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Serum Fibroblast Growth Factor 23 Levels are Associated with Vascular Smooth Muscle Dysfunction in Type 2 Diabetes

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Aim: Increased level of serum fibroblast growth factor 23 (FGF23) is a hallmark of abnormal phosphate metabolism in patients with chronic kidney disease (CKD) and is recently shown to be associated with the risk of cardiovascular disease even in those without CKD. This study investigated the association between serum FGF23 levels and vascular function in patients with type 2 diabetes.

Methods: This was a cross-sectional study involving 283 Japanese patients with type 2 diabetes. Flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) of the brachial artery were measured via ultrasonography to evaluate vascular endothelial and smooth muscle functions, respectively. Serum intact FGF23 levels were determined via a sandwich enzyme-linked immunosorbent assay.

Results: The median values of FMD, NMD, and serum FGF23 were 6.0%, 14.0%, and 27.3 pg/mL, respectively. The serum FGF23 levels were inversely associated with NMD but not with FMD, and the association was independent of atherosclerotic risk factors, estimated glomerular filtration rate (eGFR), and serum phosphate levels. Furthermore, the relationship between serum FGF23 levels and NMD was modified by kidney function, which was pronounced in subjects with normal kidney function (eGFR ≥ 60 mL/min/1.73 m^2).

Conclusion: Serum FGF23 levels are independently and inversely associated with NMD in patients with type 2 diabetes, particularly in those with normal kidney function. Our results indicate that FGF23 is involved in vascular smooth muscle dysfunction and that increased serum levels of FGF23 may serve as a novel biomarker for vascular smooth muscle dysfunction in patients with type 2 diabetes.

Key words: Fibroblast Growth Factor 23, Flow-Mediated Dilatation, Nitroglycerin-Mediated Dilatation, Type 2 Diabetes

Introduction

Bone-derived hormone fibroblast growth factor 23 (FGF23) is a key regulator of phosphate homeostasis that acts on the kidney to promote urinary phosphate excretion and inhibit vitamin D activation[1]. Serum FGF23 levels are significantly elevated with a tight correlation with serum creatinine and phosphate levels in patients with chronic kidney disease (CKD)[2]. Because serum FGF23 levels increase early in the course of CKD before serum phosphate levels rise, elevated serum FGF23 levels are considered an early marker of disordered mineral metabolism in patients with CKD[3].

Several recent studies have consistently shown a link between elevated serum FGF23 levels and the risk

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of cardiovascular diseases (CVDs) and mortality not only in patients with CKD[4, 5] but also in the general population[6, 7]. Furthermore, although the results are inconsistent, several studies have indicated an association between serum FGF23 levels and surrogate markers of CVD risk, such as carotid intima-media thickness[8, 9], arterial stiffness[10, 11], and vascular dysfunction[11], in the community-based population. These findings suggest that FGF23 is involved in structural and/or functional vascular damage regardless of the CKD status, although the underlying mechanism remains unclear.

Type 2 diabetes is a leading cause of CKD and CVDs and is often accompanied by abnormal phosphate metabolism[12, 13]. Elevated serum FGF23 levels are observed in patients with type 2 diabetes, even with preserved kidney function, compared with those without diabetes[12, 14]. Of note, several recent studies have demonstrated that elevated FGF23 levels are associated with an increased risk of CVD events and mortality in patients with type 2 diabetes and preserved kidney function[15-17]. To date, no study has demonstrated the association between serum FGF23 levels and subclinical atherosclerosis in patients with type 2 diabetes.

Here, we hypothesized that FGF23 is involved in vascular dysfunction, an important feature of atherosclerosis[18], in type 2 diabetes. Flow-mediated vasodilation (FMD) and nitroglycerin-mediated vasodilation (NMD) of the brachial artery are widely adopted to assess vascular endothelial and smooth muscle functions, respectively, and are suggested as surrogate markers for atherosclerosis and CVD outcomes[18, 19].

Aim

In this study, we measured the serum FGF23 levels and examined their association with FMD and NMD in patients with type 2 diabetes.

