Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Clinical Science

Association between serum fibroblast growth factor 21 and diabetic nephropathy

Wei-Xia Jian[a,1], Wen-Hui Peng[b,1], Jie Jin[a], Xue-Ru Chen[a], Wen-Jun Fang[a], Wei-Xing Wang[a], Li Qin[a], Yan Dong[a], Qing Su[a,*]

[a] Department of Endocrinology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China
[b] Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China

ARTICLE INFO

Article history:
Received 4 July 2011
Accepted 19 October 2011

ABSTRACT

Fibroblast growth factor 21 (FGF-21) is a new metabolic regulator with beneficial effects on lipid and glucose metabolism in animal models of diabetes mellitus. The aim of this study was to explore the relationship between FGF-21 and diabetic nephropathy in humans. Serum FGF-21 levels were determined in groups of control (n = 50) and type 2 diabetes mellitus (T2DM) patients with normoalbuminuria (n = 158), microalbuminuria (n = 68), and macroalbuminuria (n = 38) using enzyme-linked immunosorbent assay. Multiple linear regression models were used to analyze the associations between FGF-21 or other biomedical indices and urinary albumin excretion (UAE). Median serum FGF-21 levels were increased in T2DM patients compared with nondiabetic controls and were significantly higher in patients of higher UAE group. In groups of control and T2DM patients with normoalbuminuria, microalbuminuria, and macroalbuminuria, median serum (interquartile range) FGF-21 levels were 467.89 (294.59-519.56), 492.30 (354.59-640.42), 595.01 (480.49-792.31), and 665.20 (448.68-829.75) ng/L ($P < .001$), respectively. After adjustment for the confounders, FGF-21, fasting plasma glucose, and high-density lipoprotein cholesterol levels were found to be independently associated with UAE in diabetic patients. Serum FGF-21 level is independently correlated with UAE in T2DM patients, indicating that circulating FGF-21 may be involved in diabetic nephropathy.

1. Introduction

Fibroblast growth factor 21 (FGF-21), a new member of the FGF family [1], was originally isolated and identified from mouse embryos in 2000 [2]. It lacks the conventional heparin binding domain and can diffuse from its origin tissue into plasma as a detectable circulatory hormone [3-5]. Serum FGF-21 is secreted predominantly by the liver and, to a lesser degree, by the adipose tissue in humans [6,7]. Human FGF-21 is a polypeptide of 181 amino acids, with a sequence identity of 81% to the mouse ortholog; and it resembles FGF-19 and FGF-23 on the amino acid level with sequence identities of 35% and 25%, respectively [2].

Author contributions: SQ and JWX designed, coordinated, and wrote the manuscript. PWH performed the enzyme-linked immunosorbent assay and took part in manuscript writing. FWJ, WWX, QL, and DY carried out the sample collection. JJ and CXR performed data collection and statistical analysis. All authors read and approved the final manuscript.

* *Corresponding author*. Tel.: +86 21 25078999; fax: +86 21 25077532.
E-mail address: suqing_xinhua@yahoo.cn (Q. Su).

[1] These authors contributed equally to this work.

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.10.012

As a secreted cytokine, FGF-21 might associate with other adipocytokines such as leptin and adiponectin [8], and regulate metabolic homeostasis through paracrine and/or endocrine modes of action [3]. In *ob/ob* or *db/db* mice, administration of recombinant FGF-21 can reduce plasma glucose and insulin level, ameliorate insulin resistance and energy expenditure, and relieve hepatic steatosis and obesity [3,9,10]. Fibroblast growth factor 21 was also found to improve lipoprotein profile and reduce body weight of diabetic rhesus monkeys [11]. Therefore, based on the above findings in animals, it can be deduced that FGF-21 shows potential as a treatment of type 2 diabetes mellitus (T2DM) and other metabolic disorders [7]. However, the exact physiopathologic role of FGF-21 in human is yet to be elucidated. Recently, increased FGF-21 level has been identified as an independent risk factor of metabolic syndrome, as well as coronary artery disease in diabetic patients [12,13], indicating that the effect of FGF-21 on metabolic regulation in humans is different from that in animals.

