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Metabolism Clinical and Experimental 56 (2007) 451–458
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Association between plasma visfatin and vascular endothelial function in patients with type 2 diabetes mellitus

Kohzo Takebayashi*, Mariko Suetsugu, Sadao Wakabayashi, Yoshimasa Aso, Toshihiko Inukai

Department of Internal Medicine, Dokkyo Medical University Koshigaya Hospital, 2-1-50 Minami-Koshigaya, Koshigaya 343-8555, Japan

Abstract

Visfatin is a newly identified adipocytokine that mimics insulin action. However, the pathophysiological role of visfatin in diabetic patients is not fully understood. The main purpose of this study was to investigate the association of plasma visfatin with endothelial function in patients with type 2 diabetes mellitus. In addition, the relationships of visfatin with oxidative stress, low-grade inflammation, atherosclerosis, adiponectin, plasma renin activity, and aldosterone were also explored, and the effect of pioglitazone on visfatin was examined. Visfatin levels were measured in 80 patients with type 2 diabetes mellitus and in 28 age-matched healthy subjects. Endothelial function was evaluated by using flow-mediated vasodilatation (FMD), oxidative stress was assessed by the level of urinary 8-iso-prostaglandin $F_{2\alpha}$, and atherosclerosis and inflammation were measured by using the intimal-medial complex thickness and the levels of high-sensitivity C-reactive protein and fibrinogen. Pioglitazone was administered for 12 weeks at a dose of 30 mg/d in a further 20 patients with type 2 diabetes mellitus. There was a significant negative correlation between the $\log_{10}$-transformed (log) plasma visfatin concentration and FMD or creatinine clearance ($R = -0.2672$, $P = .0167$; $R = -0.2750$, $P = .0136$). Log visfatin was also positively correlated with log urinary albumin excretion ($R = 0.2305$, $P = .0397$). In addition, it was also found that visfatin had a significant negative correlation with plasma aldosterone ($R = -0.2432$, $P = .0297$). In stepwise regression analysis, creatinine clearance, log aldosterone, FMD, and sex showed a significant association with log visfatin ($P = .0040$, $P = .0069$, $P = .0444$, and $P = .0487$, respectively), and log 8-iso-prostaglandin $F_{2\alpha}$ showed a tendency for an association ($P = .0515$). Pioglitazone therapy did not affect the visfatin concentration in the 20 pioglitazone-treated patients with diabetes, although a significant elevation of visfatin was obtained in a subgroup of 11 female patients ($P = .0381$). In conclusion, the current study showed that visfatin is negatively associated with vascular endothelial function evaluated by FMD and creatinine clearance, and positively associated with log urinary albumin excretion. Visfatin was also negatively correlated with circulating aldosterone. Pioglitazone therapy for 12 weeks did not affect the plasma visfatin concentration significantly in all diabetic patients, but a significant elevation in visfatin was obtained in women only.

1. Introduction

Visfatin is an adipocytokine that was recently identified by Fukuhara et al [1]. Visfatin has a potential insulinlike action that may cause insulin sensitivity [1]. Recently, Chen et al [2] reported that plasma visfatin concentrations elevate in patients with type 2 diabetes mellitus, compared with those in healthy subjects, and that the visfatin level is positively correlated with waist-hip ratio or fasting insulin and negatively correlated with adiponectin, which is an adipocytokine related to insulin sensitivity [3,4]. In contrast to the results of Fukuhara et al, this appears to suggest that visfatin is associated with insulin resistance, rather than insulin sensitivity. Thus, the pathophysiological role of visfatin is still unclear and a more detailed analysis is needed. In particular, it is of interest to investigate how visfatin is associated with vascular endothelial function, which has also been related to insulin resistance [5,6]; furthermore, it is reported that adiponectin is protective against vascular endothelial impairment [7,8]. The association of visfatin with oxidative stress, low-grade inflammation, and atherosclerosis, all of which are related to insulin resistance [9-11], also remains unknown, and the relationship between visfatin and renal function as evaluated by creatinine clearance (Ccr) or urinary albumin excretion (UAE) is not fully apparent.

Given the above background, the main purpose of the study was to investigate the association of plasma visfatin with endothelial function measured using flow-mediated

* Corresponding author. Tel.: +81 48 965 1111; fax: +81 48 965 1127.
E-mail address: takeb@gmail.plala.or.jp (K. Takebayashi).

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doi:10.1016/j.metabol.2006.12.001

vasodilatation (FMD) [12,13] in patients with type 2 diabetes mellitus. The association between visfatin and the following parameters was also explored: the urinary 8-iso-prostaglandin (PG) $F_{2\alpha}$ level, as a marker of systemic oxidative stress [14,15]; the levels of inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) and fibrinogen; the degree of atherosclerosis assessed by the intimal-medial complex thickness (IMT) of the carotid artery [16-18]; and the adiponectin level. Furthermore, because a close association between the renin-angiotensin-aldosterone system and insulin resistance has recently been suggested [19], we also explored the association between visfatin and plasma renin activity or the plasma aldosterone concentration, in addition to the association between visfatin and renal function.