Methods

Study Design and Participants

This was a single-center, cross-sectional study. We consecutively enrolled 283 Japanese patients with type 2 diabetes from our inpatients at the Diabetes Center of the Osaka Metropolitan University Hospital who required hospitalization for glycemic control, education, and/or evaluation of diabetic complications between January 2009 and July 2014. Type 2 diabetes was diagnosed based on the criteria of the American Diabetes Association[20] and Japan Diabetes Society[21]. Patients with type 1 diabetes or other types were excluded in this study. Information of preexisting CVDs, including coronary artery disease, cerebrovascular disease, or peripheral artery disease, was corrected from medical records.

This study was conducted in accordance with the Declaration of Helsinki (1975, as revised in 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (The Japanese Ministry of Health, Labour and Welfare, 2014). The study protocol was approved by the ethics committee of the institution (Approval No. 4417), and informed consent was obtained using the opt-out method on the department website (http://www.med.osaka-cu.ac.jp/interm2/research/optout.shtml).

Physical and Laboratory Measurements

Blood pressure was measured using an automatic sphygmomanometer (Terumo Co., Ltd., Tokyo, Japan) with a conventional cuff after the subjects had rested for at least 5 min. Blood was drawn after an overnight fast, and biochemical parameters were analyzed using a standard laboratory method at the Central Clinical Laboratory of the Osaka Metropolitan University Hospital[22]. Glycated hemoglobin A1c (HbA1c) was assessed as the National Glycohemoglobin Standardization Program equivalent value (NGSP, %) according to the guidelines of the Japan Diabetes Society[21]. Estimated glomerular filtration rate (eGFR) was calculated using the Japanese GFR-estimating equation[23]. Immunoreactive insulin levels were measured for participants not receiving insulin therapy (n=161) *via* electrochemiluminescence immunoassay [cobas 8000(502/602), Roche Diagnostics, K.K., Tokyo, Japan]. The homeostasis model assessment of insulin resistance (HOMA-R) was calculated as fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405 [22].

The serum levels of human intact FGF23 were measured using sandwich enzyme-linked immunosorbent assay kits (Kainos Laboratories, Tokyo, Japan)[24]. The intra-assay coefficient of variation for human intact FGF23 was < 10%.

Assessment of Vascular Function

Vascular function was assessed by FMD and NMD of the brachial artery according to the guidelines of the International Brachial Artery Reactivity Task Force[25] and the Japanese guidelines of the Vascular Failure Workshop Group[26]. FMD and NMD were measured using a vascular ultrasound system equipped with an edge-tracking system for two-dimensional imaging and automatic measurement (UNEXEF; Unex Co. Ltd., Nagoya, Japan.), as previously described[22, 27, 28]. In brief, the diameter of

the brachial artery at rest was measured in the cubital region, and subsequently, the cuff was inflated to 50 mmHg above systolic blood pressure for 5 min and deflated. The diameter at the same point of the artery was continuously monitored, and the maximum dilatation from 45 s to 60 s after deflation was recorded.

The endothelium-independent NMD was measured following FMD measurement. After a 15-min interval for vessel recovery, 75 μg of glyceryl trinitrate was sublingually administered, and the maximum dilatation of the brachial artery at the same point was measured during at least 1 min after the initiation of maximum dilatation. The FMD and NMD values were calculated as follows: FMD or NMD (%) = (maximum diameter − diameter at rest) × 100 / diameter at rest.

The measurements were performed in a quiet, air-conditioned room at 25℃ for inpatients who had been instructed to fast overnight and abstain from alcohol, caffeine, smoking, and exercise for at least 12 h prior to the measurements[25, 26]. Regarding the reproducibility of the FMD measurement using this system, the coefficient of correlation between examinations at different institutions was reported to be 0.84, and the intraobserver coefficient of variation was 10.1%–11.2%[19].

Statistical Analysis

Data were expressed as numbers (%) or median with interquartile range. The association between vascular function and anthropometric or metabolic parameters was assessed using Spearman's rank correlation tests and Wilcoxon's rank-sum tests for continuous and categorical variables, respectively. Independent determinants of FMD and NMD after adjusting for potential confounders were determined *via* multiple regression analysis. To assess whether the effects of serum FGF23 levels on NMD are modified between participants with and without kidney dysfunction (eGFR <60 mL/min/1.73 m^2), a cross-product term between FGF23 and with/without kidney dysfunction was included in the multiple regression analysis. $P<0.05$ was considered to indicate statistical significance for all analyses. All data were statistically analyzed using JMP 14 (SAS Institute Inc., Cary, NC, USA).