Albuminuria in diabetic patients is a predominantly clinical manifestation of diabetic nephropathy; and microalbuminuria is regarded as a pivotal sign of early diabetic nephropathy, as well as a risk factor of atherosclerosis and cardiovascular disease independent of conventional risk factors [14,15]. Because FGF-21 is considered a metabolic regulator and it contributes to vascular damage in vivo, we carried out the present study postulating that FGF-21 may also associate with urinary albumin excretion (UAE) and play a role in diabetic nephropathy in diabetic patients. In the meantime, other clinical and metabolic indices in T2DM patients were also studied in this research.

2. Materials and methods

The study was approved by the Human Ethical Review Committee, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, and Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China, and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.1. Subjects

A total of 314 subjects were included, consisting of 264 patients with T2DM and 50 nondiabetic controls who were matched for age and sex for the research. All subjects were local residents of Han ethnicity in Shanghai and consecutively recruited in the Department of Endocrinology, Xinhua Hospital, Shanghai Jiaotong University, School of Medicine, and Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University, School of Medicine. T2DM was diagnosed according to 1999 World Health Organization diagnostic criteria [16]. The diagnosis of type 1 diabetes mellitus was confirmed by C-peptide level and presence of glutamic acid decarboxylase antibody. Patients with type 1 diabetes mellitus, severe kidney or liver diseases, chronic viral or bacterial infection, asthma, tumors, and connective tissue diseases were excluded. To avoid the confusing effect of renal failure on excretion of FGF-21, all diabetic patients with macroalbuminuria and (or) serum creatinine level greater than 97 μmol/L underwent glomerular filtration rate (GFR) determination by the clearance rate of technetium Tc 99m diethylenetriaminepentaacetic acid; and then patients with GFR less than 60 mL/(min 1.73 m^2) were excluded from this study. All eligible diabetic patients were divided into 3 groups: (1) normoalbuminuria (UAE <30 mg/24 h), (2) microalbuminuria (UAE 30-300 mg/24 h), and (3) macroalbuminuria (UAE >300 mg/24 h). The data of administration of hypoglycemic drugs during the recent month were obtained from the clinical records in each patient and were recorded as using oral agents, insulin, or combination of insulin and oral agents. The data of administration of lipid-lowering drugs including statins and fibrates and antihypertensive drugs including angiotensin receptor blocker and angiotensin-converting enzyme inhibitor were also recorded. Because fibrates increase serum FGF-21 levels [17], patients taking fibrates were excluded from this study. Baseline clinical information and anthropometric indices for all subjects were obtained by standard methods.

2.2. Biochemical investigation

Blood samples were collected after overnight fasting for at least 10 hours in all subjects. Fasting plasma glucose (FPG), serum liver function profile, blood urea nitrogen, creatinine, uric acid, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), lipoprotein (a), apoprotein A, apoprotein B, and fibrinogen levels were measured with standard laboratory techniques on a Hitachi 7104 Analyzer (Hitachi, Tokyo, Japan). Glycosylated hemoglobin A1c (HbA1c) was determined by high-performance liquid chromatography (Hi-AUTO HA-8150; ARKRAY, Kyoto, Japan) (reference range, 4.0%-6.0%). Serum FGF-21 concentrations were measured by a commercial enzyme-linked immunosorbent assay kit (BioVendor Laboratory Medicine, Modrice, Czech Republic). The sensitivity was 5.0 pg/mL; and the intra- and interassay variability was 4.0% and 8.0%, respectively. High-sensitivity C-reactive protein (hsCRP) was determined using a high-sensitivity enzyme-linked immunosorbent assay kit (Biocheck Laboratories, Toledo, OH). Twenty-four-hour pooled urine specimens were collected. The urine samples were stirred before analysis. Albuminuria was determined quantitatively by enzyme immunoassay method using an automated analyzer (LX-6000; Eiken Chemical, Tokyo, Japan). Renal function was evaluated by the estimated GFR using the simplified modification of diet in renal disease equation (sMDRD), as follows: $186.3 \times [\text{serum creatinine (milligrams per deciliter)}]^{-1.154} \times (\text{age})^{-0.203} \times [(0.742) \text{ if female}]$.

