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ORIGINAL RESEARCH

Fibroblast Growth Factor 21 is Related to Atherosclerosis Independent of Nonalcoholic Fatty Liver Disease and Predicts Atherosclerotic Cardiovascular Events

Liang Wu, MD*; Lingling Qian, PhD*; Lei Zhang, PhD; Jing Zhang, PhD; Jia Zhou, MD; Yuehua Li, PhD; Xuhong Hou, MD; Qichen Fang, PhD; Huating Li, MD, PhD; Weiping Jia, MD, PhD

BACKGROUND: FGF21 (fibroblast growth factor 21), a novel hepatokine regulating lipid metabolism, has been linked to atherosclerotic disease. However, whether this relationship exists in patients without nonalcoholic fatty liver disease is unclear. We assessed the association between serum FGF21 levels and atherosclerosis in patients without nonalcoholic fatty liver disease, and investigated whether baseline FGF21 could predict incident atherosclerotic cardiovascular disease in a 7-year prospective cohort.

METHODS AND RESULTS: Baseline serum FGF21 was measured in a cross-sectional cohort of 371 patients with type 2 diabetes mellitus without nonalcoholic fatty liver disease (determined by hepatic magnetic resonance spectroscopy), and in a population-based prospective cohort of 705 patients from the Shanghai Diabetes Study. In the cross-sectional study, FGF21 was significantly higher in patients with than in those without subclinical carotid atherosclerosis ($P<0.01$). The association remained significant after adjusting for demographic and traditional cardiovascular risk factors. In the prospective cohort, 80 patients developed atherosclerotic cardiovascular disease during follow-up. Baseline FGF21 was significantly higher in those who developed ischemic heart disease or cerebral infarction than in those who did not. Using a cutoff serum concentration of 232.0 pg/mL, elevated baseline FGF21 independently predicted incident total atherosclerotic cardiovascular disease events, ischemic heart disease, and cerebral infarction in a nondiabetic population (all $P<0.05$), and significantly improved the discriminatory and reclassifying abilities of our prediction model after adjustment for established cardiovascular risk factors.

CONCLUSIONS: This study provides the first evidence that FGF21 levels are elevated in patients without nonalcoholic fatty liver disease with subclinical atherosclerosis. Baseline FGF21 is an independent predictor of atherosclerotic cardiovascular disease and represents a novel biomarker for primary prevention in the general population.

Key Words: atherosclerosis ■ biomarker ■ cardiovascular events ■ fibroblast growth factor 21 ■ nonalcoholic fatty liver disease

The major cause of cardiovascular disease (CVD) is atherosclerosis, which develops insidiously throughout life and is usually advanced by the time symptoms occur.[1] In 2015, ≈17.92 million people died from CVD worldwide, mostly as a result of ischemic heart disease (IHD) and stroke.[2] The World Health Organization (WHO) has set a goal to reduce the risk of premature death from noncommunicable diseases, including CVD, by 25% by 2025.[3] Early detection and management of cardiovascular risk factors is key in the prevention of incident CVD events. However, it is difficult to identify individuals with higher CVD risk for early intervention, highlighting the need to identify additional biomarkers for CVD risk screening and stratification.

Correspondence to: Huating Li, MD, PhD, 600 Yishan Road, Shanghai 200233, China. E-mail: huarting99@sjtu.edu.cn

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*Dr Wu and Dr Qian contributed equally to this work.

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CLINICAL PERSPECTIVE

What Is New?

- We used liver proton magnetic resonance spectroscopy to exclude the confounder factor of nonalcoholic fatty liver disease, and determined that serum FGF21 (fibroblast growth factor 21) levels were still elevated in patients with subclinical atherosclerosis.
- Baseline serum FGF21 was an independent predictor for atherosclerotic cardiovascular disease, including ischemic heart disease and cerebral infarction, among patients without diabetes mellitus.

What Are the Clinical Implications?

- Circulating FGF21 could be utilized as a novel serum biomarker for risk evaluation and primary prevention of atherosclerotic cardiovascular disease in the general population.

Nonstandard Abbreviations and Acronyms

ASCVD	atherosclerotic cardiovascular disease
BMI	body mass index
C-IMT	carotid intima-media thickness
CVD	cardiovascular disease
DHS	Dallas Heart Study
DM	diabetes mellitus
eGFR	estimated glomerular filtration rate
FGF21	fibroblast growth factor 21
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
HDL-C	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment for insulin resistance index
HKDR	Hong Kong West Diabetes Registry
hsCRP	high-sensitivity C-reactive protein
IHD	ischemic heart disease
IQR	interquartile range
LDL-C	low-density lipoprotein cholesterol
MESA	Multi-Ethnic Study of Atherosclerosis
NAFLD	nonalcoholic fatty liver disease
SHDS	Shanghai Diabetes Study
TNT	Treating to New Targets study
T2DM	type 2 diabetes mellitus
WHO	World Health Organization

FGF21 (fibroblast growth factor 21) is a novel metabolic regulator predominantly produced by the liver.[4,5] As it lacks a conventional heparin-binding domain, FGF21 can be secreted into the circulation, where it acts as an endocrine hormone.[6] In animal studies, FGF21 has several beneficial metabolic effects, including anti-inflammatory, antidiabetes mellitus, antiobesity, and lipid-lowering profiles.[7–9] However, in human studies, elevated serum FGF21 levels are found in cardiometabolic diseases such as type 2 diabetes mellitus (T2DM), obesity, dyslipidemia, and, in particular, nonalcoholic fatty liver disease (NAFLD).[10–12] NAFLD is one of the biggest influences on FGF21 levels. Both hepatic and circulating FGF21 levels are significantly higher in patients with NAFLD and are strongly correlated with intrahepatic triglycerides.[12] Elevated circulating FGF21 levels in patients with impaired glucose tolerance are mediated by steatosis rather than insulin sensitivity.[13]

Despite all of this work, the association between circulating FGF21 levels and CVD remains unclear. First, contradictory results were reported in several cross-sectional studies. In some studies, serum FGF21 levels were elevated in patients with atherosclerosis or coronary heart disease,[14–17] while other studies reported negative results.[18,19] In these studies, liver steatosis, the determining factor of serum FGF21 levels and also a risk factor for atherosclerosis, was not evaluated or considered. Second, in long-term prospective studies, increased serum FGF21 levels can predict CVD events in patients with T2DM or established CVD at baseline.[20–22] However, because circulating FGF21 levels are already elevated in patients with diabetes mellitus (DM), whether it could predict incident CVD in the general population and in patients without DM remains to be explored.

