 and −2.42 for Quartiles 2, 3 and 4, respectively, P for sex

Table 1. Baseline characteristics of participants (n = 5724)

Characteristics	*n*	FGF21 levels (pg/mL)				P-value
		Quartile 1 (≤81.1)	Quartile 2 (81.2–145.6)	Quartile 3 (145.6–245.3)	Quartile 4 (≥245.4)	
n	5724	1433	1430	1428	1433	
Age (years)	5724	60.3 ± 10.2	62.5 ± 10.0	63.6 ± 10.1	64.2 ± 10.2	<0.001
Women	5724	692 (48.3)	709 (49.6)	779 (54.6)	800 (55.8)	<0.001
Race/ethnicity	5724					<0.001
Caucasian		504 (35.2)	521 (36.4)	545 (38.2)	561 (39.1)	
African American		476 (33.2)	427 (29.9)	373 (26.1)	367 (25.6)	
Hispanic American		252 (17.6)	294 (20.6)	340 (23.8)	363 (25.3)	
Chinese American		201 (14.0)	188 (13.1)	170 (11.9)	142 (9.9)	
Education	5703					<0.001
<High school		210 (14.7)	244 (17.1)	292 (20.6)	302 (21.1)	
High school		544 (38.1)	595 (41.7)	610 (43.0)	636 (44.5)	
>High school		672 (47.1)	589 (41.2)	517 (36.4)	492 (34.4)	
BMI (kg/m^2)	5724	27.2 ± 5.0	27.8 ± 5.1	28.8 ± 5.6	29.3 ± 5.7	<0.001
Waist circumference (cm)	5724	94.3 ± 13.6	96.9 ± 13.6	99.9 ± 14.4	101.6 ± 14.4	<0.001
Hip circumference (cm)	5724	103.7 ± 10.4	104.5 ± 10.6	106.5 ± 12.1	107.3 ± 12.0	<0.001
Waist:hip ratio	5724	0.91 ± 0.08	0.93 ± 0.08	0.94 ± 0.08	0.95 ± 0.08	<0.001
Height (cm)	5724	167.4 ± 9.7	166.9 ± 10.0	165.8 ± 10.4	165.2 ± 10.0	0.63
Smoking	5704					<0.001
Never		760 (53.3)	722 (50.6)	710 (50.0)	686 (48.0)	
Former		510 (35.7)	540 (37.8)	530 (37.4)	528 (36.9)	
Current		157 (11.0)	166 (11.6)	179 (12.6)	216 (15.1)	
Pack-years of smoking	5644	8.8 ± 16.4	11.3 ± 20.9	11.6 ± 20.6	13.5 ± 23.7	<0.001
Current alcohol use	5679	781 (55.1)	817 (57.5)	767 (54.2)	756 (53.0)	0.77
Physical activity (MET-hours/week)	5706	103 ± 94	99 ± 102	88 ± 92	89 ± 98	0.10
Diabetes	5724	117 (8.2)	170 (11.9)	193 (13.5)	248 (17.3)	<0.001
Systolic blood pressure (mmHg)	5723	122.8 ± 20.3	126.8 ± 21.4	128.1 ± 21.5	130.6 ± 22.0	<0.001
Diastolic blood pressure (mmHg)	5723	71.8 ± 10.1	72.2 ± 10.1	71.7 ± 10.0	72.5 ± 10.9	<0.001
Anti-hypertensive medication	5712					
RAAS		210 (14.7)	258 (18.1)	250 (17.6)	333 (23.3)	<0.001
Non-RAAS		334 (23.4)	405 (28.4)	447 (31.4)	504 (35.2)	<0.001
Lipid-lowering medication	5712	202 (14.1)	242 (16.9)	233 (16.4)	290 (20.3)	<0.001
LDL cholesterol (mg/dL)	5650	117.0 ± 31.3	117.8 ± 31.7	117.4 ± 30.4	116.3 ± 32.7	0.19
HDL cholesterol (mg/dL)	5719	53.4 ± 15.4	51.6 ± 14.8	50.3 ± 14.7	48.4 ± 14.2	<0.001
Triglycerides (mg/dL)[a]	5722	89 (64–125)	106 (78–151)	120 (84–170)	136 (91–199)	<0.001
Fasting glucose (mg/dL)[a]	5724	88 (81–95)	90 (83–99)	91 (84–101)	92 (84–105)	<0.001
Fasting insulin (mU/L)[a]	5716	7.15 (5.28–10.06	7.77 (5.65–11.39)	8.52 (6.15–12.39)	9.77 (6.65–14.01)	<0.001
HOMA2-IR[a]	5712	0.80 (0.59–1.14)	0.88 (0.64–1.30)	0.96 (0.71–1.41)	1.12 (0.75–1.63)	<0.001
Homocysteine (μmol/L)[a]	5721	8.3 (7.1–10.0)	8.6 (7.3–10.3)	8.8 (7.4–10.6)	9.2 (7.6–11.5)	<0.001
Interleukin-6 (pg/mL)[a]	5585	0.98 (0.67–1.54)	1.16 (0.75–1.84)	1.27 (0.83–1.98)	1.48 (0.96–2.27)	<0.001
C-reactive protein (mg/L)[a]	5694	1.40 (0.62–3.18)	1.71 (0.84–3.98)	2.07 (0.90–4.32)	2.55 (1.12–5.26)	<0.001
Fibrinogen (mg/dL)	5699	339 ± 73	344 ± 71	349 ± 74	354 ± 77	0.004
eGFR (mL/min/1.73 m^2)	5724	88.4 ± 15.9	85.1 ± 16.2	82.7 ± 17.2	78.0 ± 18.9	<0.001
UACR (mg/g)[a]	5724	4.8 (3.1–9.4)	5.0 (3.2–10.2)	5.6 (3.5–12.2)	6.7 (3.8–16.6)	<0.001

