Serum FGF21 Levels Are Increased in Obesity and Are Independently Associated With the Metabolic Syndrome in Humans

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OBJECTIVE—Fibroblast growth factor 21 (FGF21) is a metabolic regulator with multiple beneficial effects on glucose homeostasis and insulin sensitivity in animal models. This study aimed to investigate the relationship between its serum levels and various cardiometabolic parameters in humans.

RESEARCH DESIGN AND METHODS—A newly developed immunoassay was used to measure serum FGF21 levels in 232 Chinese subjects recruited from our previous cross-sectional studies. The mRNA expression levels of FGF21 in the liver and adipose tissues were quantified by real-time PCR.

RESULTS—Serum FGF21 levels in overweight/obese subjects were significantly higher than in lean individuals. Serum FGF21 correlated positively with adiposity, fasting insulin, and triglycerides but negatively with HDL cholesterol, after adjusting for age and BMI. Logistic regression analysis demonstrated an independent association between serum FGF21 and the metabolic syndrome. Furthermore, the increased risk of the metabolic syndrome associated with high serum FGF21 was over and above the effects of individual components of the metabolic syndrome. Our in vitro study detected a differentiation-dependent expression of FGF21 in 3T3-L1 adipocytes and human adipocytes. In *db/db* obese mice, FGF21 mRNA expression was markedly increased in both the liver and adipose tissue compared with that in their lean littermates. Furthermore, FGF21 expression in subcutaneous fat correlated well with its circulating concentrations in humans.

CONCLUSIONS—FGF21 is a novel adipokine associated with obesity-related metabolic complications in humans. The paradoxical increase of serum FGF21 in obese individuals, which may be explained by a compensatory response or resistance to FGF21, warrants further investigation. ***Diabetes*** **57:1246–1253, 2008**

The fibroblast growth factor (FGF) family is composed of 22 members with a wide range of biological functions, including cell growth, development, angiogenesis, and wound healing (1–5). More recently, three members of this family, including FGF19 (FGF15 in the mouse), FGF21, and FGF23, have emerged as endocrine factors involved in metabolic regulation (6,7). FGF23 is secreted primarily from bone and exerts its actions on the kidney to inhibit phosphate reabsorption and vitamin D biosynthesis (8–10). FGF19 is expressed by the intestinal epithelium and is involved in regulating hepatic bile acids synthesis (11) and adiposity (12). On the other hand, FGF21, a polypeptide with 210 amino acid residues produced preferentially from the liver tissue (13), has recently been shown to possess potent beneficial effects on glucose and lipid metabolism and insulin sensitivity in animal models (7).

FGF21 was first suggested as a metabolic regulator with potential antidiabetic properties during a high throughput screening for agents capable of increasing glucose uptake in 3T3-L1 adipocytes (7). Addition of recombinant FGF21 to adipocytes was found to stimulate insulin-independent glucose uptake by enhancing the expression of GLUT1 (7). A recent study has demonstrated a profound synergy between FGF21 and the antidiabetic agent rosiglitazone (a peroxisome proliferator–activated receptor γ [PPARγ] agonist) in stimulating glucose uptake (14). The metabolic effects of FGF21 in adipocytes were mediated by β-Klotho, a single-pass transmembrane protein whose expression is induced during adipogenesis (15). Transgenic mice with overexpression of FGF21 were resistant to diet-induced obesity and metabolic disturbance (7). In both *ob/ob* and *db/db* obese/diabetic mice, therapeutic intervention with recombinant FGF21 resulted in a reduction of blood glucose and triglycerides to near normal levels, without apparent mitogenicity, hypoglycemia, or weight gain (7). Furthermore, chronic treatment of diabetic rhesus monkeys with FGF21 for a period of 6 weeks could provide efficient and durable glucose control and triglyceride low-

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A-FABP, adipocyte–fatty acid–binding protein; ELISA, enzyme-linked immunosorbent assay; FGF, fibroblast growth factor; HOMA-IR, homeostasis model assessment index–insulin resistance; IL, interleukin; NCEP, National Cholesterol Education Program; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor; QUICKI, quantitative insulin sensitivity check index; TNF-α, tumor necrosis factor-α.

ering without obvious adverse effects (16). More importantly, FGF21 administration led to significant improvements in lipoprotein profiles, including decreased LDL and elevated HDL cholesterol, and beneficial changes in the circulating levels of several cardiovascular factors (16). Furthermore, FGF21 has also been shown to improve pancreatic β-cell function and survival by activation of extracellular signal–regulated kinase 1/2 and Akt signaling pathways (17). The multiple beneficial effects of FGF21 on glucose and lipid metabolism and insulin sensitivity suggest that this small–molecular weight polypeptide might represent a promising therapeutic agent for the treatment of diabetes and other obesity-related metabolic disorders (18,19).

More recent studies from three independent groups have identified FGF21 as a key downstream target of the transcription factor PPARα, involved in adaptation to starvation (20–22). The PPARα agonists Wy14643 and GW7647 increased hepatic expression of FGF21 and its circulating levels in mice (20,21). On the other hand, adenovirus-mediated downregulation 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 (22). Together, these findings demonstrate a key role of FGF21 as a hepatic hormone in the regulation of systemic lipid metabolism and also suggest that the therapeutic benefits of the PPARα agonists might be mediated, at least in part, by induction of hepatic FGF21 production.

Although these animal-based studies are certainly of pharmacological interest, the physiological role of FGF21 remains poorly understood. To explore the clinical relevance of FGF21 in humans, we measured its serum concentrations in 232 Chinese subjects recruited from our previous cross-sectional studies (23) and analyzed its association with parameters of adiposity and a cluster of metabolic parameters. Unexpectedly, our data demonstrated a significantly increased serum level of FGF21 in obese individuals and subjects with the metabolic syndrome. In line with these clinical findings, we also observed an elevated FGF21 mRNA expression in both the liver and adipose tissue in rodent models of obesity.