2.3. Statistical analysis

Continuous variables were expressed as mean ± SD. Categorical variables were presented as frequencies. Data that were not normally distributed tested by the Kolmogorov-Smirnov method were logarithmically transformed before analysis and expressed as median with interquartile range. χ^2 test, Pearson correlations, partial correlations, 1-way analysis of variance, or analysis of covariance was used as appropriate

for comparisons between or among groups; and multiple testing was corrected using the Bonferroni correction method. Multiple stepwise linear regression analysis was used to examine the association of serum FGF-21 and other clinical and biochemical parameters with UAE in diabetic patients. All models were adjusted for age, sex, duration of diabetes, smoking, HbA1c, body mass index (BMI), lipid profile, serum creatinine, and sMDRD when the relationship between FGF-21 and UAE was analyzed. All analyses were performed with Statistical Package for Social Sciences version 13.0 (SPSS, Chicago, IL). A 2-sided probability level of ≤.05 was taken as significant. The significant level for multiple testing was $P \leq .0083$.

3. Results

3.1. Clinical and biochemical characteristics of subjects

Detailed baseline clinical characteristics of all subjects are presented in Table 1. As for age, duration of diabetes, percentage of male and smokers, ratio of waist circumference to hip circumference (WHR), and blood pressure levels, there were no significant differences among the control and diabetic subgroups. BMI and percentages of drug administration of statins, hypoglycemic drugs, and antihypertensive drugs were significantly different among control and diabetic subgroups with normal albuminuria, microalbuminuria, and macroalbuminuria ($P < .05$). Biochemical measures of all subjects are shown in Table 2. After multiple testing corrected by the Bonferroni method, some biochemical findings were found to be significantly different among control and T2DM patients either with or without albuminuria. The UAE levels in the groups of T2DM with macroalbuminuria and microalbuminuria were significantly higher compared with the level in the group of T2DM with normoalbuminuria (1068.80 [498.35-1642.57], 83.85 [46.89-220.01], and 16.34 [6.92-25.57] mg/24 h, respectively; $P < .001$). The T2DM patients with macroalbuminuria showed a significant increase in serum fibrinogen (3.95 ± 0.73 vs 3.21 ± 0.74 g/L, $P = .004$) and creatinine (98.80 ± 24.62 vs 73.00 ± 16.74 μmol/L, $P < .001$) and a significant decrease in sMDRD (74.56 ± 31.50 vs 92.41 ± 29.29 mL/[min 1.73 m^2], $P = .004$) when compared with those with normoalbuminuria. HbA1c in the groups of T2DM with normoalbuminuria and microalbuminuria and TC in the T2DM group with normoalbuminuria were significantly higher than those in the control group (all $P < .008$). Other detailed results of analysis of variance for biochemical indices are listed in Table 2.

3.2. FGF-21 levels in nondiabetic control and in diabetic patients by categories of albuminuria

The median (interquartile) serum concentration of FGF-21 in controls and in T2DM with normoalbuminuria, microalbuminuria, and macroalbuminuria groups were 467.89 (294.59-519.56), 492.30 (354.59-640.42), 595.01 (480.49-792.31), and 665.20 (448.68-829.75) ng/L, respectively; and the difference among the groups was statistically significant ($F = 10.74$, $P < .001$). As shown in Table 2, the median FGF-21 level was significantly higher in the T2DM group than in the control group. The FGF-21 concentration increased accordingly with the UAE levels in diabetic patients (P value for trend < .001). After multiple testing corrected by the Bonferroni method, FGF-21 levels were found to be significantly higher in T2DM patients with microalbuminuria and macroalbuminuria compared with those with normoalbuminuria and control group (all $P < .0083$). Furthermore, significantly increased serum FGF-21 levels were still observed with increased UAE levels after adjustment for age, sex, duration of diabetes, smoking, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c serum lipid profile, serum creatinine, and sMDRD by analysis of covariance ($F = 9.719$, $P < .001$).