Finally, it has been reported that rosiglitazone, a peroxisome proliferator–activated receptor (PPAR)-γ agonist, increases the expression of visfatin messenger RNA (mRNA) in visceral fat deposits in rat [20]; however, a study in humans showed that pioglitazone therapy for 4 weeks did not affect the circulating visfatin concentration [21]. This result may be due to the relatively short observation time; therefore, we examined the effect of pioglitazone on visfatin over 12 weeks.

Based on the recent report that visfatin may be associated with insulin resistance [2], we hypothesized that circulating visfatin is negatively associated with endothelial function and the level of adiponectin, and positively associated with oxidative stress, inflammatory markers, and atherosclerosis. We further hypothesized that a 12-week course of pioglitazone therapy would elevate the plasma visfatin concentration.

Table 1
Clinical characteristics of the nondiabetic and diabetic subjects

	Nondiabetic	Diabetic	*P*
No. (male/female)	28 (10/18)	80 (49/31)	–
Age (y)	59.1 ± 11.9	62.0 ± 4.9	.0695
Duration of diabetes mellitus (year)	11.9 ± 10.4	–	–
FPG (mg/dL)	118.6 ± 24.1	96.9 ± 9.1	<.0001*
HbA_{1c} (%)	9.9 ± 2.1	5.0 ± 0.3	<.0001*
BMI (kg/m^2)	24.5 ± 4.3	21.4 ± 2.8	<.0001*
Diabetic therapy			
Insulin therapy	–	42	–
SU	–	34	–
SU1/SU2/SU3/SU4/SU5	–	12/2/9/10/1	–
Metformin and α-GI	–	1	–
Diet alone	–	3	–
Antihypertensive drugs	–	39	–
A/B/C/D/E	–	12/13/5/3/6	–
No. of smokers	–	26	–

Data are expressed as mean ± SD. Comparison of variables between 2 groups were made by use of an unpaired *t* test. SU1 indicates SU alone; SU2, SU and metformin; SU3, SU and α-GI, SU4: SU, metformin, and α-GI; SU5, SU and pioglitazone. Antihypertensive drugs: A, angiotensin-II receptor blocker (ARB) alone; B, calcium channel blocker (Ca) alone; C, Ca and ARB; D, Ca and a β- or α-blocker or methyldopa; E, Ca and an ARB and/or an angiotensin-converting enzyme inhibitor, a β- orα-blocker, and/or methyldopa.

*$P < .05$, statistically significant.

2. Patients and methods

2.1. Patients

Eighty patients with type 2 diabetes mellitus (49 men and 31 women) hospitalized for glycemic control and/or diabetes education were prospectively and consecutively enrolled in the study from August 2005 to January 2006. As controls, 30 age-matched healthy subjects were also enrolled (2 subjects were subsequently excluded because they turned out to have elevated glycosylated hemoglobin [HbA_{1c}] level). The clinical characteristics of the diabetic patients and healthy subjects are shown in Table 1.

To investigate the effect of pioglitazone on visfatin, we studied another group of 20 patients with type 2 diabetes mellitus (9 men and 11 women) who were consecutively enrolled in the study from October 2005 to November 2005 after visiting our outpatient department. Of these patients, 15 received sulfonylurea (SU) (6 received SU alone; 3, SU and α-glucosidase inhibitor [α-GI]; 6, SU and metformin), 2 were treated with α-GI alone, 1 patient had received metformin alone, and 2 patients had been controlled by dietary modification alone. No patients had received any thiazolidinediones, including pioglitazone. In addition, 10 patients who had not received thiazolidinediones and who were changed from SU to insulin therapy because of poor diabetic control were simultaneously prospectively enrolled in the study (3 patients subsequently dropped out because of poor compliance at follow-up). Patients exhibiting evidence of liver dysfunction or findings of infectious or autoimmune disease were excluded from the study.

