

Role of Circulating Fibroblast Growth Factor 21 Measurement in Primary Prevention of Coronary Heart Disease Among Chinese Patients With Type 2 Diabetes Mellitus

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Background—Fibroblast growth factor 21 (FGF21) has demonstrated beneficial effects on lipid and carbohydrate metabolism. In cross-sectional studies, an association of raised circulating FGF21 levels with coronary heart disease (CHD) was found in some but not all studies. Here we investigated prospectively whether baseline serum FGF21 levels could predict incident CHD in subjects with type 2 diabetes mellitus and no known cardiovascular diseases.

Methods and Results—Baseline serum FGF21 levels were measured in 3528 Chinese subjects with type 2 diabetes mellitus recruited from the Hong Kong West Diabetes Registry. The role of baseline serum FGF21 levels in predicting incident CHD over a median follow-up of 3.8 years was analyzed using Cox regression analysis. Among 3528 recruited subjects without known cardiovascular diseases, 147 (4.2%) developed CHD over a mean follow-up of 4 years. Baseline serum log-transformed FGF21 levels were significantly higher in those who had incident CHD than those who did not (222.7 pg/mL [92.8–438.4] versus 151.1 pg/mL [75.6–274.6]; $P < 0.001$). On multivariable Cox regression analysis, baseline serum FGF21 levels, using an optimal cutoff of 206.22 pg/mL derived from our study, independently predicted incident CHD (hazard ratio, 1.55; 95% CI, 1.10–2.19; $P = 0.013$) and significantly improved net reclassification index and integrated discrimination improvement after adjustment for conventional cardiovascular risk factors.

Conclusions—We have demonstrated, for the first time, that serum FGF21 level is an independent predictor of incident CHD and might be usefully utilized as a biomarker for identifying type 2 diabetes mellitus subjects with raised CHD risk, for primary prevention. (*J Am Heart Assoc.* 2017;6:e005344. DOI: 10.1161/JAHA.116.005344.)

Key Words: adipokine • cardiovascular disease • coronary heart disease • primary prevention • type 2 diabetes mellitus

Fibroblast growth factor 21 (FGF21) is an emerging metabolic hormone, secreted mainly from the liver, but also from the adipose tissue, muscle, and pancreas.¹ Treatment with FGF21 conferred beneficial effects on body weight, glucose, and lipid metabolism in animal studies,² and

similar benefits were demonstrated in humans treated with FGF21 analogues.^{3,4} On the other hand, raised FGF21 levels have been found in various cardiometabolic conditions in clinical studies, suggesting a reactive change to FGF21 resistance or the underlying metabolic disturbances.¹ In population studies, we and others had previously shown that high circulating FGF21 levels predicted the development of type 2 diabetes mellitus (T2DM) and were associated with carotid atherosclerosis independent of established cardiovascular risk factors.^{5–9} Among subjects with T2DM, recently, we also demonstrated that serum FGF21 levels predicted nephropathy progression, in terms of decline in estimated glomerular filtration rate (eGFR), even in the stage of normoalbuminuria and relatively preserved renal function, highlighting its potential use as an early biomarker for diabetic kidney disease, a devastating diabetic complication.¹⁰

Likewise, coronary heart disease (CHD) remains an important macrovascular complication of T2DM,¹¹ which, in turn, has been suggested as a risk equivalent of CHD.¹² Over the years, the association of circulating FGF21 with CHD in T2DM has not been clearly defined. In cross-sectional studies,

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An accompanying Table S1 is available at <http://jaha.ahajournals.org/content/6/6/e005344/DC1/embed/inline-supplementary-material-1.pdf>

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conflicting results were reported regarding circulating FGF21 levels in subjects with CHD, with an association of raised FGF21 levels with CHD reported in some studies,^{13–15} but not in others.^{16,17} The association of circulating FGF21 with CHD in T2DM, especially among those without known cardiovascular disease (CVD), remains to be defined. Therefore, we aimed to investigate longitudinally whether circulating FGF21 levels could be usefully utilized as a risk marker to predict the development of CHD in subjects with T2DM and no known CVD.

Subjects and Methods

Subjects

All subjects were recruited from the Hong Kong West Diabetes Registry, which included patients who had T2DM and were being regularly followed up at the medical specialist clinics of the Hong Kong West Cluster since 2008. All Chinese patients at enrollment to the registry had been invited to participate in a prospective cohort study to identify the risk factors, including serum and genetic biomarkers, predisposing to the development of diabetic complications. Each visit comprised of clinical assessments and laboratory investigations to determine the control of diabetes mellitus, its related cardiovascular risk factors, and the presence of diabetic complications.

In this study, subjects who were on fibrates or had known CVD at baseline were excluded. Consequently, a total of 3528 subjects were included in this study. The study protocol was approved by the institutional review board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. All recruited subjects gave written informed consent before any study-related procedures.

