Serum Fibroblast Growth Factor 21 Is Associated with Adverse Lipid Profiles and γ-Glutamyltransferase But Not Insulin Sensitivity in Chinese Subjects

Huating Li, Yuqian Bao, Aimin Xu, Xiaoping Pan, Junxi Lu, Haiya Wu, Huijuan Lu, Kunsan Xiang, and Weiping Jia

Department of Endocrinology and Metabolism (H.Li., Y.B., X.P., J.L., H.W., H.Lu., K.X., W.J.), Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Diabetes Institute, Shanghai Clinical Center of Diabetes, Shanghai 200233, China; Department of Medicine (H.Li.), Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; and Department of Medicine and Department of Pharmacology (A.X.), Research Centre of Heart, Brain, Hormone, and Healthy Aging (A.X.), University of Hong Kong, Hong Kong 999077, China

Objective: Fibroblast growth factor (FGF) 21, a hormone primarily secreted by liver, has recently been shown to have beneficial effects on glucose and lipid metabolism and insulin sensitivity in animal models. This study investigated the association of serum FGF21 levels with insulin secretion and sensitivity, as well as circulating parameters of lipid metabolism and hepatic enzymes in Chinese subjects.

Design: Serum FGF21 levels were determined by ELISA in 134 normal glucose tolerance (NGT), 101 isolated-impaired fasting glucose, and 118 isolated-impaired glucose tolerance (I-IGT) Chinese subjects, and their association with parameters of adiposity, glucose, and lipid profiles, and levels of liver injury markers was studied. In a subgroup of this study, the hyperglycemic clamp technique was performed in 31 NGT, 17 isolated-impaired fasting glucose, and 15 I-IGT subjects to measure insulin secretion and sensitivity to test the associations with serum FGF21.

Results: The serum FGF21 levels in I-IGT were significantly higher than NGT subjects [164.6 pg/ml (89.7, 261.0) *vs.* 111.8 pg/ml (58.0, 198.9); $P < 0.05$], and correlated positively with several parameters of adiposity. Multiple stepwise regression analysis showed an independent association of serum FGF21 with serum triglycerides, total cholesterol, and γ-glutamyltransferase (all $P < 0.05$). However, FGF21 did not correlate with insulin secretion and sensitivity, as measured by hyperglycemic clamp and a 75-g oral glucose tolerance test.

Conclusions: Serum levels of FGF21 are closely related to adiposity, lipid metabolism, and biomarkers of liver injury but not insulin secretion and sensitivity in humans. **(*J Clin Endocrinol Metab* 94: 2151–2156, 2009)**

The fibroblast growth factor (FGF) family comprises at least 22 members with diverse functions, such as cell growth, development, differentiation, and wound healing (1). Recently, three members of this family, including FGF19, FGF21, and FGF23, have been demonstrated as endocrine factors involved in hormone-like metabolic effects by interacting with FGF receptors (1–4). FGF21, a polypeptide with 210 amino acid residues abundantly expressed in the liver, has recently been an important regulator of glucose and lipid metabolism in animal models (2, 5). Transgenic mice with overexpression of FGF21 were resistant to diet-induced obesity and metabolic disturbance (2). The therapeutic intervention with recombinant FGF21 resulted in a re-

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Abbreviations: ALT, Alanine aminotransferase; BMI, body mass index; FGF, fibroblast growth factor; FINS, fasting serum insulin concentration; FM, fat mass; FPG, fasting plasma glucose concentration; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model of assessment; HOMA-IR, homeostasis model of assessment-insulin resistance; 2hPG, 2-h plasma glucose concentration; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; I-IFG, isolated-impaired fasting glucose; I-IGT, isolated-impaired glucose tolerance; ISI, insulin sensitivity index; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; 1PH, first-phase insulin secretion; 2PH, second-phase insulin secretion; PPARα, peroxisome proliferator-activated receptor α; TC, total cholesterol; TG, triglyceride.