In this study, we investigated the association between serum FGF21 levels and subclinical carotid atherosclerosis in a cross-sectional study, in which participants were evaluated for the presence of NAFLD by liver proton magnetic resonance spectroscopy, and patients with NAFLD were excluded. Furthermore, we investigated whether serum FGF21 could be a useful biomarker to predict the development of atherosclerotic CVD (ASCVD) events in a population-based 7-year prospective cohort.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants

Two cohorts of participants were recruited for this study. The first was a cross-sectional cohort consisting of 371 patients with T2DM without NAFLD. The patients were enrolled from the Department of

Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital between August 2015 and July 2016. All patients underwent liver proton magnetic resonance spectroscopy and carotid ultrasonography to measure their hepatic fat fractions and determine the occurrence of carotid atherosclerosis. Exclusion criteria included: (1) hepatic fat fraction ≥5.56%[23]; (2) clinical symptoms of atherosclerosis or the presence of CVD, including angina, myocardial infarction, heart failure, stroke, transient ischemic attack, or a prior invasive cardiovascular procedure; (3) age younger than 45 or older than 75 years; (4) liver or kidney dysfunction (alanine transaminase >40 U/L or estimated glomerular filtration rate [eGFR] <30 mL/min per 1.73 m^2); (5) alcoholism (≥140 g per week for men or ≥70 g per week for women); (6) known history of viral hepatitis, autoimmune hepatitis, severe infection, or cancer; and (7) current use of lipid-lowering drugs such as fenofibrate (which significantly increases hepatic FGF21 expression).[24]

The second cohort was a prospective cohort from the second study of the SHDS (Shanghai Diabetes Study), which has previously been described.[25] A flow diagram of the cohort is provided in Figure S1. Briefly, in 2004, a total of 1651 patients from Caoyang community aged 33 to 96 years were enrolled in a baseline survey. From 2010 to 2011, these patients were invited for follow-up assessments. Of the 1651, 642 patients were not included because of emigration and 112 patients died during the follow-up period as a result of non-CVD causes. Of the 897 patients enrolled in the follow-up, 63 were excluded because of known CVD at baseline and 129 were excluded because of insufficient sample volume for serum FGF21 measurement. All patients except for those taking antidiabetic drugs underwent a 75-g oral glucose tolerance test. The study was performed in accordance with the Declaration of Helsinki and the protocol was approved by the human research ethics committee of the Shanghai Sixth People's Hospital. Written informed consent was obtained from all participants.

Clinical and Biochemical Assessment

All participants were assessed after overnight fasting and underwent comprehensive physical examinations and routine biochemical analyses of blood samples. Anthropometric measurements included body weight, height, waist circumference, and blood pressure. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using standard enzymatic methods on a biochemical analyzer (7600-120; Hitachi). Plasma glucose was determined using the glucose oxidase method. Glycated hemoglobin levels, using high-performance liquid chromatography on a VARIANT II Hemoglobin Testing System (Bio-Rad Laboratories, Inc). Basal insulin resistance was evaluated using the homeostatic model assessment for insulin resistance index (HOMA-IR).[26] The eGFR was calculated using the formula developed by the Chronic Kidney Disease Epidemiology Collaboration.[27] Detailed medical, drug, and family histories, including any history of CVD, were obtained using a standardized questionnaire.

Circulating FGF21 levels were quantified using an ELISA kit. The intra-assay and interassay coefficients of variation were 4.5% and 6.9%, respectively. The serum lipocalin-2 concentration was also measured using an ELISA kit, with intra-assay and interassay variations of 3.1% and 9.0%, respectively. Serum C-reactive protein levels were measured using a hsCRP (high-sensitivity C-reactive protein) assay. The intra-assay and interassay variations were 2.0% and 9.8%, respectively. The abovementioned assays were obtained from Antibody and Immunoassay Services, The University of Hong Kong.

Carotid Ultrasonography

A high-resolution B-mode scanner (VOLUSON 730 pro, GE) and a 10.0-MHz probe were used for carotid artery scanning. An experienced ultrasound doctor blinded to the study scanned both common carotid arteries. Carotid intima-media thickness (C-IMT) was measured on the far wall of the common carotid arteries, 1 cm proximal to the carotid bulb. The C-IMT value was measured as the highest value of intima-media thickness of each carotid artery in both sides. Plaque was defined by C-IMT ≥1.3 mm or the presence of a focal protrusion into the lumen with a thickness at least 50% greater than the adjacent intima-media complex. Subclinical atherosclerosis was defined as C-IMT >1.0 mm and/or the presence of plaque without clinical manifestations.[28]

Diagnosis of NAFLD

NAFLD was diagnosed by hepatic steatosis at proton magnetic resonance spectroscopy in the absence of history of excessive alcohol consumption and any other specific causes of hepatic steatosis. Proton magnetic resonance spectroscopy was performed on an Ingenia 3T magnetic resonance system (Philips). The fat fraction was measured in a single voxel (2×2×2 cm^3) placed at the right lobe of the liver, avoiding liver edges, large vessels, and bile ducts. Hepatic steatosis was defined by fat fraction ≥5.56%, the 95th percentile of hepatic triglyceride content

among 2287 healthy participants in the DHS (Dallas Heart Study).[23]

Diagnosis of Metabolic Disorders and ASCVD

DM was diagnosed according to 1999 WHO classifications.[29] Hypertension was defined as a sitting blood pressure ≥140/90 mm Hg or current use of antihypertensive medication. Dyslipidemia was defined as having ≥1 of the following criteria: (1) triglycerides ≥1.7 mmol/L; (2) total cholesterol ≥5.2 mmol/L; (3) HDL-C <1.04 mmol/L; (4) LDL-C ≥3.4 mmol/L; and (5) current use of lipid-lowering medications.[30]

Diagnoses of ASCVD events (IHD and cerebral infarction) were obtained from individual health records, using categories I21 to 25 and I63 of the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*, respectively. Mortality information was ascertained by the Shanghai Municipal Center for Disease Control and Prevention.