Table 1 – Baseline clinical characteristics of all subjects

	Control (n = 50)	T2DM with normoalbuminuria (n = 158)	T2DM with microalbuminuria (n = 68)	T2DM with macroalbuminuria (n = 38)	P value
Duration of diabetes (y)		9.2 ± 7.1	9.4 ± 7.1	11.6 ± 6.6	.294
Sex (men, %)	24 (48.0%)	84 (53.2%)	40 (58.8%)	22 (57.9%)	.651
Age (y)	65.3 ± 8.0	65.7 ± 10.3	64.7 ± 10.1	65.7 ± 10.0	.447
Smoker (n, %)	7 (14.0%)	37 (23.4%)	14 (20.6%)	11 (29.0%)	.362
BMI (kg/m^2)	25.59 ± 3.68	24.96 ± 3.89	26.31 ± 3.96	25.33 ± 3.04	.041*
WHR	0.93 ± 0.04	0.92 ± 0.03	0.94 ± 0.06	0.93 ± 0.06	.259
SBP (mm Hg)	135.26 ± 10.12	136.06 ± 19.09	141.43 ± 20.34	136.39 ± 18.88	.242
DBP (mm Hg)	80.89 ± 6.90	77.43 ± 10.87	80.37 ± 11.12	76.05 ± 9.46	.091
ACEI or ARB use	5 (10.0%)	27 (17.1%)	33 (48.5%)	22 (57.9%)	<.001*
Statins use	5 (10.0%)	39 (24.7%)	12 (17.6%)	14 (36.8%)	.016*
Hypoglycemic therapy					
Only diet and exercise	–	15 (9.5%)	3 (4.4%)	0 (0%)	.075
Oral agents	–	109 (69.0%)	25 (36.8%)	6 (15.8%)	<.001*
Insulin	–	23 (14.6%)	7 (10.3%)	15 (39.5%)	<.001*
Oral agents + insulin	–	11 (7.0%)	33 (48.5%)	17 (44.7%)	<.001*

The numbers in the table are mean ± SD or number (percentage). ACEI indicates angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blocker.

* $P \leq .05$.

Table 2 – Biochemical characteristics and FGF-21 levels in nondiabetic controls and in diabetic patients by categories of albuminuria

	Control (n = 50)	T2DM with normoalbuminuria (n = 158)	T2DM with microalbuminuria (n = 68)	T2DM with macroalbuminuria (n = 38)	P value
Creatinine (μmol/L)	75.32 ± 9.43	73.00 ± 16.74	80.28 ± 43.61	98.80 ± 24.62 †	<.001 *
BUN (mmol/L)	6.37 ± 1.43	5.77 ± 2.33	7.23 ± 8.91	8.12 ± 4.44	.033 *
Uric acid (μmol/L)	366.33 ± 117.88	315.70 ± 97.26	317.99 ± 98.31	355.32 ± 108.25	.078
UAE (mg/24 h)[a]	–	16.34 (6.92-25.57)	83.85 (46.89-220.01) †	1068.80 (498.35-1642.57) †	<.001 *
hsCRP (mg/L)	7.18 ± 5.71	10.06 ± 20.24	7.81 ± 19.43	7.56 ± 11.83	.865
Fibrinogen (g/L)	3.28 ± 0.46	3.21 ± 0.74	3.14 ± 0.77	3.95 ± 0.73 †	.003 *
FPG (mmol/L)	5.45 ± 2.01	7.59 ± 2.71	7.58 ± 2.35	7.23 ± 2.56	.060
2hPG (mmol/L)	7.10 ± 2.23	13.46 ± 4.09 ‡	12.96 ± 3.64	12.51 ± 4.12	.022 *
HbA1c (%)	5.60 ± 1.16	7.72 ± 1.48 ‡	7.97 ± 1.68 ‡	7.68 ± 1.80	.011 *
TC (mmol/L)	4.02 ± 0.86	4.20 ± 0.87 ‡	4.33 ± 0.98	4.55 ± 1.16	.001 *
TG (mmol/L)	1.52 ± 0.63	1.63 ± 0.88	1.73 ± 0.95	1.62 ± 0.91	.798
HDL-C (mmol/L)	1.29 ± 0.24	1.19 ± 0.35	1.19 ± 0.34	1.08 ± 0.24	.117
LDL-C (mmol/L)	2.46 ± 0.76	2.50 ± 0.71	2.66 ± 0.66	2.72 ± 0.82	.104
Apoprotein A (g/L)	1.32 ± 0.15	1.22 ± 0.28	1.23 ± 0.28	1.21 ± 0.31	.616
Apoprotein B (g/L)	1.07 ± 0.33	1.21 ± 2.16	0.87 ± 0.31	1.04 ± 0.46	.555
Lipoprotein (a) (mg/L)	211.09 ± 143.05	219.29 ± 186.30	316.01 ± 243.07	260.54 ± 260.54	.052
sMDRD (mL/[min 1.73 m^2])	94.12 ± 30.01	92.41 ± 29.29	99.69 ± 45.49	74.56 ± 31.50 †	.002 *
FGF-21 (ng/L) [a]	467.89 (294.59-519.56)	492.30 (354.59-640.42)	595.01 (480.49-792.31) †‡	665.20 (448.68-829.75) †‡	<.001 *