duction of blood glucose and triglycerides (TGs) to near normal levels in both *ob/ob* and *db/db* mice without apparent mitogenicity, hypoglycemia, or weight gain. In diabetic rhesus monkeys, the therapeutic intervention with recombinant FGF21 caused a dramatic decline in fasting plasma glucose, insulin, and glucagon, further supporting the role of FGF21 as an insulin sensitizer in animals (6). In addition, FGF21 administration in the monkey led to a significant improvement in lipoprotein profiles, including decreased low-density lipoprotein cholesterol (LDL-C) and TGs, as well as elevated high-density lipoprotein cholesterol (HDL-C). FGF21 has an antilipolytic effect in human adipocytes, which could be a mechanism of promoting insulin sensitivity via reducing the release of fatty acids into the circulation (7). Moreover, FGF21 treatment increased islet insulin content and glucose-induced insulin secretion by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathway in diabetic rodents (8). More recently, FGF21 was implicated as a hormone important in the intermediate period in the adaptation to starvation (9–11). Peroxisome proliferator-activated receptor α (PPARα) regulated FGF21 transcription necessary for the fasting response in mice, and PPARα agonists increased FGF21 mRNA transcription in human hepatocytes. On the other hand, adenovirus-mediated down-regulation of FGF21 in the liver led to the development of fatty liver, dyslipidemia, and reduced serum ketones due to the altered expression of key genes involved in hepatic lipid and ketone metabolism (10). Together, these findings demonstrate an important role of FGF21 as a hepatic hormone in the regulation of lipid metabolism and also suggest that PPARα agonists might mediate the therapeutic benefits by stimulating hepatic FGF21 production (11). Although these animal-based studies are certainly of interest, the clinical relevance of FGF21 has seldom been explored so far.

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are two important categories of prediabetes that represent an intermediate stage between normal glucose tolerance (NGT) and diabetes (12, 13). Recent studies showed that IFG and IGT have different characteristics of impaired insulin secretion and sensitivity. IFG is due to impaired basal insulin secretion and hepatic insulin resistance, whereas IGT mainly results from reduced second-phase insulin release and peripheral insulin resistance (14). At the molecular level, the inactivation of the forkhead transcription factor Foxa2 by nuclear exclusion plays a crucial role in hepatic resistance in IFG (15). Interestingly, a recent study demonstrated that treatment with FGF21 increased hepatic Foxa2 expression in mice (16). However, the pathophysiological roles of FGF21 in IFG and IGT remain unknown. The objectives of the study were to investigate whether altered serum levels of FGF21 are specifically associated with either IFG or IGT in prediabetic subjects, and to explore the association of serum FGF21 levels with insulin secretion and sensitivity, as well as glucose and lipid profiles in these subjects.

Subjects and Methods

Study subjects

A total of 353 subjects of Chinese origin (Han Chinese) (116 men and 237 women) from Shanghai Diabetes Studies was recruited for this study. All subjects underwent comprehensive physical examinations and routine biochemical analyses of blood. A 75-g oral glucose tolerance test (OGTT) was also performed. Among all subjects, 134 had NGT, 101 had isolated-impaired fasting glucose (I-IFG), and 118 had isolated-impaired glucose tolerance (I-IGT) (14). Subjects with the following conditions were excluded from this study: biliary obstructive diseases, acute or chronic virus hepatitis, cirrhosis, known hyperthyroidism or hypothyroidism, presence of cancer, current treatment with systemic corticosteroids, and pregnancy. Current drinkers and ex-drinkers were excluded from the study. Current drinkers were those who drank 6 g or more alcohol/d on average for at least 1 yr, and ex-drinkers were those who had been a drinker but quit for at least half a year before the survey. The study was approved by the human research ethics committee of the hospital, and informed consent was obtained from all subjects.

Definition for IFG and IGT

The 1997 American Diabetes Association diagnostic criteria were used to characterize subjects with IFG and IGT (12). IFG was defined as a fasting plasma glucose concentration (FPG) of 100–125 mg/dl, and IGT was defined as a 2-h plasma glucose concentration (2hPG) in a 75-g OGTT of 140–199 mg/dl. I-IFG was defined as IFG with normal 2hPG, and I-IGT was defined as IGT with normal FPG.

Anthropometric and biochemical measurements

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the midaxillary line. Body fat percentage, fat mass (FM), and lean body mass were quantified with the TBF-410 Tanita Body Composition Analyzer (Tanita Corp., Tokyo, Japan).

