

ORIGINAL ARTICLE

Serum fibroblast growth factor 21 is a superior biomarker to other adipokines in predicting incident diabetes

Yu Cho Woo*, Chi Ho Lee*†, Carol H.Y. Fong*, Aimin Xu*†‡, Annette W.K. Tso*, Bernard M.Y. Cheung*†‡ and Karen S.L. Lam*†‡

*Department of Medicine, The University of Hong Kong, †State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, and ‡Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong, China

Summary

Objective Fibroblast growth factor 21 (FGF21) improves glucose and lipid metabolism, but high circulating levels are found in type 2 diabetes, suggesting FGF21 resistance. Serum FGF21 predicts incident diabetes, but its performance compared to established and emerging predictors is not known. We aimed to study the performance of FGF21 in diabetes prediction, relative to other adipokines and established risk factors including 2-h plasma glucose (2hG) during the oral glucose tolerance test (OGTT).

Design/Participants/Measurements We studied 1380 non-diabetic subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study using the second visit (2000–2004) as baseline when serum levels of FGF21 and other adipokines were measured. Glycaemic status was assessed by OGTT. Incident diabetes was defined as fasting glucose level (FG) ≥ 7 mmol/l or 2hG ≥ 11.1 mmol/l or use of antidiabetic agents, at subsequent visits.

Results A total of 123 participants developed diabetes over 9.0 years (median). On multivariable logistic regression analysis, FGF21 ($P = 0.003$), adipocyte fatty acid-binding protein ($P = 0.003$) and adiponectin ($P = 0.035$) were independent predictors of incident diabetes. FGF21 had the best change in log likelihood when added to a diabetes prediction model (DP) based on age, family history, smoking, hypertension, BMI, dyslipidaemia and FG. It also improved the area under ROC curve (AUROC) of diabetes prediction (DP) from 0.797 to 0.819 ($P = 0.0072$), rendering its performance comparable to the 'DP + 2hG' model (AUROC=0.838, $P = 0.19$).

Conclusions As a biomarker for diabetes prediction, serum FGF21 appeared to be superior to other adipokines and, on its own, could be considered as an alternative to the OGTT.

(Received 25 July 2016; returned for revision 15 August 2016; finally revised 16 August 2016; accepted 5 September 2016)

Introduction

Fibroblast growth factor 21 (FGF21) is secreted predominantly by the liver but also by other tissues involved in glucose and lipid metabolism, such as adipose tissue, pancreas and skeletal muscle,¹ with the contribution from adipose tissue being much increased in obesity. Multiple beneficial effects of FGF21 on insulin sensitivity, glucose and lipid homeostasis have been observed in animal models.² FGF21 is also possibly bioactive in humans as treatment for obese diabetic subjects with FGF21-based therapies resulted in beneficial effects on body weight, plasma lipoprotein profile and serum adiponectin levels^{3,4} and reduced fasting insulin.³ Circulating FGF21 levels, however, were elevated in obesity,⁵ as well as in obesity-related diseases such as dyslipidaemia, type 2 diabetes (T2DM), nonalcoholic fatty liver disease, carotid atherosclerosis and coronary artery disease.^{6–10} Raised serum FGF21 levels in these diseases may represent a compensatory response to the underlying metabolic stresses,¹ including insulin resistance/hyperinsulinaemia, or may be secondary to FGF21 resistance. Indeed, impaired FGF21 signalling in adipocytes has been demonstrated in mice with diet-induced obesity.¹¹ The above clinical observations have suggested the potential role of FGF21 as a biomarker for obesity-related diseases. Notably, high serum FGF21 levels have been shown to predict the development of T2DM in two 5-year prospective studies.^{8,12}

The dysregulated secretion, and hence altered circulating levels of various adipokines in obesity,¹³ has raised the possibility that serum levels of such adipokines can be used as biomarkers of obesity-related diseases such as T2DM. In the ARIC study of 4 US communities, it was found that measurement of adiponectin and leptin, but not interleukin-6 and retinol acid-binding protein 4, could provide a modest improvement in the prediction of T2DM by conventional risk factors.¹⁴ In the Chinese population, we previously found that adiponectin, tumour necrosis factor- α (TNF- α) or its soluble receptor tumour necrosis