Results

Clinical Characteristics, Serum FGF23 Levels, and Vascular Function

The clinical characteristics of the study subjects are presented in **Table 1**. The median age, duration of diabetes, body mass index, blood pressure, and HbA1c were 64 years, 11 years, 25.1 kg/m^2, 130/74 mmHg, and 8.3%, respectively. The prevalence rates of current smokers, participants with CVDs, those taking renin-angiotensin system (RAS) inhibitors, those taking calcium channel blockers, and those taking statins were 25.1%, 19.4%, 40.6%, 39.9%, and 41.3%, respectively. The prevalence rate of participants receiving insulin therapy was 41.0%. Details of other antidiabetic drugs are presented in **Table 1**. The median eGFR, serum calcium level, and serum phosphate level were 66.0 mL/min/1.73 m^2, 9.4 mg/dL, and 3.7 mg/dL, respectively.

The median values of serum FGF23 level, FMD, and NMD were 27.3 pg/mL (range, 0.006–751.0 pg/mL), 6.0% (range, 0.4%–24.6%), and 14.0% (range, 0.5%–33.7%), respectively.

Association between Serum FGF23 Levels and Vascular Function

We first examined the unadjusted correlations between FMD or NMD and cardiometabolic risk factors, serum calcium, phosphate, and FGF23 levels **(Table 2A, Fig. 1)**. The serum FGF23 levels were not ($\rho=-0.105$, $P=0.079$), but systolic blood pressure and eGFR were, significantly correlated with FMD. On the other hand, the serum FGF23 levels were significantly correlated with NMD ($\rho=-0.282$, $P<0.001$), along with systolic blood pressure, eGFR, and HbA1c. Of note, neither the serum calcium levels nor the serum phosphate levels showed a significant correlation with FMD or NMD. In participants not receiving insulin therapy (N=161), neither FMD nor NMD was correlated with HOMA-R. For categorical variables, male sex, RAS inhibitor use, and calcium channel blocker use were associated with lower FMD, whereas calcium channel blocker use and pioglitazone use were associated with lower NMD **(Table 2B)**.

Multivariate Analysis for the Determinants of FMD and NMD

We next conducted a multiple regression analysis to explore the association between serum FGF23 levels and FMD or NMD with adjustment for age, sex, serum phosphate levels, and traditional CVD risk factors **(Table 3)**. The serum FGF23 levels ($\beta=-0.131$, $P=0.046$), along with age, systolic blood pressure, and use of calcium channel blockers, were independently and negatively associated with NMD. On the other hand, male sex, RAS inhibitor use, and HbA1c, but not serum FGF23 levels ($\beta=-0.018$, $P=0.793$), were independently associated with FMD. None of the antidiabetic drugs, inulin ($\beta=-0.028$, $P=0.650$), sulfonylureas ($\beta=0.014$, $P=0.811$),

Table 1. Clinical characteristics, serum intact FGF23 levels, and vascular function in all participants and in subgroups by kidney function