Continuous variables were expressed as mean ± SD. BUN indicates blood urea nitrogen; 2hPG, 2-hour postprandial plasma glucose.
[a] Median (interquartile range).
* $P \leq .05$. vs group of T2DM with normoalbuminuria.
† $P \leq .0083$ vs group of T2DM with normoalbuminuria.
‡ $P \leq .0083$ vs control group.

3.3. *Correlations between FGF-21 and clinical and biochemical parameters*

After being logarithmically transformed for normal distribution, FGF-21 level was found to be positively correlated with serum HDL-C ($r = 0.184$, $P = .046$) in the nondiabetic control group. Selected significant results of Pearson correlation analysis for FGF-21 and other clinical indices in T2DM patients are listed in Table 3. Serum FGF-21 level positively correlated with age ($r = 0.154$, $P = .012$), serum HDL-C ($r = 0.147$, $P = .017$), and creatinine level ($r = 0.172$, $P = .006$) and negatively correlated with sMDRD ($r = -0.123$, $P = .046$) in all T2DM patients. In subgroups of diabetic patients categorized by albuminuria, significant correlations of FGF-21 with serum creatinine and sMDRD were only found in the subgroup of T2DM with macroalbuminuria. Significant correlation was not observed between FGF-21 and anthropometric indices (BMI and WHR), blood pressures, or plasma glucose levels in the control group or the diabetic groups (data not shown).

After adjusting for all confounders in the partial correlation analysis, significantly positive correlation was found between FGF-21 and UAE level ($r = 0.398$, $P < .001$). The same significant correlation was also found in T2DM subgroups with normoalbuminuria and macroalbuminuria.

3.4. *Multiple linear regression analysis for UAE*

After adjustment for age, sex, duration of diabetes, smoking, BMI, blood pressures, hsCRP, serum creatinine, and sMDRD, the multiple linear regression analysis demonstrated that

Table 3 – The significant correlations between FGF-21 and clinical indices in T2DM patients

	All T2DM patients (n = 264)		T2DM with normoalbuminuria (n = 158)		T2DM with microalbuminuria (n = 68)		T2DM with macroalbuminuria (n = 38)	
	R value	P value	R value	P value	R value	P value	R value	P value
Age (y)	0.154	.012 *	0.139	.082	0.116	.345	0.251	.129
HDL-C (mmol/L)	0.147	.017 *	0.231	.003 *	0.233	.056	–0.105	.531
Creatinine (μmol/L)	0.172	.006 *	–0.128	.121	0.069	.590	0.349	.034 *
sMDRD (mL/[min 1.73 m^2])	–0.123	.046 *	–0.056	.486	–0.084	.494	–0.333	.041 *
UAE (mg/24 h) [a]	0.398	<.001 *	0.266	.007 *	0.029	.868	0.647	.023 *

[a] Partial correlation analysis was performed between UAE and FGF-21; and age, sex, duration of diabetes, smoking, HbA1c, BMI, lipid profile, serum creatinine, and sMDRD were adjusted in the partial correlation analysis.
* $P \leq .05$.

