Atherosclerosis 287 (2019) 46–53

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Relationship of fibroblast growth factor 21 with subclinical atherosclerosis and cardiovascular events: Multi-Ethnic Study of Atherosclerosis

Kwok Leung Ong[a,b,1,*], Steven Campbell[b,1], Ben J. Wu[a], Robyn L. McClelland[c], John Kokkinos[a], Moyses Szklo[d], Joseph F. Polak[e], Matthew A. Allison[f], Kerry-Anne Rye[a,b,*]

[a] Lipid Research Group, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia
[b] Sydney Medical School, University of Sydney, Sydney, NSW, Australia
[c] Department of Biostatistics, University of Washington, Seattle, WA, USA
[d] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[e] Department of Radiology, Tufts University School of Medicine, Boston, MA, USA
[f] Department of Family Medicine and Public Health, University of California San Diego, La Jolla, CA, USA

HIGHLIGHTS

- It was not known whether FGF21 is a CVD biomarker in a general population.
- Baseline FGF21 levels were measured in MESA participants free of CVD at baseline.
- FGF21 levels were not cross-sectionally associated with carotid IMT, ABI and CAC.
- FGF21 levels were not associated with incident CVD after multiple adjustment.
- FGF21 may not be a useful CVD biomarker in people without a history of CVD.

ARTICLE INFO

Keywords:
Ankle-brachial index
Cardiovascular disease
Carotid intima-media thickness
Coronary artery calcification
Fibroblast growth factor 21

ABSTRACT

Background and aims: Fibroblast growth factor 21 (FGF21) has been suggested as a novel biomarker for cardiovascular disease (CVD), especially in people with high CVD risk. However, it is not known whether FGF21 is a CVD biomarker in an initially healthy cohort. We therefore investigated the relationship of plasma FGF21 levels with measures of subclinical atherosclerosis and cardiovascular events in Multi-Ethnic Study of Atherosclerosis participants without known CVD at baseline.

Methods: A total of 5788 participants had plasma FGF21 levels measured at the baseline exam (2000–2002). Carotid intima-media thickness (IMT), ankle-brachial index (ABI) and coronary artery calcification (CAC) were measured at baseline. Participants were followed up for incident CVD events over a median period of 14 years.

Results: In cross-sectional analyses adjusting for socio-demographic variables, participants with higher FGF21 levels had higher carotid IMT, lower ABI, and higher prevalence of CAC ($p < 0.001$). However, these associations were not significant after simultaneously adjusting for demographic, socioeconomic and lifestyle factors, traditional CVD risk factors, and biomarkers of inflammation and hemostasis. Among 5768 patients with follow-up data, 820 developed incident CVD endpoints. Higher baseline FGF21 levels were not associated with the risk for incident CVD endpoints after adjusting for multiple confounding factors (odds ratio 1.03; 95% confidence interval, 0.94–1.12, per SD increase in ln-transformed FGF21 levels).

Conclusions: Although FGF21 has been suggested as a CVD biomarker for people with high CVD risk, our findings do not support a role of FGF21 as a CVD biomarker in those without a history of CVD.

1. Introduction

Fibroblast growth factor 21 (FGF21) is a novel metabolic regulator, which plays an important role in glucose and lipid metabolism [1–3]. In mice, it is predominantly expressed in the liver, and to a lesser extent, in adipocytes and the pancreas [4]. In animal studies, FGF21 has anti-

* Corresponding authors. School of Medical Sciences, University of New South Wales, Sydney, NSW, 2052, Australia.
E-mail addresses: oklws@yahoo.com.hk (K.L. Ong), k.rye@unsw.edu.au (K.-A. Rye).
[1] These authors contributed equally first authors.

https://doi.org/10.1016/j.atherosclerosis.2019.06.898
Received 30 March 2019; Received in revised form 23 May 2019; Accepted 7 June 2019
Available online 08 June 2019
0021-9150/

inflammatory, anti-diabetic and hypolipidemic effects [1,5,6] and FGF21-transgenic mice are resistant to diet-induced obesity [1]. In mice with hyperglycemia and insulin resistance, administration of recombinant human FGF21 decreases circulating glucose, triglyceride and fasting insulin levels, and improves glucose clearance during an oral glucose tolerance test without causing mitogenicity, hypoglycemia or weight gain [1]. However, in human studies, circulating FGF21 levels are often elevated in obesity, dyslipidemia, insulin resistance, the metabolic syndrome, type 2 diabetes, non-alcoholic fatty liver disease and coronary artery disease (CAD) [7–13]. This elevation in circulating FGF21 levels may be due to FGF21 resistance, or compensatory responses to the underlying metabolic stress [4]. Given this, circulating FGF21 levels have been suggested as a potential biomarker for the early detection of these cardiometabolic disorders [4].

We have previously reported that higher plasma FGF21 levels at baseline predict a higher risk of total cardiovascular disease (CVD) events and new on-study microvascular complications during 5-years of follow-up in patients with type 2 diabetes from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study [14,15]. Moreover, we have recently demonstrated that elevated plasma FGF21 levels can also predict a higher risk of major cardiovascular events in stable CAD patients treated with atorvastatin from the Treating to New Targets [TNT] trial [16]. However, as the patients in these studies were at increased CVD risk, the results may not be generalizable to those individuals without extant disease. Given the potential role of FGF21 as a biomarker for monitoring and predicting the progress of cardiovascular risk, we investigated the relationship of plasma FGF21 levels with different measures of subclinical atherosclerosis including carotid intima-media thickness (IMT), ankle-brachial index (ABI) and coronary artery calcification (CAC), as well as incident CVD events.

2. Materials and methods

2.1. Study participants

Details of the study objectives, design, and protocol of the Multi-Ethnic Study of Atherosclerosis (MESA) have been described previously [17]. Briefly, the MESA cohort consists of 6814 men and women aged 45–84 years in four major ethnic groups (Caucasian, African American, Hispanic American, and Chinese American) [17]. All participants were recruited from six United States communities between July 2000 and August 2002, and were free of clinically apparent CVD at baseline. The Institutional Review Boards at all participating centers approved the study, which was performed in compliance with the principles of the Declaration of Helsinki. Informed written consent was provided by all participants.