Statistical Analysis

Normally distributed data were expressed as mean±SD. Data with a skewed distribution were expressed as the median with interquartile range (IQR) and were natural log-transformed before analysis. One-way ANOVA was used to compare continuous variables between groups. Chi-square tests were used to compare differences in the proportions of categorical variables between groups. Multivariable linear regression was used to examine the associations between serum FGF21 levels and various parameters. Logistic regression was used to identify independent factors of subclinical atherosclerosis. The Youden index was calculated as sensitivity+specificity–1.[31] To identify independent predictors of incident ASCVD events, baseline variables were analyzed using univariable and multivariable Cox proportion hazards regression. C-statistics were calculated using R package survC1. The incremental value of FGF21 with reference to the baseline model in predicting incident ASCVD was evaluated by net reclassification index and integrated discrimination improvement.[32] Cumulative hazard curves of incident ASCVD was tested by log-rank test. Interaction by sex was evaluated in multivariable logistic regression and Cox proportional hazards regression by including the interaction term in the fully adjusted models. No significant interaction with sex was detected. Thus, we did not stratify the results by sex. All statistical analyses were performed in SPSS 22.0 (IBM) and R software 3.3.3 (Package survC1 and PredictABEL). Two-sided $P<0.05$ values were considered statistically significant.

RESULTS

Serum FGF21 Levels are Independently Associated With Subclinical Carotid Atherosclerosis in Patients Without NAFLD: Results From the Cross-Sectional Study

A total of 371 patients with T2DM but without NAFLD were included in the cross-sectional study. Their demographic and biochemical characteristics are summarized in Table 1. The average age and BMI in this cohort were 56.5±8.9 years and 23.3±2.7 kg/m^2, respectively. Patients with subclinical carotid atherosclerosis tended to be older, men, and smokers; they also had higher BMI, C-IMT, systolic blood pressure, diastolic blood pressure, triglycerides, total cholesterol, LDL-C, and HDL-C values. After adjustment for age and sex, BMI, diastolic blood pressure, triglycerides, HDL-C, and LDL-C remained statistically different between patients with and without atherosclerosis. Notably, serum FGF21 levels were significantly higher in patients with atherosclerosis (median, 266.7 pg/mL; IQR, 135.5–415.2) versus those without (198.4 pg/mL; IQR, 99.9–373.6 [$P=0.005$]), and this remained statistically significant after age and sex adjustment ($P=0.002$).

We next analyzed the association of serum FGF21 levels with related factors. Serum FGF21 levels were higher in patients with dyslipidemia (303.1 pg/mL; IQR, 154.1–459.7) versus without (168.2 pg/mL; IQR, 93.1–295.5 [$P<0.001$]) and with hypertension (268.0 pg/mL; IQR, 136.0–440.3) versus without (208.9 pg/mL; IQR, 107.4–374.2 [$P=0.017$]), but no significant difference was observed between smokers and nonsmokers (Table S1). In linear regression analysis of log-transformed serum FGF21 levels with other parameters, FGF21 levels had a positive correlation with triglycerides and an inverse correlation with HDL-C after adjusted for age and BMI (both $P<0.001$, Table S2).

To evaluate independent risk factors for carotid atherosclerosis, we conducted a multivariable logistic regression model with log-transformed serum FGF21 levels and other conventional atherosclerosis risk factors, including sex, age, BMI, waist circumference, HOMA-IR, hsCRP, smoking, hypertension, and dyslipidemia. Only FGF21 (odds ratio [OR], 1.315; 95% CI, 1.033–1.674 [$P=0.026$]), age (OR, 1.062; 95% CI, 1.033–1.091 [$P<0.001$]), and smoking (OR, 2.007; 95% CI, 1.099–3.665 [$P=0.023$]) were significantly correlated with carotid atherosclerosis (Table 2).

Serum FGF21 Levels are a Predictor of Incident CVD Events in a Community-Based Population: Results From the 7-Year Prospective Study

In the prospective cohort, there were no significant differences in age, sex, BMI, or rate of DM at baseline