Correspondence: Karen Lam or Bernard Cheung, Department of Medicine, University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China. Tel.: +852 2255-4783; Fax: +852 2816-2863; E-mail: kslam@hku.hk or mycheung@hku.hk

factor-alpha receptor 2 (TNF- α R2), and adipocyte fatty acid-binding protein (A-FABP), could all independently predict incident diabetes.^{15,16} We also found that the combined use of serum adiponectin and TNF- α R2 levels, when added to conventional risk factors, had comparable performance to 2-h plasma glucose (2hG) during the oral glucose tolerance test (OGTT), in predicting incident diabetes.¹⁵ With increasing data on FGF21 as a metabolic regulator in humans^{3,4} and its role as a novel biomarker for predicting T2DM,^{8,12} it appeared timely that we should assess its performance in the prediction of incident diabetes, in comparison with other adipokines and established predictors of T2DM, including the OGTT. This was conducted using the 9-year prospective data from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS).

Materials and methods

Subjects were recruited from CRISPS, a long-term, population-based, prospective study on the development of cardiovascular risk factors in Hong Kong. In 1995–1996 (CRISPS1), 2,895 unrelated Chinese subjects were invited randomly by their telephone numbers to undergo a detailed assessment.¹⁷ Subjects returned for the reassessment visits in 2000–2004 (CRISPS2), 2005–2008 (CRISPS3) and 2010–2012 (CRISPS4). Details of medical history, anthropometric and biochemical parameter measurements were described elsewhere.¹⁸ Subjects attended the visits in the morning, and all blood taking was performed at 9am after an overnight fast to avoid the effect of diurnal changes and meals on circulating adipokine levels.¹⁹ Serum levels of the adipokines potentially predictive of T2DM were measured from stored serum samples collected at CRISPS2; hence, this visit was used as the baseline of this study. Subjects who had been started on antidiabetic medications, or with fasting glucose (FG) ≥ 7 mmol/l or 2-hG ≥ 11.1 mmol/l, were considered to have diabetes. Subjects without diabetes at baseline visit were followed for their glycaemic status at CRISPS3 and CRISPS4. The incident diabetes group included all subjects with incident diabetes when screened at CRISPS3 or CRISPS4, whilst subjects in the nondiabetes group were those who remained nondiabetic at CRISPS4. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or on hypertensive treatment. The presence of dyslipidaemia was defined as: triglycerides ≥ 1.7 mmol/l; HDL cholesterol < 1.0 mmol/l for men and 1.3 for women; LDL cholesterol ≥ 3.4 mmol/l; or on lipid-lowering treatment. Insulin resistance was estimated using the homoeostasis model assessment index of insulin resistance (HOMA-IR), calculated by the formula (FG in mmol/l \times fasting insulin in mIU/l/22.5). The study was approved by the ethics committee of the Faculty of Medicine, University of Hong Kong. Informed consent was obtained from all subjects.

Detailed information on the measurement of serum A-FABP, TNF- α R2 and total adiponectin levels was described previously.¹⁵ TNF- α R2 was measured as a surrogate marker for TNF- α because soluble TNF- α R2 can be measured in frozen plasma with greater sensitivity and reliability than TNF- α .²⁰

Serum leptin was measured using a commercial ELISA kit (BioVendor Laboratory Medicine, Brno, Czech). Serum FGF21 was measured with an in-house ELISA kit (Antibody and Immunoassay Services, University of Hong Kong) as previously described.^{5,8,21} The intra- and interassay variations were 4.5% and 6.8%, respectively.

All statistical analyses were performed with SPSS Statistics 19 (SPSS, Chicago, IL). Results were presented as mean \pm SD or median with interquartile range (IQR) as appropriate. For data that were not normally distributed, natural logarithmic transformation was applied before analyses. In univariate analyses, variables were compared between groups by one-way ANOVA for continuous data and Chi-square test for categorical data, respectively. Biomarkers including A-FABP, adiponectin, leptin and TNF- α R2 with circulating levels showing gender-specific dimorphisms were sex-adjusted. They were categorised as high or low level with reference to their optimal cut-offs identified by Youden index.²² Multivariable logistic regression was used to test for significant independent variables for incident diabetes. To compare the relative performance of biomarkers, a basic model based on conventional risk factors and common biochemical parameters, which were independent predictors or of clinical relevance, was constructed. Log-likelihood ratio tests were used to compare the change in log likelihood (-2LL) before and after addition of biomarkers to the basic model using multivariable logistic regression analysis. The biomarker with the best -2LL was tested for the change in the area under the curve of receiver operating characteristics (AUROC) when added to the basic model and compared with the AUROC with 2hG added to the basic model. Differences in AUROC were assessed using Delong's test.²³