2.2. Plasma FGF21 measurement

At the baseline visit, venous blood was drawn from all participants using standardized venipuncture procedures after a 12-h fast. Stored plasma samples obtained at baseline were used to measure FGF21 levels as described previously [14–16]. These samples were stored at −70 °C for more than 14–16 years before FGF21 measurement. The intra- and inter-assay coefficients of variation were < 10%. In our pilot study, FGF21 levels were stable after 1–6 freeze-thaw cycles with the coefficient of variation being 8.1% [18].

2.3. Measures of subclinical atherosclerosis

At baseline, carotid IMT assessment was performed using high-resolution B-mode ultrasound with the Logiq 700 ultrasound device (General Electric Medical Systems, Waukesha, Wisconsin) as previously described [19–21]. The maximal IMT of the common carotid artery (CCA) and internal carotid artery (ICA) was measured as the mean of the maximum IMT of the far and near walls of the left and right sides [20,21]. The same set of blinded replicate scans were used to assess the reproducibility [22]. For CCA IMT, the correlation coefficient between readings from the same reader was 0.91, and inter-reader correlation coefficient was 0.82. For ICA IMT, the correlation coefficient between readings from the same reader was 0.91 [22].

ABI was calculated by measuring systolic blood pressure (SBP) with an appropriate-sized cuff in both arms and both legs using a continuous-wave Doppler ultrasound probe after a 5-min supine rest [23]. SBP in dorsalis pedis and posterior tibial arteries were measured for each leg. The leg-specific ABI was calculated as maximum SBP in the dorsalis pedis and posterior tibial arteries divided by the mean of the right and left brachial pressures. The higher brachial pressure was used when the right and left brachial pressures differed by ≥ 10 mm Hg. For this analysis, the lower of the two leg-specific ABIs was utilized. Participants with ABI ≥1.40 were excluded from the analysis, as this indicated poorly compressible leg arteries and an inability to gauge arterial obstructive disease accurately. All ABI measurements have intraclass correlation coefficients > 0.9 and technical error of measurement < 5% [24].

At baseline, all participants underwent computed tomography (CT) scans of the chest for CAC as described previously [25]. Calcification was defined as the presence of a plaque of ≥1 mm^2 with a density of ≥130 Hounsfield units. The Agatston scoring method was used to quantify the extent of calcification [26]. There was a high agreement for the presence of coronary artery calcification between consecutive CT scans (kappa = 0.92) and between CT image analysts (kappa = 0.90) [25].

2.4. Event ascertainment

Details on CVD event ascertainment have been described previously [20]. Participants were followed up for CVD events until death, loss to follow-up, or 31 December 2015, whichever came first, with a median follow-up period of 14 years. In this analysis, the outcome of incident total CVD included myocardial infarction, resuscitated cardiac arrest, definite angina, probable angina associated with coronary revascularization, stroke, stroke death, coronary heart disease (CHD) death, other atherosclerotic death, and other CVD death. Hard CVD endpoints included myocardial infarction, resuscitated cardiac arrest, stroke, CHD death, and stroke death. Hard CHD endpoints included myocardial infarction, resuscitated cardiac arrest and CHD death. The criteria for “all CHD endpoints” also included probable angina (if followed by revascularization) and definite angina.

2.5. Other covariates of interest

Information on race/ethnicity, age, education, family income, alcohol use, smoking, physical activity, medical history and medication use was obtained from standardized questionnaires. Physical activity was measured as the total number of hours per week of vigorous and moderate activity multiplied by metabolic equivalent (MET) level [23]. Body mass index (BMI) was measured as the weight in kilograms divided by height in meters squared. Resting blood pressure was measured three times in a seated position and the mean of the last two readings was used in the analyses. Diabetes was defined as the use of insulin or oral hypoglycemic medications, or fasting glucose ≥126 mg/dL. Low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, homeostasis model assessment index of insulin resistance (HOMA-IR), estimated glomerular filtration rate (eGFR), interleukin-6 (IL-6), fibrinogen, and high-sensitivity C-reactive protein (CRP) were measured as described previously [27,28].

2.6. Statistical analysis

Data were presented as mean (standard deviation [SD]) and percentage (number), or median (interquartile range). For variables with a

Table 1
Baseline characteristics of participants according to FGF21 quartiles.