RESEARCH DESIGN AND METHODS

We measured serum FGF21 levels in 232 subjects from the community-based Hong Kong Cardiovascular Risk Factor Prevalence Study (24–26). The clinical characteristics of these subjects are summarized in Supplementary Table 1. Participants were selected from our database on the basis of their BMI and included 105 lean (BMI <25 kg/m^2), 98 overweight (BMI 25–29.9 kg/m^2), and 29 obese (BMI ≥ 30 kg/m^2) participants such that a study sample covering a wide range of adiposity was available to examine the relationship between the circulating FGF21 levels and various cardiometabolic parameters. In addition, we obtained subcutaneous fat tissue from 29 healthy premenopausal Chinese women (age 42.5 ± 6.7 years) undergoing abdominal surgery for benign gynecological conditions (such as uterine fibroids or ovarian cysts) to investigate the association of FGF21 expression with its circulating concentrations. All subjects gave informed consent, and all the procedures were approved by the local ethics committee.

Anthropometric and biochemical measurements. All subjects were assessed after overnight fasting for at least 10 h. The details of anthropometric measurements (height, weight, BMI, waist circumference, and blood pressure) and the methods for assay of biochemical variables (fasting glucose, glucose 2 h after oral glucose tolerance test [OGTT], insulin, total cholesterol, triglycerides, and LDL and HDL cholesterol) were reported previously (24–26). Insulin resistance was estimated using homeostasis model assessment index–insulin resistance (HOMA-IR) (27). Body fat was quantified with the TBF-410 Tanita Body Composition Analyzer (Tanita, Tokyo).

Subjects were classified as having either normal glucose tolerance (NGT) or diabetes according to World Health Organization 1998 diagnostic criteria (28). Hypertension was defined as a sitting blood pressure of $\geq$130/85 mmHg, taken as a mean of two readings obtained after resting for at least 10 min, or regularly taking antihypertensive medications (29). The metabolic syndrome was defined according to the U.S. National Cholesterol Education Program (NCEP) Adult Treatment Panel III guidelines (29) and modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement (30) by adopting the Asian criteria for waist circumference and a lower cutoff for fasting glucose. The metabolic syndrome was defined as having three or more of the following metabolic risk factors: *1*) central obesity (waist circumference $\geq$80 cm in women and $\geq$90 cm in men); *2*) hypertriglyceridemia (fasting triglyceride $\geq$1.69 mmol/l); *3*) low HDL cholesterol (fasting HDL $<$1.29 mmol/l in women and $<$1.04 mmol/l in men); *4*) hyperglycemia (fasting glucose $\geq$5.6 mmol/l or already taking oral hypoglycemic agents for treatment of type 2 diabetes); and *5*) hypertension (sitting blood pressure $\geq$130/85 mmHg, taken as a mean of two readings taken after resting for at least 10 min, or taking regular antihypertensive medications).

Measurements of serum adipokines and FGF21. Human adipocyte–fatty acid–binding protein (A-FABP) and human FGF21 enzyme-linked immunosorbent assay (ELISA) kits were obtained from BioVendor Laboratory Medicine (Modrice, Czech Republic). Serum A-FABP concentrations were analyzed as we previously described (23,31,32). A panel of circulating factors, including other members of the human FGF family (FGF1, -2, -3, -5, -6, -7, -9, -10, -12, -16, -17, -19, -20, -22, and -23), basic FGF, adiponectin, A-FABP, lipocalin-2, leptin, retinol-binding protein 4, tumor necrosis factor-α (TNF-α), and interleukin (IL)-6, was prepared at 50 ng/ml in a calibrator diluent and assayed with the human FGF21 ELISA kit for cross-reactivity. In addition, preparations of the above factors at 50 ng/ml in a mid-range FGF21 standard control or in human serum samples were assayed for interference. Sera of several mammalian species (dog, bovine, hamster, monkey, pig, rat, mouse, sheep, and goat) were also analyzed with human FGF21 ELISA for cross-reactivity. For the measurement of FGF21, serum samples were diluted 1:3 before the assay and then 100 μl diluted sera, calibrators, and quality controls were added to 96-well microtiter plates coated with an affinity-purified polyclonal anti-human FGF21 antibody. The assay was conducted according to the manufacturer's protocol. A calibration curve was constructed by plotting the absorbance values at 450 nm versus the FGF21 concentrations of the calibrators, and concentrations of unknown samples were determined by using this calibration curve. The intra- and interassay variations were 5.4–7.6 and 4.3–7.7%, respectively. High-sensitivity C-reactive protein was measured with a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) (25). Serum adiponectin levels were determined with our in-house sandwich ELISA (26,33).

Animal experiments. C57BL/6J male mice were purchased from the Animal Unit of the University of Hong Kong. Twelve-week-old male C57BL/KsJ-lepr(db)/lepr(db) diabetic mice and age-matched lean littermates *db*/+ mice were obtained from The Jackson Laboratories. The mice were housed in a room under controlled temperature (23 ± 1°C) with water and standard mouse chow ad libitum. All of the experiments were conducted under the University of Hong Kong guidelines for the humane treatment of laboratory animals.