Results

A total of 123 subjects developed incident diabetes over a median of 9.0 years (IRQ: 6.6 – 9.7). Those who developed diabetes were older, with higher body mass index (BMI), waist circumference (WC), FG, fasting insulin and HOMA-IR (all $P < 0.001$) at baseline visit. More had a history of smoking, family history of diabetes (both $P = 0.005$), central obesity, hypertension and dyslipidaemia (all $P < 0.001$). The levels of all biomarkers were significantly different between the incident diabetes and nondiabetes groups in univariate analysis (Table 1). The optimal cut-offs of the biomarkers were as follows: 178.2 pg/ml for FGF21; 29.8 ng/ml in men and 22.4 ng/ml in women for A-FABP; 6.19 μ g/ml in men and 6.03 μ g/ml in women for adiponectin; 2051.1 ng/ml in men and 2134.6 ng/ml in women for TNF- α R2; and 4.666 ng/ml for men and 10.227 ng/ml for women for leptin. On multiple logistic regression analysis, smoking history, family history of diabetes, WC and BMI were the clinical parameters independently predictive of diabetes, whilst FG was the independent biochemical predictor. Of the biomarkers, high FGF21 and A-FABP, but not TNF- α R2 and leptin, were independent predictors of diabetes, whereas high adiponectin was associated with a reduced risk of incident diabetes (Table 2). Addition of FGF21 to the basic diabetes prediction model (DP)

Table 1. Baseline clinical and biochemical characteristics of subjects with and without incident Type 2 diabetes in 9.0 (6.6–9.7) years

Baseline variables	Cumulative DM	Non-DM	P-value
N	123	1257	–
Age, years	55.2 ± 10.4	49.8 ± 10.9	<0.001
Men, %	51.6	44.7	0.139
Ever smoker, %	34.7	23.4	0.005
Physical activity, %	30.8	27.4	0.430
Family history of diabetes, %	25.8	16.0	0.005
Central obesity, %	47.6	23.4	<0.001
Waist circumference, cm			
Male	88.5 ± 8.89	82.8 ± 8.31	<0.001*
Female	80.8 ± 9.05	74.7 ± 8.57	
Body mass index, kg/m ²	25.9 ± 3.69	23.6 ± 3.22	<0.001
Hypertension, %	33.9	19.3	<0.001
Systolic blood pressure, mmHg	128 ± 19	119 ± 17	<0.001
Diastolic blood pressure, mmHg	79 ± 9	74 ± 10	<0.001
IGT/IFG, %	66.9	22.9	<0.001
Fasting glucose, mmol/l	5.45 ± 0.58	5.01 ± 0.47	<0.001
Fasting insulin†, mIU/l	8.9 (6.6–15.0)	6.9 (5.0–9.7)	<0.001
HOMA-IR†	2.30 (1.49–2.30)	1.52 (1.11–2.19)	<0.001
Dyslipidaemia, %	82.3	58.9	<0.001
Total cholesterol, mmol/l	5.51 ± 1.00	5.24 ± 0.87	0.002
Triglycerides†, mmol/l	1.3 (1.0–2.0)	1.1 (0.7–1.5)	<0.001
HDL cholesterol, mmol/l	1.29 ± 0.33	1.44 ± 0.38	<0.001
LDL cholesterol, mmol/l	3.50 ± 0.91	3.23 ± 0.77	<0.001
Adiponectin†, ug/ml			
Men	4.53 (3.04–6.06)	5.90 (3.83–9.35)	<0.001*
Women	7.12 (4.42–11.3)	8.16 (5.76–11.8)	
FGF21†, pg/ml			
Men	234.7 (154.1–408.9)	155.3 (84.4–261.3)	<0.001*
Women	266.6 (186.5–322.6)	131.1 (78.1–233.1)	
A-FABP†, ng/ml			
Men	21.8 (14.8–31.9)	18.2 (13.2–24.4)	<0.001*
Women	29.3 (23.4–37.0)	22.0 (16.4–30.3)	
TNF-alpha R2†, ng/ml			
Men	2153.8 (1864.4–2654.6)	1934.9 (1683.7–2282.0)	<0.001*
Women	1897.3 (1557.6–2335.2)	1758.8 (1520.1–2067.4)	
Leptin†, ng/ml			
Men	5.78 (3.21–8.73)	4.01 (2.02–6.48)	<0.001*
Women	14.4 (10.4–20.7)	11.0 (7.75–15.7)	

Data are presented as mean ± standard deviation, median (interquartile range) or percentage as appropriate.