Characteristic	n	FGF21 levels, pg/mL				*p* for trend
		Quartile 1 (≤81.2)	Quartile 2 (81.2–145.9)	Quartile 3 (145.9–245.1)	Quartile 4 (≥245.1)	
n		1447	1447	1447	1447	
Age, years	5788	60.3 (10.2)	62.5 (10.0)	63.5 (10.1)	64.2 (10.2)	< 0.001
Women, %	5788	48.3 (699)	49.6 (718)	54.7 (792)	55.6 (805)	< 0.001
Race/ethnicity, %						
Caucasian	2153	35.3 (511)	36.6 (529)	38.0 (550)	38.9 (563)	0.039
African American	1669	33.3 (482)	29.9 (433)	26.4 (382)	25.7 (372)	< 0.001
Hispanic American	1261	17.4 (252)	20.4 (295)	23.9 (346)	25.4 (368)	< 0.001
Chinese American	705	14.0 (202)	13.1 (190)	11.7 (169)	10.0 (144)	< 0.001
Education, %						
< High school	1055	14.7 (211)	17.1 (247)	20.4 (293)	21.1 (304)	< 0.001
High school	2416	37.9 (546)	41.7 (602)	43.3 (623)	44.7 (645)	< 0.001
> High school	2296	47.4 (683)	41.2 (596)	36.3 (522)	34.3 (495)	< 0.001
Total gross family income, %						
< $30 000	2114	31.5 (439)	36.5 (507)	40.5 (564)	44.1 (604)	< 0.001
$30 000- $74 999	2188	40.6 (566)	39.5 (548)	39.0 (543)	38.8 (531)	0.367
≥$75 000	1243	28.0 (390)	24.0 (334)	20.5 (285)	17.1 (234)	< 0.001
Smoking, %						
Never	2904	53.2 (767)	50.5 (730)	49.8 (716)	47.8 (691)	0.007
Former	2139	35.9 (517)	38.0 (549)	37.4 (538)	37.0 (535)	0.785
Current	729	11.0 (158)	11.5 (167)	12.9 (185)	15.2 (219)	< 0.001
Pack-years of smoking	5706	8.8 (16.4)	11.2 (20.8)	11.6 (20.6)	13.5 (23.7)	< 0.001
Current alcohol intake, %	5743	55.1 (789)	57.6 (828)	54.1 (775)	53.1 (765)	0.078
Physical activity, MET-hours/weeks	5770	102.6 (94.0)	98.6 (101.4)	87.8 (92.0)	89.0 (97.9)	< 0.001
BMI, kg/m^2	5788	27.2 (5.0)	27.8 (5.1)	28.9 (5.7)	29.4 (5.7)	< 0.001
Heart rate, beats per minute	5745	62.1 (9.4)	62.6 (9.3)	63.2 (9.7)	64.4 (10.1)	< 0.001
Diabetes, %	5778	8.2 (119)	12.0 (173)	13.7 (198)	17.2 (249)	< 0.001
HOMA-IR[a]	5762	0.8 (0.6–1.1)	0.9 (0.6–1.3)	1.0 (0.7–1.4)	1.1 (0.8–1.6)	< 0.001
Anti-hypertensive medication, %	5786	30.1 (436)	36.6 (529)	39.3 (569)	45.1 (652)	< 0.001
SBP, mm Hg	5786	122.8 (20.3)	126.7 (21.4)	128.1 (21.5)	130.5 (22.0)	< 0.001
DBP, mm Hg	5786	71.8 (10.1)	72.2 (10.1)	71.6 (9.9)	72.4 (10.8)	0.142
Lipid-lowering medications, %	5776	14.1 (204)	16.7 (242)	16.4 (236)	20.1 (290)	< 0.001
LDL cholesterol, mg/dL	5710	117.0 (31.3)	117.8 (31.7)	117.4 (30.4)	116.3 (32.7)	0.409
HDL cholesterol, mg/dL	5778	53.4 (15.4)	51.7 (14.9)	50.2 (14.6)	48.4 (14.1)	< 0.001
Triglycerides, mg/dL	5781	89 (64–124)	105 (78–151)	121 (84–170)	136 (92–199)	< 0.001
eGFR, mL/min/1.73 m^2	5778	80.8 (15.2)	78.5 (15.6)	77.5 (16.2)	74.3 (18.0)	< 0.001
CRP, mg/L[a]	5755	1.4 (0.6–3.2)	1.7 (0.8–4.0)	2.1 (0.9–4.4)	2.5 (1.1–5.3)	< 0.001
Fibrinogen, mg/dL	5759	339.2 (72.9)	344.1 (71.5)	349.2 (73.6)	353.8 (77.0)	< 0.001
IL-6, pg/mL[a]	5642	1.0 (0.7–1.5)	1.2 (0.8–1.8)	1.3 (0.8–2.0)	1.5 (1.0–2.3)	< 0.001
IMT, mm						
CCA	5711	0.84 (0.18)	0.87 (0.20)	0.89 (0.20)	0.89 (0.20)	< 0.001
ICA	5624	1.00 (0.55)	1.08 (0.61)	1.10 (0.60)	1.16 (0.68)	< 0.001
Mean	5615	0.92 (0.33)	0.97 (0.36)	0.99 (0.35)	1.03 (0.39)	< 0.001
ABI[b]	5675	1.12 (0.11)	1.11 (0.12)	1.11 (0.12)	1.09 (0.12)	< 0.001
CAC						
Presence, % (n)	5788	45.1 (653)	49.6 (717)	50.7 (734)	57.8 (836)	< 0.001
Score[a,c]	2940	83.1 (21.5–273.4)	88.3 (19.9–305.1)	95.7 (24.0–283.9)	105.3 (25.7–353.3)	0.021

Data are expressed as mean (SD), percent (n), or median (interquartile range). Data were compared by chi-square test for categorical variables and ANOVA for continuous variables.

[a] *p* values were estimated using ln-transformed data.

[b] Among 5715 participants with data on ABI, 40 participants with a value of ≥1.40 were excluded from analysis, as this indicates poorly compressible leg arteries and an inability to gauge arterial obstructive disease accurately.

[c] Data were estimated among subjects with non-zero CAC score.

skewed distribution, data were presented as median (interquartile range) and natural log (ln)-transformed before analysis. Distributions of demographic data, cardiovascular risk factors, and baseline measures of subclinical atherosclerosis were compared across FGF21 quartiles at baseline using a linear trend test by fitting an univariable linear or logistic regression model with each characteristic as a dependent variable and a discrete variable derived using the median FGF21 level of each quartile (as the ranges of FGF21 level within each quartile were not equal) as the independent variable. Variables that showed increasing or decreasing trends with FGF21 levels ($p < 0.2$) were included as covariates in subsequent regression analysis.

In all regression analysis, data were adjusted for demographic, socioeconomic and lifestyle factors, including age, sex, race/ethnicity (Caucasian, African American, Hispanic American, and Chinese American), family income (< $30 000, $30 000-$74 999, and ≥$75 000), physical activity, and education (< high school, high school, and > high school) in model 1. In model 2, data were further adjusted for other traditional CVD risk factors including body mass index (BMI), heart rate, use of lipid lowering medications, HDL cholesterol, triglycerides, HOMR-IR, eGFR, smoking status (never, former, and current smokers), pack-years of smoking, diabetes, SBP and use of anti-hypertensive medications. In model 3, data were further adjusted for inflammation and hemostasis biomarkers, including CRP, fibrinogen and IL-6 levels. As plasma FGF21 levels were highly skewed, data were ln-transformed to prevent unstable associations since extreme values may have undue influence on the estimate of the regression coefficient.

The association of FGF21 levels with carotid IMT, ABI and CAC scores (among those with non-zero scores) at baseline was assessed by multivariable linear regression using robust standard error estimation after adjusting for confounding variables. In all of these analyses, no

multi-collinearity issue was detected (all variance inflation factors < 3.0). In this analysis, an abnormal carotid IMT was defined as a value in the highest quintile [29] and a low ABI was defined as a value < 0.9 [30]. FGF21 in quartiles was also modeled in a separate analysis.

In a separate analysis, we also assessed the association of FGF21 levels with abnormal IMT, low ABI and the presence of CAC. As there was a high prevalence of calcification in the cohort, the prevalence odds ratio from the logistic regression did not approximate the prevalence ratio. Therefore, for the presence of CAC, abnormal IMT and low ABI, prevalence ratios (PR) were estimated using a regression model $y = \exp(X^{T}\beta)$ with robust standard error estimates [31].

We also assessed the non-linear relationship of FGF21 levels with different measures of subclinical atherosclerosis and CVD outcomes by using regression splines to model potentially nonlinear relationships. The relationship for the FGF21 levels with the measures of interest was allowed to be nonlinear with the assumption of linear relationship of FGF21 levels with other confounding variables. When nonlinearity was detected, the approximate knot positions were used to fit regression analyses within strata defined by these thresholds.