	All participants	eGFR ≥ 60 mL/min/1.73 m²	eGFR <60 mL/min/1.73 m²	*p*
N (male/female)	283 (170/113)	172 (95/77)	111 (75/36)	0.039
Age (year)	64 [55–70]	62 [51–68]	68 [61–73]	<0.001
Duration of diabetes (year)	11 [5–20]	10 [2–17]	15 [9–20]	<0.001
Body mass index (kg/m²)	25.1 [22.1–27.9]	25.3 [21.8–28.5]	24.9 [22.5–27.7]	0.943
Systolic blood pressure (mmHg)	130 [118–144]	125 [115–135]	139 [124–156]	<0.001
Diastolic blood pressure (mmHg)	74 [69–81]	74 [68–79]	76 [69–84]	0.047
Current smoker (*n*, %)	71 (25.1)	43 (25.0)	28 (25.2)	0.966
Cardiovascular diseases (*n*, %)	55 (19.4)	23 (13.4)	32 (28.8)	0.001
Creatinine (mg/dL)	0.82 [0.67–1.07]	0.70 [0.62–0.81]	1.20 [1.01–1.82]	<0.001
eGFR (mL/min/1.73 m²)	66.0 [51.1–79.0]	76.9 [68.9–87.3]	43.2 [26.5–55.9]	<0.001
Serum calcium (mg/dL)	9.4 [9.1–9.7]	9.5 [9.2–9.7]	9.3 [9.0–9.7]	0.004
Serum phosphate (mg/dL)	3.7 [3.4–4.1]	3.7 [3.4–4.1]	3.6 [3.4–4.2]	0.752
Fasting glucose (mg/dL)	121 [104–147]	127 [106–149]	121 [100–145]	0.307
HbA1c (%)	8.3 [7.3–9.7]	8.6 [7.5–9.9]	8.0 [7.1–9.2]	0.012
Immunoreactive insulin (μU/mL)*	7.1 [4.9–10.2]	7.0 [4.9–10.4]	7.4 [4.6–10.1]	0.953
HOMA-R*	2.2 [1.4–3.1]	2.2 [1.4–3.1]	2.0 [1.4–3.1]	0.576
Triglycerides (mg/dL)	117 [91–154]	114 [86–143]	134 [97–183]	0.002
Total cholesterol (mg/dL)	178 [151–202]	180 [153–199]	174 [148–203]	0.688
HDL-cholesterol (mg/dL)	40 [35–55]	42 [36–52]	39 [34–47]	0.015
LDL-cholesterol (mg/dL)	107 [86–131]	107 [88–134]	105 [86–129]	0.426
RAS inhibitors (*n*, %)	115 (40.6)	57 (33.1)	58 (52.7)	0.001
Calcium channel blockers (*n*, %)	113 (39.9)	48 (27.9)	65 (58.6)	<0.001
Statins (*n*, %)	117 (41.3)	69 (40.1)	48 (43.2)	0.602
Sulfonylureas (*n*, %)	105 (37.1)	67 (39.0)	38 (34.2)	0.422
Metformin (*n*, %)	97 (34.3)	74 (43.0)	23 (20.7)	<0.001
DPP-4 inhibitors (*n*, %)	77 (27.2)	47 (27.3)	30 (27.0)	0.956
Pioglitazone (*n*, %)	32 (11.3)	22 (12.8)	10 (9.0)	0.327
α-Glucosidase inhibitors (*n*, %)	44 (15.5)	29 (16.9)	15 (13.5)	0.448
Insulin (*n*, %)	116 (41.0)	54 (31.4)	62 (55.9)	<0.001
Oral vitamin D (*n*, %)	10 (3.5)	5 (2.9)	5 (4.5)	0.477
FGF23 (pg/mL)	27.3 [12.4–48.3]	20.6 [9.6–35.4]	41.9 [21.0–72.6]	<0.001
FMD (%)	6.0 [3.8–8.4]	6.6 [3.9–9.3]	5.7 [3.4–7.7]	0.028
NMD (%)	14.0 [9.4–19.3]	15.6 [11.2–20.1]	10.6 [7.5–16.3]	<0.001

Data are median [interquartile range] or *n* (%). *P*-values were determined by χ^2-test or Wilcoxon rank-sum test for comparison between the group with normal kidney function and the group with impaired kidney function.
Abbreviations: eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HOMA-R, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RAS, renin-angiotensin system; DPP, dipeptidyl peptidase; FGF, fibroblast growth factor; FMD, flow-mediated vasodilatation; NMD, nitroglycerin-mediated vasodilatation. *, N=161 for participants not receiving insulin therapy

metformin (β=0.014, P=0.813), DPP-4 inhibitors (β=0.004, P=0.947), pioglitazone (β=−0.110, P=0.060), or α-glucosidase inhibitors (β=0.057, P=0.307), showed a significant effect on NMD, nor did they virtually affect the relationship between serum FGF23 levels and NMD.