*Sex-adjusted *P*-value.

†log-transformed before analysis. Central obesity was defined as waist circumference ≥ 90 cm for men and 80 for women; hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or on hypertensive treatment. Dyslipidaemia: triglycerides ≥ 1.7 mmol/l; HDL cholesterol < 1.0 mmol/l for men and 1.3 for women; LDL cholesterol ≥ 3.4 mmol/l; or on lipid-lowering treatment.

consisting of age, smoking history, BMI, family history, hypertension, dyslipidaemia and FG resulted in the best -2LL of the model, followed by A-FABP and adiponectin, whilst addition of TNF- α R2 or leptin did not result in significant changes in -2LL (Table 3). The AUROC of using FGF21 alone in diabetes prediction was 0.708. When added to DP, the model consisting of established diabetes risk factors, FGF21 significantly improved the AUROC of DP from 0.797 to 0.819 ($P = 0.0072$). Likewise, 2hG significantly improved the AUROC of DP to 0.838. However, there was no significant difference observed in the AUROC of the two models: 'FGF21 + DP' versus '2hG+DP' (0.819 vs 0.838, $P = 0.19$) (Table 4, Figure 1). Using FGF21 levels in the

form of continuous data instead of applying the cut-off value did not affect the results (Tables 3 and 4). Addition of adiponectin or A-FABP to the same DP model did not show significant improvement in the AUROC (Table 4). In our previous report, the combination of ADP + TNF- α R2 had a significant improvement when added to the basic model.¹⁵ However, in this cohort with a longer follow-up duration and more cases of incident diabetes, adding the same combination of biomarkers to the DP did not show significant improvement in AUROC (0.807 vs 0.797, $P = 0.09$), in keeping with the superior predictive performance of serum FGF21 as a biomarker of incident diabetes, even when used on its own.

Table 2. Multivariable logistic regression for significant independent variables which predicted the development of DM

Baseline variables	Adjusted OR (95% CI)	P-value
Age (years)	1.02 (1.00–1.04)	0.039
Ever smoked	1.81 (1.14–2.85)	0.011
Family history of diabetes	2.37 (1.45–3.85)	0.001
BMI (kg/m ²)	1.09 (1.02–1.16)	0.008
HT (%)	1.10 (0.70–1.72)	0.682
FG (mmol/l)	3.63 (2.39–5.50)	<0.001
Dyslipidaemia	1.61 (0.96–2.71)	0.071
FGF21 (pg/ml)	1.60 (1.18–2.16)	0.003
A-FABP* (ng/ml)	2.03 (1.28–3.20)	0.003
Adiponectin* (ug/ml)	0.62 (0.40–0.97)	0.035

*Sex specific; BMI, body mass index; HT, hypertension; FG, fasting glucose; FGF21, fibroblast growth factor 21; A-FABP, adipocyte fatty acid-binding protein.

Values in bold, statistically significant

Table 3. Log-likelihood ratio tests comparing the change before and after addition of adipokines using multivariable logistic regression analysis

Diabetes Prediction Model	Biomarkers	Change in -2LL from basic model		P-value
		-2LL	model	
Age, FH of DM, smoking, BMI, HT, FG and dyslipid		682.562	–	
	+ FGF21		15.070	<0.001
	+ FGF21*		21.805	<0.001
	+ A-FABP*		11.969	<0.001
	+ Adiponectin*		4.323	0.038
	+ TNF- α R2*		1.558	0.212
	+ Leptin*		1.515	0.218

FGF21 was log-transformed before analysis, unless otherwise specified.

*Sex-specific optimal cut-off points applied.

-2LL, -2 log Likelihood; FH of DM, family history of diabetes; smoking, history of ever smoking; BMI, body mass index; HT, hypertension; FG, fasting glucose; dyslipid, dyslipidaemia; FGF21, fibroblast growth factor 21; A-FABP, adipocyte fatty acid-binding protein; TNF- α R2: tumour necrosis factor-alpha receptor 2.