Cox proportional hazard analysis was used to estimate the hazards ratios (HR) for the association of baseline FGF21 levels with incident CVD events. The proportional hazard assumption was checked by using Schoenfeld residuals, and no significant violation was found.

In all analyses, we investigated whether there was an interaction by sex and race/ethnicity. *P* for interaction was estimated by including the interaction term in the regression models in the full sample after adjusting for the main effects of the covariates. However, we did not find any significant interaction with sex and race/ethnicity in any of the analyses. Therefore, we did not stratify the results by sex and race/ethnicity. All data analyses were performed using SPSS 25 (IBM, Armonk, NY) or STATA 14 (StataCorp, College Station, TX). Participants with missing data were excluded. A two-tailed $p < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

Among 6814 participants at the baseline exam, FGF21 levels were available for 5792 participants. As shown in Supplementary Table 1, participants with FGF21 measurements were older, more likely to be African American and Chinese Americans, and less likely to be women and Caucasian than those without these measurements. After further excluding 4 participants due to the presence of pre-existing CVD events, which were not identified at baseline, a total of 5788 participants were included in this analysis.

Table 1 shows the baseline characteristics of participants according to quartiles of baseline FGF21 levels. Participants with higher FGF21 levels were more likely to be older, women, Caucasian or Hispanic American, less educated, current smoker with higher pack-years of smoking, less physically active and more obese, have lower family income and higher heart rate, and were less likely to be African or Chinese American than those with lower FGF21 levels. Participants with higher FGF21 levels were also more likely to be insulin resistant, have diabetes or hypertension, taking lipid-lowering therapy, and have higher triglyceride, CRP, fibrinogen and IL-6 levels, and lower HDL cholesterol and estimated GFR than those with lower FGF21 levels. All these above relationships were in a graded ("dose-response") fashion.

3.2. Carotid IMT and ABI

As shown in Table 1, higher FGF21 levels were related to higher carotid IMT and lower ABI in a graded fashion (all $p < 0.01$). These associations remained significant after adjustment for age, sex, race/ethnicity, family income, physical activity, and education (all $p < 0.01$, Model 1, Table 2). However, these associations were not significant after further adjustment for other CVD risk factors (Model 3, Table 2). Similar non-significant results were obtained when assessing elevated carotid IMT and low ABI as binary categorical variables in the full adjustment model (Table 3).

In a separate analysis, we assessed the non-linear association of FGF21 levels with different measures of subclinical atherosclerosis. The regression spline analysis suggested that there may be a positive association between FGF21 levels and CCA IMT for FGF21 levels between 80.1 and 242.6 pg/mL and an inverse association with a threshold FGF21 level at approximately ≥242.7 pg/mL, although the association did not reach statistical significance within strata defined by these thresholds (Table 2). A similar non-linear association was found for elevated CCA IMT, in which the positive association between FGF21 levels and CCA IMT for FGF21 levels between 80.1 and 242.6 pg/mL did reach statistical significance, but not the inverse association at FGF21 levels ≥242.7 pg/mL (Table 3). No non-linear associations were found for continuous variables of ICA IMT, mean IMT and ABI, or categorical variables of elevated ICA IMT, elevated mean IMT and low ABI.

3.3. CAC

As shown in Table 1, following graded patterns, the percentage of participants with a non-zero CAC score tended to be greater in participants with higher FGF21 levels ($p < 0.01$). Among participants with a non-zero CAC score, there was also a significant graded trend in the extent of calcified atherosclerosis with FGF21 levels ($p = 0.02$, Table 1). The association of FGF21 levels with CAC at baseline remained significant after adjustment for age, sex, race/ethnicity, family income, physical activity, and education (Model 1, Table 3). However, these associations were not significant after further adjustment for other CVD risk factors. Similar non-significant results were obtained when assessing ln-transformed CAC score among participants with CAC in the full adjustment model (Table 2). No non-linear associations were found for the presence of CAC, nor ln-transformed CAC scores among participants with CAC.

3.4. Cardiovascular events

Among all the 5788 participants, 20 participants were lost to follow-up. Of the remaining 5768 patients, 820 (14.2%) developed incident total CVD during follow-up. As shown in Table 4, higher FGF21 levels at baseline were associated with higher risk of total CVD after adjusting for age, sex, race/ethnicity, family income, physical activity, and education ($p < 0.01$, Model 1). However, such an association did not remain significant after adjusting for other CVD risk factors. In a separate analysis, we assessed different CVD endpoints: hard CVD, all CHD and hard CHD endpoints, but no significant results were obtained (Table 4).

In a separate analysis, we assessed the non-linear association of baseline FGF21 levels with different CVD endpoints. The regression spline analysis suggested that there may be a positive association of FGF21 levels with higher risk of hard CVD and hard CHD endpoints at higher baseline FGF21 levels with a threshold at approximately 80.0 mg/dL, although the association did not reach statistical significance within strata defined by this threshold (Table 4). No non-linear associations were found for total CVD and all CHD endpoints.

3.5. Sensitivity analysis with exclusion of participants with diabetes

In a sensitivity analysis, we excluded 739 participants with diabetes and 10 participants with unknown diabetes status at baseline, leaving a total of 5039 participants for analysis. Similar results were obtained. FGF21 levels were not associated with IMT and ABI (Supplementary Tables 2 and 3). A similar non-linear association was found with continuous CCA IMT or elevated CCA IMT with similar FGF21 cut-off points in the full adjustment model. Baseline FGF21 levels were not

Table 2
Absolute difference in carotid IMT, ABI and ln-transformed CAC score related to a difference of one standard deviation of FGF21 level at baseline.