Isolation, differentiation, and culture of adipocytes. 3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum. Cells were grown to confluence and induced to differentiate into adipocytes, as described previously (33). Human mature adipocytes and preadipocytes were isolated from subcutaneous adipose tissue biopsies as described previously (34). In brief, adipose tissue was minced and digested with collagenase for 1 h at 37°C with constant shaking. Cells were then suspended, filtered through a 250-μm nylon membrane, and spun for 10 min at 380*g*. Adipocytes were decanted, washed three times in an isolation medium without collagenase, resuspended in the adipocyte medium, plated onto sixwell culture dishes, and then grown at 37°C in humidified atmosphere with 5% CO_2. Stromal vascular pellets were resuspended in an erythrocyte lysis buffer (Sigma) for isolation of preadipocytes as described previously (34). Isolated preadipocytes were induced for differentiation in Dulbecco's modified Eagle's medium/Ham's F12 (1:1) supplemented with 1 μmol/l insulin, 1 nmol/l triiodothyronine, and 100 nmol/l hydrocortisone. For the first 3 days of the differentiation period, 0.25 mmol/l methyl-isobutylxanthine was also added. Media were changed every 3 days. Fifteen days after differentiation, 70–80% cells became mature adipocytes.

RNA extraction and quantitative real-time PCR. Total RNA from various mouse tissues and 3T3-L1 cells was isolated using TRIzol reagent (Invitrogen) and treated with RNase-free DNase (Promega, Madison, WI) at 37°C for 30 min to remove genomic DNA. For reverse transcription, 1 μg total RNA was converted to first-strand complementary DNA in 20-μl reactions using a cDNA synthesis kit (Promega). Quantitative real-time PCR was performed in duplicate in a total reaction volume of 20 μl with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI Prism 7000 instrument

(Applied Biosystems) with the following parameters: 50°C for 2 min and 95°C for 10 min, followed by 35 two-step cycles at 95°C for 15 s and 60°C for 1 min. The forward and reverse primers used for PCR amplification of mouse FGF21 are 5′-AGATCAGGGAGGATGGAACA-3′ and 5′-TCAAAGTGAGGCGATC CATA-3′. The mRNA abundance of FGF21 in human adipose tissue was quantified using assay-on-demand TaqMan primers and probes from Applied Biosystem (assay ID hs00173927).

Statistical analysis. All analyses were performed with Statistical Package for Social Sciences version 14.0 (SPSS, Chicago, IL). Data are expressed as mean ± SD or median with interquartile range. Data that were not normally distributed, as determined using Kolmogorox-Smirnov test, were logarithmically transformed before analysis. A-FABP and adiponectin levels were adjusted for sex in all analyses because of the higher levels of these adipokines in women (23,31–33). Student's unpaired t test was used for comparison between two groups. Pearson's correlations or one-way ANOVA were used as appropriate for comparisons between groups, and multiple testing was corrected using Bonferroni correction. Logistic regression analyses were used in calculating the association of the odds ratios (ORs) for the association with the metabolic syndrome in subjects with raised baseline FGF21 (second and third tertiles) compared with those with low FGF21 (lowest tertile as the reference group), with additional adjustment for each component of the metabolic syndrome. Stepwise logistic regression analysis was used to examine the association of serum FGF21 and other parameters with the metabolic syndrome. The variables selected to enter into stepwise regression were those that correlated significantly with serum FGF21 (after Bonferroni correction for multiple testing). In all statistical tests, P values <0.05 were considered significant.

RESULTS

Serum FGF21 levels are significantly elevated in overweight/obese subjects and correlate closely with a cluster of cardiometabolic risk factors. We first confirmed the specificity of human FGF21 ELISA kits from the manufacturer and found that the assay was highly specific to human FGF21 and did not cross-react with other members of the human FGF family (FGF1, -2, -3, -5, -6, -7, -9, -10, -12, -16, -17, -19, -20, -22, and -23), basic FGF, adiponectin, A-FABP, lipocalin-2, leptin, retinol-binding protein 4, TNF-α, and IL-6. Except for a weak reactivity to monkey serum, no cross-reactivity with sera of other mammalian species was observed. This assay was then used to measure serum FGF21 levels in 232 Chinese subjects listed in Supplementary Table 1. Serum FGF21 levels ranged from 45.3 to 795.2 ng/l. There was no sex difference in serum FGF21 levels (men [n = 115], median 221.5 ng/l [interquartile range 134.0–432.3] vs. women [n = 117], 236.5 ng/l [130.6–389.3], P = 0.767). Interestingly, overweight/obese subjects (291.8 ng/l [144.5–512.0]; n = 127) had significantly higher ($P < 0.001$) serum FGF21 levels than the lean individuals (208.7 ng/l [94.4–325.7]; n = 105) (Supplementary Table 1). Subjects with hypertension (289.9 ng/l [175.1–474.3]; n = 138) had significantly higher ($P < 0.001$) serum FGF21 levels than those without hypertension (156.5 ng/l [72.8–314.9]; n = 94). No significant difference in serum FGF21 levels was observed between nondiabetic (normal glucose tolerant) and diabetic subjects (nondiabetic [n = 125], 218.0 ng/l [134.4–403.7] vs. diabetic [n = 107], 260.2 ng/l [128.1–428.7], P = 0.853). Among the 107 diabetic subjects in this cohort, 62 were treated with metformin and sulfonylurea (n = 46) or sulfonylurea alone (n = 16), 14 were on diet control alone, and 31 were newly diagnosed from OGTT screening. The duration of diabetes was 9.6 ± 4.5 years (mean ± SD) in the patients on drug treatment and 1.0 ± 1.5 years in those on no antidiabetic drugs. Serum FGF21 levels were not significantly influenced by the use of antidiabetic drugs (279.0 ng/l [101.5–422.9] in the treated patients vs. 329.7 ng/l [134.2–442.9] in patients on no drugs; P = 0.178).