[a]Data are expressed as mean ± SD, *n* (%) or median (IQR). MET, metabolic equivalent; HDL, high-density lipoprotein; LDL, low-density lipoprotein. P for trend was estimated from multivariable linear regression model with continuous ln-transformed FGF21 levels as the dependent variable after adjusting for age, sex and race/ethnicity. P-values were estimated using ln-transformed data.

interaction = 0.029). No significant interaction with diabetes was found with UACR, reduced eGFR or albuminuria (P > 0.05).

Association of FGF21 levels with eGFR progression

After excluding 542 participants with reduced eGFR at baseline and a further 523 participants with missing data on incident reduced eGFR, a total of 4659 participants were included in the analysis of incident CKD by eGFR. Participants in higher FGF21 quartiles were more likely to have incident CKD by eGFR (Table 3). However, in multivariable Cox regression analysis, the association of both ln-transformed FGF21 levels and FGF21 quartile with incident CKD by eGFR was not significant after adjusting for confounding factors (Table 3). In a separate analysis, incident rapid kidney function decline was assessed among 5098 MESA participants after excluding 886 participants with missing data on incident rapid kidney function decline. As shown in Table 4, both ln-transformed FGF21 levels and FGF21 quartiles were not significantly associated with the incident rapid kidney function decline in the full adjustment model. No significant interaction with sex, race/ethnicity and diabetes was found in any of the above analyses (P > 0.05).

Association of FGF21 levels with progression of albuminuria

For UACR progression over the follow-up period, analysis was performed among 3563 participants with valid data on

Table 2. Association of FGF21 levels with UACR and eGFR at baseline using multivariable linear regression analysis (n = 5724)