Parameter	n	Model 1		Model 2		Model 3	
		B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
CCA IMT	5711	0.010 (0.006, 0.015)	< 0.001	0.003 (−0.002, 0.008)	0.270	0.002 (−0.003, 0.007)	0.456
FGF21 subgroup							
≤80.0 pg/mL	1417	0.005 (−0.003, 0.012)	0.254	0.000 (−0.007, 0.008)	0.929	0.000 (−0.008, 0.008)	0.924
80.1–242.6 pg/mL	2840	0.042 (0.013, 0.070)	0.005	0.026 (−0.002, 0.054)	0.072	0.023 (−0.005, 0.051)	0.117
≥242.7 pg/mL	1454	−0.025 (−0.048, −0.003)	0.027	−0.021 (−0.043, 0.001)	0.067	−0.020 (−0.043, 0.002)	0.074
ICA IMT	5624	0.032 (0.019, 0.046)	< 0.001	0.009 (−0.005, 0.023)	0.009	0.007 (−0.007, 0.022)	0.318
Mean IMT	5615	0.022 (0.014, 0.030)	< 0.001	0.006 (−0.002, 0.014)	0.126	0.005 (−0.003, 0.013)	0.255
ABI[a]	5675	−0.004 (−0.006, −0.001)	0.009	−0.001 (−0.004, 0.002)	0.381	0.000 (−0.003, 0.002)	0.743
ln (CAC score)[b]	2940	0.057 (−0.014, 0.129)	0.117	−0.006 (−0.082, 0.069)	0.871	−0.017 (−0.094, 0.061)	0.674

Regression coefficient (B): absolute change in carotid IMT and ABI associated with one SD (1.36) increase in ln-transformed FGF21 level.
Model 1: Adjusted for age, sex, race/ethnicity (Caucasian, African American, Hispanic American, and Chinese American), family income (< $30 000, $30 000- $74 999, and ≥$75 000), physical activity, and education (< high school, high school, and > high school).
Model 2: Further adjusted for BMI, heart rate, use of lipid lowering medications, HDL cholesterol, triglycerides (ln-transformed), HOMR-IR (ln-transformed), eGFR, smoking status (never, former, and current smokers), pack-years of smoking, current alcohol intake, diabetes, SBP, and use of anti-hypertensive medications.
Model 3: Further adjusted for CRP (ln-transformed), fibrinogen, and IL-6 (ln-transformed).
[a] For ABI, participants with a value of ≥1.40 were excluded, as this indicates poorly compressible leg arteries resulting in inability to gauge arterial obstructive disease accurately.
[b] Data were analyzed among participants with the presence of CAC.

Table 3
Baseline prevalence ratios of elevated carotid IMT, low ABI and CAC related to one SD change in FGF21 levels at baseline.

Parameter	n	Case, % (n)	Model 1		Model 2		Model 3	
			PR (95% CI)	***p***	**PR (95% CI)**	***p***	**PR (95% CI)**	***p***
Elevated CCA IMT	5711	20.2 (1154)	1.05 (0.98-1.11)	0.158	1.01 (0.94-1.07)	0.871	1.00 (0.94-1.07)	0.926
FGF21 subgroup								
≤80.0 pg/mL	1417	15.0 (213)	0.94 (0.84-1.05)	0.298	0.92 (0.82-1.04)	0.200	0.93 (0.82-1.06)	0.259
80.1-242.6 pg/mL	2840	21.3 (605)	1.41 (1.03-1.93)	0.031	1.38 (0.99-1.92)	0.059	1.49 (1.06-2.08)	0.021
≥242.7 pg/mL	1454	23.1 (336)	0.82 (0.66-1.02)	0.077	0.79 (0.61-1.02)	0.070	0.78 (0.60-1.01)	0.060
Elevated ICA IMT	5624	20.0 (1123)	1.13 (1.05-1.20)	< 0.001	1.06 (1.00-1.12)	0.052	1.06 (1.00-1.12)	0.043
Elevated Mean IMT	5615	20.0 (1122)	1.12 (1.05-1.21)	< 0.001	1.06 (0.99-1.12)	0.076	1.06 (0.99-1.12)	0.081
Low ABI[a]	5675	4.0 (225)	1.34 (1.02-1.78)	0.038	1.03 (0.81-1.32)	0.790	1.02 (0.78-1.34)	0.877
CAC	5788	50.8 (2940)	1.04 (1.01-1.07)	0.003	1.01 (0.98-1.03)	0.584	1.01 (0.98-1.04)	0.402

For continuous FGF21 levels, data are expressed as change in the PR (95% CI) related to one SD (1.36) increase in ln-transformed FGF21 levels (pg/mL).
Model 1: Adjusted for age, sex, race/ethnicity (Caucasian, African American, Hispanic American, and Chinese American), family income (< $30 000, $30 000-$74 999, and ≥$75 000), physical activity, and education (< high school, high school, and > high school).
Model 2: Further adjusted for BMI, heart rate, use of lipid lowering medications, HDL cholesterol, triglycerides, HOMR-IR, eGFR, smoking status (never, former, and current smokers), pack-years of smoking, current alcohol intake, diabetes, SBP and use of anti-hypertensive medications.
Model 3: Further adjusted for CRP, fibrinogen, and IL-6.
[a] For ABI, participants with a value of ≥1.40 were excluded, as this indicates poorly compressible leg arteries resulting in inability to gauge arterial obstructive disease accurately.

associated with ln-transformed CAC score among participants with a non-zero CAC score, nor the presence of CAC in the full adjustment model. Baseline FGF21 levels were not associated with any CVD endpoints in the full adjustment model (Supplementary Table 4). No non-linear associations were found for other measures of subclinical atherosclerosis, or any CVD composite endpoint.

4. Discussion

The present study assessed the relationship of FGF21 levels with different measures of subclinical atherosclerosis and incident CVD events in the MESA study. We did not observe significant association of FGF21 levels with several different measures of subclinical atherosclerosis or CVD events after adjusting for other CVD risk factors.

Circulating FGF21 levels have been reported to be associated with different cardiovascular risk factors such as obesity, metabolic syndrome, type 2 diabetes, hypertriglyceridemia, hyperinsulinemia, pericardial fat volume, hypertension, and renal function [4,7–13]. This is consistent with our previous analysis, showing that elevated FGF21 levels are associated with both prevalent and incident metabolic syndrome in the MESA study [28].

Although the role of FGF21 in atherosclerosis has been demonstrated using a genetic knockout mouse model [32], the association of circulating FGF21 levels with carotid IMT is less well-established. In a cross-sectional study of 670 Chinese subjects, a high plasma FGF21 level was found to be an independent predictor of CCA IMT in women, but not in men [33]. In the present study, however, we did not find any sex interaction for the association of FGF21 levels with carotid IMT. Moreover, in this cohort, there is a high proportion of people with dysglycemia (60.9%, defined as fasting glucose ≥110 mg/dL, 2-h glucose ≥140 mg/dL after 75-g oral glucose tolerance test, or use of anti-diabetic medications) and use of anti-diabetic medications (26.3%). In a study of 120 patients with type 2 diabetes, FGF21 levels were found to independently predict subclinical atherosclerosis defined as a carotid

Table 4
Cardiovascular disease hazard ratios according to FGF21 levels.