2.2. Methods

2.2.1. Measurement of FMD and endothelium-independent nitroglycerin-mediated vasodilatation of the brachial artery

Flow-mediated vasodilatation was measured by a single specialist who had more than 6 months' experience with the FMD measurement procedure. FMD was measured in the morning after at least a 10-hour overnight fast and was performed while diabetes was under control after admission. During the measurement, patients were required to maintain a supine position, and the right brachial artery 5 to 10 cm above the elbow was longitudinally scanned by ultrasonography (SSA-550A NEMIO, Toshiba Medical Systems, Tokyo, Japan) with a 14-MHz probe (PLM-703-AT Toshiba Medical Systems). The lead II electrocardiographic signal was simultaneously measured to synchronize the image capture with the moment when the R wave was most elevated to diminish variability between cardiac cycles. After the lumen diameter of the right brachial artery was measured at baseline, the cuff placed above the measurement point was inflated to a pressure 30 mm Hg greater than the patient's systolic blood pressure (SBP) to occlude the brachial artery completely. After confirmation of complete occlusion of the brachial artery, continuous occlusion was maintained for 5 minutes. The cuff was then released and the artery image was

continuously recorded on videotape for 3 minutes. FMD was calculated according to the formula: FMD (%) = [(maximal artery lumen diameter after cuff release − artery lumen diameter at baseline)/artery lumen diameter at baseline] × 100. To confirm the reproducibility of this test, 6 young healthy subjects (mean age, 30.7 ± 6.4 years) underwent 2 consecutive FMD measurements with a 30-minute interval, and the change in FMD value was shown to be less than 5% in each subject (mean change, 1.1% ± 1.8%).

To determine the nitroglycerin-mediated (NMD) vasodilatation of the artery, the patients were required to maintain a supine position at rest for an additional 15 minutes after the FMD test. After recovery of the lumen diameter of the brachial artery to its baseline value, patients received 25 μg of nitroglycerin sublingually. After 5 minutes, the brachial artery diameter was measured again, and NMD was calculated similarly to FMD.

2.2.2. Measurement of the IMT of the carotid artery

The IMT was assessed by using the ultrasonography device used in the FMD measurement, based on the method described by Kawamori et al [22]. A suitable portion of the extracarotid artery (common carotid artery) in the neck was selected and scanned in the longitudinal projection on the right side in an anterior oblique position. A plaque was defined as a locally thickened IMT of more than 1.1 cm, following the criteria of Handa et al [23]. The IMT was measured with calipers at the thickest portion of the scanning area with a 1-cm interval on each side, not including plaques. The mean of 3 IMT measurements was recorded as the IMT for the patient. Maximum IMT was calculated in a similar manner using data from the most thickened portion of the artery, including plaques. IMT was measured in 70 of the 80 patients in the study.

2.2.3. Blood and urine tests

All venous blood and urine samples for biochemical tests were simultaneously collected from patients in the morning before breakfast on the day when the FMD measurement was performed. After collection, the blood was rapidly centrifuged at 1500 rpm for 5 minutes to separate sera or plasma from the clot-containing blood cells. These samples were stored at −70°C until analysis.

2.2.4. Plasma visfatin assay

Plasma visfatin was measured using frozen plasma in a test tube containing disodium EDTA (EDTA-2Na), with a visfatin C-terminal (Human) enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, CA). Each value was recorded as a mean of duplicate measurements in the same plasma sample. The intra- and interassay coefficients of variation (CVs) of this kit are less than 5% and less than 14%, respectively.

2.2.5. Serum adiponectin assay

Serum adiponectin was measured by using an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceuticals, Tokyo, Japan); the intra- and interassay CVs were 4.06% and 4.69%, respectively. Adiponectin was measured in 76 of the 80 patients.

2.2.6. Serum hsCRP

A BNI IN High-Sensitivity CRP assay (Dade Behring, Marburg, Germany) was used to measure serum hsCRP. The intra- and interassay CVs in this assay were 1.72% and 2.80%, respectively, and the lowest detectable concentration of hsCRP was 0.05 mg/L. Only 2 patients had a value of hsCRP below 0.05 mg/L, and for analysis this value was taken to be 0.05 mg/L.

2.2.7. Measurement of plasma glucose, HbA1c, and serum lipid concentrations

Fasting plasma glucose was evaluated by the automated glucose oxidase method (Glucose Auto Stat GA1160; Arkray, Kyoto, Japan). HbA_{1c} was measured by high-performance liquid chromatography (HPLC; Hi-auto A1C, HA8150, Arkray). Only HbA_{1c} was detected by this method, and the normal range was 4.3% to 5.8%. Serum total (TC), low-density (LDL-C), and high-density lipoprotein cholesterol (HDL-C) and serum triglyceride (TG) concentrations were measured by using enzymatic assays. The Determiner L TC II and Determiner L TG reagents (Kyowa Medics, Tokyo, Japan) were used for measurement of TC and TG, respectively. HDL-C was measured directly by a method based on the selective solubilizing effect of a proprietary detergent of the different lipoproteins using the Cholestest N HDL-C reagent (Daiichi Pure Chemicals, Tokyo, Japan). LDL-C was also measured directly by a homogenous method using the Cholestest LDL reagent (Daiichi Pure Chemicals), and not by using the Friedewald equation.

