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Original Article

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Vascular Endothelial Function Is Associated with eGFR Slope in Female and Non-Smoking Male Individuals with Cardiovascular Risk Factors: A Pilot Study on the Predictive Value of FMD for Renal Prognosis

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Aims: It is known that there are sex differences in vascular endothelial function and the development of chronic kidney diseases; however, it remains unclear whether sex differences influence the association between vascular endothelial function and renal prognosis.

Methods: To clarify the relationship between vascular endothelial function and longitudinal eGFR changes in male and female patients with cardiovascular risk factors, we retrospectively evaluated 341 patients (176 males and 165 females) with cardiovascular risk factors in whom vascular function was assessed by flow-mediated dilation (FMD) and brachial-ankle pulse wave velocity (baPWV) and in whom 24-month longitudinal eGFR values were recorded after the vascular function examinations. Associations of values of FMD and baPWV with values of eGFR slope were statistically analyzed.

Results: Simple regression analysis showed that the value of FMD was positively associated with eGFR slope in females (p=0.001) and non-smoking males (p=0.033) but not in smoking males. Multiple regression analysis showed that the value of FMD remains a positive contributor for eGFR slope in females (p=0.001) and non-smoking males (p=0.045) but not in smoking males. In contrast, values of baPWV had no significant association with eGFR slope regardless of sex and cigarette smoking.

Conclusions: In individuals with cardiovascular risk factors, evaluation of vascular endothelial function enables prediction of renal prognosis in females and non-smoking males.

Key words: Sex difference, FMD, baPWV, eGFR slope, Cigarette smoking

Introduction

Since chronic kidney disease (CKD) is not only a risk for the development of end-stage renal disease but also a strong risk factor for the development of cardiovascular disease (CVD), even in cases of mild renal dysfunction and albuminuria[1)], much attention has been given to the concept of cardiorenal syndrome (CRS)[2, 3)].

It is well known that vascular endothelial damage is not only involved in the progression of CVD but is also an aggravating factor for the progression of albuminuria and renal damage[4)]. Endothelial cells respond to blood flow-induced increases in vascular

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wall shear stress by increasing vasodilatory autocoid synthesis, leading to vascular smooth muscle relaxation [flow-mediated dilation (FMD)][5]. Endothelial dysfunction, as indicated by reduced brachial artery FMD, is considered to be a key early disorder in the development of atherosclerosis[6] and it is well known that there is a gender axdifference in the value of FMD[7, 8].

Although previous studies have revealed an association between estimated glomerular filtration rate (eGFR) and endothelial function[9-13], it has not been determined whether FMD can predict future eGFR changes in individuals with cardiovascular risk factors regardless of sex.

Aim

To clarify the influence of sex differences on the predictive value of FMD for prognosis of renal function, we retrospectively analyzed the correlations between clinical parameters including FMD value and eGFR slope in individuals with lifestyle-related diseases including hypertension, dyslipidemia, diabetes and obesity.

Materials

Study Design, Subjects and Ethics Statement

We retrospectively analyzed 341 Japanese adult individuals (176 males and 165 females) who were inpatients and outpatients for treatment of cardiovascular risk factors including hypertension, dyslipidemia, diabetes and obesity and who underwent two examinations of vascular function including FMD measurement and brachial-ankle pulse wave velocity (baPWV) measurement within 16 weeks prior to or after FMD examination and in whom eGFR was measured at a minimum of 8 time points including baseline with 8-12-week intervals during a period of 24 months after the vascular function evaluation in the Department of Endocrinology and Metabolism at Tokushima University Hospital and Anan Medical Center, Tokushima, Japan between April 2015 and March 2020 **(Supplemental Fig. 1)**. All of the subjects underwent a standardized interview and a physical examination in the baseline period.