We next investigated the relationship between serum FGF21 levels and a cluster of anthropometric parameters and cardiometabolic risk factors. This analysis showed a significant positive association of serum FGF21 levels with age and several parameters of adiposity, including BMI (Fig. 1; Table 1), waist circumference, waist-to-hip ratio, and fat percentage. When the diabetic and nondiabetic subjects were analyzed separately, the association of serum FGF21 levels and BMI, waist circumference, and waist-to-hip ratio remained significant in both groups before and after adjustment for age. There was also a positive association between serum FGF21 and fat percentage in the nondiabetic group (n = 107). However, the association of serum FGF21 with fat percentage was not significant in the diabetic group (n = 38 only).

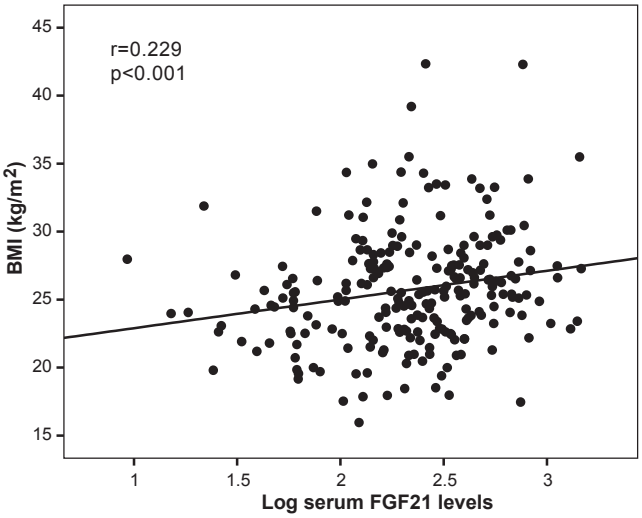

FIG. 1. Correlation between serum levels of FGF21 and BMI in 232 Chinese subjects. BMI in these subjects was normally distributed.

Serum FGF21 levels correlated positively with fasting insulin, the insulin resistance index (HOMA-IR), triglycerides, and serum A-FABP levels (Table 1). The positive correlation of serum FGF21 with these parameters, except for fat percentage and HOMA-IR, remained significant even after adjustment for age and BMI (Table 1). Interestingly, adiponectin was found to be negatively associated with age-adjusted serum FGF21 levels. In addition, serum FGF21 concentrations correlated negatively with quantitative insulin sensitivity check index (QUICKI) and HDL cholesterol. However, after age and BMI adjustment, significant negative correlation was only observed in HDL cholesterol (Table 1).

Elevated serum FGF21 levels are independently associated with increased risk of the metabolic syndrome. On the basis of the updated NCEP criteria (30), the metabolic syndrome was diagnosed in 41.8% of the 232 individuals studied. Serum FGF21 levels were significantly higher in subjects with the metabolic syndrome (n = 97), 336.1 ng/l (199.5–540.6), versus subjects without the metabolic syndrome (n = 135), 179.4 ng/l (95.5–311.7); $P <$ 0.001. Furthermore, there was a significant and progressive increase in serum FGF21 levels with increasing components of the metabolic syndrome. Serum FGF21 levels for those with zero, one, two, three, and four or more components of the metabolic syndrome were 215.7 ± 47.9, 208.6 ± 24.6, 268.5 ± 24.4, 398.1 ± 40.6, and 434.0 ± 47.0 ng/l, respectively ($P < 0.001$, ANOVA adjusted for multiple testing). To determine whether serum FGF21 was independently associated with the metabolic syndrome, step-

TABLE 1
Correlations of serum FGF21* levels with cardiometabolic risk factors

	Serum FGF21		Serum FGF21 (age-adjusted)		Serum FGF21 (age-and BMI-adjusted)	
	r	*P*	*r*	*P*	*r*	*P*
Age	0.168	0.010				
BMI	0.200	0.002	0.229	<0.001		
Waist circumference	0.244	<0.001	0.249	<0.001	0.135	0.041
Waist-to-hip ratio	0.256	<0.001	0.236	<0.001	0.161	0.014
Fat percentage†	0.220	0.010	0.230	0.007	0.107	0.218
Fasting glucose‡	0.024	0.201	0.145	0.136	0.133	0.172
2-h glucose‡	0.038	0.695	0.050	0.606	0.045	0.648
Fasting insulin*‡	0.273	0.001	0.288	0.001	0.184	0.016
HOMA-IR*‡	0.292	0.001	0.304	0.004	0.205	0.087
QUICKI‡	−0.273	0.002	−0.281	0.002	−0.178	0.051
Free fatty acids*§	0.069	0.365	0.022	0.773	0.000	0.999
Triglycerides*§	0.510	<0.001	0.485	<0.001	0.457	<0.001
LDL cholesterol§	0.108	0.103	0.080	0.300	0.050	0.518
HDL cholesterol§	−0.286	<0.001	−0.280	<0.001	−0.250	0.001
Systolic blood pressure¶	0.181	0.053	0.168	0.075	0.139	0.143
Diastolic blood pressure¶	0.033	0.729	0.045	0.636	0.005	0.958
Serum A-FABP*	0.327	<0.001	0.299	<0.001	0.205	0.002
Serum hsCRP*	0.130	0.070	0.104	0.149	0.060	0.405
Serum adiponectin*	−0.098	0.141	−0.133	0.046	−0.087	0.195

*Log transformed before analysis. †Included 63 men and 73 women with available data. ‡Included 63 men and 62 women not on diabetic treatment. §Included 94 men and 96 women not on lipid-lowering drug. ¶Included 62 men and 54 women not on antihypertensive drug.

wise logistic regression analysis involving the parameters with significant correlations with serum FGF21 was performed. Serum FGF21 was found to be independently associated with the metabolic syndrome (OR 6.01 [95% CI 2.18–16.59], $P = 0.001$), together with BMI and serum A-FABP (Table 2).