Table 1. Characteristics of Patients in the Cross-Sectional Cohort

Variables	Total N=371	Nonatherosclerosis n=185	Atherosclerosis n=186	*P* Value*
Age, y	56.5±8.9	54.4±9.0	58.6±8.4	<0.001
Men	210 (56.6)	95 (51.4)	115 (61.8)	0.042
BMI, kg/m^2	23.3±2.7	23.0±2.6	23.7±2.8	0.023
Waist circumference, cm	87.1±8.6	85.9±8.3	88.3±8.8	0.070
C-IMT, mm	0.79±0.16	0.73±0.13	0.87±0.16	<0.001
SBP, mm Hg†	125 (120–135)	120 (118–132)	129 (120–135)	0.012
DBP, mm Hg†	78 (70–80)	78 (70–80)	79 (70–80)	0.030
FBG, mmol/L†	6.7 (5.5–8.6)	6.5 (5.3–8.6)	6.8 (5.7–8.5)	0.403
2hBG, mmol/L†	11.5 (8.0–14.8)	11.5 (7.5–14.7)	11.6 (8.4–15.0)	0.395
Glycated hemoglobin, mmol/mol†	7.80 (6.525–9.70)	7.70 (6.30–9.90)	7.85 (6.70–9.60)	0.757
Fasting insulin, mmol/L†	9.7 (5.1–17.1)	9.9 (5.2–16.5)	9.2 (4.9–17.9)	0.994
HOMA-IR†	2.9 (1.4–5.7)	2.9 (1.4–5.7)	3.0 (1.5–6.3)	0.777
Triglycerides, mmol/L†	1.1 (0.8–1.5)	1.0 (0.7–1.4)	1.1 (0.8–1.6)	0.032
Total cholesterol, mmol/L†	4.4 (3.8–5.1)	4.2 (3.7–4.9)	4.5 (3.9–5.3)	0.013
HDL-C, mmol/L†	1.1 (0.9–1.4)	1.2 (1.0–1.4)	1.1 (0.9–1.3)	0.042
LDL-C, mmol/L†	2.6 (2.1–3.3)	2.4 (2.1–3.1)	2.8 (2.3–3.3)	0.005
hsCRP, mg/L†	0.60 (0.29–1.34)	0.59 (0.30–1.32)	0.61 (0.28–1.55)	0.147
Ever smoker	137 (36.9)	55 (29.7)	82 (44.1)	0.004
Dyslipidemia	215 (58.0)	99 (53.5)	116 (62.4)	0.090
Hypertension	153 (41.2)	69 (37.3)	84 (45.2)	0.140
FGF21, pg/mL†	233.5 (117.6–390.2)	198.4 (99.9–373.6)	266.7 (135.5–415.2)	0.005

Data are presented as mean±SD, median (interquartile range), or number (percentage). 2hBG indicates 2-hour blood glucose; BMI, body mass index; C-IMT, carotid intima-media thickness; DBP, diastolic blood pressure; FBG, fasting blood glucose; FGF21, fibroblast growth factor 21; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance index; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; and SBP, systolic blood pressure.

*Atherosclerosis group vs nonatherosclerosis group.

†Log-transformed before analysis.

between the patients included in the analysis and the 946 excluded patients. Of the 705 patients with complete follow-up information and sufficient baseline serum samples, 97 patients (13.8%) developed CVD events during the follow-up period, with a median follow-up duration of 74 months. Among these patients, 80 developed ASCVD; 44 with cerebral infarction and 36 with IHD. Baseline characteristics of this cohort are shown in Table 3. Compared with patients without incident CVD, patients who developed ASCVD were older; had higher blood glucose, glycated hemoglobin, systolic blood pressure, and hsCRP levels; and were more likely to have DM and hypertension. In addition, patients who developed IHD had higher waist circumference and lipocalin-2 and lower eGFR. Serum FGF21 levels were significantly elevated both in patients with incident IHD (479.5 pg/mL, 302.4–627.0; *P*=0.004) and those with incident cerebral infarction (401.6 pg/mL, 238.3–616.4; *P*=0.021) compared with patients without incident CVD (325.2 pg/mL, 189.0–498.9). In linear regression analysis of log-transformed serum FGF21 levels with other parameters, FGF21 levels had a positive correlation with triglycerides and an inverse correlation with HDL-C after adjusted for age and BMI (both *P*<0.001, Table S3).

Table 2. Multivariable Logistic Regression Showing the Independent Factors for Subclinical Atherosclerosis

Variables	OR (95% CI)	*P* Value
FGF21*	1.315 (1.033–1.674)	0.026
Age	1.062 (1.033–1.091)	<0.001
Ever smoker	2.007 (1.099–3.665)	0.023
Men	1.060 (0.585–1.921)	0.847
Hypertension	0.951 (0.588–1.538)	0.837
Dyslipidemia	1.005 (0.625–1.617)	0.984
BMI	1.077 (0.958–1.211)	0.214
Waist circumference	1.005 (0.968–1.043)	0.812
HOMA-IR	0.991 (0.953–1.030)	0.634
hsCRP	1.012 (0.990–1.034)	0.291

BMI indicates body mass index; HOMA-IR, homeostatic model assessment for insulin resistance index; and hsCRP, high-sensitivity C-reactive protein.

*Odds ratios (ORs) were expressed as per SD increase in log-transformed fibroblast growth factor 21 (FGF21).

Table 3. Baseline Characteristics of Patients in the Prospective Cohort

Variables	No Incident CVD (n=608)	Developed IHD (n=36)	Developed Cerebral Infarction (n=44)
Age, y	57.6±13.5	71.6±14.1†	71.4±10.6†
Men	236 (38.8)	20 (55.6)	18 (40.9)
BMI, kg/m²‡	24.1 (21.7–26.2)	24.9 (22.5–26.6)	23.4 (21.6–25.9)
Waist circumference, cm	82.2±9.7	86.1±8.4*	83.6±10.8
SBP, mm Hg‡	120 (110–134)	132 (119–150)†	130 (120–140)†
DBP, mm Hg‡	75 (70–80)	79 (71–86)	80 (71–80)
Hypertension	189 (31.1)	22 (61.1)†	27 (61.4)
Ever smoker	155 (25.5%)	10 (27.8%)	8 (18.2%)
DM	73 (12.0%)	9 (25.0%)*	16 (36.4%)†
FBG, mmol/L‡	5.19 (4.85–5.60)	5.54 (5.15–6.35)*	5.46 (5.17–6.81)†
2hBG, mmol/L‡	6.20 (5.10–7.21)	6.75 (6.27–8.20)*	6.75 (6.17–8.75)†
HOMA-IR‡	0.84 (0.50–1.42)	0.96 (0.68–1.43)	0.97 (0.56–1.60)
Glycated hemoglobin, %‡	5.7 (5.3–6.1)	6.0 (5.7–6.6)†	5.8 (5.5–6.6)†
Triglycerides, mmol/L‡	1.45 (1.02–2.25)	1.87 (1.24–2.83)	1.66 (1.14–2.79)*
Total cholesterol, mmol/L‡	4.80 (4.20–5.50)	4.95 (4.33–5.70)	5.20 (4.25–5.95)
HDL-C, mmol/L‡	1.32 (1.10–1.55)	1.24 (1.05–1.42)	1.25 (1.10–1.48)
LDL-C, mmol/L	2.75±0.77	3.01±0.99	2.81±0.80
Dyslipidemia	349 (57.4)	24 (66.7)	32 (72.7)
eGFR, mL/min per 1.73 m²‡	100.3 (85.6–114.7)	80.1 (60.5–102.6)†	89.6 (79.0–114.2)
hsCRP, mg/L‡	0.9 (0.4–2.2)	1.5 (0.7–7.1)†	2.4 (0.9–5.4)†
Lipocalin-2, pg/mL‡	15.4 (10.6–22.7)	18.6 (13.6–27.8)†	18.5 (9.9–25.0)
FGF21, pg/mL‡	325.2 (189.0–498.9)	479.5 (302.4–627.0)†	401.6 (238.3–616.4)*