2.2.8. Measurement of plasma renin activity and plasma aldosterone

Plasma renin activity (PRA) and plasma aldosterone were measured by radioimmunoassay using reagent kits: a plasma renin activity SRL kit (SRL, Tokyo, Japan) and a SPAC-C aldosterone kit (SRL), respectively.

2.2.9. Measurement of UAE

UAE was measured in a 24-hr urine specimen kept at 4 °C, in an immunoturbidimetric assay using the urinary albumin RD reagent kit (Roche Diagnostics, Tokyo, Japan). Albumin values were corrected for the urinary creatinine concentration.

2.2.10. Urinary 8-iso-$PGF_{2\alpha}$ assay

Urinary 8-iso-$PGF_{2\alpha}$ was measured in morning urine using an EIA kit (ACE EIA; Cayman Chemical Company, Ann Arbor, MI). The intra- and interassay CVs were both less than 10%, based on actual values. This assay has been reported to show no difference in measurements using morning urine samples analyzed immediately and stored for 24 hours [24].

2.2.11. Measurement of Ccr

Ccr was directly measured by using urine samples stored for 24 hours.

2.2.12. Pioglitazone and insulin therapy

These therapies were continued for more than 12 weeks. Blood tests were performed at baseline and after 12 weeks, in each case before breakfast after at least a 10-hour period of overnight fasting. All patients in the pioglitazone-treated group received 30 mg/d of pioglitazone. For patients who changed from SU to insulin therapy, insulin was initiated at a dose of approximately the patient's body weight (kg) × 0.2 U, and then the dose was adjusted based on the degree of glycemic control. No change in administration of any drug occurred for any patient during the 12-week investigational period.

2.2.13. Ethical considerations

All subjects gave informed consent to inclusion in the study, which was performed according to the guidelines proposed in the Declaration of Helsinki. The study was approved by the Dokkyo Medical Ethics Committee (Koshigaya, Japan).

Table 2
The results of linear regression analysis between log visfatin and various variables, and stepwise regression analysis for log visfatin as a dependent variables

	Simple		Multiple	
	R	*P*	*β*	*P*
Sex	0.0979	.3905	−0.2167	.0487*
Age (y)	0.0329	.7714	–	–
Duration (y)	0.1731	.1246	–	–
Smoking (±)	0.1359	.2294	–	–
BMI (kg/m^2)	0.0596	.5995	–	–
FPG (mg/dL)	0.0985	.3848	0.1561	.1439
HbA_{1c} (%)	0.0283	.8035	–	–
SBP (mm Hg)	0.1031	.3628	–	–
DBP (mm Hg)	0.0136	.9043	–	–
TC (mg/dL)	0.1069	.3450	–	–
TG (mg/dL)	0.1866	.0975	–	–
HDL-C (mg/dL)	−0.0559	.6224	–	–
LDL-C (mg/dL)	0.0731	.5191	–	–
Ccr (mL/min)	−0.2750	.0136*	−0.3331	.0040*
Log UAE (mg/g Cr)	0.2305	.0397*	–	–
Log PRA (ng/mL · h)	−0.2023	.0739	–	–
Log aldosterone (ng/dL)	−0.2432	.0297*	−0.3053	.0069*
Log CRP (mg/L)	−0.0062	.9564	–	–
Fibrinogen (mg/dL)	−0.0058	.9593	–	–
Log 8-iso-$PGF_{2\alpha}$ (pg/mL)	−0.0502	.6581	0.2273	.0515
Adiponectin(μg/mL) (n = 76)	0.0827	.4777	–	–
FMD (%)	−0.2672	.0167*	−0.2217	.0444*
NMD (%)	−0.1662	.1432	–	–
IMT (mm) (n = 70)	0.1606	.1841	–	–
Maximum IMT (mm) (n = 70)	−0.0283	.8162	–	

Linear regression analysis was performed in 80 patients with type 2 diabetes mellitus except for adiponectin (n = 76), IMT (n = 70), and maximum IMT (n = 70). In stepwise regression analysis (n = 70), independent variables tested initially were age, sex, smoking, duration of diabetes, BMI, FPG, HbA_{1c}, LDL-C, HDL-C, TG, SBP, DBP, log CRP, log 8-iso-$PGF_{2\alpha}$, log aldosterone, Ccr, IMT, and FMD. Each variable with an F value less than 2 was excluded.

* $P < .05$, statistically significant.