Clinical and Biochemical Assessments

Subjects attended each visit after an overnight fasting of at least 8 hours. At the baseline visit, demographic data, including age, sex, occupation, smoking, alcohol consumption, and physical activity, were obtained. Detailed family, medical, and drug histories were ascertained using a standardized questionnaire. Anthropometric parameters, including body weight, height, body mass index, waist circumference, and blood pressure, were measured. Fasting blood was drawn for plasma glucose, lipids, and glycated hemoglobin. Serum creatinine was measured and the eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation as described previously.¹⁸ Serum high-sensitivity C-reactive protein was measured with a high-sensitivity, particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany) and serum FGF21 was

measured with ELISA kits (Antibody and Immunoassay Services, University of Hong Kong, Hong Kong, China).^{19,20}

Definitions of Clinical Variables and Outcomes

Presence of dyslipidemia was defined as fasting triglycerides (TG) ≥ 1.69 mmol/L, high-density lipoprotein cholesterol (HDL-C) < 1.04 mmol/L in men and < 1.29 mmol/L in women, low-density lipoprotein cholesterol ≥ 3.4 mmol/L, or on lipid-lowering agents. Hypertension was defined as blood pressure $\geq 140/90$ mm Hg or on antihypertensive medications. Incident CHD events, the primary outcome of interest in this study, was defined as the first recorded diagnosis of myocardial infarction and/or coronary revascularization, verified from the Hospital Authority database or their private practitioners as of March 31, 2016. Known CVD at baseline, the exclusion criteria of the study, was defined as any history of myocardial infarction, stroke, transient ischemic attack, heart failure, and coronary or other arterial revascularization. Diagnoses of CHD events and other CVD were based on The International Classification of Diseases, Ninth Revision codes (402, 404, 410–414, 425–447, and 518.4). In this study, the medical diagnoses were adjudicated and reviewed by 2 physicians independently, and disagreements between them were resolved by a third.

Statistical Analysis

All data were analyzed with IBM SPSS Statistics (version 23.0; IBM Corp, Armonk, NY) and R software (Version 3.3.1; Package *survC1* and *survIDINRI*; R Foundation for Statistical Computing, Vienna, Austria). Data that were not normally distributed as determined using Kolmogorov–Smirnov test, which included serum FGF21, TG, and high-sensitivity C-reactive protein, were natural-logarithmically transformed to obtain near normality before analysis. Values were reported as means \pm SD, medians with interquartile range, or percentages, as appropriate. Chi-square test and ANOVA test were used for comparisons of categorical and continuous variables, respectively. Pearson correlation and multiple linear regression analysis were used to evaluate the determinants of serum FGF21 levels at baseline. To evaluate an optimal serum FGF21 cutoff to predict incident CHD event, the point with the maximum Youden index (J) was determined on the receiver-operating characteristic operation curve with $J = \text{sensitivity} + \text{specificity} - 1$.²¹ Multivariable Cox regression analysis was used to evaluate the association between baseline circulating FGF21 levels and incident CHD events. The variables included in Cox regression models were those that were statistically significant in the univariate analysis and with minimal overlap in representation. Discrimination of serum FGF21 levels in predicting incident CHD was analyzed using

Table 1. Baseline Characteristics of the 3528 Subjects by Incident CHD

Baseline Variables	Incident CHD	No Incident CHD	P Value
N	147	3381	...
Men, %	66.7	51.1	<0.001*
Age, y	65.6±12.1	60.6±12.8	<0.001*
Ever smoker, %	34.7	28.7	0.023*
Duration of diabetes mellitus, years	14.9±9.23	11.6±8.67	<0.001*
Body mass index, kg/m ²	26.1±4.19	26.0±4.65	0.940
Waist circumference, cm			0.438 [†]
Men	90.9±11.9	90.4±12.3	
Women	86.2±12.1	85.3±12.4	
Systolic blood pressure, mmol/L	143±21	136±20	<0.001*
Diastolic blood pressure, mmol/L	75±11	75±10	0.587
Antihypertensive drug, %	88.4	78.0	0.003*
Hypertension, %	91.8	82.5	0.003*
Fasting glucose, mmol/L	8.57±2.79	7.83±2.51	<0.001*
Glycated hemoglobin, % (mmol/mol)	8.02±1.64 (64.2±17.9)	7.62±1.43 (59.8±15.7)	0.001*
PPAR-γ [‡] , %	2.0	1.1	0.243
Estimated glomerular filtration rate (CKD-EPI), mL/min per 1.73 m ²	63.6±26.9	79.2±24.7	<0.001*
HDL-cholesterol, mmol/L	1.18±0.38	1.26±0.38	0.012*
LDL-cholesterol, mmol/L	2.73±0.98	2.52±0.80	0.002*
Triglyceride [§] , mmol/L	1.42 (1.03–2.24)	1.26 (0.90–1.79)	0.001*
Lipid-lowering drug, %	53.1	44.8	0.047*
Dyslipidemia	85.7	78.4	0.034*
Highly sensitive C-reactive protein [§] , mg/mL	1.34 (0.60–4.05)	1.24 (0.50–2.99)	0.190
FGF21 [§] , pg/mL	222.7 (92.8–438.4)	151.1 (75.6–274.6)	<0.001*