Values in bold, statistically significant

Discussions

It has been established that the development of diabetes can be effectively delayed or prevented in a cost-effective manner in high-risk subjects with impaired glucose tolerance.²⁴ Despite requiring the inconvenient and cumbersome OGTT, 2hG remains a very strong predictor for diabetes development, which predicts diabetes on its own and provides additional value to other prediction models.^{15,25} In this study, we showed that FGF21, a novel metabolic regulator of glucose and lipid metabolism and a potential therapeutic agent for metabolic disorders, could also be a

useful biomarker for the prediction of incident diabetes. As a single biomarker, it significantly enhanced the prediction of diabetes development by established risk factors, with a performance that was comparable to 2hG, and appeared to be superior to other adipokines previously studied in our CRISPS cohort.

Obesity indices are good predictors of incident diabetes. Simple diabetes prediction models usually rely on obesity indices as their prerequisite parameters. In our cohort, the obesity indices, BMI and WC, were also independent predictors. However, it is noteworthy that only 20% or fewer obese Caucasians will progress to diabetes over subsequent years.²⁶ Likewise, in this long-term prospective Chinese cohort, only 12.1% subjects with BMI \geq 23 and 20.5% of subjects with BMI \geq 27.5 developed diabetes in 9 years' time. Hence, prediction models usually also include other parameters to improve overall performance. In the basic model we constructed for testing the performances of different biomarkers, we did not include both BMI and WC because of their strong correlation. BMI was preferred to WC in the model because the measurement of body weight and height should be more precise than WC, which is heavily influenced by the anatomic location of measurement.²⁷ Taking our basic model as an example, incorporation of other clinical risk factors, such as age, hypertension, family history, smoking history as well as readily measurable metabolic parameters, including fasting glucose and lipid profiles to BMI, on their own resulted in a reasonably good predictive model with an AUROC at 0.797. Hence, it has been argued that a prediction model based on simple and clinically available variables might do even better than performing the OGTT.²⁵ In our cohort, however, the addition of the 2hG measurement to the multivariable model, which included FG and dyslipidaemia in addition to clinically available risk factors, increased the AUC of the prediction model still further, in this and a previous study.¹⁵ On the other hand, being an inconvenient test lacking in reproducibility, the OGTT is far from ideal as a screening tool and a replacement with comparable predictability of diabetes would be welcome. We did demonstrate in our previous study, with a shorter follow-up period and fewer incident diabetes cases, that the addition of a combination of two obesity-related biomarkers, namely adiponectin and TNF- α R2, could be comparable to 2hG in improving diabetes prediction by established risk factors, dyslipidaemia and FG.¹⁵ These observations may suggest that 2hG remains a strong predictor for future diabetes because it represents part of the pathophysiology of T2DM that cannot be reflected by FG but can be attributed at least partially to the effects of circulating adipokines. The findings in the current study suggest that FGF21, as a single obesity-related biomarker, appears to have a prediction performance that is closest to 2hG, compared to others that have been studied in our population.

FGF21 has multiple beneficial effects on insulin sensitivity, glucose and lipid homeostasis in animal models. However, raised FGF 21 levels have been generally observed in obesity and conditions associated with obesity or increased insulin resistance. Whilst insulin resistance is well known to appear before the onset of diabetes, FGF21 resistance also precedes dysglycaemia.^{7,12} The raised FGF21 levels in these conditions may be due to a compensatory response to metabolic stresses resulting from increased

Table 4. Comparisons of AUROCs of different diabetes prediction models.

Model	Additional variable(s)	AUROC (95% CI)	Delong P-value [Referent: basic model]	Delong P-value [Referent: 2-h glucose]
Age, BMI, Fhx of DM, ever smoking, HT, FG and Dyslip	–	0.797 (0.773–0.817)	Referent	–
	+ 2-h glucose	0.838 (0.817–0.857)	0.0010	Referent
	+ FGF21	0.813 (0.792–0.833)	0.0083	0.0622
	+ FGF21*	0.819 (0.798–0.839)	0.0072	0.1882
	+ A-FABP*	0.808 (0.786–0.828)	0.0743	0.0290
	+ ADP*	0.799 (0.777–0.820)	0.4319	0.0023
	+ TNF- α R2*	0.804 (0.782–0.825)	0.0782	0.0099
	+ ADP* + TNF- α R2*	0.807 (0.785–0.828)	0.0906	0.0203

FGF21 was log-transformed before analysis, unless otherwise specified.