Data are presented as mean±SD, median (interquartile range), or number (percentage). 2hBG indicates 2-hour blood glucose; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; FGF21, fibroblast growth factor 21; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance index; hsCRP, high-sensitivity C-reactive protein; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; and SBP, systolic blood pressure.

*P<0.05 and †P<0.01 compared with no incident cardiovascular disease (CVD) group.

‡Log-transformed before analysis.

The predictive ability of baseline serum FGF21 concentrations for incident ASCVD, IHD, and cerebral infarction during the follow-up period was evaluated with Cox proportional hazard models (Table 4). For the 705 patients in this cohort, using an optimal FGF21 level cutoff of 232.0 pg/mL (obtained from the Youden index), elevated serum FGF21 was associated with incident ASCVD, IHD, and cerebral infarction in the unadjusted model. In the fully adjusted model, after adjusting for other conventional cardiovascular risk factors (age, sex, BMI, fasting blood glucose, glycated hemoglobin, HOMA-IR, eGFR, hsCRP, smoking status, dyslipidemia, and hypertension) and lipocalin-2 levels, higher serum FGF21 concentration remained an independent predictor for incident total ASCVD and IHD. In our study, 602 of 705 patients did not have DM at baseline. In this subsample of patients, serum FGF21 concentration remained an independent predictor for all atherosclerotic outcomes in the fully adjusted model. However, as there were only 103 patients with DM, we did not observe a statistically significant association among patients with DM, presumably because of inadequate sample size.

Figure shows the Kaplan–Meier curve of the cumulative hazard for total incident ASCVD in the entire cohort, stratified by serum FGF21 concentration. For patients with baseline serum FGF21 levels >232.0 pg/mL, the overall incident rate for ASCVD events was 14.4%, significantly higher than in patients with lower baseline FGF21 levels (5.6%; log-rank test, P=0.001).

The ability of serum FGF21 measurements to improve discrimination and reclassification for total ASCVD events is shown in Table 5. The addition of FGF21 level to a baseline model containing traditional CVD risk factors (age, sex, BMI, eGFR, and hsCRP) and the novel biomarker lipocalin-2, as either a continuous variable or optimal cutoff, did not result in significant improvement in the C-statistics for the prediction of incident ASCVD events. However, it did significantly improve the net reclassification index of the baseline model (net reclassification index +0.240 and 0.389, P=0.041 and P<0.001, as a continuous variable and as an optimal cutoff, respectively). In addition, using serum FGF21 levels as an optimal cutoff resulted in a modest but significant improvement in the integrated

Table 4. HRs of Incident ASCVD, IHD, and Cerebral Infarction Related to Elevated Baseline Circulating FGF21 Levels of >232.0 pg/mL

	Cases, No. (%)	Unadjusted		Fully Adjusted Model	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Whole cohort (N=705)					
Total ASCVD	80 (11.3)	2.984 (1.607–5.540)	0.001	2.176 (1.156–4.096)	0.016
IHD	36 (5.10)	5.691 (1.736–18.654)	0.004	3.904 (1.153–13.213)	0.029
Cerebral infarction	44 (6.2)	2.276 (1.057–4.900)	0.036	1.918 (0.862–4.266)	0.11
Patients without DM at baseline (n=602)					
Total ASCVD	55 (9.1)	4.602 (1.958–10.813)	<0.001	3.185 (1.319–7.693)	0.01
IHD	27 (4.5)	6.497 (1.534–27.515)	0.011	4.536 (1.013–20.307)	0.048
Cerebral infarction	28 (4.7)	4.523 (1.364–15.000)	0.014	4.188 (1.193–14.704)	0.025
Patients with DM at baseline (n=103)					
Total ASCVD	25 (24.3)	1.098 (0.430–2.803)	0.845	1.398 0.478–4.086)	0.541
IHD	9 (8.7)	3.622 (0.423–31.018)	0.24	14.317 (0.281–730.75)	0.185
Cerebral infarction	16 (15.5)	0.691 (0.240–1.990)	0.494	0.696 (0.186–2.603)	0.59

Fully adjusted model: adjusted for age, sex, body mass index, fasting blood glucose, glycated hemoglobin, homeostatic model assessment for insulin resistance index, estimated glomerular filtration rate, high-sensitivity C-reactive protein, lipocalin-2, smoking status (never, ever-smoker), dyslipidemia (yes/no), and hypertension (yes/no). ASCVD indicates atherosclerotic cardiovascular disease; DM, diabetes mellitus; FGF21, fibroblast growth factor 21; HR, hazard ratio; and IHD, ischemic heart disease.

discrimination improvement for incident ASCVD prediction (integrated discrimination improvement +0.008, *P*=0.037).