Data are presented as mean±SD or median (interquartile range); chi-square test and ANOVA test were used for comparisons of categorical and continuous variables, respectively. CHD indicates coronary heart disease; CKD-EPI, chronic kidney disease epidemiology collaboration equation; FGF21, fibroblast growth factor 21.

*P<0.05.

[†]Sex-adjusted P value.

[‡]PPAR-γ, peroxisome proliferator-agonist receptor gamma.

[§]Log-transformed before analysis.

^{||}Dyslipidemia was defined as fasting triglycerides (TG) ≥1.69 mmol/L, high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L in men and <1.29 mmol/L in women, low-density lipoprotein cholesterol (LDL-C) ≥3.4 mmol/L, or on lipid-lowering agents.

C-statistics.²² The incremental value of serum FGF21 levels with reference to a baseline clinical model of conventional cardiovascular risk factors in predicting incident CHD was assessed by the integrated discrimination improvement and the category-free net reclassification index. CHD-free survival curves were plotted by the Kaplan–Meier method and subsequently compared by log-rank test. In all statistical tests, 2-sided P<0.05 was considered significant.

Results

A total of 3528 Chinese subjects with T2DM and no known CVD were included in this prospective study. Table 1 summarizes their baseline characteristics. Over a median

follow-up of 3.8 (2.8–5.0) years, 147 subjects (4.2%) developed the first CHD event, with a cumulative incidence of 10.1 per 1000 person-years. Subjects who had incident CHD tended to be men, older, ever smoker, hypertensive with higher systolic blood pressure, and longer duration of diabetes mellitus with poorer glycemic control, as evident by their higher fasting glucose and glycated hemoglobin levels, and more of them had dyslipidemia and lower eGFR than those who did not develop CHD. Whereas their serum high-sensitivity C-reactive protein levels did not differ between groups with and without incident CHD (1.34 mg/mL [0.60–4.05] versus 1.24 mg/mL [0.50–2.99], respectively; P=0.190), circulating log-transformed FGF21 levels were significantly higher in those who developed CHD compared

Table 2. Multivariable Cox Proportional Hazard Regression Model Showing the Association of Serum FGF21 Levels With Incident CHD

Baseline Variables	Model 1		Model 2	
	Adjusted Hazard Ratio (95% CI)	P Value	Adjusted Hazard Ratio (95% CI)	P Value
Sex	2.17 (1.53–3.06)	<0.001*	2.04 (1.37–3.05)	<0.001*
Age, y	1.05 (1.03–1.06)	<0.001*	1.03 (1.01–1.05)	0.003*
Ever smoker	1.07 (0.73–1.56)	0.727
Duration of diabetes mellitus, years	1.01 (0.99–1.03)	0.235
HbA1c, %	1.07 (0.96–1.19)	0.220
SBP, mm Hg	1.01 (1.00–1.02)	0.075
Antihypertensive drug, %	1.07 (0.62–1.86)	0.806
Estimated glomerular filtration rate (CKD-EPI), mL/min per 1.73 m ²	0.986 (0.979–0.993)	<0.001*
HDL-C, mmol/L	0.68 (0.40–1.13)	0.135
LDL-C, mmol/L	1.39 (1.15–1.68)	0.001*
Lipid-lowering drug, %	1.61 (1.13–2.29)	0.008*
FGF21 level ≥206.22 pg/mL [†]	1.98 (1.42–2.74)	<0.001*	1.55 (1.10–2.19)	0.013*

CHD indicates coronary heart disease; CKD-EPI, chronic kidney disease epidemiology collaboration equation; FGF21, fibroblast growth factor 21; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

*P<0.05.

[†]Optimal serum FGF21 cutoff obtained by Youden Index.

with those who did not (222.7 pg/mL [92.8–438.4] versus 151.1 pg/mL [75.6–274.6], respectively; P<0.001).