Outcome	n	Event, % (n)	Model 1		Model 2		Model 3	
			HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Total CVD	5768	14.2 (820)	1.12 (1.03-1.22)	0.007	1.03 (0.95-1.12)	0.497	1.03 (0.94-1.12)	0.549
Hard CVD	5767	10.4 (597)	1.19 (1.07-1.32)	0.001	1.10 (0.99-1.23)	0.077	1.09 (0.98-1.22)	0.123
FGF21 subgroup								
≤80.0 pg/mL	1424	7.0 (99)	0.91 (0.77-1.08)	0.276	0.89 (0.75-1.06)	0.202	0.89 (0.74-1.06)	0.201
≥80.1 pg/mL	4343	11.5 (498)	1.34 (1.12-1.60)	0.001	1.20 (1.00-1.45)	0.052	1.18 (0.97-1.43)	0.095
All CHD	5767	9.6 (551)	1.12 (1.01-1.24)	0.034	1.00 (0.90-1.10)	0.952	1.00 (0.90-1.11)	0.980
Hard CHD	5767	6.5 (374)	1.21 (1.06-1.38)	0.005	1.08 (0.95-1.24)	0.237	1.08 (0.94-1.25)	0.261
FGF21 subgroup								
≤80.0 pg/mL	1430	4.6 (65)	0.92 (0.75-1.13)	0.412	0.85 (0.69-1.05)	0.141	0.84 (0.68-1.05)	0.123
≥80.1 pg/mL	4343	7.1 (309)	1.46 (1.19-1.80)	< 0.001	1.26 (1.00-1.57)	0.045	1.25 (0.99-1.58)	0.063

For continuous FGF21 levels, data are expressed as change of HR (95% CI) in relation to a change of one SD (1.36) increase in ln-transformed FGF21 levels (pg/mL).
Model 1: Adjusted for age, sex, race/ethnicity (Caucasian, African American, Hispanic American, and Chinese American), family income (< $30 000, $30 000-$74 999, and ≥$75 000), physical activity, and education (< high school, high school, and > high school).
Model 2: Further adjusted for BMI, heart rate, use of lipid lowering medications, HDL cholesterol, triglycerides, HOMR-IR, eGFR, smoking status (never, former, and current smokers), pack-years of smoking, current alcohol intake, diabetes, and SBP and use of anti-hypertensive medications.
Model 3: Further adjusted for CRP, fibrinogen, and IL-6.

IMT > 9.0 mm and/or ABI < 0.9 [34]. Another study of 141 patients with type 2 diabetes has reported a significant association of higher FGF21 levels with carotid artery plaque after adjusting for age, sex and BMI [35]. In a study of 212 patients with newly diagnosed type 2 diabetes, FGF21 levels were also associated with IMT of several different arteries [36]. It should be noted that patients with dysglycemia or type 2 diabetes are at a higher CVD risk and have elevated FGF21 levels [7]. Therefore, findings from these studies may not be generalizable to a healthy population. Our results also suggest there may be a non-linear relationship between FGF21 levels and CCA IMT. However, such a potential non-linear relationship was moderate, and needs to be confirmed in other independent cohorts.

There are also very limited studies on relationship of circulating FGF21 level with CAC and ABI. In a study of 417 patients undergoing coronary angiography, FGF21 was reported to be an independent predictor for peripheral artery disease (defined as ABI < 0.9), but not CAD [37]. However, this study was limited by the low number of patients with PAD (n = 38). FGF21 has recently been reported to have a protective effect on vascular calcification in rats [38]. Although we did not find any association of FGF21 levels with the presence and severity CAC in the present study, it may be worthwhile to study such an association in people with type 2 diabetes in the future.

FGF21 has anti-inflammatory, anti-oxidative and anti-apoptotic properties [4], which may explain its protection against CVD events, including cardiac hypertrophy and myocardial injury, in experimental mouse models [39–42]. This is consistent with some cross-sectional human studies, where elevated circulating FGF21 levels have been reported in patients with CHD [12], acute myocardial infarction [43], unstable angina pectoris [44], and CAD [45]. We have previously reported that higher FGF21 levels can predict a higher risk of CVD events in patients with type 2 diabetes [14], as well as in patients with stable CAD and treated with atorvastatin [16]. In another study of 169 patients with CAD at baseline, higher FGF21 levels were associated with higher risk of major adverse cardiovascular events [46]. However, in the present study of apparently healthy participants, we did not find a significant independent association of FGF21 levels with different CVD events. The association of FGF21 levels with measures of subclinical atherosclerosis and CVD events can be explained by the association of FGF21 levels with other CVD risk factors. Taking the findings from our previous studies, circulating FGF21 is more likely to be a useful biomarker for CVD events in people with high CVD risk, such as those with type 2 diabetes and CAD, of whom their circulating FGF21 levels are already elevated due to FGF21 resistance, but not in those who are less likely to have FGF21 resistance. The analysis of non-linear associations in the present study also suggests that elevated FGF21 levels may be associated with higher risks of hard CVD and hard CHD endpoints, but only among participants with higher FGF21 levels. Nevertheless, further studies in other independent cohorts of apparently healthy people are needed to confirm this hypothesis.

The present study has several strengths: a large and well-characterized multi-ethnic sample of participants free of clinical CVD at the time of recruitment, stringent quality control measures, long follow-up period, and availability of data on several measures of subclinical atherosclerosis. However, there are also some limitations in this study that need to be considered. The longitudinal analysis of the change in FGF21 levels was not possible, as FGF21 was measured only at baseline. Thus, as for all cross-sectional analyses, temporal and selection biases may have occurred. In addition, we cannot exclude the possibility of misclassification due to long-term sample storage, although we did not find any correlation between FGF21 levels and length of sample storage (Spearman correlation coefficient = 0.008, $p = 0.72$) in our previous study using stored plasma samples from the TNT trial [16]. However, such misclassification would likely be nondifferential.