Because serum FGF23 levels are strongly affected by kidney function[29, 30], an interaction analysis was conducted to determine whether the presence or absence of kidney dysfunction modifies the association between serum FGF23 levels and NMD. The interaction analysis indicated a potential effect modification by the presence or absence of kidney dysfunction on the association between serum FGF23 levels and NMD (P for interaction =0.015). Therefore, the association between FGF23 levels and NMD was then separately analyzed for the group with normal kidney function (eGFR ≥ 60 mL/min/1.73 m²) and the group with impaired kidney function (eGFR <60 mL/min/1.73 m²). As expected, the impaired kidney function group exhibited significantly higher serum FGF23 levels and lower NMD than the

Table 2. Associations of FMD and NMD with cardiometabolic risk factors and medications

	FMD		NMD	
	ρ	p	ρ	p
A. Continuous variables				
Age	–0.074	0.217	–0.241	<0.001
Body mass index	–0.019	0.752	–0.027	0.648
Systolic blood pressure	–0.117	0.049	–0.304	<0.001
eGFR	0.132	0.026	0.296	<0.001
Serum calcium	0.078	0.193	0.066	0.272
Serum phosphate	0.086	0.138	–0.024	0.685
HOMA-R*	0.011	0.885	0.065	0.371
LDL-cholesterol	–0.057	0.342	0.033	0.585
Triglycerides	–0.011	0.858	–0.045	0.448
HDL-cholesterol	–0.005	0.936	0.009	0.880
HbA1c	–0.102	0.086	0.129	0.030
FGF23	–0.105	0.079	–0.282	<0.001
B. Categorical variables				
	FMD (medians, %)		NMD (medians, %)	
	Yes	No	p	Yes	No	p
Male	5.6	6.9	0.007	13.9	14.6	0.565
Current smoker	5.8	6.1	0.412	14.2	14.0	0.468
RAS inhibitors	5.3	6.6	0.021	13.4	14.2	0.186
Calcium channel blockers	5.6	6.4	0.022	11.0	15.4	<0.001
Insulin therapy	6.1	5.9	0.648	13.0	14.5	0.218
Sulfonylureas	5.9	6.2	0.458	13.2	14.2	0.935
Metformin	6.3	5.9	0.137	14.8	13.7	0.089
DPP-4 inhibitors	5.4	6.1	0.133	13.2	14.2	0.969
Pioglitazone	5.7	6.1	0.296	10.6	14.3	0.049
α-Glucosidase inhibitors	7.2	5.8	0.110	12.6	14.4	0.315

ρ, Spearman's rank correlation coefficient. *P* values were determined by Spearman's rank correlation test and Wilcoxon rank sum test for continuous variables and categorical variables, respectively.
Abbreviations are as in Table 1. *, N = 161 for participants not receiving insulin therapy

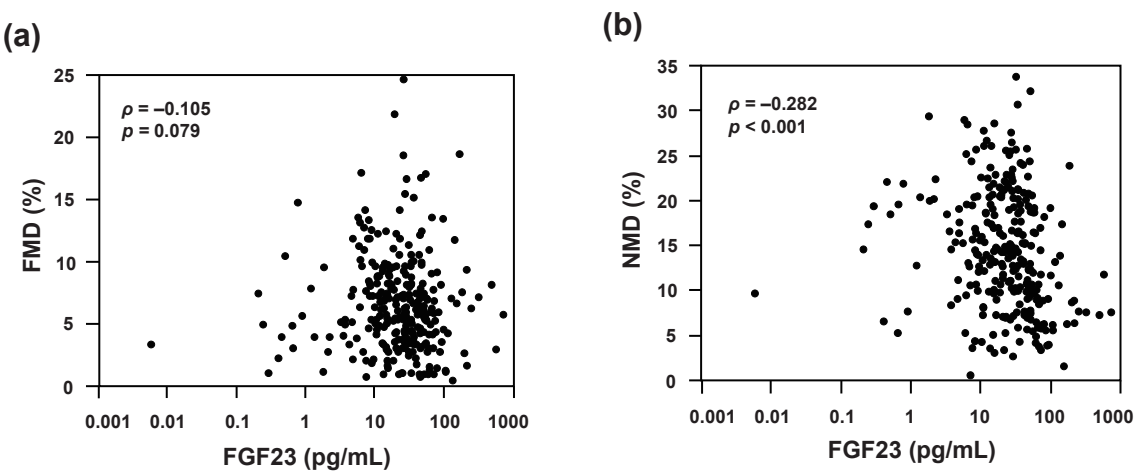

Fig. 1. Correlation between serum FGF23 levels and FMD (a) and NMD (b) in all participants