All the biochemical indexes were measured on a Hitachi 7600 analyzer (Hitachi, Ltd., Tokyo, Japan). FPG and 2hPG were measured by the hexokinase method. Serum levels of total cholesterol (TC), TG, HDL-C, and LDL-C were determined enzymatically. Alanine aminotransferase (ALT) was measured by the UV method. γ-Glutamyltransferase (GGT) was measured by the Szasz-Persijn method.

Hyperglycemic clamp

A total of 31 NGT, 17 I-IFG, and 15 I-IGT subjects underwent hyperglycemic clamp. None of the subjects was taking any medications known to affect glucose and lipid metabolism. Each subject was asked to abstain from alcohol and excessive physical exercise for 2 d before the clamp study. Hyperglycemic clamp was performed as previously described (17). Subjects were asked to have a standard dinner (10 kcal/kg: 50% carbohydrate, 35% fat, and 15% protein) 12 h before the experiments. Apart from water, subjects fasted thereafter until the experiments were completed. The following morning, a dorsal hand vein was cannulated in a retrograde fashion and kept in a thermoregulated box at 65 C for sampling arterialized venous blood. An antecubital vein was cannulated for infusion of 20% glucose. After a 30-min baseline period, plasma glucose concentrations were increased to 234 mg/dl and subsequently maintained at this level for 150 min using the glucose clamp technique. During the experiments, blood samples for serum insulin determinations were obtained at −30, −15, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 min.

Calculations

First-phase insulin secretion (1PH) was considered the sum of the serum insulin concentration from 2–10 min of the hyperglycemic clamp. Second-phase insulin secretion (2PH) was calculated as the average serum insulin concentration from 20–150 min of the clamp. The insulin sensitivity index (ISI) was determined as the average glucose metabolized rate during the last 30 min of the hyperglycemic clamp divided by the average serum insulin concentration during the same interval. Basal insulin secretion was assessed by homeostasis model of assessment (HOMA)-B, which was calculated as: [fasting serum insulin concentration (FINS) (mU/liter) $\times$ 6 − 3.33)]/[FPG (mmol/liter) − 3.5]. HOMA-

TABLE 1. Anthropometric parameters and biochemical indexes among subjects with NGT, I-IFG, and I-IGT

Variables	NGT (n = 134)	I-IFG (n = 101)	I-IGT (n = 118)	*P* value					
				Adjust for age and gender			Adjust for age, gender, and BMI		
				NGT *vs.* I-IFG	NGT *vs.* I-IGT	I-IFG *vs.* I-IGT	NGT *vs.* I-IFG	NGT *vs.* I-IGT	I-IFG *vs.* I-IGT
M/F	41/93	33/68	42/76						
Age (yr)	46.0 ± 12.1	50.0 ± 12.3	50.8 ± 10.3						
BMI (kg/m^2)	22.1 ± 2.3	25.2 ± 3.4	24.9 ± 3.3	<0.001	<0.001	NS			
Waist circumference (cm)	74.8 ± 5.8	83.2 ± 10.1	83.8 ± 9.5	<0.001	<0.001	NS	NS	<0.001	NS
Fat (%)	27.6 ± 6.8	33.0 ± 8.4	31.9 ± 8.0	<0.001	<0.001	NS	NS	NS	NS
FM (kg)	16.6 ± 4.9	21.6 ± 8.3	20.9 ± 7.6	<0.001	<0.001	NS	NS	NS	NS
LBM (kg)	42.4 ± 6.5	43.1 ± 7.2	43.9 ± 8.4	NS	NS	NS	NS	NS	NS
ALT (IU/liter)[a]	16.0 (11.8, 22.0)	19.0 (14.0, 27.0)	23.0 (16.0, 33.0)	NS	<0.001	0.029	NS	NS	0.005
GGT (IU/liter)[a]	15.0 (12.0, 20.0)	18.0 (14.0, 26.0)	23.0 (17.0, 32.5)	0.005	<0.001	0.036	NS	0.003	0.014
TC (mmol/liter)	4.7 ± 0.9	4.8 ± 1.0	5.0 ± 0.9	NS	0.034	0.029	NS	NS	0.028
TG (mmol/liter)	1.2 ± 0.9	1.4 ± 0.8	2.0 ± 1.5	NS	<0.001	0.001	NS	0.007	<0.001
HDL-C (mmol/liter)	1.5 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	<0.001	<0.001	NS	NS	0.003	NS
LDL-C (mmol/liter)	2.9 ± 0.7	3.1 ± 0.8	3.2 ± 0.8	NS	NS	NS	NS	NS	NS
FPG (mmol/liter)	5.2 ± 0.6	6.4 ± 0.2	5.3 ± 0.6	<0.001	NS	<0.001	<0.001	NS	<0.001
2hPG (mmol/liter)	5.3 ± 1.0	6.0 ± 1.1	8.7 ± 0.8	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Data are means ± SD or median (interquartile range). F, Female; LBM, lean body mass; M, male; NS, not significant.