DISCUSSION

In the cross-sectional study of patients with T2DM, we demonstrated that elevated serum FGF21 is independently associated with subclinical carotid atherosclerosis in patients without NAFLD. In the prospective study, we provided the first evidence that baseline circulating FGF21 levels can independently predict incident ASCVD events in a community-based cohort of patients without known CVD at baseline.

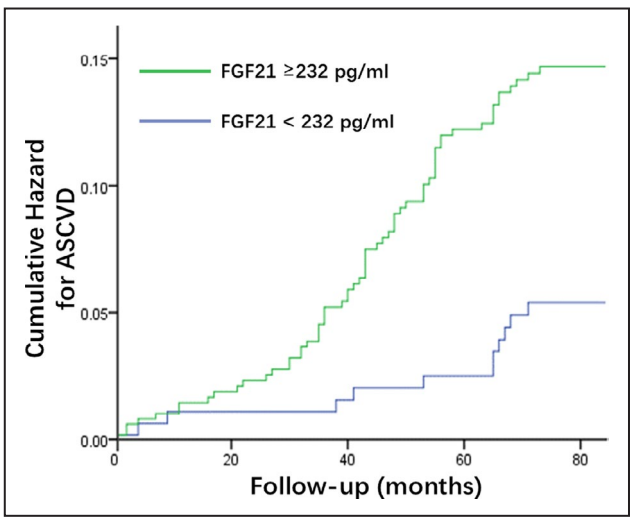

Figure. Cumulative hazard of incident atherosclerotic cardiovascular disease (ASCVD) in patients below and above the cutoff values of fibroblast growth factor 21 (FGF21).
Green line: above the FGF21 cutoff; blue line: below the FGF21 cutoff. Log-rank test: *P*<0.001.

Our study explores the association between FGF21 levels and atherosclerosis, the subject of conflicting results in recent years. Several cross-sectional studies showed that serum FGF21 levels were significantly elevated in patients with compared with those without coronary heart disease.[14,15] In a study of 212 newly diagnosed patients with T2DM, serum FGF21 was independently associated with the presence of carotid or iliac lesions in patients with atherosclerosis.[16] Another study showed that circulating FGF21 was associated with carotid atherosclerosis and C-IMT, independent of established risk factors.[17] However, it was reported that serum FGF21 levels were not significantly correlated with coronary heart disease in BMI- or glucose status–matched cohorts.[18,19] A major limitation of these studies is that they did not measure the degree of hepatic steatosis, and, hence, lacked adjustment for NAFLD.[15–19] NAFLD is a major determinant of serum FGF21 levels.[12,33] Circulating FGF21 levels are 2-fold higher in patients with NAFLD than in controls, and hepatic FGF21 mRNA expression positively correlates with intrahepatic triglyceride levels.[12] A 3-year prospective study of 712 Chinese patients suggested that high serum FGF21 level is a predictor of NAFLD.[33] As NAFLD is a significant risk factor for CVD,[34] further investigation is required to determine whether elevated FGF21 levels are the result of higher NAFLD prevalence in patients with subclinical atherosclerosis. In

Table 5. Discrimination and Reclassification Performance of the Addition of Circulating FGF21 Levels in Predicting Incident ASCVD

Model	C-Statistics (95% CI)	*P* Value	NRI (95% CI)	*P* Value	IDI (95% CI)	*P* Value
Baseline model*	0.827 (0.782–0.871)	···	···	···	···	···
+ FGF21 levels†	0.828 (0.785–0.871)	0.795	0.240 (0.009–0.471)	0.042	0.005 (–0.002 to 0.012)	0.162
+ FGF21 >232 pg/mL	0.828 (0.784–0.872)	0.822	0.389 (0.210–0.568)	<0.001	0.008 (0.001–0.0163)	0.037

ASCVD indicates atherosclerotic cardiovascular disease; FGF21, fibroblast growth factor 21; IDI, integrated discrimination improvement; and NRI, net reclassification index.

*Baseline model included age, sex, body mass index, smoking status, high-sensitivity C-reactive protein, estimated glomerular filtration rate, and lipocalin-2.

†Log-transformed before analysis.

this study, we applied proton magnetic resonance spectroscopy to precisely measure patients' liver fat fractions and used this information to entirely exclude the confounding effects of NAFLD. Our results demonstrate that elevated serum FGF21 levels are indeed associated with subclinical carotid atherosclerosis, independent of liver steatosis and other cardiovascular risk factors.

In the past decade, the beneficial metabolic effects of FGF21 have been extensively studied. In mice, administration of recombinant FGF21 resulted in weight loss, improved insulin sensitivity, reduction of triglyceride and LDL-C levels, and amelioration of liver steatosis.[7,8] In human studies, injection of FGF21 analogs led to significant improvement in the lipid profiles of patients with T2DM.[9,35] As FGF21 exerts favorable metabolic effects, it may seem contradictory that higher serum FGF21 levels were observed in patients with atherosclerosis. FGF21 elevation could be explained by a compensatory response to underlying metabolic stress, or by FGF21 resistance as a result of impaired FGF21 signaling. As with insulin resistance, this may suggest a requirement for a supraphysiological dose of FGF21 to meet concentration demands.[36] FGF21 resistance has been reported in several cardiometabolic diseases and may occur well in advance of clinical manifestation.[17,33,37,38] Therefore, underlying FGF21 resistance might lead to multiple CVD risk factors, such as dyslipidemia and DM, which then promote the development of atherosclerosis.

Animal studies have demonstrated direct or indirect protective effects of FGF21 on atherosclerotic disease. Exogenous FGF21 inhibited apoptosis in rat cardiac microvascular endothelial cells in atherosclerosis-like conditions, suggesting a protective effect during early-stage atherosclerosis.[39] Apolipoprotein E and FGF21 double-knockout mice demonstrated accelerated atherosclerotic plaque formation, worsened hypercholesterolemia, and increased expression of proinflammatory factors compared with mice with apolipoprotein E deficiency alone.[40] Treatment with recombinant FGF21 showed a significant reduction in plaque formation. This antiatherosclerotic effect could be caused by increased expression of adiponectin, a downstream factor of FGF21 that suppresses the proliferation and migration of smooth muscle cells and reduces oxidized LDL-C uptake by macrophages,[41] and/or direct inhibition of sterol regulatory element-binding protein 2.[40] The increased serum FGF21 observed in our study may be a protective response to metabolic stress underlying the atherosclerotic process.

