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

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OBJECTIVE—To investigate whether circulating levels of fibroblast growth factor 21 (FGF21), which previously has been shown to be elevated in obesity, could predict the development of type 2 diabetes in a 5.4-year, population-based, prospective study.

RESEARCH DESIGN AND METHODS—Baseline plasma FGF21 levels were measured using an enzyme-linked immunosorbent assay in 1,900 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS). The prospective association of FGF21 with diabetes development over 5.4 years was analyzed using multiple logistic regression.

RESULTS—At baseline, plasma levels of FGF21 increased progressively with worsening dysglycemia from normal glucose tolerance, through prediabetes, to diabetes (global trend, $P < 0.001$). Of 1,292 subjects without diabetes at baseline, a high baseline FGF21 level was a strong independent predictor for diabetes development (odds ratio 1.792; $P < 0.01$), together with waist circumference and fasting plasma glucose levels.

CONCLUSIONS—Plasma FGF21 levels were significantly increased in subjects with prediabetes and diabetes and predicted the development of diabetes in humans.

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Recent evidence from animal studies suggests that fibroblast growth factor 21 (FGF21), a member of the FGF19 subfamily, acts in an endocrine fashion (1) to regulate glucose and lipid metabolism and overall energy balance (2,3). Despite the multiple beneficial effects of FGF21 on glucose and lipid homeostasis and insulin sensitivity in animal models, circulating FGF21 levels are elevated in obese humans and animals with diet-induced and genetic obesity (4), suggesting the presence of FGF21 resistance in obesity (5). Circulating levels of FGF21 also are elevated in patients with obesity-related disorders, including the metabolic syndrome (4), type 2 diabetes (6), and nonalcoholic fatty liver disease (7), in cross-sectional studies. We hypothesized, therefore, that elevated FGF21 levels could predict the development of type 2 diabetes and investigated this possibility prospectively in a long-term follow-up study involving a population-based cohort comprising 1,900 Chinese subjects.

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RESEARCH DESIGN AND METHODS

—All subjects were from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) (8,9) (Supplementary Data). The study protocol was approved by the ethics committee of the University of Hong Kong. Subjects returned after a median interval of 5.4 years for reassessment but were under the care of their primary care physicians between visits. Plasma FGF21 was measured using an in-house ELISA (10) (Antibody and Immunoassay Services, University of Hong Kong, Hong Kong). All statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL). Two-sided P values < 0.05 were considered significant (Supplementary Data).

RESULTS

—At baseline, there were 1,900 subjects with complete demographic and biochemical data. Of these, 1,044 had normal glucose tolerance (NGT), 558 had impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), and 298 subjects had diabetes (11) (Supplementary Table A1). FGF21 levels increased progressively with worsening dysglycemia from NGT, to prediabetes (IGT/IFG), to diabetes (global trend, $P < 0.001$) (Supplementary Fig. A1). Plasma FGF21 concentrations were higher in the groups with IGT/IFG and diabetes (IGT/IFG vs. NGT, median 197.1 vs. 133.2 pg/mL, $P < 0.001$; diabetes vs. IGT/IFG, median 234.0 vs. 197.1 pg/mL, $P = 0.012$).

Plasma levels of FGF21 were similar between men and women (median 172.2 vs. 161.3 pg/mL, $P = 0.106$). Baseline FGF21 levels correlated positively with age, BMI, waist circumference, diastolic and systolic blood pressure (SBP), fasting plasma glucose (FPG), 2-h glucose, fasting insulin, homeostasis model assessment of insulin resistance, total cholesterol, LDL cholesterol, triglycerides, and high-sensitivity C-reactive protein (hsCRP) but negatively with HDL cholesterol (all $P < 0.001$) (Supplementary Table A2). Partial correlation between FGF21 and these parameters remained unchanged after adjustment for

age, sex, and waist circumference/BMI (Supplementary Table A2). In a multiple linear regression analysis, triglycerides ($\beta = 0.244$), age ($\beta = 0.160$), hsCRP ($\beta = 0.102$) (all $P < 0.001$), SBP ($\beta = 0.083$, $P = 0.001$), LDL cholesterol ($\beta = 0.065$, $P = 0.003$), 2-h glucose ($\beta = 0.061$, $P = 0.008$), and waist circumference ($\beta = 0.051$, $P = 0.042$) were independently related to plasma FGF21 levels. The model also included FPG ($P = 0.525$), fasting insulin ($P = 0.664$), and HDL cholesterol ($P = 0.679$).