*Sex-specific optimal cut-off points applied.

AUROC, Area under the curve of receiver operating characteristics; BMI, body mass index; HT, hypertension; Fhx of DM, family history of diabetes; FG, fasting glucose; dyslip, dyslipidaemia; FGF21, fibroblast growth factor 21; A-FABP, adipocyte fatty acid-binding protein; ADP, adiponectin; TNF- α R2; tumour necrosis factor-alpha receptor 2.

Values in bold, statistically significant

circulating free fatty acids and insulin, which are known to stimulate increased FGF21 expression.¹ Moreover, adipose tissue inflammation in obesity, which involves the c-Jun NH2-terminal kinase 1 pathway, could result in the suppression of β -Klotho expression by TNF- α and hence impaired FGF21 action in adipocytes.¹¹ FGF21 is also the upstream regulator of adiponectin. It induces expression and secretion of adiponectin in adipocytes to confer glucose-lowering and insulin-sensitizing effects.²⁸ Hence, FGF21 resistance in obesity can potentially lead to insulin resistance through hypo adiponectinaemia. Whereas the role of hypo adiponectinaemia in diabetes development is well established,^{13–15} the mechanism linking A-FABP with glucose homeostasis is less clear. A recent study, however, has demonstrated that A-FABP is a secreted adipokine regulating hepatic glucose production, and its immune neutralization *in vivo* led to reduced gluconeogenesis and the correction of a diabetic phenotype in obese mice.²⁹ Our study suggested that leptin and TNF- α , both pro-inflammatory adipokines with increased expression in obesity,¹⁵ are not by themselves the major players in diabetes development in our cohort. In a nutshell, FGF21 has been found to be involved in the interplay with various other hormones in the pathogenesis of T2DM, rendering it a good candidate as a biomarker for diabetes prediction and a possible alternative to the inconvenient OGTT. FGF21 was also found to be useful as a biomarker in other diseases related to obesity. Serum FGF21 levels were found to be significantly increased in nonalcoholic fatty liver disease and were positively correlated with intrahepatic triglyceride. Measurement of FGF21 might have the additional benefit of detecting mild steatosis, whilst the diagnostic sensitivity of ultrasonography decreased sharply if the degree of steatosis was less than 30% on biopsy.³⁰ There may be additional advantage of measurement of FGF21 in assessing or predicting other diseases related to obesity and insulin resistance.

Our study has the strengths of being a long-term study with prospective data on incident diabetes over 9 years in a

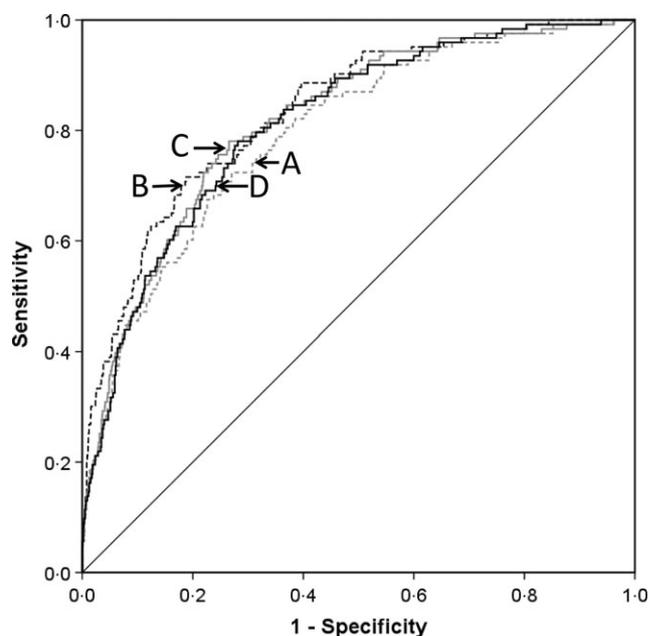


Fig. 1 Comparisons of AUROCs of different diabetes prediction models. A: Diabetes prediction model (DP) based on age, body mass index, family history of diabetes, ever smoking history, hypertension, fasting glucose and dyslipidaemia; B: DP + 2-h glucose; C: DP + FGF21 (with cut-off); D: FGF21 (continuous data); FGF21, fibroblast growth factor 21.