The close correlations between FGF21, the pathophysiological process of atherosclerosis, and cardiometabolic disorders make FGF21 a promising biomarker for ASCVD risk. At present, only limited prospective studies have examined the relationship between baseline serum FGF21 levels and incident CVD, and most of these studies focused on patients with T2DM or established CVD. As circulating FGF21 levels are increased in patients with T2DM and established CVD, the result of these studies may not translate well to use with the general population. In the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, baseline circulating FGF21 levels were measured in 9697 patients with T2DM. Over a follow-up period of 5 years, plasma FGF21 levels were associated with total CVD events, stroke, and coronary/carotid revascularization, but not incident coronary heart disease events, after adjustment for conventional CVD risk factors.[20] Another study of 3538 Chinese patients with T2DM from the HKDR (Hong Kong West Diabetes Registry) reported that baseline serum FGF21 level was an independent predictor for coronary heart disease events over a median follow-up period of 3.8 years.[21] The TNT (Treating to New Targets) study reported that higher plasma FGF21 levels were associated with higher CVD risk in high-risk patients treated with statins.[22] A recent study of the MESA (Multi-Ethnic Study of Atherosclerosis) cohort did not observe a significant association between baseline FGF21 levels with incident total CVD events.[42] In this study, some end point events not related to atherosclerosis were not excluded, such as hemorrhagic stroke. Our study provides the evidence of an independent, prospective relationship between increased circulating FGF21 levels and incident ASCVD events in the general population. The optimal cutoff for serum FGF21, 232.0 pg/mL, could be used as an independent

predictor for both IHD and cerebral infarction among patients without DM.

Our previous study demonstrated that lipocalin-2 is an independent predictor for incident CVD events in men.[25] In this study, the addition of serum FGF21 levels to the previously established model consisting of conventional cardiovascular risk factors and lipocalin-2 resulted in significant improvements in the model's discriminatory and reclassifying abilities to predict incident ASCVD events. Our results suggest that application of those novel biomarkers could improve risk stratification ability in the primary prevention of CVD.

A major limitation of this study is that hepatic steatosis measurements were not routinely performed in our prospective cohort, preventing further evaluation of the impact of NAFLD on the relationship between serum FGF21 levels and incident ASCVD events. Additionally, this was a single-centered study of Chinese patients. Our cohort is relatively small and has lower mean BMI compared with Western patients. For this reason, the optimal cutoff for serum FGF21 should be interpreted carefully, and the results may not be generalizable to other groups of patients. To obtain more robust results, future studies should be performed using larger cohorts of multiethnic patients with well-documented baseline information on liver steatosis.

CONCLUSIONS

Our study demonstrates that elevated serum FGF21 occurs in subclinical carotid atherosclerosis in patients without NAFLD, independent of established risk factors. Baseline serum FGF21 level is an independent predictor of future ASCVD events in the general population and is a promising novel biomarker for use in the primary prevention of CVD.

ARTICLE INFORMATION

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Affiliations

From the Departments of Endocrinology and Metabolism (L.W., L.Q., L.Z., J. Zhang, X.H., Q.F., H.L., W.J.) and Radiology (J. Zhou, Y.L.), Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China; Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Center of Diabetes, Shanghai, China (L.W., L.Q., L.Z., J. Zhang, X.H., Q.F., H.L., W.J.); Department of Medicine, Shanghai Jiao Tong University School of Medicine, Shanghai, China (L.Q., L.Z., J. Zhang).

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Author contributions: H. Li and W. Jia conceived and designed the study. L. Wu, L. Qian, L. Zhang, and J. Zhang contributed to FGF21 level measurement. J. Zhou and Y. Li contributed to the measurement of hepatic fat fraction. L. Wu and L. Qian contributed to data analysis. L. Wu, L. Qian, X. Hou, Q. Fang, and H. Li contributed to data interpretation. L. Wu and H. Li drafted the article. All authors approved the final version of the article.

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Disclosures

None.

Supplementary Materials

Tables S1–S3
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Association between serum FGF21 levels and clinical parameters.

	n	FGF21 level, pg/ml	P value
Ever smoker			0.119
yes	137	233.9 (113.3-405.5)	-
no	234	227.4 (122.2-407.8)	-
Dyslipidemia			<0.001
yes	215	303.1 (154.1-459.7)	-
no	156	168.2 (93.1-295.5)	-
Hypertension			0.017
yes	153	268.0 (136.0-440.3)	-
no	218	208.9 (107.4-374.2)	-

Data are presented as median (interquartile range).

FGF21, fibroblast growth factor 21.

Table S2. Absolute difference related to the increase of one standard deviation of FGF21 level.

Variables	B (95% CI)	P Value
Waist circumference	0.001 (-0.015, 0.016)	0.918
C-IMT	-0.063 (-0.679, 0.552)	0.840
SBP	-0.001 (-0.008, 0.006)	0.744
DBP	-0.002 (-0.014, 0.011)	0.778
FBG	-0.014 (-0.051, 0.023)	0.459
2hBG	-0.004 (-0.026, 0.017)	0.702
Fasting insulin	-0.004 (-0.012, 0.003)	0.272
HOMA-IR	-0.007 (-0.025, 0.010)	0.422
Triglycerides	**0.477 (0.336, 0.617)**	**<0.001**
Total cholesterol	0.072 (-0.023, 0.168)	0.135
HDL-C	**-0.650 (-0.976, -0.324)**	**<0.001**
LDL-C	0.080 (-0.033, 0.193)	0.166
hsCRP	-0.003 (-0.012, 0.006)	0.520

Regression coefficient (B): absolute changes in the variables associated with one standard deviation increase in ln-transformed FGF21 level.