In conclusion, in the present study of an apparently healthy multi-ethnic cohort, circulating FGF21 levels were not cross-sectionally associated with measures of subclinical atherosclerosis, including carotid IMT, ABI and CAC, and were not predictive of incident CVD events. Our findings do not support FGF21 as a CVD biomarker in an ethnically diverse and healthy population, and further studies are needed to confirm its role as CVD biomarker in different clinical settings.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

Kwok Leung Ong was supported by the Vice-Chancellor's Postdoctoral Fellowship from the University of New South Wales (RG134592) and the Australian National Health and Medical Research Council Career Development Fellowship (1122854). The measurement of FGF21 levels in MESA samples was supported by the NSW CVRN Research Development Project Grant (100715) from the National Heart

Foundation of Australia to Dr. Kwok Leung Ong. The MESA study was supported by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, and N01-HC-95169 from the National Heart, Lung, and Blood Institute, United States, and by grants UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420 from National Center for Advancing Translational Sciences, United States.

Author contributions

K.L.O. and S.C. participated in data analysis and wrote the manuscript; K.L.O., R.L.M., M.A.A., and K.A.R. participated in the study design. K.L.O., B.J.W. and J.K. measured FGF21 levels in MESA samples. All authors participated in data interpretation and critical revision of the manuscript.

Acknowledgements

The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.06.898.

References

[1] A. Kharitonenkov, T.L. Shiyanova, A. Koester, A.M. Ford, R. Micanovic, E.J. Galbreath, et al., FGF-21 as a novel metabolic regulator, J. Clin. Investig. 115 (2005) 1627–1635.
[2] M.K. Badman, P. Pissios, A.R. Kennedy, G. Koukos, J.S. Flier, E. Maratos-Flier, Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states, Cell Metabol. 5 (2007) 426–437.
[3] T. Inagaki, P. Dutchak, G. Zhao, X. Ding, L. Gautron, V. Parameswara, et al., Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21, Cell Metabol. 5 (2007) 415–425.
[4] Y.C. Woo, A. Xu, Y. Wang, K.S. Lam, Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives, Clin. Endocrinol. 78 (2013) 489–496.
[5] W. Wente, A.M. Efanov, M. Brenner, A. Kharitonenkov, A. Köster, G.E. Sandusky, et al., Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways, Diabetes 55 (2006) 2470–2478.
[6] A. Kharitonenkov, V.J. Wroblewski, A. Koester, Y.F. Chen, C.K. Clutinger, X.T. Tigno, et al., The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21, Endocrinology 148 (2007) 774–781.
[7] A.O. Chavez, M. Molina-Carrion, M.A. Abdul-Ghani, F. Folli, R.A. Defronzo, D. Tripathy, Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance, Diabetes Care 32 (2009) 1542–1546.
[8] M. Mraz, M. Bartlova, Z. Lacinova, D. Michalsky, M. Kasalicky, D. Haluzikova, et al., Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor-21 in patients with type 2 diabetes and obesity, Clin. Endocrinol. 71 (2009) 369–375.
[9] X. Zhang, D.C. Yeung, M. Karpisek, D. Stejskal, Z.G. Zhou, F. Liu, et al., Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans, Diabetes 57 (2008) 1246–1253.
[10] H. Li, Y. Bao, A. Xu, X. Pan, J. Lu, H. Wu, et al., Serum fibroblast growth factor 21 is associated with adverse lipid profiles and gamma-glutamyltransferase but not insulin sensitivity in Chinese subjects, J. Clin. Endocrinol. Metab. 94 (2009) 2151–2156.
[11] H. Li, Q. Fang, F. Gao, J. Fan, J. Zhou, X. Wang, et al., Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride, J. Hepatol. 53 (2010) 934–940.
[12] Z. Lin, Z. Wu, X. Yin, Y. Liu, X. Yan, S. Lin, et al., Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile, PLoS One 5 (2010) e15534.
[13] C. Chen, B.M. Cheung, A.W. Tso, Y. Wang, L.S. Law, K.L. Ong, et al., High plasma level of fibroblast growth factor 21 is an Independent predictor of type 2 diabetes: a 5.4-year population-based prospective study in Chinese subjects, Diabetes Care 34 (2011) 2113–2115.
[14] K.L. Ong, A.S. Januszewski, R. O'Connell, A.J. Jenkins, A. Xu, D.R. Sullivan, et al., The relationship of fibroblast growth factor 21 with cardiovascular outcome events in the Fenofibrate Intervention and Event Lowering in Diabetes study, Diabetologia 58 (2015) 464–473.
[15] K.L. Ong, A.S. Januszewski, R. O'Connell, L. Buizen, A.J. Jenkins, A. Xu, et al., Relationship of fibroblast growth factor 21 with baseline and new on-study microvascular disease in the Fenofibrate Intervention and Event Lowering in Diabetes study, Diabetologia 58 (2015) 2035–2044.
[16] K.L. Ong, N. Hui, A.S. Januszewski, N.O. Kaakoush, A. Xu, R. Fayyad, et al., High plasma FGF21 levels predicts major cardiovascular events in patients treated with atorvastatin (from the Treating to New Targets [TNT] Study), Metabolism 93 (2019) 93–99.
[17] D.E. Bild, D.A. Bluemke, G.L. Burke, R. Detrano, A.V. Diez Roux, A.R. Folsom, et al., Multi-ethnic study of atherosclerosis: objectives and design, Am. J. Epidemiol. 156 (2002) 871–881.
[18] K.L. Ong, K.A. Rye, R. O'Connell, A.J. Jenkins, C. Brown, A. Xu, et al., Long-term fenofibrate therapy increases fibroblast growth factor 21 and retinol-binding protein 4 in subjects with type 2 diabetes, J. Clin. Endocrinol. Metab. 97 (2012) 4701–4708.
[19] D.H. O'Leary, J.F. Polak, R.A. Kronmal, T.A. Manolio, G.L. Burke, S.K. Wolfson Jr., Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group, N. Engl. J. Med. 340 (1999) 14–22.
[20] A.R. Folsom, R.A. Kronmal, R.C. Detrano, D.H. O'Leary, D.E. Bild, D.A. Bluemke, et al., Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA), Arch. Intern. Med. 168 (2008) 1333–1339.
[21] E.Z. Soliman, J. Ding, F.C. Hsu, J.J. Carr, J.F. Polak, D.C. Goff Jr., Association between carotid intima-media thickness and pericardial fat in the Multi-Ethnic Study of Atherosclerosis (MESA), J. Stroke Cerebrovasc. Dis. 19 (2010) 58–65.
[22] J.F. Polak, D.H. O'Leary, Carotid intima-media thickness as surrogate for and predictor of CVD, Glob. Heart 11 (2016) 295–312 e3.
[23] A.G. Bertoni, M.C. Whitt-Glover, H. Chung, K.Y. Le, R.G. Barr, M. Mahesh, et al., The association between physical activity and subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis, Am. J. Epidemiol. 169 (2009) 444–454.
[24] G.H. Tison, C.E. Ndumele, G. Gerstenblith, M.A. Allison, J.F. Polak, M. Szklo, Usefulness of baseline obesity to predict development of a high ankle brachial index (from the Multi-Ethnic Study of Atherosclerosis), Am. J. Cardiol. 107 (2011) 1386–1391.
[25] J.J. Carr, J.C. Nelson, N.D. Wong, M. McNitt-Gray, Y. Arad, D.R. Jacobs Jr.et al., Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study, Radiology 234 (2005) 35–43.
[26] A.S. Agatston, W.R. Janowitz, F.J. Hildner, N.R. Zusmer, M. Viamonte Jr., R. Detrano, Quantification of coronary artery calcium using ultrafast computed tomography, J. Am. Coll. Cardiol. 15 (1990) 827–832.
[27] V. Veeranna, S.K. Zalawadiya, A. Niraj, A. Kumar, B. Ference, L. Afonso, Association of novel biomarkers with future cardiovascular events is influenced by ethnicity: results from a multi-ethnic cohort, Int. J. Cardiol. 166 (2013) 487–493.
[28] K.L. Ong, R.L. McClelland, M.A. Allison, J. Kokkinos, B.J. Wu, P.J. Barter, et al., Association of elevated circulating fibroblast growth factor 21 levels with prevalent and incident metabolic syndrome: the Multi-Ethnic Study of Atherosclerosis, Atherosclerosis 281 (2019) 200–206.
[29] P.K. Garg, R.L. McClelland, N.S. Jenny, M. Criqui, K. Liu, J.F. Polak, et al., Association of lipoprotein-associated phospholipase A(2) and endothelial function in the Multi-Ethnic Study of Atherosclerosis (MESA), Vasc. Med. 16 (2011) 247–252.
[30] A.B. Newman, D.S. Siscovick, T.A. Manolio, J. Polak, L.P. Fried, N.O. Borhani, et al., Ankle-arm index as a marker of atherosclerosis in the cardiovascular Health study. Cardiovascular heart study (CHS) collaborative Research group, Circulation 88 (1993) 837–845.
[31] K.L. Ong, R.L. McClelland, K.A. Rye, B.M. Cheung, W.S. Post, D. Vaidya, et al., The relationship between insulin resistance and vascular calcification in coronary arteries, and the thoracic and abdominal aorta: the Multi-Ethnic Study of Atherosclerosis, Atherosclerosis 236 (2014) 257–262.
[32] Z. Lin, X. Pan, F. Wu, D. Ye, Y. Zhang, Y. Wang, et al., Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice, Circulation 131 (2015) 1861–1871.
[33] W.S. Chow, A. Xu, Y.C. Woo, A.W. Tso, S.C. Cheung, C.H. Fong, et al., Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 2454–2459.
[34] S. Yafei, F. Elsewy, E. Youssef, M. Ayman, M. El-Shafei, Fibroblast growth factor 21 association with subclinical atherosclerosis and arterial stiffness in type 2 diabetes, Diabetes Metab. Syndr. 13 (2019) 882–888.
[35] S.Y. An, M.S. Lee, S.A. Yi, E.S. Ha, S.J. Han, H.J. Kim, et al., Serum fibroblast growth factor 21 was elevated in subjects with type 2 diabetes mellitus and was associated with the presence of carotid artery plaques, Diabetes Res. Clin. Pract. 96 (2012) 196–203.
[36] Y. Xiao, L. Liu, A. Xu, P. Zhou, Z. Long, Y. Tu, et al., Serum fibroblast growth factor 21 levels are related to subclinical atherosclerosis in patients with type 2 diabetes, Cardiovasc. Diabetol. 14 (2015) 72.
[37] Y. Miyazaki, E. Saita, Y. Kishimoto, S. Ibe, T. Seki, K. Miura, et al., Low plasma levels of fibroblast growth factor-21 in patients with peripheral artery disease, J. Atheroscler. Thromb. 25 (2018) 821–828.
[38] Y. Shi, W. Lu, Y. Hou, K. Fu, F. Gan, J. Liu, Fibroblast growth factor 21 ameliorates