Biomarkers	Model 1[a]		Model 2[b]		Model 3[c]	
	B (95% CI)	P-value	*B* (95% CI)	P-value	*B* (95% CI)	P-value
eGFR (mL/min/1.73 m^2)						
ln-transformed FGF21	−1.88 (−2.25 to −1.50)	<0.001	−0.54 (−0.89 to −0.18)	0.003	−0.54 (−0.89 to −0.18)	0.003
FGF21 quartile						
1	Reference		Reference		Reference	
2	−1.16 (−2.20 to −0.11)	0.030	−0.08 (−1.02 to 0.86)	0.87	−0.08 (−1.02 to 0.87)	0.87
3	−2.32 (−3.37 to −1.27)	<0.001	−0.53 (−1.50 to 0.44)	0.28	−0.53 (−1.50 to 0.43)	0.28
4	−6.30 (−7.35 to −5.24)	<0.001	−2.62 (−3.63 to −1.61)	<0.001	−2.63 (−3.64 to −1.62)	<0.001
Overall P-value		<0.001		<0.001		<0.001
UACR [ln (mg/g)]						
ln-transformed FGF21	0.119 (0.090–0.148)	<0.001	0.032 (0.003–0.060)	0.029	0.032 (0.003–0.061)	0.028
FGF21 quartile						
1	Reference		Reference		Reference	
2	0.062 (−0.018 to 0.143)	0.13	−0.031 (−0.106 to 0.045)	0.43	−0.030 (−0.106 to 0.045)	0.43
3	0.163 (0.082–0.244)	<0.001	0.030 (−0.047 to 0.108)	0.44	0.031 (−0.047 to 0.108)	0.44
4	0.381 (0.299–0.462)	<0.001	0.133 (0.052–0.214)	0.001	0.134 (0.053–0.215)	0.001
Overall P-value		<0.001		<0.001		<0.001

Data are expressed as regression coefficient (95% CI) in terms of per SD (1.35) increase in ln-transformed FGF21 levels. *B*, regression coefficients; HDL, high-density lipoprotein.
[a]Adjusted for age, sex and race/ethnicity.
[b]Further adjusted for BMI, education, smoking, pack-years of smoking, diabetes, systolic blood pressure, use of antihypertensive medication acting on RAAS, use of other antihypertensive medication, use of lipid-lowering medication, HDL cholesterol, triglycerides (ln-transformed), HOMA2-IR (ln-transformed), homocysteine (ln-transformed), interleukin-6 (ln-transformed), C-reactive protein (ln-transformed) and fibrinogen.
[c]Further adjusted for eGFR for UACR analysis and UACR (ln-transformed) for eGFR analysis.

Table 3. Associations of FGF21 levels with incident CKD by eGFR over the follow-up period

Biomarkers	*n*		Model 1[a]		Model 2[b]		Model 3[c]	
	Total	Event	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
ln-transformed FGF21 levels	4659	837	1.08 (0.98–1.20)	0.13	0.93 (0.84–1.02)	0.14	0.91 (0.83–1.01)	0.066
FGF21 quartile								
Quartile 1	1163	136	Reference		Reference		Reference	
Quartile 2	1164	183	1.02 (0.78–1.33)	0.88	0.89 (0.68–1.17)	0.41	0.87 (0.66–1.14)	0.32
Quartile 3	1166	236	1.15 (0.90–1.48)	0.27	0.91 (0.70–1.19)	0.50	0.93 (0.72–1.21)	0.61
Quartile 4	1166	282	1.43 (1.12–1.83)	0.004	1.01 (0.78–1.31)	0.95	0.90 (0.69–1.17)	0.43
Overall P-value				0.006		0.68		0.78

For continuous FGF21 levels, data are expressed as HR (95% CI) in terms of per SD (1.38) increase in ln-transformed levels. Quartiles 1, 2, 3 and 4 were defined as FGF21 levels of ≤76.1, 76.2–139.3, 139.2–231.1 and ≥231.2 pg/mL, respectively.
[a]Adjusted for age, sex and race/ethnicity.
[b]Further adjusted for BMI, education, smoking, pack-years of smoking, diabetes, systolic blood pressure, use of antihypertensive medication acting on RAAS, use of other antihypertensive medication, use of lipid-lowering medication, high-density lipoprotein cholesterol, triglycerides, HOMA2-IR, homocysteine, interleukin-6, C-reactive protein and fibrinogen.
[c]Further adjusted for baseline eGFR and UACR.