Table 4 – Results of multiple linear regression analysis for UAE in T2DM patients

	Not adjusted by drug use			Adjusted by drug use		
	β value	T value	P value	β value	T value	P value
Sex	0.171	1.577	.118	0.101	0.937	.351
Age (y)	−0.033	−0.293	.770	−0.062	−0.575	.566
Duration of diabetes (y)	−0.003	−0.040	.968	−0.034	−0.399	.691
Smoking	0.088	0.957	.340	0.077	0.861	.391
BMI (kg/m^2)	0.038	0.389	.698	0.005	0.053	.958
SBP (mm Hg)	0.166	1.717	.089	0.087	0.889	.376
DBP (mm Hg)	0.027	0.284	.777	0.005	0.049	.961
FPG (mmol/L)	0.269	2.224	.028*	0.229	1.939	.050*
2hPG (mmol/L)	−0.145	−1.291	.199	−0.138	−1.263	.209
HbA1c (%)	−0.202	−1.922	.057	−0.165	−1.606	.111
TC (mmol/L)	0.030	0.119	.905	−0.032	−0.131	.896
TG (mmol/L)	−0.113	−1.151	.252	−0.066	−0.681	.497
HDL-C (mmol/L)	−0.388	−2.850	.005*	−0.361	−2.724	.007*
LDL-C (mmol/L)	0.165	0.706	.482	0.191	0.842	.401
HsCRP (mg/L)	−0.038	−0.459	.647	−0.018	−0.218	.828
Creatinine (μmol/L)	0.162	1.270	.206	0.143	1.178	.241
sMDRD (mL/[min 1.73 m^2])	−0.136	−1.255	.212	−0.099	−0.931	.354
FGF-21 (ng/L)	0.370	4.236	<.001*	0.361	4.266	<.001*

* $P \leq .05$.

FPG, HDL-C, and FGF-21 levels were the significantly independent determinant variables for variance of UAE. When the regression equation was further adjusted by drug use, the same above-mentioned variables were found to be significantly associated with variation of UAE. All the detailed results are shown in Table 4. These findings showed that UAE increased together with elevation of serum FGF-21 and FPG and with reduction of serum HDL-C.

4. Discussion

UAE in diabetic patients is an indicator of renal dysfunction and a predictor for cardiovascular mortality and morbidity [14,15]. Up to now, there is no study investigating the role of FGF-21 in diabetic nephropathy. In this study, we explored the relationship between FGF-21, a novel hepatoadipokine capable of regulating metabolic process, and UAE in Chinese T2DM patients. We found that serum FGF-21 level was significantly higher in diabetic patients compared with nondiabetic subjects and that FGF-21 level increased along with UAE in diabetic patients, which had never been reported before. After adjustment for the confounders, FGF-21, FPG, and HDL-C levels were found to be independently associated with UAE in diabetic patients.

Raised serum FGF-21 level was associated with increased serum creatinine and decreased sMDRD levels in T2DM patients with macroalbuminuria. This finding was consistent with results of the study of Stein et al [18]. The latter study found that circulating FGF-21 increased in patients undergoing long-term hemodialysis as compared with that in control subjects. Previous studies indicated that FGF-21 was a circulatory cytokine eliminated by kidney and thus correlated with parameters of renal function [19-21]. In our study, we found that FGF-21 was independently correlated with UAE not only in T2DM patients with macroalbuminuria but also in those with microalbuminuria. Because the biochemical renal function profile and GFR of the T2DM group with microalbuminuria were still relatively normal, we could reasonably infer that FGF-21 may be one of the early serum indicators of elevated UAE in pre- or subclinical diabetic nephropathy. The real role of FGF-21 in diabetes and diabetic nephropathy remains unclear at present. We speculate that the increased serum FGF-21 level in diabetes and diabetic nephropathy may be a compensation for the abnormal metabolic process. Fibroblast growth factor 21 may play a role in diabetic nephropathy by improving lipid profile or other beneficial metabolic effects.

Fibroblast growth factor 23 (FGF-23) resembles FGF-21 on amino acid level [2]. FGF-23 is a bone-derived hormone that induces phosphaturia and inhibits conversion of 25 (OH) vitamin D to its active form [22]. Raised FGF-23 level was associated with mortality in patients with end-stage renal disease [23], and FGF-23 was also found to be associated with kidney function and proteinuria in diabetic patients. It was observed that FGF-23 was a significantly independent predictor of renal outcome in patients with macroalbuminuric diabetic nephropathy in a prospective study [24]. As a hepatoadipokine, FGF-21 may play a role in diabetic nephropathy in a different way than FGF-23.