vascular calcification by inhibiting osteogenic transition in vitamin D3 plus nicotine-treated rats, Biochem. Biophys. Res. Commun. 495 (2018) 2448–2455.
[39] A. Planavila, I. Redondo, E. Hondares, M. Vinciguerra, C. Munts, R. Iglesias, et al., Fibroblast growth factor 21 protects against cardiac hypertrophy in mice, Nat. Commun. 4 (2013) 2019.
[40] V. Patel, R. Adya, J. Chen, M. Ramanjaneya, M.F. Bari, S.K. Bhudia, et al., Novel insights into the cardio-protective effects of FGF21 in lean and obese rat hearts, PLoS One 9 (2014) e87102.
[41] S.Q. Liu, D. Roberts, A. Kharitonenkov, B. Zhang, S.M. Hanson, Y.C. Li, et al., Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue, Sci. Rep. 3 (2013) 2767.
[42] A. Planavila, I. Redondo-Angulo, F. Ribas, G. Garrabou, J. Casademont, M. Giralt, et al., Fibroblast growth factor 21 protects the heart from oxidative stress, Cardiovasc. Res. 106 (2014) 19–31.
[43] W. Zhang, S. Chu, W. Ding, F. Wang, Serum level of fibroblast growth factor 21 is independently associated with acute myocardial infarction, PLoS One 10 (2015) e0129791.
[44] J. Cheng, X. Su, L. Qiao, C. Zhai, W. Chen, Circulating level of fibroblast growth factor 21 is independently associated with the risks of unstable angina pectoris, Biosci. Rep. 38 (2018) BSR20181099.
[45] Y. Shen, X. Ma, J. Zhou, X. Pan, Y. Hao, M. Zhou, et al., Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease, Cardiovasc. Diabetol. 12 (2013) 124.
[46] Y. Shen, X. Zhang, Y. Xu, Q. Xiong, Z. Lu, X. Ma, et al., Serum FGF21 is associated with future cardiovascular events in patients with coronary artery disease, Cardiology 139 (2018) 212–218.