In logistic regression analysis, subjects in the second tertile of serum FGF21 had OR 3.02 (95% CI 1.48–6.14) ($P = 0.002$) of the association with the metabolic syndrome compared with subjects in the lowest tertile of serum FGF21. For those with serum FGF21 in the highest tertile, the OR was 5.97 (2.92–12.22) versus those in the lowest tertile ($P < 0.001$) (Supplementary Table 2). These ORs remained statistically significant even after adjusting for individual components of the metabolic syndrome, except for hypertension with the second tertile of serum FGF21 and triglyceride with the highest tertile of serum FGF21. Furthermore, the introduction of serum FGF21 tertiles into the logistic regression model significantly increased the likelihood of developing the metabolic syndrome associated with each component of the metabolic syndrome, as suggested by the highly significant likelihood ratios ($P < 0.001$) (Supplementary Table 3). Taken together, these data suggest that high serum FGF21 is an independent risk factor for the metabolic syndrome in humans, over and above the effects of individual components of the metabolic syndrome.

Differentiation-dependent expression of FGF21 in 3T3-L1 murine adipocytes and human adipocytes. The FGF21 gene was originally reported to be predominantly expressed in the liver (13). However, whether FGF21 is expressed in adipose tissue has never been studied. Given the strong positive association between serum FGF levels and parameters of adiposity, we next examined FGF21 expression in 3T3-L1 adipocytes. RT-PCR analysis detected mRNA expression in both undifferentiated preadipocytes and mature adipocytes (Fig. 2*A*). Notably, quantitative real-time PCR analysis revealed a progressive increase of FGF21 mRNA expression during adipogenesis (Fig. 2*B*). A significant elevation of FGF21 mRNA expression over basal level was observed at day 5. At day 8 after differentiation, the steady-state mRNA abundance of FGF21 was ~fivefold higher than that in preadipocytes, suggesting that FGF21 expression is induced during adipocyte conversion of 3T3-L1 cells. Because of the lack of commercially available immunoassays specific for mouse FGF21, we were unable to quantify FGF21 protein levels produced in 3T3-L1 adipocytes at this stage. Consistent with the finding in 3T3-L1 murine adipocytes, both FGF21 mRNA expression and its protein release into the conditioned medium were also markedly increased during conversion of human preadipocytes into mature adipocytes (Fig. 2*C* and *D*). Furthermore, chronic treatment with the PPARγ agonist rosiglitazone caused a dramatic induction of FGF21 production in both 3T3-L1 adipocytes and human adipocytes isolated from subcutaneous fat (Fig. 3). In contrast, the PPARα agonist fenofibrate had no obvious effect on either FGF21 mRNA expression or its protein release.

FGF21 expression is increased in both liver and adipose tissues of *db/db* obese mice. To further explore the physiological relevance of the above in vitro findings, we next compared FGF21 expression between various

TABLE 2
Stepwise logistic regression analysis showing factors independently associated with the metabolic syndrome

Variable	OR (95% CI)	*P* value
Serum FGF21*	6.01 (2.18–16.59)	0.001
BMI	1.14 (1.02–1.26)	0.018
Serum A-FABP*	7.27 (1.06–49.86)	0.043
Fasting insulin	4.16 (0.87–19.94)	0.075

The analysis also included age, which was excluded in the final model. *Log transformed.