genetically homogeneous Chinese population. Our findings should be of considerable value in diabetes prediction in the heavily populated major cities of Mainland China. The glycaemic status of all participants, except those who had been taking antidiabetic agents, was reliably verified by OGTT. We did not use HbA1c as a diagnostic criterion for diabetes as the diagnostic criteria have changed over time, with the HbA1c criterion only being accepted after 2011. Had the HbA1c criterion been adopted, an additional number of subjects would have been

screened to have diabetes as shown by our results at CRISPS4.³¹ On the other hand, the data of this study are also limited by the drawback of a very long-term prospective study as we unavoidably had participants lost to follow-up due to various reasons. Participants who returned for follow-up visits up until CRISPS4 might well be the more health conscious subjects and might not be truly representative of the general population. Application of the FGF21 level for diabetic risk prediction requires integration with other risk factors to formulate a risk score which requires multistep calculations and may be inconvenient to use when compared with prediction models based on simple measurable parameters. However, this clumsy calculation procedure is not difficult to overcome due to the relatively easy access to computers or even smartphones nowadays. Lastly, our participants were predominantly middle-aged individuals with a mean age of 50.3 years; the youngest participant was 30 years old at CRISPS2 baseline. Our conclusions may thus not be applicable to younger individuals.

In summary, we have demonstrated that, as a single biomarker, FGF21 adds value to established risk factors when used in the construction of diabetes prediction models. In this regard, its performance appears to be superior to other obesity-related biomarkers and is comparable to the 2-h post-OGTT glucose. Further studies in other populations are warranted to validate its use as a biomarker to identify high-risk subjects for interventional measures to prevent diabetes development.

Funding

Research Grant Council, Hong Kong (CRF HKU02/12R).

Disclosure statement

The authors have nothing to disclose.