Studies in human subjects suggested that FGF-21 may be a metabolic regulator that plays an important role in metabolic process such as lipid and energy metabolism. Two cross-sectional studies reported increased FGF-21 levels in patients with obesity and T2DM [12,25]. Furthermore, FGF-21 level was shown to be associated with several symptoms of metabolic syndrome [12]. Recently, FGF-21 has been identified as an independent risk factor for coronary artery disease in diabetic patients [13]. In our study, we did not find any correlation between serum FGF-21 and obesity-associated parameters or plasma glucose level, which is in accordance with the results of the study of Galman et al [17]. However, other studies revealed that FGF-21 was positively associated with metabolic disorders, including dyslipidemia, obesity, high plasma glucose level, and insulin resistance [8,26,27]. The cause of these discrepancies is still unclear, adding to the existing paradoxical findings about FGF-21 in human and other mammals. The results of our study differed considerably from those obtained in mice, suggesting that the physiologic role of FGF-21 in humans may differ from that in animals.

In this study, we found that FGF-21 level positively correlated with HDL-C level, which was consistent with the beneficial metabolic function of FGF-21 in rodents [10]. However, we should be prudent in interpreting the correlation between FGF-21 and HDL-C because of the confounding factor of administration of statin agents in some of the diabetic patients. Previous studies have found that treatment of hypertriglyceridemia with the PPAR-α agonist fenofibrate affects serum FGF-21 levels [17,19], and the effect of

administration of statins on serum FGF-21 variation in diabetic patients is still unclear. In this study, declined HDL-C levels were found to be an independent determinant for variance of UAE, which was accordant with the fact that administration of lipid-lowering agent in T2DM patients had beneficial effects such as reducing albuminuria and slowing down the decline of GFR and progression of diabetic nephropathy [28,29]. Dyslipidemia and lipotoxicity may play roles in endothelial injury and microalbuminuria in diabetic patients. Therefore, early diagnosis and prompt treatment of dyslipidemia can be beneficial to T2DM patients. It is still unknown at present whether or not the administration of hypolipidemic drugs in diabetic patients would increase serum FGF-21 level and thus allow it to perform its protective function indirectly through metabolic amelioration. A prospective study on this issue in diabetic patients will shed light upon this question.

This study has the following limitations. First, in this article, UAE was studied as a surrogate variable for diabetic nephropathy. Although we excluded patients with other possible acute or chronic kidney diseases or with history of other kidney diseases except diabetic nephropathy according to clinical and biochemical findings, ideally, patients with diabetic nephropathy should have been diagnosed by renal biopsy. Second, this research was a cross-sectional study; so we could not conclude that increased serum FGF-21 level was a cause or subsequent result of albuminuria in diabetic patients. Third, we measured UAE only once on patients' admission. Ideally, we should have evaluated the correlation between UAE and serum FGF-21 level using the mean UAE from repeated urine tests. However, in this study, we excluded patients with GFR less than 60 mL/(min 1.73 m^2) to minimize the confounding effect of renal excretion of FGF-21. Therefore, despite the above-mentioned limitations, we can still draw the conclusion that FGF-21 is significantly positively associated with UAE in T2DM patients.

In summary, the findings of the present study suggest that circulating FGF-21 may associate with diabetic nephropathy as a sign of microangiopathy. However, the underlying pathophysiological role of FGF-21 in diabetic nephropathy is yet to be elucidated by further prospective studies.

Funding

This study was partially supported by National Natural Science Foundation of China (30900699), Shanghai Rising-Star Program (10QA1405500), and Specialized Research Fund for the Doctoral Program of Higher Education (20090073120095).

Acknowledgment

We are very grateful to all volunteers who participated in this study. In addition, we express our appreciation to Ms Hai-Ling Li for her assistance in recruiting the study subjects and to Prof Getu Zhaori for his help in improvement of the language.

Conflict of Interest

There are no conflicts of interest relevant to this article.