In our study, baseline serum log-transformed FGF21 levels correlated positively with body mass index (r=0.16), waist circumference (r=0.19), systolic blood pressure (r=0.18), serum TG (r=0.36), and high-sensitivity C-reactive protein levels (r=0.20) and negatively with HDL-C (r=-0.22) and eGFR levels (r=-0.27; all P<0.001). All remained significant independent determinants of baseline serum log-transformed FGF21 levels on multiple linear regression analysis (Table S1). On multivariable Cox regression analysis, using an optimal serum FGF21 cutoff of 206.22 pg/mL obtained by Youden index, circulating FGF21 level was confirmed to be an independent predictor of incident CHD in subjects with T2DM without known CVD (hazard ratio [HR], 1.55; 95% confidence interval [95% CI], 1.10–2.19; P=0.013), together with sex (HR, 2.04; 95% CI, 1.37–3.05; P<0.001), age (HR, 1.03; 95% CI, 1.01–1.05; P=0.003), baseline eGFR (HR, 0.986; 95% CI, 0.979–0.993), low-density lipoprotein cholesterol (HR, 1.39; 95% CI, 1.15–1.68; P=0.001), and lipid-lowering drugs (HR, 1.61; 95% CI, 1.13–2.29; P=0.008), in a model that also included smoking status, duration of diabetes mellitus, use of antihypertensive drugs, baseline glycated hemoglobin, systolic blood pressure, and HDL-C (Table 2). Importantly, its association with incident CHD remained statistically significant irrespective of whether serum FGF21 level was expressed as a continuous variable (log-transformed FGF21:

HR, 1.21; 95% CI, 1.02–1.43; P=0.026) or as a tertile cutoff of 224.22 pg/mL, comparing the highest with the lower 2 serum FGF21 tertiles (HR, 1.47; 95% CI, 1.04–2.07; P=0.029). Figure depicts the Kaplan–Meier analysis of incident CHD among

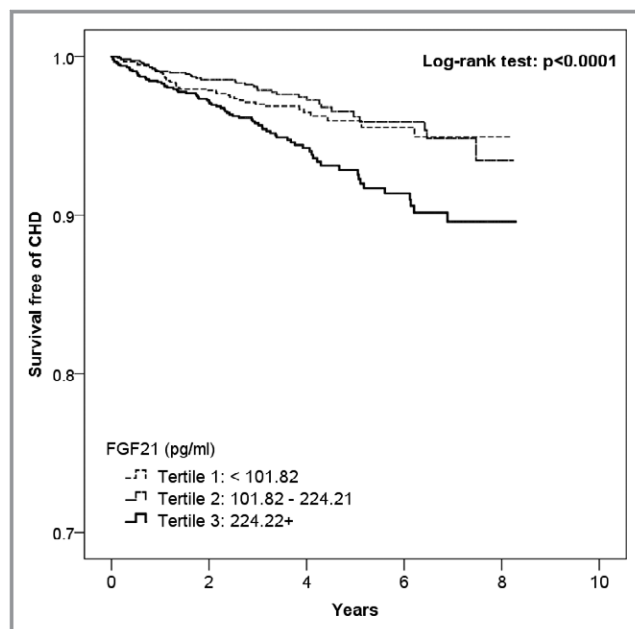


Figure. Kaplan–Meier analysis of incident coronary heart disease (CHD) among subjects stratified by tertiles of their serum fibroblast growth factor 21 (FGF21) levels at baseline.

Table 3. Discrimination and Reclassification Performance of the Addition of Circulating FGF21 Levels in Predicting Incident CHD, Based on C-Statistics, NRI, and IDI

Model	Addition of Circulating FGF21 Levels to the Baseline Clinical Model	C-Statistics (95% CI)	P Value	Category Free NRI (95% CI)	P Value	IDI (95% CI)	P Value
Baseline clinical model*		0.7449 (0.6830–0.8067)
	FGF21 levels [†]	0.7458 (0.6830–0.8067)	0.442	0.051 (–0.068 to 0.158)	0.342	0.004 (0.0001–0.017)	0.018 [‡]
	FGF21 ≥206.22 pg/mL [§]	0.7467 (0.6817–0.8117)	0.343	0.179 (0.039–0.273)	0.032 [‡]	0.004 (0.0001–0.013)	0.020 [‡]
	FGF21 ≥224.22 pg/mL	0.7457 (0.6813–0.8102)	0.389	0.170 (0.003–0.263)	0.048 [‡]	0.003 (0.0001–0.012)	0.026 [‡]

CHD indicates coronary heart disease; FGF21, fibroblast growth factor 21; HDL, high-density lipoprotein cholesterol; IDI, integrated discrimination improvement; LDL-C, low-density lipoprotein cholesterol; NRI, net reclassification index.

*Baseline clinical model included sex, age, smoking status, duration of diabetes mellitus, glycated hemoglobin, systolic blood pressure, use of antihypertensive drugs, estimated glomerular filtration rate, HDL-C, LDL-C, and use of lipid-lowering drugs.

[†]Circulating FGF21 levels as a log-transformed variable.

[‡]P<0.05.