[a] Log transformed before analysis.

insulin resistance (HOMA-IR) was calculated as: FINS (mU/liter) × FPG (mmol/liter)/22.5 (18). The ISI in the OGTT was calculated according to Cederholm formula (ISI-Cederholm) (19).

Measurement of FGF21 and insulin in human serum

Serum concentrations of FGF21 were quantified using the ELISA kits (BioVendor Laboratory Medicine, Inc., Modrice, Czech Republic) as described previously (20). The assay was proven to be highly specific to human FGF21 and did not cross-react with other members of the FGF family. The intraassay and interassay variations were 5.5 and 6.3%, respectively. Circulating levels of insulin were measured by RIA (LINCO Research, Inc., St. Charles, MO).

Statistical analysis

All analyses were performed with Statistical Package for the Social Sciences version 11.0 (SPSS, Inc., Chicago, IL). Normally distributed data were expressed as mean ± SD. Data that were not normally distributed, as determined using the Kolmogorox-Smirnov test, were logarithmically transformed before analysis and expressed as median with interquartile range. The Student's unpaired *t* test was used for comparison between two groups. Pearson's correlations or one-way ANOVA was used as appropriate for comparisons between groups, and multiple testing was corrected using Bonferroni correction. Multiple stepwise regression analysis was used to examine the association of serum FGF21 and other parameters. The variables that correlated significantly with serum FGF21 (after Bonferroni correction for multiple testing) were selected to enter into stepwise regression. In all statistical tests, *P* values less than 0.05 were considered significant.

Results

Characteristics of subjects

The clinical characteristics of the study subjects are shown in Table 1. Compared with the NGT subjects, I-IFG and I-IGT subjects were older ($P < 0.05$). No significant differences in the male to female ratio were observed in I-IGT and I-IFG subjects in comparison with NGT. After adjustment for age and gender, both subjects with I-IFG and I-IGT had higher BMI, waist circumference, fat percentage, and FM than those with NGT (all $P < 0.001$). There were no significant differences in these parameters of adiposity between I-IFG and I-IGT groups. Serum levels of ALT, TC, TG, and GGT in subjects with I-IGT were significantly higher than those with NGT (all $P < 0.05$). By contrast, there were no obvious differences in ALT, TC, and TG between I-IFG and NGT subjects. GGT levels in I-IFG individuals were also elevated but lower than those in I-IGT.

Serum FGF21 levels in I-IGT and I-IFG groups

In 353 Chinese subjects, serum concentrations of FGF21 ranged from 11.0–930.0 pg/ml. There were no sex differences in serum FGF21 levels: men (n = 116), median 125.4 pg/ml (interquartile range 58.5, 250.1) *vs.* women (n = 237), 135.1 pg/ml (79.4, 218.3) ($P = 0.698$). Interestingly, I-IGT subjects had significantly higher serum FGF21 levels [164.6 pg/ml (89.7, 261.0)] than NGT individuals [111.8 pg/ml (58.0, 198.9)] after adjustment for age and gender ($P < 0.01$) (Fig. 1). However, no significant difference in FGF21 levels was observed between I-IFG