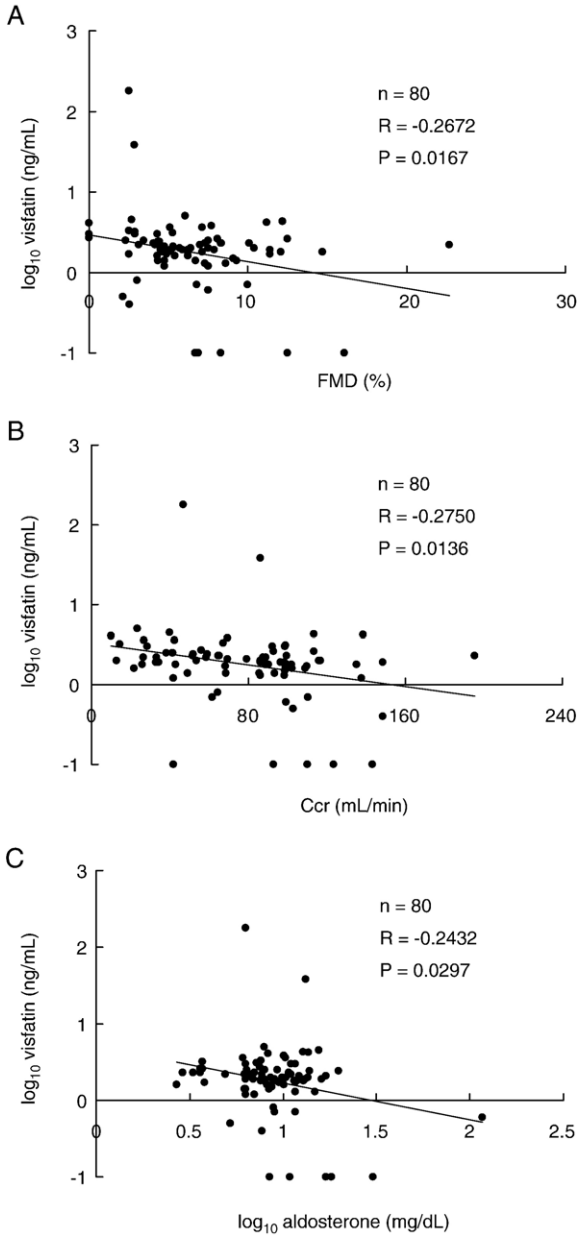

Fig. 1. Correlation between $\log_{10}$ transformed plasma visfatin and FMD (A), Ccr (B), and log plasma aldosterone (C) in 80 patients with type 2 diabetes mellitus.

2.2.14. Statistical methods

All data except those for visfatin concentration are presented as means ± SD. The visfatin concentration is expressed as median and interquartile ranges (25th and 75th percentiles) because of its skewed distribution. The significance of correlations between 2 variables was determined by simple regression analysis. Data for visfatin, UAE, PRA, aldosterone, hsCRP, and 8-iso-$PGF_{2\alpha}$ were $\log_{10}$ transformed because of skewed distributions. Multiple regression analysis with log visfatin as the dependent variable was performed by

using stepwise regression analysis. The independent variables that were tested initially were age, sex, smoking, duration of diabetes, body mass index (BMI), fasting plasma glucose (FPG), HbA_{1c}, LDL-C, HDL-C, TG, SBP, diastolic blood pressure (DBP), log CRP, log 8-iso-$PGF_{2\alpha}$, log aldosterone, Ccr, IMT and FMD (because IMT was measured in only 70 patients, this analysis was also performed for these 70 patients). No combination with a strong correlation (R >0.9) was found among these variables. Each variable with an F value less than 2 was excluded, and multiple regression analysis was performed among the remaining independent variables. Comparison of 2 time points for an individual was performed by a paired t test except for visfatin, for which a Wilcoxon signed rank test was used as a nonparametric test. Comparison of visfatin levels between the 2 groups was performed with a Wilcoxon rank sum test or unpaired t test after data showing markedly high values were excluded. A P value of less than .05 was considered to indicate statistical significance in all analyses.

3. Results

No statistically significant difference in the plasma visfatin concentration was observed in the 80 diabetic patients compared with the 28 age-matched healthy subjects (2 [1.525, 2.5] vs 2.45 [1.7, 2.9] ng/mL, P = .0680). Markedly elevated visfatin levels were found in 2 diabetic patients (38.0 and 179.0 ng/mL) and 3 healthy subjects (64.3, 112.0, and 200 ng/mL); all others had visfatin levels less than 5.0 pg/mL. When these data were excluded, the mean values for diabetic patients and for healthy subjects were 2.0 ± 1.0 and 2.0 ± 0.9 pg/mL, respectively (P = .8981). No sex-related difference in visfatin concentration was observed (2 [1.65, 2.4] ng/mL for men vs 2 [1.3, 3] ng/mL for women, P = .4862); however, in multiple regression analysis, sex had a weak but significant association with log visfatin. No significant difference in visfatin was obtained between patients who had and had not received antihypertensive drugs (2 [1.6, 3] vs 1.9 [1.4, 2.3] ng/mL, P = .2077) or between smokers and nonsmokers (1.9 [1.4, 2.6] vs 2 [1.8, 2.35] ng/mL, P = .3484]. In the diabetic patients, correlations between log visfatin and several variables were examined in simple regression analysis, and stepwise regression analysis was performed with log visfatin as a dependent variable. Log visfatin showed a significant positive correlation with log UAE and a negative correlation with Ccr, log aldosterone, and FMD. These results are summarized in Table 2, and correlations of log visfatin with FMD, Ccr, and log aldosterone are shown in Fig. 1A-C. Because marked elevation of visfatin was observed in 2 patients, the association between visfatin and variables that showed significant correlations with log visfatin was reanalyzed in the other 78 patients. Significant negative correlations of log visfatin were found with Ccr and log aldosterone (R = −0.2943, P = .0089; R = −0.2942, P = .0089, respectively), and a tendency for a correlation was found between log visfatin and FMD (R = −0.2144, P = .0593). However, the significant correlation between log visfatin and log UAE vanished in the new analysis (R = −0.1779, P = .1190).