[§]Optimal serum FGF21 cutoff obtained by Youden Index.

^{||}Serum FGF21 tertile cutoff obtained by comparing the highest with the lower 2 serum FGF21 tertiles as referent.

subjects stratified by tertiles of their serum FGF21 levels at baseline. In view of the strong correlation between serum log-transformed FGF21 and TG levels (Table S1), and that increased serum TG together with low HDL-C levels have been found to be highly associated with incident CHD in subjects with T2DM,²³ we further adjusted for the effect of TG and found that serum FGF21 remained an independent predictor of incident CHD (HR, 1.48; 95% CI, 1.04–2.11; P=0.031) using an optimal serum FGF21 cutoff of 206.22 pg/mL obtained by Youden index.

The discriminatory and reclassifying abilities of circulating FGF21 levels are shown in Table 3. The addition of serum FGF21 level, whether as a log-transformed variable, optimal or tertile cutoff, to a baseline clinical model consisting of conventional cardiovascular risk factors, including sex, age, smoking status, duration of diabetes mellitus, glycated hemoglobin, systolic blood pressure, use of antihypertensive drugs, eGFR, HDL-C, low-density lipoprotein cholesterol, and use of lipid-lowering drugs, resulted in comparable C-statistics and therefore did not improve discrimination with regard to the prediction of incident CHD. However, in contrast, in terms of reclassification, the addition of serum FGF21 level, irrespective of using it as a log-transformed variable, optimal or tertile cut-ff, all provided significant improvements in integrated discrimination improvement to the baseline clinical model (integrated discrimination improvement +0.004, +0.004, and +0.003; P=0.018, 0.020, and 0.026, respectively). Furthermore, both optimal and tertile serum FGF21 cutoffs resulted in a modest, yet significant, increase in category-free net reclassification index in predicting incident CHD (net reclassification index +0.179 and +0.170; P=0.032

and 0.048, respectively). Taken together, among Chinese subjects with T2DM and no known CVD, the addition of circulating FGF21 levels, preferably using an optimal cutoff of 206.22 pg/mL, to conventional cardiovascular risk factors, improved reclassification and retained considerable discrimination in CHD prediction.

Discussions

In this prospective study, we demonstrated, for the first time, that, circulating FGF21 levels independently predicted the development of CHD in subjects with T2DM and no known CVD. Our findings have expanded the current understanding on the relationship between serum FGF21 and CHD. Several cross-sectional studies had shown that serum FGF21 levels were significantly higher in subjects with CHD than those in controls.^{13,14} In a study involving 100 Chinese subjects, with 38% of them having T2DM, serum FGF21 levels were independently associated with the presence of acute myocardial infarction and were significantly higher in those who developed reinfarction within 30 days.¹⁵ However, conflicting results were also reported from a few studies, which showed a lack of significant association after adjusting for body mass index and glycemic status.^{16,17} Given that circulating FGF21 levels were higher in T2DM,^{6,9} and most cross-sectional studies consisted of relatively small sample size and therefore a limited number of subjects with T2DM, the association of serum FGF21 with CHD in T2DM could only be, at most, speculative. Nonetheless, to our knowledge, 2 prospective studies were available that looked into the relationship between circulating FGF21 levels and CVD in

subjects with T2DM. As part of the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, plasma FGF21 levels were measured at baseline in 9697 subjects with T2DM. Circulating FGF21 levels were found to be predictive of the primary outcome of total CVD over a median follow-up of 5 years. However, analyses of secondary outcomes showed that the associations were positive for stroke, coronary, and carotid revascularization, but not CHD events, after adjusting for established CVD risk factors.²⁰ A small study on 87 T2DM subjects from Poland also reported a predictive role of serum FGF21 levels with incident cardiovascular morbidity and mortality over 2 years,²⁴ but no information on the prediction of incident CHD was provided. Notably, both studies consisted of a significant number of subjects with known CVD at baseline, 22% in FIELD and 64.4% in the Polish study, and around half of the subjects in the FIELD study were on fenofibrate, a peroxisome proliferator-agonist receptor alpha agonist that enhances FGF21 expression.¹ Thus, our study was the first one to demonstrate a prospective, independent relationship between raised serum FGF21 levels with incident CHD. Our findings would suggest its potential application as a biomarker for risk stratification in primary prevention of CHD among subjects with T2DM.