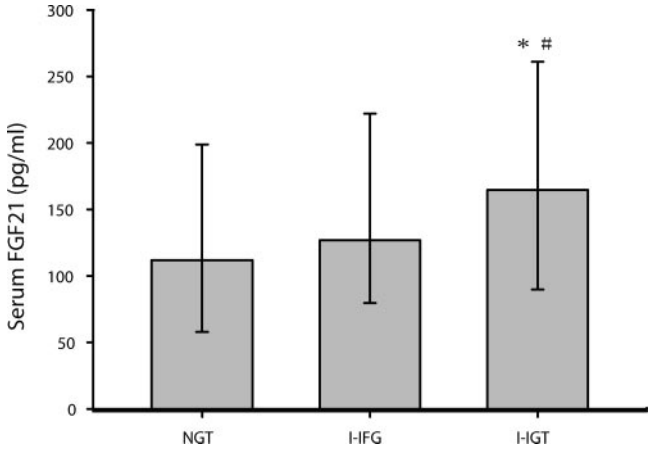

FIG. 1. Serum FGF21 concentrations in subjects with NGT (n = 134), I-IFG (n = 101), and I-IGT (n = 118). Median (interquartile range) serum levels of FGF21 in subjects with NGT, I-IFG, and I-IGT are displayed. *, $P < 0.01$, I-IGT *vs.* NGT after age and gender adjustment; #, $P < 0.05$, I-IGT *vs.* NGT after age, gender, and BMI adjustment.

TABLE 2. Correlations of serum FGF21 levels with insulin secretion and sensitivity indexes in 31 NGT, 17 I-IFG, and 15 I-IGT subjects by hyperglycemia clamp and the OGTT

				Hyperglycemia clamp						OGTT							
		Age		1PH		2PH		ISI		FINS		HOMA-percent B		HOMA-IR		ISI-Cederholm	
	Group	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Serum FGF21	NGT	0.314	0.047	0.052	NS	−0.024	NS	0.190	NS	0.104	NS	0.040	NS	0.163	NS	0.061	NS
	I-IFG	0.020	NS	−0.154	NS	−0.192	NS	0.191	NS	−0.043	NS	−0.136	NS	0.089	NS	−0.282	NS
	I-IGT	0.434	NS	0.494	NS	0.224	NS	−0.211	NS	0.238	NS	0.290	NS	0.192	NS	−0.262	NS
Serum FGF21 (age adjusted)	NGT			0.114	NS	0.016	NS	−0.087	NS	0.207	NS	0.307	NS	0.131	NS	0.006	NS
	I-IFG			−0.159	NS	−0.199	NS	0.200	NS	−0.041	NS	−0.136	NS	0.094	NS	−0.283	NS
	I-IGT			0.470	NS	0.346	NS	−0.275	NS	0.369	NS	0.387	NS	0.264	NS	−0.068	NS

NS, Not significant.

[126.9 pg/ml (79.8, 221.9)] and NGT subjects. After adjustment for age, gender, and BMI, the serum FGF21 levels in I-IGT subjects were still higher than those in NGT subjects ($P < 0.05$).

Association of serum FGF21 levels with insulin secretion and sensitivity in NGT, I-IFG, and I-IGT subjects through hyperglycemic clamp and the OGTT

The association analyses between serum FGF21 levels and the variables about insulin secretion and sensitivity through hyperglycemic clamp and the OGTT were performed respectively in 31 NGT, 17 I-IFG, and 15 I-IGT subjects (Table 2). In NGT subjects the correlation analysis showed a significant positive association of serum FGF21 levels with age (r = 0.314; $P < 0.05$). However, no significant associations were found between serum FGF21 and such variables, including 1PH, 2PH, ISI, FINS, HOMA-IR, HOMA-B, and ISI-Cederholm in each group before and after age adjustment.

Association of serum FGF21 levels with TG, TC, and GGT

We next investigated the relationship between serum FGF21 levels and a cluster of anthropometric parameters and biochemical indexes. The results are shown in Table 3 and Fig. 2. The analysis demonstrated a significant positive association of serum FGF21 with age (r = 0.373; $P < 0.001$). After adjustment for age, we found a significant positive association of serum FGF21 levels with ALT, GGT, TC, TG, LDL-C, and several parameters of adiposity, including BMI, waist circumference, fat percentage, and FM (all $P < 0.05$). A negative association of age-adjusted FGF21 and HDL-C levels was also observed ($P < 0.01$). After age and BMI adjustment, GGT, TC, TG (all $P < 0.001$), and LDL-C ($P < 0.05$) remained positively correlated with serum FGF21 levels.

To determine which parameters were independently associated with serum FGF21, multiple stepwise regression analysis was performed (Table 4). The analysis involved glucose tolerance status and the aforementioned parameters with significant correlations with serum FGF21, including age, BMI, waist circumference, fat percentage, FM, ALT, GGT, TC, TG, HDL-C, and LDL-C. TG, TC, and GGT were found to be independently associated with serum FGF21 in addition to age (all $P < 0.05$). However, glucose tolerance status was not a significant independent determinant. Moreover, the differences in FGF21 levels between I-IGT and NGT became insignificant after adjustment for age, gender, and TG or GGT (all $P > 0.05$).