Current smokers were defined as subjects who had habitually smoked within the past 2 years in the baseline examination period. Body mass index was calculated as an index of obesity. Blood pressure was measured twice and averaged. Pulse pressure (PP) was determined by the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP). Hypertensive patients were defined as those with SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or those receiving antihypertensive agents. Patients with dyslipidemia were defined as those with low-density lipoprotein cholesterol (LDL-C) ≥ 140 mg/dl or triglyceride (TG) level ≥ 150 mg/dl or high-density lipoprotein cholesterol (HDL-C) less than 40 mg/dl or those receiving lipid-lowering agents. Diabetic patients were defined as individuals who were receiving hypoglycemic agents or individuals with glycosylated hemoglobin A1c (HbA1c) ≥ 6.5%. Exclusion criteria included patients with serum creatinine (Cr) above 2.0 mg / dl at entry, patients undergoing hemodialysis, patients with serious infections, patients with known malignancy, and patients who had undergone surgery under the control of anesthesia.

Our retrospective observational study followed the institutional guidelines of each hospital (Tokushima University Hospital and Anan Medical Center) regarding human experimentation and was in accordance with the Helsinki Declaration and it was approved by each hospital's Institutional Review Board.

Measurements of FMD and baPWV

Subjects fasted the previous night and abstained from consuming alcohol, cigarette smoking and consuming caffeine on the day of the examination. The subjects rested for at least 15 min in the supine position before FMD measurements. Using a 10-MHz linear array transducer probe positioned with a hybrid probe holder, a longitudinal image of the right brachial artery was recorded at baseline and then a forearm-cuff was inflated for 5 min at 50 mmHg above systolic blood pressure just prior to FMD measurement[8, 14]. After cuff deflation, the diastolic diameter of the brachial artery was semi-automatically recorded for 2.0 min using an instrument equipped with software for monitoring brachial artery diameter (UNEXEF 18VG; UNEX Co. Ltd., Nagoya, Japan). Then FMD was estimated as the percent change in the vessel diameter over the baseline value at maximal dilation during reactive hyperemia.

baPWV was measured after a 15-min rest period

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in the supine position in an air-conditioned room using a vascular testing device (Form PWV/ABI, Omron Healthcare, Kyoto, Japan). The four cuffs of this volume-plethysmographic device fitted with oscillometric sensors were wrapped around the upper arms and ankles and then inflated automatically and simultaneously. Patients with an ankle-brachial index less than 0.9 were excluded. Means of the right and left baPWV values were used for analysis. The validity and reproducibility of measurements using this device were assessed in previous studies[8, 14, 15].

Urinalysis

Qualitative analysis of spot urine protein was performed by a urine test (Uropaper α III®, EIKEN Chemical Co, LTD. Tokyo Japan) and each result of proteinuria was allocated to an ordinal variable by the following scale: (-)=0, (±)=0.5, (1+)=1.0, (2+)=2.0, (3+)=3.0.

Biochemical Analyses

Blood and single spot urine samples were collected from each patient and used for determining blood cell counts, fasting plasma glucose (FPG), HbA1c, and serum biochemical parameters including LDL-C, TG, HDL-C, uric acid (UA) and serum creatinine (Cr). FPG and serum levels of LDL-C, TG, HDL-C, UA and Cr were measured by enzymatic methods. HbA1c was assayed by performing latex agglutination assays.

Determination eGFR Slope

eGFR was calculated according to the following formula from the Japanese Society of Nephrology: $eGFR\ (mL/min/1.73m^2) = 194 \times Cr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ if female)[22]. Values of eGFR during a period of 24 months after the baseline FMD measurement were selected at 8 time points including baseline with 8-12-week intervals between eGFR examinations, and eGFR slope (expressed in mL/min/1.73 m^2/year) was determined by linear regression analysis[16-18].