The upper panel (a) indicates that serum FGF23 levels were not significantly correlated with FMD. The lower panel (b) indicates that serum FGF23 levels were significantly correlated with NMD.
Abbreviations: FGF, fibroblast growth factor; FMD, flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation; ρ, Spearman's rank correlation coefficient

Table 3. Multivariate analyses for the determinants of FMD and NMD in all participants

	FMD		NMD	
	β	p	β	p
Age	–0.137	0.076	–0.187	0.012
Sex (male=1, female=0)	–0.132	0.043	–0.068	0.281
Body mass index	–0.106	0.120	–0.082	0.216
Systolic blood pressure	–0.107	0.102	–0.167	0.008
Current smoker (yes=1, no=0)	–0.066	0.279	–0.004	0.942
RAS inhibitors (yes=1, no=0)	–0.135	0.028	0.020	0.731
Calcium channel blockers (yes=1, no=0)	–0.043	0.516	–0.130	0.045
Insulin therapy (yes=1, no=0)	0.069	0.290	–0.028	0.650
eGFR	0.114	0.149	0.061	0.421
Serum calcium	0.039	0.518	0.024	0.680
Serum phosphate	0.049	0.472	0.078	0.236
HbA1c	–0.212	0.001	0.035	0.576
LDL-cholesterol	–0.028	0.675	0.035	0.583
Triglycerides	–0.008	0.909	–0.081	0.236
HDL-cholesterol	–0.050	0.463	–0.054	0.410
FGF23	–0.018	0.793	–0.131	0.046
R^2 (p)	0.126 (0.002)		0.191 (<0.001)	

β, standardized regression coefficient by multiple regression analysis; R^2, multiple coefficients of determination. Abbreviations are the same as Table 1.

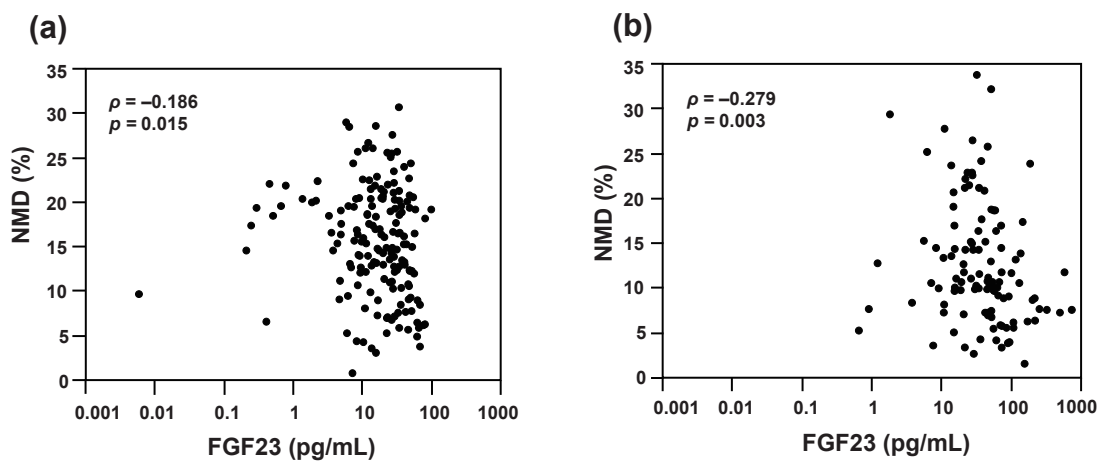

Fig. 2. Correlation between serum FGF23 levels and NMD stratified by kidney function

Serum FGF23 levels were significantly and inversely correlated with NMD in both the group with eGFR ≥ 60 mL/min/1.73 m^2 (N=172) (a) and the group with eGFR <60 mL/min/1.73 m^2 (N=111) (b).
Abbreviations: FGF, fibroblast growth factor; NMD, nitroglycerin-mediated dilatation; ρ, Spearman's rank correlation coefficient

normal group (Table 1). The unadjusted correlation between serum FGF23 levels and NMD was found to be statistically significant in both groups (Fig. 2). Finally, multivariate analysis by subgroup revealed that, although both were statistically significant, the association between serum FGF23 levels and NMD was more pronounced in the group with normal kidney function (β=–0.237, P=0.001) than in the group with impaired kidney function (β=–0.247, P=0.040) (Table 4).