Adjusted for age and BMI.

2hBG, 2-hour blood glucose; BMI, body mass index; C-IMT, carotid intima-media thickness; DBP, diastolic blood pressure; FGF21, fibroblast growth factor 21; HDL-C, high-density lipoproteins cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance index; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Table S3. Absolute difference related to the increase of one standard deviation of FGF21 level at baseline.

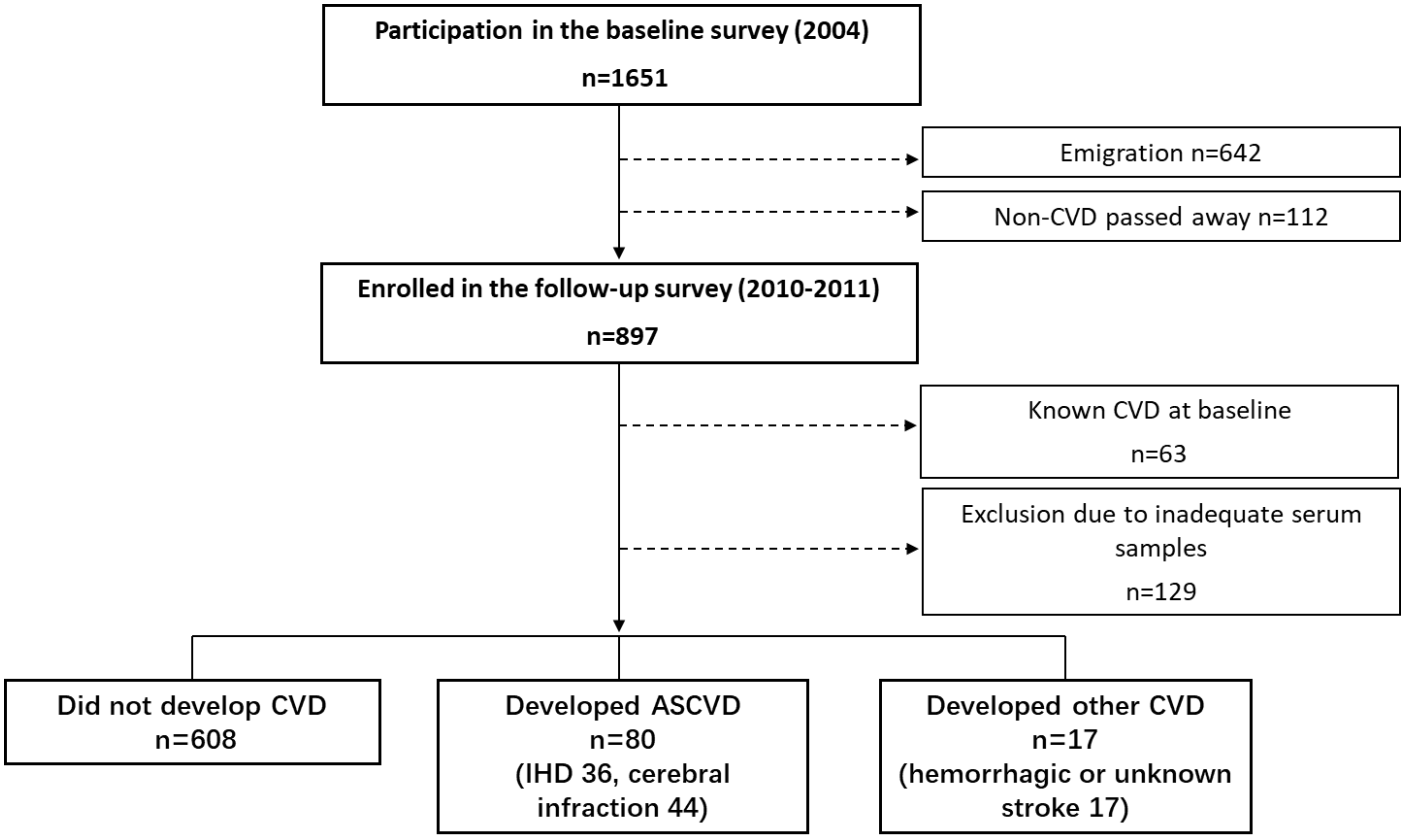
Variables	B (95%CI)	P Value
Waist circumference	0.010 (-0.003, 0.023)	0.122
SBP	0.004 (0.000, 0.009)	0.053
DBP	0.006 (-0.002, 0.014)	0.128
FBG	0.000 (-0.049, 0.049)	0.990
2hBG	0.035 (0.005, 0.065)	0.024
HOMA-IR	0.038 (-0.04, 0.115)	0.341
HbA1c	0.046 (-0.026, 0.118)	0.211
Triglycerides	**0.116 (0.071, 0.162)**	**<0.001**
Total cholesterol	0.059 (-0.017, 0.135)	0.128
HDL-C	**-0.532 (-0.739, -0.325)**	**<0.001**
LDL-C	0.001 (-0.09, 0.092)	0.986
eGFR	-0.002 (-0.005, 0.001)	0.262
hsCRP	0.004 (-0.032, 0.039)	0.836
Lipocalin-2	0.005 (-0.002, 0.012)	0.196

Regression coefficient (B): absolute change in the variables associated with one standard deviation increase in ln-transformed FGF21 level.

Adjusted for age and BMI.

2hBG, 2-hour blood glucose; BMI, body mass index; C-IMT, carotid intima-media thickness; DBP, diastolic blood pressure; FGF21, fibroblast growth factor 21; HDL-C, high-density lipoproteins cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance index; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Figure S1. Flow chart of the prospective study population.



ASCVD, atherosclerotic cardiovascular disease. CVD, cardiovascular disease.