Changes in variables after pioglitazone (n = 20) and insulin (n = 7) therapy for 12 weeks are shown in Table 3, and changes in visfatin in the entire pioglitazone-treated group and in male and female patients who received pioglitazone therapy are presented in Fig. 2A-C. In the 20 diabetic patients treated with pioglitazone, no significant correlation was observed between log visfatin and FPG or HbA_{1c} at baseline. However, the ratio of the visfatin concentration (the value at the second time point divided by the value at the first time

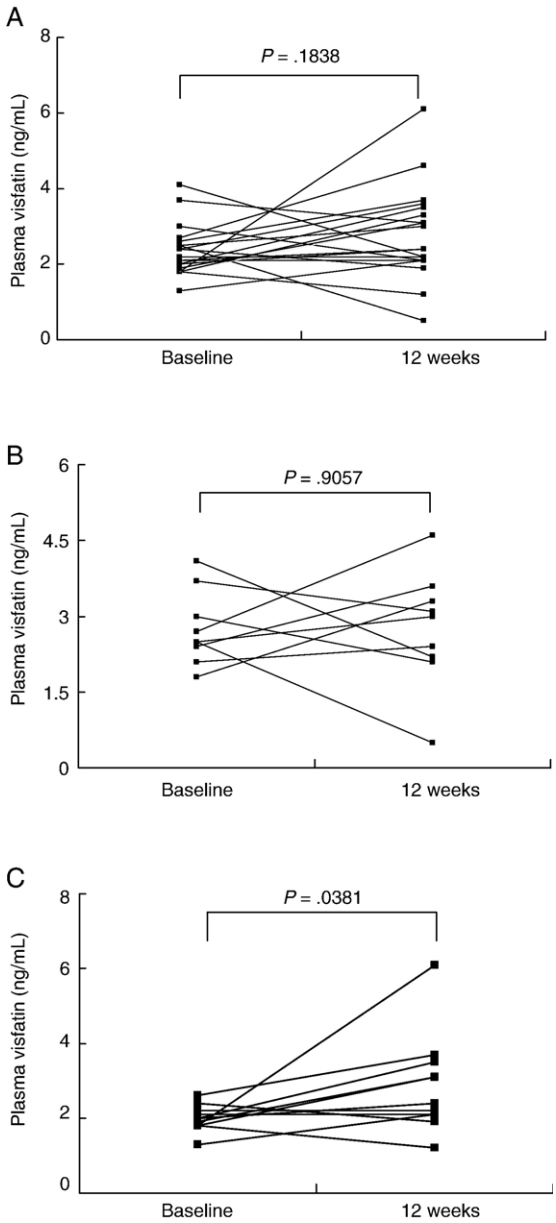

Fig. 2. Changes in plasma visfatin concentration levels by 12-week pioglitazone (30 mg/d) therapy in the 20 patients: total (A), 9 men (B), and 11 women (C).

Table 3
Changes in various variables at baseline and 12 weeks after pioglitazone or insulin therapy