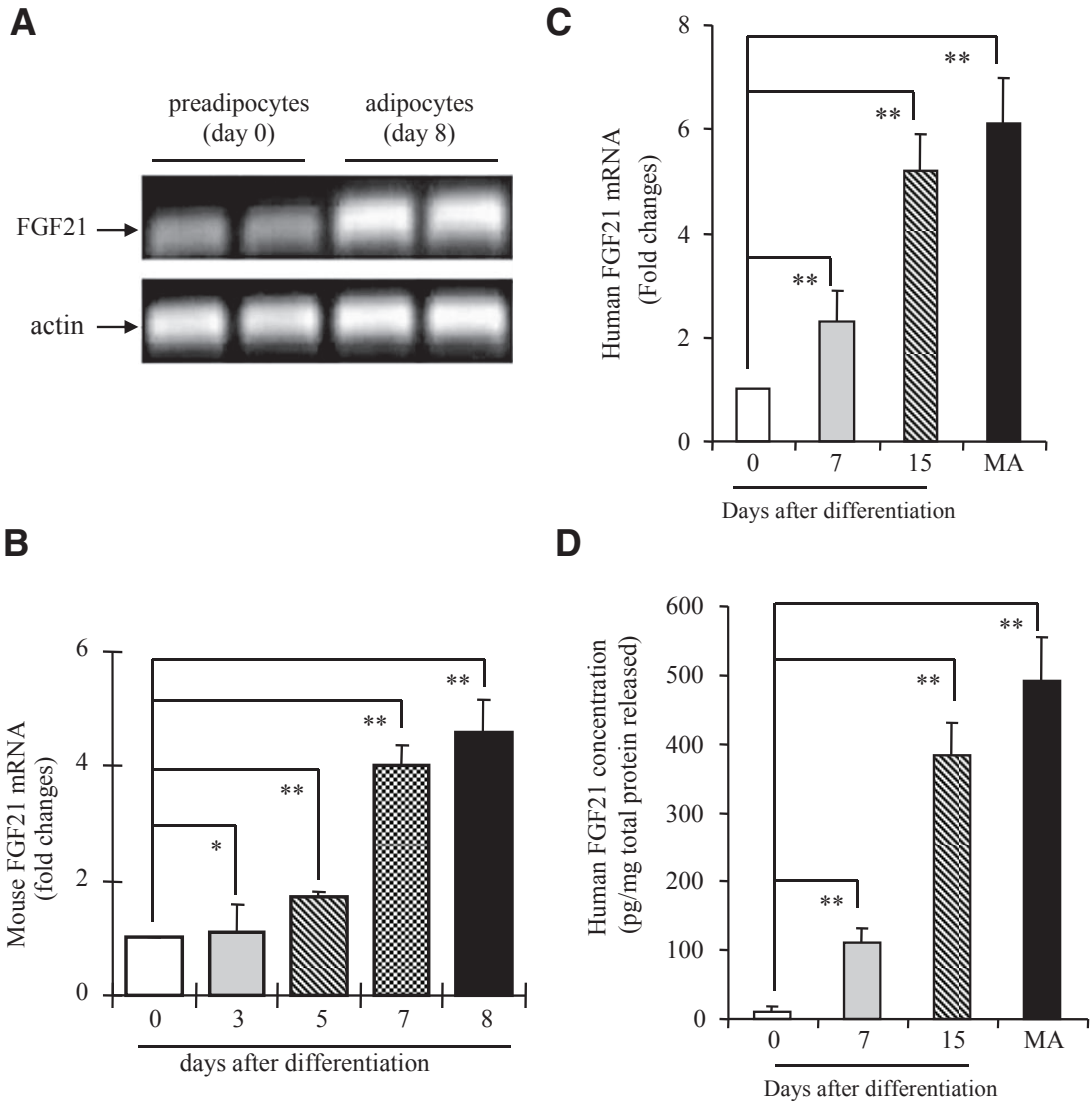

FIG. 2. F21 is expressed in both 3T3-L1 adipocytes and primary human adipocytes in a differentiation-dependent manner. Total RNA from 3T3-L1 cells at different days after induction of differentiation was subjected to either RT-PCR (*A*) or quantitative real-time PCR (*B*) analysis for mouse FGF21 mRNA expression. Human preadipocytes separated from subcutaneous fat were induced for differentiation. *C*: Human FGF21 mRNA from cells at different days after differentiation or from mature adipocytes (MA) directly isolated from subcutaneous fat was quantified by real-time PCR. Human FGF21 protein released into the conditioned medium was measured with the human FGF21 ELISA kit. All data are presented as means ± SD. *$P < 0.05$; **$P < 0.01$ ($n = 4–6$).

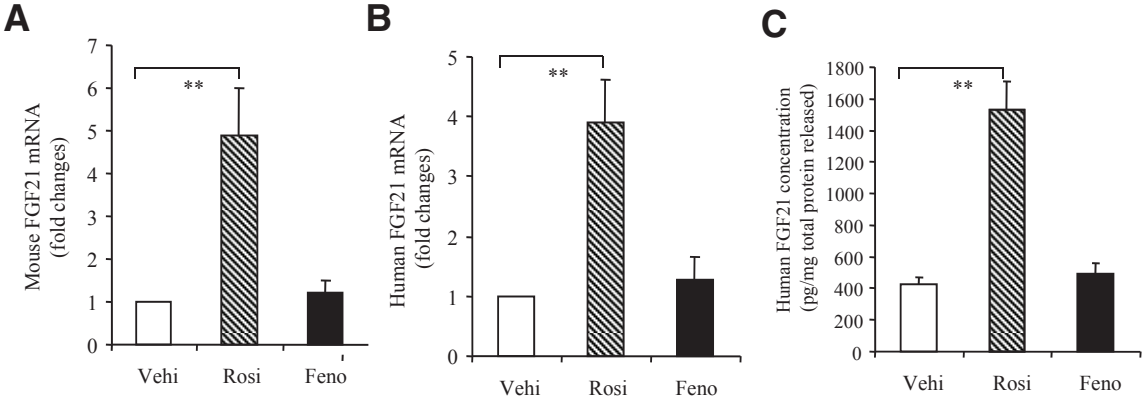

FIG. 3. Effects of the PPARγ and PPARα agonists on FGF21 expression in adipocytes. Mature 3T3-L1 adipocytes (*A*) or human adipocytes (*B* and *C*) were treated with vehicle control (Vehi), 10 μmol/l rosiglitazone (Rosi), or 50 μmol/l fenofibrate (Feno) for 48 h to quantify mRNA expression levels and protein concentrations released into the conditioned media as described in Fig. 2. **$P < 0.01$ ($n = 5–6$).