Comparison of Actual eGFR Values and Calculated eGFR Values based on the eGFR Slope

Since the accuracy of a linear regression model is greatly affected by the values of outliers and since muscle mass in patients with end-stage renal disease decreases due to accelerated catabolism and malnutrition, which may result in a smaller annual decline in the eGFR slope, the validity of the eGFR slope in this study is a critical issue for evaluating the predictive value of FMD for renal prognosis. Therefore, to assess the accuracy of the eGFR slope based on eGFR data obtained during a period of 24 months, we investigated the associations between actual eGFR values and calculated eGFR values based on the eGFR slope at 36, 48 and 60 months after the baseline examination in individuals who had eGFR records at those time points (36M, 48M and 60M).

Statistical Analysis

Continuous variables were averaged and expressed as means ± standard deviation (SD). Categorical variables were compared by performing the χ^2 test or Fisher's exact test. For comparisons between two groups, we performed the Mann-Whitney U test or Student's t-test for numeric variables depending on the variables' distribution. The degrees of associations between eGFR slope calculated by using the following 24-month longitudinal eGFR values and variables including sex, age, BMI, SBP, PP, serum lipid parameters, UA, Cr, FPG, HbA1c, history of current smoking, hypertension, diabetes mellitus, and dyslipidemia were measured by means of simple linear regression analysis. To compare the trends of the association between FMD and eGFR slope in the male and female subjects, analysis of covariance (ANCOVA) was performed. In addition, we performed multiple linear regression analysis with the significant variables determined by the simple linear regression analysis. Since it was considered that relatively high percentage of male current smokers would have an influence on the results, we conducted those analyses in total subjects, male subjects, male subjects with or without a current smoking habit and female subjects. These analyses were performed using Microsoft Office Excel 2019 (Microsoft, Richmond, CA), GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) and EZR (Saitama Medical Center, Jichi Medical University, Japan). The threshold for statistical significance was set at $p < 0.05$.

Results

Baseline Characteristics of Total Subjects, Male Subjects and Female Subjects

The physical and laboratory-determined characteristics of subjects enrolled in this study are shown in **Table 1**. Males were older than females. There was no significant difference in BMI, SBP, FPG, HbA1c, eGFR slope or severity of proteinuria between males and females. On average, the females enrolled in this study had greater PP and higher serum levels of HDL-C, LDL-C and eGFR than did the males. The male patients showed higher serum levels of TG, UA and Cr than those in the female patients. The percentage of male patients who were current smokers was significantly higher than that of female

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Table 1. Baseline Clinical Characteristics of Total subjects, Male Subjects and Female Subjects in This Study