UACR at Examinations 1, 2, 3 and 5 who did not have severely increased UACR at baseline. As shown in Table 5, participants with higher FGF21 levels had a higher risk of UACR progression after adjusting for age, sex and race/ethnicity (overall $P < 0.001$ for both ln-transformed FGF21 levels and FGF21 quartiles, Model 1). However, the association was borderline significant for ln-transformed FGF21 levels ($P = 0.048$) and was not significant for FGF21 quartile ($P = 0.32$) in the full adjustment model (Model 4; Table 5). No significant interaction with sex, race/ethnicity and diabetes was found in any of the above analyses ($P > 0.05$).

Association of FGF21 levels with rates of changes in UACR and eGFR

In a separate analysis we assessed the relationship of baseline FGF21 levels with changes in eGFR and ln-transformed UACR per year using a linear mixed-effects model. As shown in Supplementary data, Tables S4 and S5, both ln-transformed FGF21 levels and FGF21 quartiles ($P = 0.098$) at baseline were not significantly associated with the rate of change in eGFR and ln-transformed UACR over time. There was no significant interaction with sex and race/ethnicity in any of the above analyses ($P > 0.05$). Although a significant interaction with diabetes was found for the rate of change in ln-transformed UACR, which was robust for both ln-transformed FGF21 levels and FGF21 quartiles (P for interaction = 0.032 and 0.006, respectively), no significant association of ln-transformed FGF21 levels and FGF21 quartiles with the rate of change in ln-transformed UACR was found in participants with or without diabetes ($P > 0.05$). No significant interaction with diabetes was found with the rate of change in eGFR ($P > 0.05$).

DISCUSSION

In this multiethnic cohort of participants who were free of clinically apparent CVD at baseline, higher plasma FGF21 levels

Table 4. Associations of FGF21 levels with incident rapid kidney function decline over follow-up period

Biomarkers	*n*		Model 1[a]		Model 2[b]		Model 3[c]	
	Total	Event	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
ln-transformed FGF21 levels	5098	886	1.25 (1.04–1.50)	0.016	1.11 (0.92–1.34)	0.27	1.11 (0.92–1.34)	0.29
FGF21 quartile								
Quartile 1	1274	185	Reference		Reference		Reference	
Quartile 2	1275	213	0.98 (0.63–1.54)	0.95	0.88 (0.55–1.39)	0.57	0.87 (0.55–1.38)	0.55
Quartile 3	1275	229	1.29 (0.85–1.98)	0.23	1.12 (0.72–1.73)	0.63	1.11 (0.71–1.72)	0.65
Quartile 4	1274	259	1.65 (1.10–2.48)	0.015	1.33 (0.86–2.06)	0.19	1.33 (0.86–2.05)	0.20
Overall P-value				0.024		0.24		0.24

For continuous FGF21 levels, data are expressed as HR (95% CI) in terms of per SD (1.36) increase in ln-transformed levels. Quartiles 1, 2, 3 and 4 were defined as FGF21 levels of ≤79.4, 79.5–144.0, 144.1–241.9 and ≥242.0 pg/mL, respectively.

[a]Adjusted for age, sex and race/ethnicity.

[b]Further adjusted for BMI, education, smoking, smoking pack-years, diabetes, systolic blood pressure, use of antihypertensive medication acting on RAAS, use of other antihypertensive medication, use of lipid-lowering medication, high-density lipoprotein cholesterol, triglycerides, HOMA2-IR, homocysteine, interleukin-6, C-reactive protein and fibrinogen.

[c]Further adjusted for baseline UACR and eGFR.

were associated with lower eGFR and higher UACR. However, in a longitudinal analysis, FGF21 levels did not predict eGFR decline and UACR progression over a follow-up period of 10 years after adjusting for demographic factors and other potential confounding factors.