Our findings of elevated circulating FGF21 levels in subjects who developed CHD could be explained, at least in part, by FGF21 resistance that had also been reported in other cardiometabolic conditions.^{6,8,10,20,25} The increase in circulating FGF21 levels might also reflect a protective compensatory response to insulin resistance or hyperinsulinemia, hyperlipidemia, and the increase in systemic inflammation in patients with atherosclerotic diseases.^{26,27} In fact, multiple beneficial effects of FGF21 in the context of CHD had been demonstrated in preclinical studies. Mice with combined apolipoprotein E and FGF21 deficiency had worsened hyperlipidemia, increased expressions of both local and systemic proinflammatory chemokines and cytokines, with resultant acceleration of aortic plaque formation,²⁸ compared with mice with apolipoprotein E deficiency only. Although these antiatherosclerotic properties of FGF21 could be explained by adiponectin, which had been shown to mediate the effects of FGF21 on glucose homeostasis and insulin sensitivity in mice,²⁹ FGF21 could also ameliorate atherosclerosis independent of adiponectin, through suppression of cholesterol biosynthesis by directly inhibiting sterol regulatory element-binding protein-2.²⁸ In another study using FGF21 knockout mice and cultured rat neonatal cardiomyocytes, FGF21 deficiency enhanced isoproterenol induced cardiac hypertrophy, increased cardiac expressions of proinflammatory genes, as well as markers of cardiac hypertrophy and fibrosis. Importantly, FGF21 treatment reversed the above adverse effects both in vitro and in vivo.³⁰ Moreover, in vitro studies

using THP1 macrophage derived foam cells also demonstrated that FGF21 could decrease oxidized low-density lipoprotein cholesterol-related foam cell formation and apoptosis.³¹ Taken together, the elevation of circulating FGF21 levels observed in our study might also reflect a protective bodily response to myocardial ischemia or injury during CHD development, similar to that observed in acetaminophen-induced hepatotoxicity we described previously.³²

Not until recently, FGF21 is regarded as a major hepatokine, and also an adipokine and myokine, though to a lesser extent. In response to myocardial ischemia in mice, both hepatic and adipocyte-derived FGF21 expression and secretion were shown to be upregulated, highlighting a cardioprotective response of FGF21 in an endocrine manner.³³ Recently, both cardiac FGF21 expression and secretion were also found to be increased significantly with isoproterenol treatment in mice and phenylephrine treatment in neonatal cardiomyocytes, respectively.³⁰ Furthermore, cardiac ischemia was able to induce significant increases in cardiac FGF21 mRNA, protein expression, and secretion ex vivo in a rat heart Langendorff model, suggesting possible autocrine or paracrine cardioprotective effects during myocardial ischemia.³⁴ However, the extent of contribution from this cardiac production of FGF21 to the elevated circulating FGF21 levels observed in our subjects with T2DM and incident CHD remains uncertain.

In the current study, not only have we identified a predictive role of serum FGF21 level on incident CHD in subjects with T2DM and no known CVD, but we also demonstrated that the addition of serum FGF21 above 206.22 pg/mL, an optimal cutoff derived from our cohort, to a baseline clinical model consisting of conventional cardiovascular risk factors, resulted in a modest, yet significant, improvement in both category-free NRI and IDI. Our findings have provided useful information from a clinician's perspective. Current clinical guidelines do not advocate routine screening for CHD in asymptomatic patients with T2DM, given that risk factor-based screening strategy is not sensitive and some noninvasive coronary assessment carries radiation risks.³⁵ Therefore, in the setting of primary prevention of CHD in T2DM, our findings would suggest the need to validate the potential use of FGF21, a serum biomarker that can be readily assessed using commercially available assay, for coronary risk stratification.

Our study, however, is limited by the relatively low event rate and short duration of follow-up. Furthermore, attributed to registry design and recommendations from current clinical guidelines,³⁵ a screening test for myocardial ischemia was not routinely performed in our asymptomatic subjects with T2DM. Nonetheless, although silent myocardial ischemia is not uncommon in T2DM, there have also been data showing that

it might reverse over time,³⁶ and adverse cardiac outcomes were similar in those who had silent myocardial ischemia compared with those who did not.³⁷ Notwithstanding these limitations, we have provided the first demonstration that elevated circulating FGF21 level was an independent predictor of incident CHD in patients with T2DM and no known CVD. In recent years, despite the more-liberal use of statins in T2DM, in particular, moderate-to-high intensity statins as driven by the latest guidelines,³⁵ substantial health burden from CHD still exists. We previously showed that circulating FGF21 had the potential to become a novel marker for nephropathy development in early stages of diabetic kidney disease; our current findings further proposed that it might also be usefully utilized in risk stratification in the setting of primary CHD prevention among subjects with T2DM.

Author Contributions

Lee researched the data and wrote the manuscript. Woo, Cheung, Chow and Yuen researched the data. Fong performed statistical analyses. Xu, Tse, and Lam critically reviewed and edited the manuscript. Tse and Lam initiated and supervised the study. They are the guarantor of this work and as such had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosures

None.