Discussion

In this cross-sectional study, we investigated the association between serum FGF23 levels and vascular function in patients with type 2 diabetes. Our results indicated that serum FGF23 levels were inversely associated with NMD, independently of age, sex,

Table 4. Multivariate analysis for the determinants of NMD stratified by kidney function

	eGFR ≥ 60 mL/min/1.73 m² (N = 172)		eGFR < 60 mL/min/1.73 m² (N = 111)	
	β	*p*	*β*	*p*
Age	–0.426	< 0.001	0.056	0.635
Sex (male = 1, female = 0)	–0.129	0.122	0.061	0.578
Body mass index	–0.259	0.003	0.174	0.125
Systolic blood pressure	–0.132	0.064	–0.153	0.153
Current smoker (yes = 1, no = 0)	–0.005	0.944	–0.038	0.711
RAS inhibitors (yes = 1, no = 0)	–0.005	0.944	0.102	0.331
Calcium channel blockers (yes = 1, no = 0)	–0.141	0.076	–0.105	0.336
Insulin therapy (yes = 1, no = 0)	–0.081	0.290	0.021	0.843
Serum calcium	0.061	0.377	0.030	0.779
Serum phosphate	0.053	0.501	0.040	0.727
HbA1c	0.082	0.307	0.007	0.947
LDL-cholesterol	–0.060	0.450	0.176	0.140
Triglycerides	–0.175	0.036	0.040	0.741
HDL-cholesterol	–0.051	0.515	–0.053	0.656
FGF23	–0.237	0.001	–0.247	0.040
R^2 (*p*)	0.306 (< 0.001)		0.149 (0.356)	

β, standardized regression coefficient by multiple regression analysis; R^2, multiple coefficients of determination. Abbreviations are the same as Table 1.

serum phosphate levels, and traditional CVD risk factors. Furthermore, the association between FGF23 and NMD was more pronounced in those with normal kidney function than in those with impaired kidney function. On the other hand, no significant association was observed between serum FGF23 levels and FMD. To the best of our knowledge, this is the first study to associate increased levels of FGF23 with vascular smooth muscle dysfunction in patients with type 2 diabetes.

This study first demonstrated an independent association between higher levels of serum FGF23 and higher NMD, a marker of vascular smooth muscle function, in patients with type 2 diabetes. A few studies previously investigated the association between FGF23 and vasodilatory function in a community[11] or a hospital[31, 32] setting. Our results are consistent with a previous report by Mirza, *et al.*[11], which associated higher serum FGF23 levels with impaired endothelium-independent vasodilatation in a community-based elderly cohort. Unlike our study, the study by Yilmaz, *et al.*[32] failed to find an association between serum FGF23 levels and NMD, probably because the study included only patients with severe CKD (stages 3–4). One previous study in patients with type 2 diabetes, although the method of assessing smooth muscle function was different from ours, associated higher FGF23 levels with impaired myocardial blood flow following vasodilator-induced stress by adenosine[31], which may support our results.

Contrary to the prior studies[11, 32], our study failed to identify the association between FGF23 and FMD, an endothelium-dependent vasodilatation. Evidence from experimental studies suggests that FGF23 directly impairs endothelium-dependent vasodilatation by reducing nitric oxide bioavailability[33-35]. The association between increased serum FGF23 levels and endothelial dysfunction was demonstrated in the general population[11]. Considering that our study only included patients with type 2 diabetes with inadequate glycemic control and that hyperglycemia is a major factor that attenuates nitric oxide bioavailability in the endothelium[18], we might have failed to associate serum FGF23 levels with FMD as endothelial function was significantly impaired due to chronic hyperglycemia. The association between FGF23 and FMD was also shown in patients with advanced CKD[32]. Although CKD is a risk factor for endothelial dysfunction[36, 37], the patients in the study[32] were younger (mean age, 45 vs. 64 years) and had greater FMD (mean, 6.8% vs. 6.0%) than those in our study. Thus, the association between FGF23 and endothelial function may differ depending on the age and/or medical conditions related to the CVD risk of the study subjects.