In animal studies, the effect of FGF21 administration has been reported to have beneficial effects on kidney function. FGF21 reduces renal lipid accumulation, fibrosis, inflammation and oxidative stress, thereby preventing diabetic renal injury, while deficiency of FGF21 aggravates these conditions [27]. Interestingly, FGF21 can directly suppress triglyceride levels and lipid accumulation in kidney tissues without suppressing plasma triglyceride and lipid levels [27]. This suggests that the renoprotective effect of FGF21 was largely due to its lipid-lowering effects specifically in the kidney rather than via systematic lipid reduction. FGF21 can also prevent hyperglycemia-induced fibrogenesis in renal mesangial cells [28], suggesting FGF21 may protect against renal fibrosis, which is often found in CKD patients. In diabetic *db*/*db* mice, daily administration of a small dose of FGF21 can significantly ameliorate renal function and morphological glomerular abnormalities [29]. Expression of the FGF21 receptor complex is up-regulated in diabetic kidney and FGF21 administration can significantly suppress its expression. Nonetheless, the role of FGF21 in these animal models may not be an accurate representation of FGF21 function in humans.

Several studies have reported the association of circulating FGF21 levels with different kidney diseases and treatments in humans. In patients receiving chronic hemodialysis, plasma FGF21 levels were elevated 15-fold compared with control subjects with eGFR >50 mL/min/1.73 m^2 [30]. In another study of 135 men and women, plasma FGF21 levels were increased 8-fold in patients receiving peritoneal dialysis compared with healthy control subjects [31]. In agreement with these findings, plasma FGF21 levels have been reported to gradually increase as CKD progresses from early to end-stage disease in a small cross-sectional study of only 240 CKD and healthy participants [32]. In a longitudinal study of 9697 participants with type 2 diabetes, plasma FGF21 levels correlated inversely with eGFR and elevated baseline FGF21 levels predicted the development of new, on-study, reduced eGFR over 5 years and albuminuria over 2 years [14]. Similarly, in another longitudinal study of 1136 participants with type 2 diabetes and normoalbuminuria, elevated FGF21 levels predicted eGFR decline [15]. However, as these studies only investigated participants with type 2 diabetes, the results may not be generalizable to a healthy population. This is particularly important given that FGF21 levels are elevated in people with diabetes [10].

In the present study, we did not show a significant association of baseline FGF21 levels with progression of eGFR and albuminuria in healthy participants, and there was no significant interaction with gender and race/ethnicity. One of the possibilities that may explain this discrepancy between the present study and the previous two studies [14, 15] may be that the association of FGF21 levels with kidney function and albuminuria is more pronounced in participants with type 2 diabetes and/or diabetic nephropathy. In our study, we did find a significant interaction with diabetes in which the association of higher FGF21 levels with lower eGFR at baseline was stronger in participants with diabetes than in those without diabetes. However, we did not find any robust and meaningful interaction with diabetes in any longitudinal analysis. This could be due to insufficient study power, as only 728 (12.7%) participants had diabetes in the present study. Moreover, the previous two studies used the Modification of Diet in Renal Disease Study formula for eGFR estimation [14, 15]. Moreover, the definition of eGFR decline was different between these studies and the present study. In the present study, we included the analysis of the rate of change in eGFR and UACR without the use of any cut-point. Further investigation is needed to clarify whether FGF21 could be a better biomarker for kidney disease in patients with type 2 diabetes than in those without type 2 diabetes. Nevertheless, our cross-sectional study findings suggest that lower renal clearance rate is associated with higher FGF21 levels. The nonsignificant finding in the longitudinal analysis suggests that FGF21 may not play a direct causal role in the development of kidney function decline and albuminuria and the elevated FGF21 levels commonly found in CKD patients could be a consequence of the underlying kidney injury.