TABLE 3. Correlations of serum FGF21 levels with anthropometric parameters, and biochemical indexes in 353 subjects

	Serum FGF21		Serum FGF21 (age adjusted)		Serum FGF21 (age and BMI adjusted)	
Variables	r	*P*	r	*P*	r	*P*
Age	0.373	<0.001				
BMI	0.179	0.001	0.149	0.005		
Waist circumference	0.221	<0.001	0.173	0.001	0.102	NS
Fat percentage	0.175	0.001	0.124	0.023	0.022	NS
FM	0.145	0.007	0.139	0.011	0.001	NS
ALT	0.205	<0.001	0.142	0.008	0.100	NS
GGT	0.329	<0.001	0.245	<0.001	0.215	<0.001
TC	0.336	<0.001	0.191	<0.001	0.205	<0.001
TG	0.351	<0.001	0.278	<0.001	0.263	<0.001
HDL-C	−0.110	0.027	−0.150	0.005	0.104	NS
LDL-C	0.264	<0.001	0.111	0.038	0.113	0.035

NS, Not significant.

Discussion

Although many recent animal-based studies suggest FGF21 as a potent metabolic regulator with multiple beneficial effects on obesity, hyperlipidemia, and diabetes (2, 6), the clinical relevance of FGF21 remains poorly characterized. In this study we provided the clinical evidence revealing that serum concentrations of FGF21 were elevated in subjects with I-IGT. Elevated concentrations of circulating FGF21 have also been found in obese subjects, hypertriglyceridemic patients, and newly diagnosed patients with diabetes (20–22). Furthermore, serum FGF21 has been positively associated with the increased risk of the metabolic syndrome (20). These findings raise the possibility that the pathophysiological role of FGF21 in humans might be different from that in animals.

FGF21 was found to have beneficial effects on islet insulin content and glucose-induced insulin secretion in diabetic animals (8). Immunohistochemistry analysis showed that treatment of *db/db* mice increases the intensity of insulin staining, as well as the number of islets per pancreas section and of insulin-positive cells per islet. Serum FGF21 in humans correlated positively with FINS after adjusting for age and BMI (20). However, FINS could not reflect the β-cell function accurately. The relevance of FGF21 and β-cell function in humans has never been explored. In this

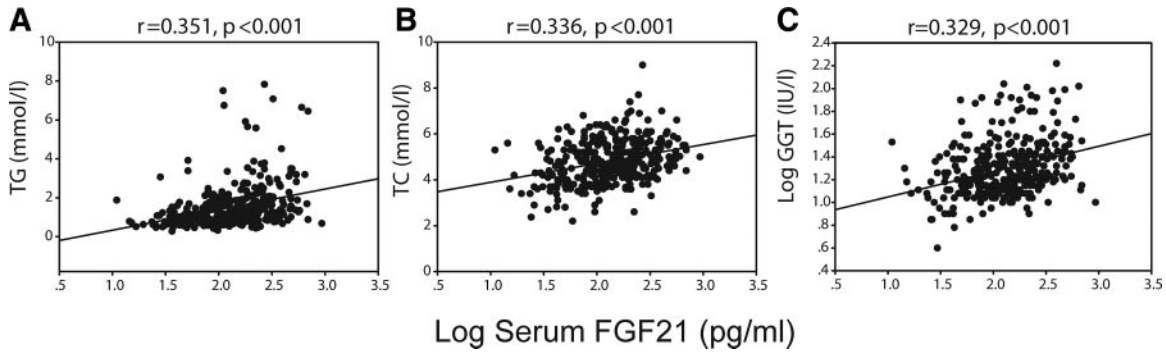

FIG. 2. Correlations of serum levels of FGF21 (log transformed) with TG (A), TC (B), and GGT (log transformed) (C) in 353 Chinese subjects.

study we performed the hyperglycemic clamp to investigate the relationship between serum FGF21 levels and β-cell function. Our study demonstrated that serum FGF21 levels in humans were not related to FINS and glucose-induced insulin secretion, including 1PH and 2PH.