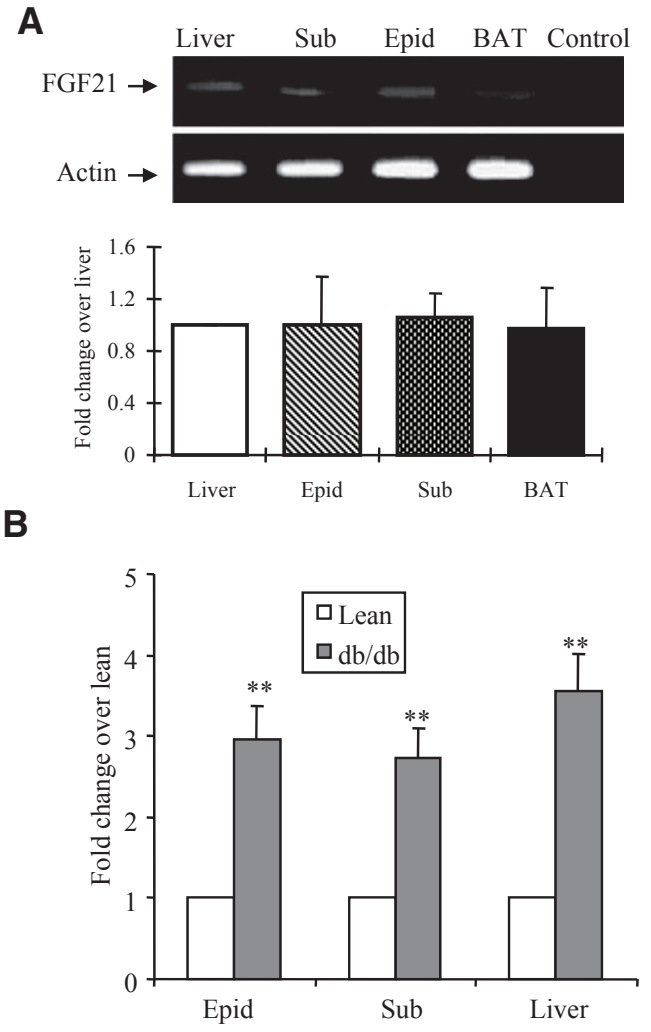

FIG. 4. GF21 is expressed in adipose tissue and is elevated in *db/db* obese mice. *A*: Detection and comparison of FGF21 mRNA expression in various fat depots and liver tissue of C57BL/6 mice ($n = 4$). *B*: Comparison of FGF21 gene expression profiles between *db/db* obese mice and their lean littermates. **$P < 0.01$ ($n = 5$). Epid, epididymal; Sub, subcutaneous; BAT, brown adipose tissue.

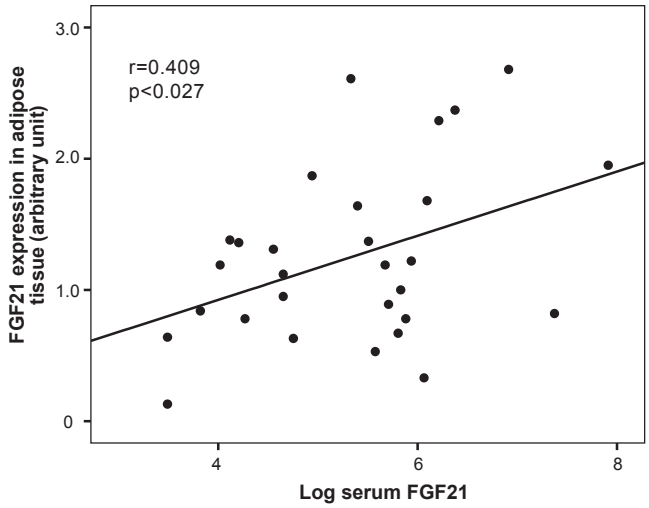

FIG. 5. Correlation between FGF21 adipose tissue expression and its serum concentrations (log transformed) in 29 Chinese individuals.

adipose tissues and the liver in C57BL/6 mice. Interestingly, RT-PCR detected mRNA expression of FGF21 not only in the liver but also in several types of adipose tissues from different anatomical locations, including subcutaneous and epididymal fat pads and brown adipose tissue (Fig. 4*A*). Furthermore, quantitative real-time PCR analysis revealed that the steady-state mRNA levels of FGF21 in adipose tissue were comparable with those expressed in the liver, suggesting that adipose tissue is also an important contributor to the production of circulating FGF21 in animal models.

The observation that FGF21 mRNA was expressed in the adipose tissue of wild-type C57BL/6 mice led us to examine whether FGF21 expression was altered in obesity. To this end, we compared the FGF21 expression in both liver and various adipose tissues between 12-week-old male *db/db* obese mice and their lean littermates. Notably, the FGF21 expression in both adipose tissue and the liver was markedly elevated in obese mice compared with lean mice (Fig. 4*B*). Furthermore, the magnitude of increase in FGF21 mRNA expression was comparable between these two tissues. These data are in agreement with our clinical observation showing increased serum levels of FGF21 in obese individuals. In high-fat diet–induced obese mice, FGF21 expression in both adipose tissue and liver was also significantly elevated compared with that in the lean controls (data not shown).

FGF21 mRNA expression in subcutaneous fat is positively associated with its serum concentrations in humans. To further explore the clinical relevance of the above animal-based findings, we quantified FGF21 gene expression in adipose tissue obtained from 29 Chinese individuals undergoing abdominal surgery for benign gynecological conditions. Correlation analysis revealed a significant positive correlation between FGF21 mRNA expression in subcutaneous fat and its serum protein concentration (Fig. 5), and this significance remained even after adjustment for age and BMI ($r = 0.412$, $P = 0.033$), suggesting that adipose tissue contributed, at least in part, to the elevated circulating FGF21 levels observed in obese subjects.

DISCUSSION

Mounting evidence from animal-based studies suggests FGF21 as a potent metabolic regulator with multiple beneficial effects on obesity and diabetes (18,19). Direct administration of recombinant FGF21 has been shown to alleviate hyperglycemia, hyperinsulinemia, and dyslipidemia in *ob/ob* and *db/db* obese mice and in diabetic monkeys (7,16). However, the clinical relevance of these findings has never been explored. In this study, we provide the first clinical evidence showing that serum levels of FGF21, which has been suggested as a potential candidate for the treatment of diabetes, are increased in obesity. In addition, our correlation analysis has demonstrated a strong positive association of serum FGF21 levels with age, several parameters of adiposity (BMI, waist circumference, waist-to-hip ratio, and fat percentage), insulin resistance (increased fasting insulin and HOMA-IR and decreased QUICKI), and adverse lipid profiles (increased triglycerides and decreased HDL cholesterol). The positive association of serum FGF21 with HOMA-IR and its negative association with QUICKI become insignificant after adjustment for BMI, suggesting that elevated serum FGF21 level is not a direct contributor to insulin resistance and might be secondary to the effects of obesity on insulin and/or