This study has several strengths. MESA is a large multiethnic cohort with a long follow-up period of ∼10 years. The present study has the advantage of making use of MESA data with good quality control as part of a large well-characterized sample of clinically apparently healthy participants. Both eGFR

Table 5. Association of FGF21 levels with progression of albuminuria over the follow-up period

Outcome	*n*	Model 1[a]		Model 2[b]		Model 3[c]	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
ln-transformed FGF21 levels							
No UACR progression	2270	Reference		Reference		Reference	
Intermediate UACR progression	1020	1.08 (0.99–1.17)	0.067	0.95 (0.87–1.03)	0.19	0.95 (0.87–1.03)	0.20
Definite UACR progression	273	1.44 (1.21–1.72)	<0.001	1.18 (0.98–1.41)	0.073	1.19 (0.99–1.42)	0.064
Overall P-value			<0.001		0.045		0.040
FGF21 quartile							
Intermediate UACR progression							
Quartile 1	234	Reference		Reference		Reference	
Quartile 2	226	0.88 (0.71–1.10)	0.25	0.77 (0.61–0.97)	0.025	0.77 (0.61–0.97)	0.025
Quartile 3	266	1.13 (0.91–1.40)	0.26	0.88 (0.70–1.11)	0.29	0.88 (0.70–1.11)	0.29
Quartile 4	294	1.35 (1.09–1.68)	0.006	0.92 (0.73–1.18)	0.52	0.93 (0.73–1.18)	0.54
Definite UACR progression							
Quartile 1	49	Reference		Reference		Reference	
Quartile 2	59	1.07 (0.72–1.61)	0.73	0.86 (0.56–1.32)	0.49	0.87 (0.57–1.32)	0.51
Quartile 3	75	1.50 (1.02–2.20)	0.041	1.08 (0.71–1.62)	0.73	1.08 (0.72–1.63)	0.71
Quartile 4	90	1.98 (1.35–2.89)	<0.001	1.20 (0.79–1.82)	0.39	1.23 (0.81–1.87)	0.34
Overall P-value			<0.001		0.27		0.25

For continuous FGF21 levels, data are expressed as OR (95% CI) in terms of per SD (1.37) increase in ln-transformed levels. FGF21 Quartiles 1, 2, 3 and 4 were defined as FGF21 levels of ≤76.3, 76.4–138.4, 138.5–231.0 and ≥231.1 pg/mL, respectively.

[a]Adjusted for age, sex and race/ethnicity.

[b]Further adjusted for BMI, education, smoking, pack-years of smoking, diabetes, systolic blood pressure, use of antihypertensive medication acting on RAAS, use of other antihypertensive medication, use of lipid-lowering medication, high-density lipoprotein cholesterol, triglycerides, HOMA2-IR, homocysteine, interleukin-6, C-reactive protein and fibrinogen.

[c]Further adjusted for baseline UACR and eGFR.

and UACR were analyzed to assess kidney function and their data were available in follow-up visits that allow the causal and temporal relationship between baseline FGF21 levels and kidney function to be assessed. However, our study also has several limitations. eGFR was used in the analysis instead of the more accurate direct measurement of GFR. Nevertheless, we used the CKD-EPI creatinine–cystatin C formula, which estimates GFR more accurately compared with other widely used formulas [20]. Another limitation is that FGF21 levels were only measured at the baseline examination and not in subsequent follow-up visits. These samples were stored for ∼15 years before FGF21 measurement and thus we cannot exclude the possibility of confounding effects due to long-term sample storage. Furthermore, we may also have adjusted for variables that may act as mediators rather than confounders in the association of baseline FGF21 and progression of eGFR and albuminuria.

In conclusion, higher FGF21 levels were associated with lower eGFR and higher UACR at baseline. However, baseline FGF21 levels did not predict the progression of eGFR and UACR over 10 years of follow-up. Our study does not support FGF21 as a biomarker for predicting kidney function decline and albuminuria, independent of other traditional risk factors, at least in the people free of clinically apparent CVD at baseline.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

K.L.O., M.A.A., M.G.S., H.K. and K.-A.R were responsible for the research idea and study design. R.L.M., S.T., L.H. and K.L.O did the data acquisition. S.A., R.L.M., and K.L.O did the data analysis/interpretation. S.A. and K.L.O were responsible for the statistical analysis. M.A.A., K.-A.R. and K.L.O provided study supervision or mentorship. S.A. and K.L.O drafted the manuscript. All authors did a critical revision of the manuscript for intellectual content.

CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

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