FGF21 has been shown as a potent regulator of glucose uptake and insulin sensitivity by enhancing the expression of glucose transporter 1 in 3T3-L1 adipocytes and primary human adipocytes (7). However, our study demonstrated that serum concentrations of FGF21 in humans were not associated with insulin sensitivity as measured by both clamp and the OGTT. Notably, the lack of an independent association between FGF21 and adiponectin, which is a well-characterized adipokine with potent insulin-sensitizing activity, suggested that FGF21 might not be closely related to insulin sensitivity but secondary to the effects of obesity (20).

We found a significant positive association of FGF21 levels with several parameters of obesity, including BMI, waist circumference, fat percentage, and FM, which was similar to the result of the previous study (20). In addition, four parameters, including age, TG, TC, and GGT, were found to be independently associated with serum FGF21 by multiple stepwise regression analysis. Therefore, our data suggest that serum FGF21 levels are more related to lipid metabolism instead of insulin secretion and sensitivity. Direct administration of recombinant FGF21 has alleviated dyslipidemia in *ob/ob* and *db/db* mice and diabetic monkeys (2, 6). By contrast, serum FGF21 in humans was positively correlated with TG after adjusting for age and BMI. Consistent with our findings, a recent report showed a 2-fold increase of serum FGF21 in hypertriglyceridemic nondiabetic patients (21). This phenomenon might be similar to hyperinsulinemia and hyperleptinemia in obesity, both of which are considered to be the consequence of increased production in compensation for insulin and leptin resistance (23). A possible explanation is that the elevated serum FGF21 observed in subjects with hypertriglyceridemia might be a compensatory response to the decreased FGF21 sensitivity, a scenario reminiscent of leptin and insulin.

TABLE 4. Multiple stepwise regression analysis showing variables independently associated with the serum level of FGF21

Independent variables	Standardized β	*t*	*P* value
Age	0.210	3.742	<0.001
TG	0.208	3.789	<0.001
TC	0.171	3.074	0.002
GGT	0.146	2.602	0.010

The analysis also included BMI, waist circumference, fat percentage, FM, ALT, HDL-C, LDL-C, and glucose tolerance status, which were all excluded in the final model.

Another novel observation of this study was the independent positive association of GGT with FGF21 in humans. In addition, ALT also had a positive correlation with age-adjusted FGF21, and a trend of significant association with FGF21 after the adjustment for age and BMI (r = 0.100; $P = 0.065$). GGT and ALT are well-accepted noninvasive biochemical markers of liver injury (24). GGT has been proven to have better sensitivity than ALT in chronic liver injury (25). Because subjects with biliary obstructive diseases, hepatitis, alcoholism, and cirrhosis were excluded, the elevations of GGT and ALT in such subjects were probably caused by nonalcoholic fatty liver disease (NAFLD). As a protein primarily produced in liver, FGF21 has been suggested to have a close relationship with NAFLD and its related liver injuries. Adenovirus knockdown of liver FGF21 expression in mice caused fatty liver disease (10). A recent study also reported that systemic administration of FGF21 for 2 wk caused a significant decrease in hepatic steatosis in diet-induced obese mice (16). The paradoxical increase of serum FGF21 during the early liver injury in NAFLD subjects might be a defensive response of human body to counteract the metabolic stress, or simply a reflection of FGF21 resistance, leading to its compensatory up-regulation.

There are several limitations of this study, including a relatively limited sample size. The study did not address the cause-effect relationship between FGF21 and dyslipidemia as well as liver injury. Further prospective studies are warranted to determine whether elevated serum FGF21 is causally related to obesity, dyslipidemia, and NAFLD or a compensatory increase in response to these diseases.

In summary, this study provides clinical evidence revealing that serum concentrations of FGF21, which has been regarded as a potential candidate for treatment of diabetes, are increased in I-IGT subjects. Our data demonstrate that serum concentrations of FGF21 in humans are not related to insulin secretion and sensitivity. Instead, it is more related to lipid metabolism and early liver injury. TG, TC, and GGT are found independently associated with serum FGF21 by multiple stepwise regression analysis.

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Address all correspondence and requests for reprints to: Weiping Jia, M.D., Ph.D., Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai 200233, China. E-mail: wpjia@sjtu.edu.cn.

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