	Pioglitazone (30 mg/d), n = 20, 9 men, 11 women			Insulin (initial, BW × 0.2 U), n = 7, 3 men, 4 women		
	Baseline	12 wk	*P*	Baseline	12 wk	*P*
Visfatin (ng/mL)	2.125 (1.825, 2.575)	2.7 (2.1, 3.45)	.1838	2 (0.1, 2.3)	2 (1.7, 2.9)	.3105
Men	2.5 (2.25, 3.35)	3 (2.15, 3.45)	.9057	1.8 (0.1, 2.3)	2 (1.2, 4.6)	.2851
Women	2 (1.8, 2.2)	2.4 (2.1, 3.5)	.0381*	2.15 (0.575, 3.275)	2.15 (1.7, 2.825)	.7150
FPG (mg/dL)	191.5 ± 52.1	147.9 ± 33.4	.0073*	186.9 ± 24.1	124.4 ± 52.7	.0495*
Men	177.2 ± 28.7	167.2 ± 33.9	.3571	197.3 ± 6.5	82.3 ± 25.4	.0146*
Women	203.2 ± 64.5	132 ± 24.3	.0093*	179 ± 30.6	156 ± 45.1	.5111
HbA_{1c} (%)	8.9 ± 1.6	7.6 ± 1.4	.0091*	10.3 ± 1.3	8.2 ± 1.6	.0317*
Men	8.7 ± 1.0	8.5 ± 1.6	.4872	10.8 ± 1.7	6.9 ± 1.2	.0774
Women	9.1 ± 2.0	6.9 ± 0.9	.0089*	9.9 ± 1.0	9.1 ± 1.2	.1119
TC (mg/dL)	201.3 ± 45.2	207.3 ± 39.4	.4580	175.1 ± 27.9	183.3 ± 39.9	.4416
HDL-C (mg/dL)	45.3 ± 9.7	56.3 ± 15.8	<.0001*	52.2 ± 23.7	60.3 ± 17.7	.1428
LDL-C (mg/dL)	119.8 ± 41.0	128.2 ± 34.8	.3536	94.3 ± 20.4	98 ± 31.6	.4250
TG (mg/dL)	198.8 ± 143.6	115.5 ± 68.7	.0003*	120.7 ± 76.8	83.3 ± 40.8	.0849
SBP (mm Hg)	126.5 ± 10.2	128.4 ± 11.9	.2693	135 ± 3.4	128.3 ± 13.2	.2278
DBP (mm Hg)	71.7 ± 6.4	71.5 ± 8.1	.8506	71.7 ± 12.1	69.7 ± 5.2	.5549
BW (kg)	67.2 ± 15.7	68.5 ± 15.9	.0218*	56.2 ± 18.9	56.8 ± 19.0	.6611
IRI (μU/mL)	23.3 ± 28.4	11.2 ± 6.1	.0483*	–	–	–
HOMA-R	10.7 ± 11.8	3.8 ± 1.6	.0106*	–	–	–

Data are expressed as mean ± SD, except for visfatin, which are expressed as medians with interquartile ranges (25th and 75th percentiles). IRI indicates immunoreactive insulin; HOMA-R, homeostasis model assessment ratio.

* $P < .05$, statistically significant.

point) was significantly negatively correlated with the ratio of FPG and HbA_{1c} ($R = -0.476$, $P = .0376$; $R = -0.4606$, $P = .0409$, respectively). Furthermore, in the 11 female patients the visfatin ratio showed a significant correlation with the FPG ratio ($R = -0.7020$, $P = .0160$) and a tendency for a correlation with the HbA_{1c} ratio ($R = -0.5220$, $P = .0995$); in contrast, no correlation was obtained in the 9 male patients. In all 20 patients, no correlations were found between the visfatin ratio and ratios calculated for body weight (BW), TC, TG, LDL-C, HDL-C, SBP, DBP, insulin, and the homeostasis model assessment ratio (data not shown).

4. Discussion

In the current study, we first investigated the association between plasma visfatin and vascular endothelial function evaluated by FMD. As expected, we found a significant negative correlation between log visfatin and FMD, suggesting that visfatin may be related to impaired endothelial function. However, it has also been reported that adiponectin, an insulin-sensitive adipocytokine, is positively associated with FMD [25]; this result is supported by the association of adiponectin with plasma metabolic levels of endothelial nitric acid (NO) synthetase (eNOS) or eNOS mRNA expression, and with plasma or mRNA levels of prostaglandin I_2 synthetase [26], and FMD is known to be NO-dependent [27]. Therefore, if visfatin is really related to an insulin-resistant state, in contrast to adiponectin, it might be possible that visfatin affects FMD via an inhibitory effect on NO; however, whether visfatin is associated with insulin resistance or obesity is still controversial [1,2,28,29]. These suggestions are still tentative and further analysis of the link between visfatin and FMD is required.

The plasma visfatin concentration was found to be negatively associated with Ccr and positively associated with UAE, although the correlation between visfatin and log UAE vanished when patients with markedly elevated visfatin (n = 2) were excluded. Our data suggest that visfatin levels are influenced by renal function. We speculate that the elevated visfatin level is due to a decrease in clearance, but whether elevated visfatin can itself influence the progression of renal impairment is unknown. We also investigated the association between visfatin and urinary 8-iso-$PGF_{2\alpha}$ (a marker of oxidative stress). A recent study showed that oxidative stress influences other adipocytokines, such as adiponectin, tumor necrosis factor α, and plasminogen activator inhibitor 1 [30]. However, in the current study, no correlation between visfatin and urinary 8-iso-$PGF_{2\alpha}$ was obtained. Accordingly, our result may suggest that, unlike other adipocytokines, the level of visfatin is not affected by oxidative stress, at least not to an extent that is clinically significant. Furthermore, we explored the association of visfatin with inflammatory markers, such as hsCRP and fibrinogen, and with atherosclerosis, as evaluated by IMT. However, no correlation was observed between log visfatin and these parameters, showing that visfatin is not a surrogate marker for inflammation or atherosclerosis.