Among 1,602 subjects without diabetes in CRISPS2 (baseline), 1,292 subjects completed the reassessment (CRISPS3) after a median follow-up of 5.4 years (follow-up rate 80.6%); 73 subjects had developed diabetes (36 men and 37 women), with 3 subjects diagnosed and treated with medications before CRISPS3. Their baseline characteristics are shown in Supplementary Table A3. The baseline FGF21 concentration was significantly higher in subjects who had progressed to diabetes at 5.4 years compared with subjects who did not develop diabetes (median 259.5 vs. 144.0 pg/mL, $P < 0.001$).

Independent predictors for diabetes development were identified using a multiple logistic regression model that included age, sex, waist circumference, FPG, fasting insulin, SBP, triglycerides, HDL cholesterol, smoking status, hsCRP, and FGF21 (Table 1). Baseline FGF21 was a significant independent predictor of diabetes (odds ratio 1.792 [95% CI 1.215–2.642]; $P < 0.01$), together with waist circumference and FPG (both $P < 0.01$) (model 1A). When triglycerides, HDL cholesterol, and SBP were replaced with categorical variables, the results were similar (model 1B). If baseline FPG was replaced by baseline 2-h glucose or the presence of IGT/IFG in the model, FGF21 remained a significant independent predictor of diabetes (both $P < 0.01$) (models 2 and 3). In all models, similar findings were obtained if BMI replaced waist circumference.

Table 1—*Baseline parameters predictive of the development of type 2 diabetes over 5.4 years, examined using multiple logistic regression*

Baseline variables	Model 1A	Model 1B	Model 2A	Model 2B	Model 3A	Model 3B
Age	1.023 (0.996–1.051)	1.027 (1.001–1.054)*	1.032 (1.004–1.060)*	1.035 (1.009–1.061)†	1.028 (1.001–1.056)*	1.032 (1.006–1.058)*
Male	0.722 (0.397–1.310)	0.687 (0.384–1.232)	0.990 (0.542–1.808)	0.882 (0.490–1.588)	0.797 (0.439–1.448)	0.730 (0.407–1.309)
Waist circumference	1.047 (1.013–1.083)†	1.048 (1.014–1.084)†	1.038 (1.003–1.074)*	1.040 (1.004–1.076)*	1.042 (1.008–1.078)*	1.044 (1.009–1.080)*
Ln(fasting insulin)	1.087 (0.612–1.931)	1.059 (0.611–1.835)	1.170 (0.675–2.028)	1.046 (0.614–1.780)	1.150 (0.663–1.996)	1.061 (0.623–1.806)
Fasting glucose	3.023 (1.785–5.118)‡	2.998 (1.779–5.052)‡	—	—	—	—
2-h glucose	—	—	1.739 (1.466–2.063)‡	1.718 (1.451–2.034)‡	—	—
IFG/IGT	—	—	—	—	5.184 (2.832–9.940)‡	5.018 (2.759–9.127)‡
Hypertension	—	0.892 (0.496–1.604)	—	0.774 (0.429–1.395)	—	0.871 (0.488–1.554)
SBP	1.002 (0.986–1.018)	—	0.997 (0.981–1.014)	—	0.999 (0.983–1.016)	—
Hypertension treatment	1.034 (0.525–2.036)	—	1.232 (0.622–2.442)	—	1.259 (0.638–2.484)	—
Dyslipidemia	—	1.073 (0.579–1.990)	—	1.184 (0.635–2.211)	—	1.111 (0.600–2.057)
Ln(triglycerides)	0.859 (0.456–1.619)	—	0.635 (0.333–1.210)	—	0.685 (0.360–1.305)	—
LDL cholesterol	0.955 (0.681–1.339)	—	1.027 (0.740–1.426)	—	1.053 (0.757–1.466)	—
Lipid treatment	0.451 (0.120–1.702)	—	0.511 (0.135–1.935)	—	0.645 (0.170–2.451)	—
Current/past smoker	0.860 (0.384–1.916)	0.893 (0.398–2.002)	0.829 (0.362–1.900)	0.891 (0.393–2.023)	0.872 (0.387–1.963)	0.905 (0.403–2.031)
Ln(hsCRP)	1.180 (0.901–1.544)	1.171 (0.896–1.530)	1.109 (0.840–1.464)	1.111 (0.842–1.466)	1.156 (0.881–1.518)	1.165 (0.890–1.526)
Ln(FGF21)	1.792 (1.215–2.642)†	1.730 (1.194–2.505)†	1.739 (1.172–2.580)†	1.635 (1.115–2.398)*	1.836 (1.243–2.710)†	1.758 (1.206–2.562)†