References

- 1 Woo, Y.C., Xu, A., Wang, Y. *et al.* (2013) Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. *Clinical Endocrinology*, **78**, 489–496.
- 2 Kharitonov, A. & DiMarchi, R. (2015) FGF21 Revolutions: Recent Advances Illuminating FGF21 Biology and Medicinal Properties. *Trends in Endocrinology and Metabolism*, **26**, 608–617.
- 3 Gaich, G., Chien, J.Y., Fu, H. *et al.* (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. *Cell Metabolism*, **18**, 333–340.
- 4 Talukdar, S., Zhou, Y., Li, D. *et al.* (2016) A Long-Acting FGF21 Molecule, PF-05231023, Decreases Body Weight and Improves Lipid Profile in Non-human Primates and Type 2 Diabetic Subjects. *Cell Metabolism*, **23**, 427–440.
- 5 Zhang, X., Yeung, D.C., Karpisek, M. *et al.* (2008) Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*, **57**, 1246–1253.
- 6 Inagaki, T. (2015) Research Perspectives on the Regulation and Physiological Functions of FGF21 and its Association with NAFLD. *Frontiers in Endocrinology*, **6**, 147.
- 7 Li, H., Bao, Y., Xu, A. *et al.* (2009) Serum fibroblast growth factor 21 is associated with adverse lipid profiles and gamma-glutamyltransferase but not insulin sensitivity in Chinese subjects. *Journal of Clinical Endocrinology and Metabolism*, **94**, 2151–2156.
- 8 Chen, C., Cheung, B.M., Tso, A.W. *et al.* (2011) High plasma level of fibroblast growth factor 21 is an Independent predictor of type 2 diabetes: a 5.4-year population-based prospective study in Chinese subjects. *Diabetes Care*, **34**, 2113–2115.
- 9 Chow, W.S., Xu, A., Woo, Y.C. *et al.* (2013) Serum fibroblast growth factor-21 levels are associated with carotid atherosclerosis independent of established cardiovascular risk factors. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **33**, 2454–2459.
- 10 Lin, Z., Wu, Z., Yin, X. *et al.* (2010) Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. *PLoS ONE*, **5**, e15534.
- 11 Diaz-Delfin, J., Hondares, E., Iglesias, R. *et al.* (2012) TNF-alpha represses beta-Klotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway. *Endocrinology*, **153**, 4238–4245.
- 12 Bobbert, T., Schwarz, F., Fischer-Rosinsky, A. *et al.* (2013) Fibroblast growth factor 21 predicts the metabolic syndrome and type 2 diabetes in Caucasians. *Diabetes Care*, **36**, 145–149.
- 13 Tso, A.W., Xu, A., Chow, W.S. *et al.* (2008) Adipose tissue and the metabolic syndrome: focusing on adiponectin and several novel adipokines. *Biomarkers in Medicine*, **2**, 239–252.
- 14 Raynor, L.A., Pankow, J.S., Duncan, B.B. *et al.* (2013) Novel risk factors and the prediction of type 2 diabetes in the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care*, **36**, 70–76.
- 15 Woo, Y.C., Tso, A.W., Xu, A. *et al.* (2012) Combined use of serum adiponectin and tumor necrosis factor-alpha receptor 2 levels was comparable to 2-hour post-load glucose in diabetes prediction. *PLoS ONE*, **7**, e36868.
- 16 Tso, A.W., Xu, A., Sham, P.C. *et al.* (2007) Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care*, **30**, 2667–2672.
- 17 Janus, E.D., Watt, N.M., Lam, K.S. *et al.* (2000) The prevalence of diabetes, association with cardiovascular risk factors and implications of diagnostic criteria (ADA 1997 and WHO 1998) in a 1996 community-based population study in Hong Kong Chinese. Hong Kong Cardiovascular Risk Factor Steering Committee. American Diabetes Association. *Diabetic Medicine*, **17**, 741–745.
- 18 Cheung, B.M., Wat, N.M., Man, Y.B. *et al.* (2007) Development of diabetes in Chinese with the metabolic syndrome: a 6-year prospective study. *Diabetes Care*, **30**, 1430–1436.
- 19 Yu, H., Xia, F., Lam, K.S. *et al.* (2011) Circadian rhythm of circulating fibroblast growth factor 21 is related to diurnal changes in fatty acids in humans. *Clinical Chemistry*, **57**, 691–700.
- 20 Liu, S., Tinker, L., Song, Y. *et al.* (2007) A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Archives of Internal Medicine*, **167**, 1676–1685.
- 21 Lee, C.H., Hui, E.Y., Woo, Y.C. *et al.* (2015) Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. *Journal of Clinical Endocrinology and Metabolism*, **100**, 1368–1375.
- 22 Perkins, N.J. & Schisterman, E.F. (2006) The inconsistency of “optimal” cutpoints obtained using two criteria based on the

- receiver operating characteristic curve. *American Journal of Epidemiology*, **163**, 670–675.
- 23 DeLong, E.R., DeLong, D.M. & Clarke-Pearson, D.L. (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, **44**, 837–845.
 - 24 Ratner, R.E.. Diabetes Prevention Program, R. (2006) An update on the Diabetes Prevention Program. *Endocrine Practice*, **12** (Suppl 1), 20–24.
 - 25 Stern, M.P., Williams, K. & Haffner, S.M. (2002) Identification of persons at high risk for type 2 diabetes mellitus: do we need the oral glucose tolerance test? *Annals of Internal Medicine*, **136**, 575–581.
 - 26 Meigs, J.B., Wilson, P.W., Fox, C.S. *et al.* (2006) Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *Journal of Clinical Endocrinology and Metabolism*, **91**, 2906–2912.
 - 27 Mason, C. & Katzmarzyk, P.T. (2009) Variability in waist circumference measurements according to anatomic measurement site. *Obesity*, **17**, 1789–1795.
 - 28 Lin, Z., Tian, H., Lam, K.S. *et al.* (2013) Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metabolism*, **17**, 779–789.
 - 29 Cao, H., Sekiya, M., Ertunc, M.E. *et al.* (2013) Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metabolism*, **17**, 768–778.
 - 30 Li, H., Fang, Q., Gao, F. *et al.* (2010) Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *Journal of Hepatology*, **53**, 934–940.
 - 31 Woo, Y.C., Cheung, B.M., Yeung, C.Y. *et al.* (2015) Cardiometabolic risk profile of participants with prediabetes diagnosed by HbA1c criteria in an urban Hong Kong Chinese population over 40 years of age. *Diabetic Medicine*, **32**, 1207–1211.