References

- Woo YC, Xu A, Wang Y, Lam KS. Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clin Endocrinol (Oxf)*. 2013;78:489–496.
- Kharitonov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, Hansen BC, Shanfelt AB, Etgen GJ. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology*. 2007;148:774–781.
- Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonov A, Bumol T, Schilke HK, Moller DE. The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metab*. 2013;18:333–340.
- Talukdar S, Zhou Y, Li D, Rossulek M, Dong J, Somayaji V, Weng Y, Clark R, Lanba A, Owen BM, Brenner MB, Trimmer JK, Gropp KE, Chabot JR, Erion DM, Rolph TP, Goodwin B, Calle RA. A long-acting FGF21 molecule, PF-05231023, decreases body weight and improves lipid profile in non-human primates and type 2 diabetic subjects. *Cell Metab*. 2016;23:427–440.
- Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, Defronzo RA, Tripathy D. 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*. 2009;32:1542–1546.
- Chen C, Cheung BM, Tso AW, Wang Y, Law LS, Ong KL, Wat NM, Xu A, Lam KS. 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*. 2011;34:2113–2115.
- An SY, Lee MS, Yi SA, Ha ES, Han SJ, Kim HJ, Kim DJ, Lee KW. 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*. 2012;96:196–203.
- Chow WS, Xu A, Woo YC, Tso AW, Cheung SC, Fong CH, Tse HF, Chau MT, Cheung BM, Lam KS. Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arterioscler Thromb Vasc Biol*. 2013;33:2454–2459.
- Woo YC, Lee CH, Fong CH, Xu A, Tso AW, Cheung BM, Lam KS. Serum fibroblast growth factor 21 is a superior biomarker to other adipokines in predicting incident diabetes. *Clin Endocrinol (Oxf)*. 2016;86:37–43.
- Lee C, Hui E, Woo Y, Yeung C, Chow W, Yuen M, Fong C, Xu A, Lam K. Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. *J Clin Endocrinol Metab*. 2015;100:1368–1375.
- Gregg EW, Li Y, Wang J, Burrows NR, Ali MK, Rolka D, Williams DE, Geiss L. Changes in diabetes-related complications in the United States, 1990–2010. *N Engl J Med*. 2014;370:1514–1523.
- Schramm TK, Gislason GH, Kober L, Rasmussen S, Rasmussen JN, Abildstrom SZ, Hansen ML, Folke F, Buch P, Madsen M, Vaag A, Torp-Pedersen C. Diabetes patients requiring glucose-lowering therapy and nondiabetics with a prior myocardial infarction carry the same cardiovascular risk: a population study of 3.3 million people. *Circulation*. 2008;117:1945–1954.
- Lin Z, Wu Z, Yin X, Liu Y, Yan X, Lin S, Xiao J, Wang X, Feng W, Li X. Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. *PLoS One*. 2010;5:e15534.
- Shen Y, Ma X, Zhou J, Pan X, Hao Y, Zhou M, Lu Z, Gao M, Bao Y, Jia W. Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. *Cardiovasc Diabetol*. 2013;12:124.
- Zhang W, Chu S, Ding W, Wang F. Serum level of fibroblast growth factor 21 is independently associated with acute myocardial infarction. *PLoS One*. 2015;10:e0129791. <https://doi.org/10.1371/journal.pone.0129791>.
- Lee Y, Lim S, Hong ES, Kim JH, Moon MK, Chun EJ, Choi SI, Kim YB, Park YJ, Park KS, Jang HC, Choi SH. Serum FGF21 concentration is associated with hypertriglyceridaemia, hyperinsulinaemia and pericardial fat accumulation, independently of obesity, but not with current coronary artery status. *Clin Endocrinol (Oxf)*. 2014;80:57–64.
- Kim WJ, Kim SS, Lee HC, Song SH, Bae MJ, Yi YS, Jeon YK, Kim BH, Kim YK, Kim IJ. Association between serum fibroblast growth factor 21 and coronary artery disease in patients with type 2 diabetes. *J Korean Med Sci*. 2015;30:586–590.
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF III, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, Ckd EPI. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
- Ong KL, Rye KA, O'Connell R, Jenkins AJ, Brown C, Xu A, Sullivan DR, Barter PJ, Keech AC; Investigators Fs. Long-term fenofibrate therapy increases fibroblast growth factor 21 and retinol-binding protein 4 in subjects with type 2 diabetes. *J Clin Endocrinol Metab*. 2012;97:4701–4708.
- Ong KL, Januszewski AS, O'Connell R, Jenkins AJ, Xu A, Sullivan DR, Barter PJ, Hung WT, Scott RS, Taskinen MR, Keech AC, Rye KA. The relationship of fibroblast growth factor 21 with cardiovascular outcome events in the Fenofibrate Intervention and Event Lowering in Diabetes study. *Diabetologia*. 2015;58:464–473.
- Perkins NJ, Schisterman EF. The inconsistency of “optimal” cutpoints obtained using two criteria based on the receiver operating characteristic curve. *Am J Epidemiol*. 2006;163:670–675.
- Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Stat Med*. 2011;30:1105–1117.
- Lee JS, Chang PY, Zhang Y, Kizer JR, Best LG, Howard BV. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the Strong Heart Study. *Diabetes Care*. 2017;40:529–537.
- Lenart-Lipinska M, Matyjczek-Matuszek B, Gernand W, Nowakowski A, Solski J. Serum fibroblast growth factor 21 is predictive of combined cardiovascular morbidity and mortality in patients with type 2 diabetes at a relatively short-term follow-up. *Diabetes Res Clin Pract*. 2013;101:194–200.
- Li H, Dong K, Fang Q, Hou X, Zhou M, Bao Y, Xiang K, Xu A, Jia W. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol*. 2013;58:557–563.