Data are odds ratio (OR) (95% CI). Model 1A: $OR_{FGF21} = 1.792$ (1.215–2.642), $P = 0.003$ for SBP plus 10 mmHg if on hypertension treatment (SBP_{adj}) replacing SBP and hypertension treatment; $OR_{FGF21} = 1.552$ (1.012–2.382), $P = 0.044$, if excluding those on hypertension treatment. Model 2A: $OR_{FGF21} = 1.741$ (1.173–2.583), $P = 0.006$ for SBP_{adj} replacing SBP and hypertension treatment; $OR_{FGF21} = 1.557$ (1.002–2.420), $P = 0.049$, if excluding those on hypertension treatment. Model 3A: $OR_{FGF21} = 1.841$ (1.247–2.718), $P = 0.002$ for SBP_{adj} replacing SBP and hypertension treatment; $OR_{FGF21} = 1.663$ (1.082–2.558), $P = 0.020$, if excluding those on hypertension treatment. Ln, natural logarithm. *$P < 0.05$. †$P < 0.01$. ‡$P < 0.001$.

CONCLUSIONS—Previous studies have demonstrated that circulating FGF21 levels are elevated in obesity and its related metabolic disorders, including insulin resistance, type 2 diabetes, and nonalcoholic fatty liver disease (4,6,7,12). However, these studies cannot address the cause-and-effect relationship between FGF21 and such disorders because of their cross-sectional design and relatively small sample sizes. In this study, we demonstrated, for the first time, that plasma

FGF21 levels increased with the increasing degree of dysglycemia in 1,900 CRISPS2 subjects. In addition, this study was the first report showing an independent association of raised circulating FGF21 levels with the development of type 2 diabetes in a 5.4-year prospective study.

In this study cohort, we demonstrated an independent association between serum FGF21 levels and triglycerides, LDL cholesterol, and SBP, using multiple linear regression analysis, whereas fasting glucose and insulin were not present in the final model. Our findings agree with previous reports on a positive correlation between serum FGF21 and triglyceride levels (4,13) and suggest a function of FGF21 in lipid metabolism. Because FGF21 is known to exert beneficial effects on lipid profiles in animals (5,14,15), the independent association of serum FGF21 with triglycerides and LDL cholesterol in humans may represent a compensatory response to protect the body from the adverse effects of hyperlipidemia.

In our multiple logistic regression analyses, we found that FGF21 was a strong predictor of diabetes in this prospective cohort, second only to FPG, 2-h glucose, or prediabetes (IGT/IFG), the classical risk factors predictive of diabetes development. These findings further support the notion that the elevation in circulating FGF21 occurs at a very early stage during the deterioration of glucose homeostasis, well before the occurrence of overt diabetes. This phenomenon mirrors closely that of hyperinsulinemia, suggesting that FGF21 resistance, like insulin resistance, may be associated with the pathogenesis of diabetes in humans.

Our study is limited by the relatively small number of subjects with incident diabetes and an attrition rate of almost 20%, largely attributed to emigration. Additional prospective studies in other populations are needed to support our hypothesis that FGF21 resistance precedes the development of diabetes in humans.

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C.C. researched data, ran statistical analyses, and wrote the manuscript. B.M.Y.C. (principal investigator of CRISPS2) and A.W.K.T. were investigators of CRISPS, contributed to discussion, and reviewed and edited the manuscript. Y.W. researched data. L.S.C.L. and K.L.O. advised on statistical analyses and contributed to discussion. N.M.S.W. was an investigator of CRISPS, contributed to discussion, and reviewed and edited the manuscript. A.X. initiated the FGF21 project (funded by HKU3/CRF/09) and provided overall supervision, including the writing of the manuscript. K.S.L.L. (principal investigator of CRISPS3) was an investigator of CRISPS, contributed to discussion, reviewed and edited the manuscript, initiated the FGF21 project (funded by HKU3/CRF/09), and provided overall supervision, including the writing of the manuscript.

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