REFERENCES

[1] Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001;2 REVIEWS3005.
[2] Nishimura T, Nakatake Y, Konishi M, et al. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta 2000;1492:203-6.
[3] Kharitonenkov A, Shiyanova TL, Koester A, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115: 1627-35.
[4] Wente W, Efanov AM, Brenner M, et al. Fibroblast growth factor–21 improves pancreatic beta-cell function and survival by activation of extracellular signal–regulated kinase 1/2 and Akt signaling pathways. Diabetes 2006;55: 2470-8.
[5] Moyers JS, Shiyanova TL, Mehrbod F, et al. Molecular determinants of FGF-21 activity-synergy and cross-talk with PPARgamma signaling. J Cell Physiol 2007;210:1-6.
[6] Dostalova I, Haluzikova D, Haluzik M. Fibroblast growth factor 21: a novel metabolic regulator with potential therapeutic properties in obesity/type 2 diabetes mellitus. Physiol Res 2009;58:1-7.
[7] Kharitonenkov A, Shanafelt AB. Fibroblast growth factor–21 as a therapeutic agent for metabolic diseases. BioDrugs 2008;22:37-44.
[8] Dostalova I, Kavalkova P, Haluzikova D, et al. Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. J Clin Endocrinol Metab 2008;93:3627-32.
[9] Berglund ED, Li CY, Bina HA, et al. Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. Endocrinology 2009;150:4084-93.
[10] Coskun T, Bina HA, Schneider MA, et al. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 2008;149: 6018-27.
[11] Kharitonenkov A, Wroblewski VJ, Koester A, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor–21. Endocrinology 2007;148:774-81.
[12] Zhang X, Yeung DC, Karpisek M, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 2008;57: 1246-53.
[13] Lin Z, Wu Z, Yin X, et al. Serum levels of FGF-21 are increased in coronary heart disease patients and are independently associated with adverse lipid profile. PLoS One 2010;e15534:5.
[14] Stehouwer CD, Gall MA, Twisk JW, et al. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes 2002;51:1157-65.
[15] Mykkanen L, Zaccaro DJ, O'Leary DH, et al. Microalbuminuria and carotid artery intima-media thickness in nondiabetic and NIDDM subjects. The Insulin Resistance Atherosclerosis Study (IRAS). Stroke 1997;28:1710-6.
[16] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15: 539-53.
[17] Galman C, Lundasen T, Kharitonenkov A, et al. The circulating metabolic regulator FGF21 is induced by prolonged

fasting and PPARalpha activation in man. Cell Metab 2008;8: 169-74.
[18] Stein S, Bachmann A, Lossner U, et al. Serum levels of the adipokine FGF21 depend on renal function. Diabetes Care 2009;32:126-8.
[19] Eto K, Tumenbayar B, Nagashima SI, et al. Distinct association of serum FGF21 or adiponectin levels with clinical parameters in patients with type 2 diabetes. Diabetes Res Clin Pract 2010;89:52-7.
[20] Matuszek B, Lenart-Lipinska M, Duma D, et al. Evaluation of concentrations of FGF-21—a new adipocytokine in type 2 diabetes. Endokrynol Pol 2010;61:50-4.
[21] Lin Z, Zhou Z, Liu Y, et al. Circulating FGF21 levels are progressively increased from the early to end stages of chronic kidney diseases and are associated with renal function in Chinese. PLoS One 2011;e18398:6.
[22] Liu S, Quarles LD. How fibroblast growth factor 23 works. J Am Soc Nephrol 2007;18:1637-47.
[23] Gutierrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008;359:584-92.
[24] Titan SM, Zatz R, Graciolli FG, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. Clin J Am Soc Nephrol 2011;6:241-7.
[25] Chen WW, Li L, Yang GY, et al. Circulating FGF-21 levels in normal subjects and in newly diagnose patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2008;116:65-8.
[26] Chavez AO, Molina-Carrion M, Abdul-Ghani MA, et al. Circulating fibroblast growth factor–21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 2009;32:1542-6.
[27] Mai K, Schwarz F, Bobbert T, et al. Relation between fibroblast growth factor-21, adiposity, metabolism, and weight reduction. Metabolism 2011;60:306-11.
[28] Velussi M, Cernigoi AM, Tortul C, et al. Atorvastatin for the management of type 2 diabetic patients with dyslipidaemia. A mid-term (9 months) treatment experience. Diabetes Nutr Metab 1999;12:407-12.
[29] Elisaf M, Mikhailidis DP. Statins and renal function. Angiology 2002;53:493-502.