26. Fisher FM, Chui PC, Antonellis PJ, Bina HA, Kharitonov A, Flier JS, Maratos-Flier E. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes*. 2010;59:2781–2789.
27. Straczkowski M, Karczewska-Kupczewska M, Adamska A, Otziomek E, Kowalska I, Nikolajuk A. Serum fibroblast growth factor 21 in human obesity: regulation by insulin infusion and relationship with glucose and lipid oxidation. *Int J Obes (Lond)*. 2013;37:1386–1390.
28. Lin Z, Pan X, Wu F, Ye D, Zhang Y, Wang Y, Jin L, Lian Q, Huang Y, Ding H, Triggie C, Wang K, Li X, Xu A. Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice. *Circulation*. 2015;131:1861–1871.
29. Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, Itoh N, Wang Y, Bornstein SR, Xu A, Li X. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab*. 2013;17:779–789.
30. Planavila A, Redondo I, Hondares E, Vinciguerra M, Munts C, Iglesias R, Gabrielli LA, Sitges M, Giralt M, van Bilsen M, Villarroya F. Fibroblast growth factor 21 protects against cardiac hypertrophy in mice. *Nat Commun*. 2013;4:2019.
31. Li E, Wang T, Wang F, Wang T, Sun LQ, Li L, Niu SH, Zhang JY. FGF21 protects against ox-LDL induced apoptosis through suppressing CHOP expression in THP1 macrophage derived foam cells. *BMC Cardiovasc Disord*. 2015;15:80.
32. Ye D, Wang Y, Li H, Jia W, Man K, Lo CM, Wang Y, Lam KS, Xu A. FGF21 protects against acetaminophen-induced hepatotoxicity by potentiating PGC-1alpha-mediated antioxidant capacity in mice. *Hepatology*. 2014;60:977–989.
33. Liu SQ, Roberts D, Kharitonov A, Zhang B, Hanson SM, Li YC, Zhang LQ, Wu YH. Endocrine protection of ischemic myocardium by FGF21 from the liver and adipose tissue. *Sci Rep*. 2013;3:2767.
34. Patel V, Adya R, Chen J, Ramanjaneya M, Bari MF, Bhudia SK, Hillhouse EW, Tan BK, Randeve HS. Novel insights into the cardio-protective effects of FGF21 in lean and obese rat hearts. *PLoS One*. 2014;9:e87102.
35. American Diabetes A. 8. Cardiovascular disease and risk management. *Diabetes Care*. 2016;39(suppl 1):S60–S71.
36. Wackers FJ, Chyun DA, Young LH, Heller GV, Iskandrian AE, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Filipchuk N, Ratner RE, Inzucchi SE; Detection of Ischemia in Asymptomatic Diabetics I. Resolution of asymptomatic myocardial ischemia in patients with type 2 diabetes in the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study. *Diabetes Care*. 2007;30:2892–2898.
37. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Heller GV, Iskandrian AE, Wittlin SD, Filipchuk N, Ratner RE, Inzucchi SE; Investigators D. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. *JAMA*. 2009;301:1547–1555.

SUPPLEMENTAL MATERIAL

Table S1. Multiple linear regression analysis showing the determinants of serum log-transformed fibroblast growth factor 21 levels at baseline

Variable at baseline	Standardized beta	p-value
TG*	0.300	<0.001
eGFR (EPI)	0.192	<0.001
hsCRP*	0.088	<0.001
Age	0.067	0.001
SBP	0.057	0.001
BMI#	0.053	0.002
HDL-C	-0.044	0.012

FGF21 indicates fibroblast growth factor 21; TG, triglyceride; eGFR, estimated glomerular filtration rate; hsCRP, high sensitivity C reactive protein; SBP, systolic blood pressure; BMI, body mass index; HDL-C, high density lipoprotein cholesterol.

*Natural log-transformed before analysis

#Result remained the same when BMI replaced by waist circumference