FGF21 sensitivity. The paradoxical increase of serum FGF21 in obesity-related disorders might be a defensive response of the human body to counteract the metabolic stress imposed by obesity. Alternatively, obesity may cause resistance to FGF21 actions, leading to its compensatory upregulation. This scenario is reminiscent of hyperinsulinemia and hyperleptinemia, both of which are thought to be the consequence of increased production in compensation for obesity-associated resistance to insulin and leptin (35). However, a recent study demonstrated that therapeutic administration of FGF21 reduced blood glucose and serum triglycerides to near normal levels in both *ob/ob* and *db/db* mice (7), suggesting that the maximal effect of FGF21 in these models remains unaffected. Nevertheless, these data cannot exclude the possibility of the decreased sensitivity of the target tissues in response to physiological concentrations of FGF21 in these obese mice. Further detailed dose-dependent studies are warranted to investigate whether obese subjects or animal models exhibit decreased FGF21 sensitivity and impaired receptor or postreceptor signaling in its target tissues.

Another novel observation of this study is the intimate association of FGF21 with the metabolic syndrome in humans. Serum FGF21 levels in subjects with the metabolic syndrome were much higher than those without this disorder. A progressive increase in serum FGF21 levels was also observed when an increasing number of components of the metabolic syndrome were found. More importantly, serum FGF21 was shown to be independently associated with the metabolic syndrome. This result suggests that serum FGF21 can be potentially used as a biochemical parameter for risk stratification of the metabolic syndrome. However, it is worthy to note that the association of serum FGF21 with the metabolic syndrome is not as strong as that of other biomarkers such as fasting insulin and C-peptide. Further study is needed to evaluate whether serum FGF21 has any value additive to that of other classical biomarkers and/or risk factors in prediction of the metabolic syndrome.

FGF21 was previously thought to be a hepatic hormone preferentially expressed in the liver (13). In this study, we provide several lines of evidence demonstrating that adipose tissue is also an important source of FGF21 production. First, our in vitro study has observed a differentiation-dependent expression of FGF21 in both 3T3-L1 murine adipocytes and human adipocytes (Fig. 2), a typical expression pattern for many adipocyte-derived adipokines. Second, our animal-based study has shown the level of FGF21 expression in several types of adipose tissues to be comparable with that in the liver, and its expressions in both tissues were markedly increased in obese mice. Third, our clinical studies have demonstrated a positive correlation between FGF21 mRNA expression in the subcutaneous fat and circulating serum concentrations of FGF21. Furthermore, serum levels of FGF21 correlated strongly with several parameters of adiposity, suggesting that the augmented production of FGF21 from adipose tissue is perhaps an important source for the elevated serum FGF21 in obesity. However, the positive association of serum FGF21 levels with BMI and fat percentage is weaker than other adipokines predominantly produced from adipose tissue, such as leptin and A-FABP (31,32), implying that organs other than adipose tissue largely contribute to the variability in circulating FGF21. The precise contribution of adipose tissue and the liver to circulating FGF21 in humans remains to be further clarified.

The role of FGF21 as an adipokine was also supported by the close association of FGF21 with several other adipocyte-derived factors. In particular, we observed a very strong positive correlation between serum levels of FGF21 and A-FABP, an adipocyte-derived circulating factor that has been demonstrated as an independent risk factor for the metabolic syndrome, type 2 diabetes, and cardiovascular diseases in both animal and clinical studies (31). Interestingly, both FGF21 and A-FABP have been shown to play a role in modulating lipolysis in adipose tissue (21,36). On the other hand, we found a weak negative correlation between age-adjusted serum FGF21 and adiponectin, but the significance was lost after adjustment for BMI. Adiponectin is a well-characterized adipokine with potent insulin-sensitizing, anti-inflammatory, and anti-atherosclerotic activities (37). Unlike FGF21, serum levels of adiponectin are decreased in obesity and its related medical complications (37). The lack of an independent association between FGF21 and adiponectin suggests that the function of these two factors might not be closely related. Alternatively, the production of these two factors might be regulated through distinct mechanisms. Notably, FGF21 has been shown as a potent regulator of glucose uptake and insulin sensitivity in 3T3-L1 adipocytes and primary human adipocytes (7,14). The FGF21 receptor and its coreceptors have recently been identified in adipocytes (15). Therefore, FGF21 might also act in an autocrine and paracrine manner to regulate adipocyte biology.

In summary, this study provides the first clinical demonstration showing the associations of serum FGF21 levels with obesity and its related cardiometabolic risk factors in humans. Although our data support the role of FGF21 as a metabolic regulator, they emphasize the complexity of FGF21 biology and suggest that the beneficial metabolic effects of FGF21 protein-based intervention therapy observed in animal models may not be directly applicable in humans. In addition, our finding that FGF21 is an adipocyte-derived adipokine highlights the multifaceted functional nature of this molecule. Further elucidation of its regulation and functions in different species and investigation of its clinical relevance are warranted to evaluate the precise pathophysiological role of FGF21 in obesity-related pathologies.

There are several limitations in our study, including a relatively small sample size, indirect estimates of insulin sensitivity, and crude measurements of body fat percentage. Because we had to select subjects with a wide range of BMI, inclusion of a large number of overweight/obese individuals resulted in a relatively high prevalence of the metabolic syndrome and diabetes in our cohort, which was not representative of the general population. In addition, our study design was cross-sectional and did not address the cause-effect relationship between serum FGF21 and obesity-related medical disorders. Further prospective studies in different ethnic groups should help address whether elevated serum FGF21 is causally associated with obesity and its related cardiometabolic complications or simply a compensatory upregulation in response to these diseases.

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