This study further demonstrated that kidney function affected the relationship between FGF23 levels and NMD, which was more prominent in those with normal kidney function than in those with

impaired kidney function. Previous studies have also shown results similar to ours[11, 32]. In a study by Mirza, *et al.*[11], serum FGF23 levels were associated with endothelium-independent vasodilatation in a subgroup of 759 patients with normal kidney function (eGFR ≥ 60 mL/min/1.73 m^2) but not in 208 patients with impaired kidney function. Another study by Yilmaz, *et al.*[32] failed to find an association between serum FGF23 levels and NMD in 183 patients with stage 3–4 CKD (mean eGFR, 33 mL/min/1.73 m^2). Of importance, we previously reported that the advanced CKD stage preferentially and adversely affected NMD, rather than FMD, in patients with type 2 diabetes[27]. Collectively, the attenuated association between FGF23 and NMD in the group with kidney dysfunction could be explained by a loss of power due to subgrouping or by significantly impaired vascular smooth muscle function due to CKD **(Table 1)**.

There are several possible mechanisms for the close relationship between increased FGF23 levels and vascular smooth muscle dysfunction suggested in this study. Although the direct effect of FGF23 on cardiovascular tissue is still controversial, there are reports indicating the expression of fibroblast growth factor (FGF) receptors and its coreceptor, Klotho, in human aortic tissue and vascular smooth muscle cells[34, 38]. The FGF receptor signaling in vascular smooth muscle cells is shown to induce conversion from the normal contractile phenotype to the proliferative phenotype, which plays a central role in the pathogenesis of atherosclerosis[39, 40]. Furthermore, in a study evaluating the vasoreactivity of the mouse aortic ring, FGF23 stimulated aortic contractions and increased reactive oxygen species in vascular smooth muscle cells[41]. These reports suggest that FGF23 plays a direct role in the impairment of dilatory function in vascular smooth muscle cells.

Considering that FGF23 is a hallmark of abnormal phosphate metabolism[12, 30], factors related to phosphate metabolism may also be involved in the impairment of vascular smooth muscle function. First, postprandial increase in serum phosphate levels due to high phosphate intake, which increases circulating FGF23 levels[42], may also contribute to vascular dilatory dysfunction[43]. The experimental evidence has indicated a direct and adverse effect of excess phosphate in calcification[44, 45] and *de novo* cholesterol synthesis[46] in vascular smooth muscle cells. Second, an excess of phosphate and calcium in circulation promotes the formation of calciprotein particles (CPPs) that can induce inflammation and calcification in vascular smooth muscle cells in individuals with CKD[47, 48]. Considering a recent report indicating that serum calcification propensity, or the maturation time of CPPs in serum, is associated with HbA1c in patients with type 2 diabetes[49], CPPs may play a role in vascular smooth muscle dysfunction in type 2 diabetes even without CKD. Third, vitamin D and parathyroid hormone, which are major regulators of calcium–phosphate metabolism in CKD, might have contributed to vascular dysfunction[50]. Fourth, increased inflammatory cytokines[42] and dysregulated adipocytokines[51] are reportedly associated with increased FGF23 levels in the context of obesity and may also contribute to the impairment of smooth muscle function.

This study has several limitations. First, we cannot exclude the possibility that unmeasured factors related to abnormal phosphate metabolism, such as dietary phosphate intake, CPPs, vitamin D, parathyroid hormone, inflammatory cytokines, and adipocytokines, affected NMD. Second, our results should be carefully interpreted because the sample size was rather small to draw reliable conclusions, particularly in the multivariate analysis stratified by kidney function. Third, because our study consecutively included older adults with type 2 diabetes, poor glycemic control, and a relevant prevalence of macrovascular complications, our results are not applicable to the entire population of patients with type 2 diabetes. Lastly, due to the cross-sectional nature of this study, a causal relationship cannot be drawn.

Conclusion

This study demonstrated that serum FGF23 levels are independently and negatively associated with NMD in patients with type 2 diabetes and that the association is prominent in those with normal kidney function. Our data suggest that FGF23 is involved in the impairment of vascular smooth muscle function and that increased serum levels of FGF23 may serve as a novel biomarker for vascular smooth muscle dysfunction in patients with type 2 diabetes. Further interventional studies are warranted to determine whether dietary phosphate restriction or phosphate-lowering agents improve serum FGF23 levels and NMD in patients with type 2 diabetes.

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Conflict of Interest

The authors declare no conflict of interest related to this study.

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