We investigated the association between visfatin and adiponectin, but failed to find a significant association, in contrast to a previous report demonstrating a significant negative correlation [2]. However, in the report, multiple regression analysis with visfatin as the dependent variable did not show any significant association with adiponectin, and we note that no correlation between the levels of visfatin and adiponectin was found in another very recent study [31]. Therefore, we speculate that the reported association between

adiponectin and visfatin may not be robust. We also investigated the association between visfatin and aldosterone and found a significant negative association, and not a positive association. It is difficult to explain this result, as aldosterone has recently been shown to be related to insulin resistance [19], and, therefore, additional study is needed to confirm the validity of our result.

We found no significant difference in plasma visfatin levels between type 2 diabetic patients and healthy subjects. This result differed from that of the previous report, in which diabetic patients had higher plasma visfatin concentration [2,28]. However, we note that the sample sizes for diabetic patients and especially for healthy subjects were small, and the number of subjects in these groups was unbalanced. Furthermore, visfatin showed marked elevation in 2 diabetic patients and in 3 healthy subjects; although the exact reason for this is unclear, visfatin is known to be secreted from organs such as bone marrow, liver and muscle [32], and we speculate that visfatin produced in these organs might have influenced the high plasma levels. Considering the skewed distribution of the visfatin levels, an additional analysis using larger samples with a balanced design would be of importance to examine the difference in visfatin levels between diabetic patients and healthy subjects. Moreover, repeated measurement of visfatin levels in the patients with marked elevation of visfatin would have been useful to determine if the elevation was transient or continuous. We emphasize that these are major limitations of the study.

The effect of pioglitazone on the visfatin level was also examined in patients with type 2 diabetes mellitus. Expression of mRNAs for visfatin and adiponectin in visceral fat deposits in rat has been shown to increase after treatment with rosiglitazone (a thiazolidinedione) [20]; however, in contrast it was very recently shown that administration of pioglitazone for 4 weeks in 7 patients with type 2 diabetes mellitus did not affect expression of visfatin mRNA in adipose tissue or isolated adipocytes, or change the serum visfatin concentration [21]. However, pioglitazone generally requires a treatment course of more than 3 months to exert a full effect on glycemic control [33]; therefore, in the current study we studied the effect of pioglitazone therapy for 12 weeks. Contrary to our expectations, the 12-week course of pioglitazone did not significantly affect the plasma visfatin concentrations in the 20 diabetic patients, although a significant increase was observed in female patients receiving pioglitazone. A lack of change in the visfatin level was also seen in a parallel insulin-treatment group. The reason for the sex-related difference in the effect of pioglitazone on visfatin is unclear. However, pioglitazone is reported to be more effective for glycemic control in women, although the detailed mechanism is still unknown [34]. In the current study, pioglitazone was more effective for glycemic control and had more beneficial effects on HDL-C and TG in women (data not shown). Therefore, it may be possible that pioglitazone has sex-specific effects on visfatin levels, as well as on circulating glucose and lipids levels. Interestingly, the changes in visfatin and FPG in women (but not in men) were very closely correlated. Accordingly, the sex-related effect of pioglitazone on glucose levels may at least in part be associated with an effect of this compound on visfatin. However, it is also important to note that the effect of pioglitazone was relatively weak, even in female patients, compared with the effects of pioglitazone on other adipocytokines such as adiponectin [35,36]. Furthermore, the numbers of patients receiving pioglitazone and insulin therapy were small and unbalanced; it would have been preferable to investigate the effect of pioglitazone on visfatin with a larger number of patients and a balanced design.

As a final limitation of the study, we note that BMI was used to evaluate obesity, but we did not measure the waist-hip ratio or the percent body fat. Because the waist-hip ratio is closely associated with parameters related to insulin resistance [37] and visceral fat is more strongly associated with insulin resistance than subcutaneous fat [38], measurement of the waist-hip ratio and percent body fat may have been preferable to measurement of BMI.

In conclusion, we found a significant negative correlation between log plasma visfatin and FMD, Ccr, and plasma aldosterone, and a significant positive correlation between log visfatin and log UAE in patients with type 2 diabetes mellitus. Pioglitazone therapy for 12 weeks did not significantly affect the plasma visfatin concentration in patients with type 2 diabetes mellitus, although significant elevation of visfatin was observed in a female subgroup of these patients. More detailed analysis is required regarding the interpretation of these results.

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