	Total (n=341)	Male (n=176)	Female (n=165)	p value (M vs F)
Cardiovascular Risk Factors				
Age (years)	63.9 ± 12.0	65.4 ± 11.4	62.3 ± 12.5	**0.018**
Body Mass Index: BMI (kg/m^2)	25.2 ± 5.1	25.2 ± 5.0	25.3 ± 5.2	0.796
Systolic Blood Pressure: SBP (mmHg)	131.5 ± 16.2	130.2 ± 13.5	132.8 ± 18.5	0.150
Pulse Pressure: PP (mmHg)	55.0 ± 11.3	53.2 ± 10.1	56.8 ± 12.2	**0.003**
Triglyceride: TG (mg/dL)	123.6 ± 74.2	129.6 ± 83.4	117.2 ± 62.1	<**0.001**
High-density Lipoprotein Cholesterol: HDL-C (mg/dL)	59.4 ± 17.0	54.2 ± 14.3	64.9 ± 17.8	<**0.001**
Low-density Lipoprotein Cholesterol: LDL-C (mg/dL)	104.1 ± 32.4	98.2 ± 30.7	110.4 ± 32.9	**0.001**
Uric Acid: UA (mg/dL)	5.3 ± 1.2	5.7 ± 1.2	4.9 ± 1.2	<**0.001**
Fasting Plasma Glucose: FPG (mg/dL)	120.6 ± 36.5	121.8 ± 32.4	119.3 ± 40.4	0.544
Hemoglobin A1c: HbA1c (%)	6.4 ± 1.0	6.4 ± 0.8	6.5 ± 1.1	0.392
Kidney Function Parameters				
Creatinine: Cr (mg/dL)	0.80 ± 0.28	0.93 ± 0.27	0.67 ± 0.21	<**0.001**
estimated Glomerular Filtration Rate: eGFR (ml/min/1.73m^2)	71.8 ± 21.9	68.9 ± 18.9	74.9 ± 24.3	**0.012**
eGFR Slope (mL/min/1.73m^2/year)	-1.07 ± 3.85	-1.33 ± 3.69	-0.81 ± 4.01	0.216
Proteinuria Score ((-):0, (±):0.5, (1+):1.0, (2+):2.0, (3+):3.0)	0.22 ± 0.51	0.36 ± 0.20	0.19 ± 0.51	0.226
Smoking Status and Comorbidities				
Current Smoker (n, (%))	80 (23)	70 (40)	10 (6)	<**0.001**
Hypertension (n, (%))	236 (69)	126 (72)	110 (66)	0.325
Dyslipidemia (n, (%))	238 (70)	118 (67)	120 (72)	0.254
Diabetes Mellitus (n, (%))	218 (64)	120 (68)	98 (59)	0.092
Vascular Examinations				
Flow-Mediated Dilation: FMD (%)	4.91 ± 2.83	4.52 ± 2.35	5.31 ± 3.22	**0.011**
brachial-ankle pulse wave velocity: baPWV (cm/sec)	1661.6 ± 373.3	1693.0 ± 364.9	1628.1 ± 379.2	0.110
Medications Used				
Angiotensin II Receptor Blocker:	174 (51)	93 (53)	81 (49)	0.489
ARB or Angiotensin-Converting Enzyme Inhibitor: ACEi (n, (%))				
Calcium Channel Blocker: CCB (n, (%))	142 (42)	77 (44)	65 (39)	0.415
β Blocker (n, (%))	39 (11)	22 (13)	17 (10)	0.524
Mineralocorticoid Receptor Antagonist: MR Antagonist (n, (%))	14 (4)	7 (4)	7 (4)	0.902
Statin (n, (%))	178 (52)	85 (48)	93 (56)	0.136
Ezetimibe (n, (%))	30 (9)	11 (6)	19 (11)	0.086
Other Lipid-lowering Drugs (n, (%))	40 (12)	25 (14)	15 (9)	0.816
Antiplatelets (n, (%))	64 (19)	42 (24)	22 (13)	**0.013**
Sulfonyl Urea: SU or Glinide (n, (%))	29 (9)	17 (10)	12 (7)	0.407
Dipeptidyl Peptidase-4 inhibitor: DPP-4i (n, (%))	140 (41)	76 (43)	64 (39)	0.410
Metformin (n, (%))	95 (28)	50 (28)	45 (27)	0.815
alpha-glucosidase inhibitor: αGI (n, (%))	55 (16)	35 (20)	20 (12)	0.051
Pioglitazone (n, (%))	14 (4)	10 (6)	4 (2)	0.130
Sodium Glucose Cotransporter 2 Inhibitor: SGLT2i (n, (%))	35 (10)	18 (10)	17 (10)	0.982
Insulin (n, (%))	47 (14)	25 (14)	22 (13)	0.816
Glucagon-like Peptide-1 Receptor Agonist: GLP-1RA (n, (%))	21 (6)	9 (5)	12 (7)	0.143

Values are presented as mean ± SD or n (%).

patients. There were significant differences in values of FMD but not in values of baPWV between males and females as shown in Table 1. A greater percentage of male patients were antiplatelet drug users.

Associations between Actual eGFR Values and Calculated eGFR Values based on the eGFR Slope

As shown in Supplemental Fig. 2, we found that there were close relationships between the actual and calculated eGFR values (R^2=0.8413, p<0.0001

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Table 2. Simple Linear Regression Analysis for Determinants of eGFR Slope in Total Subjects, Male Subjects and Female Subjects

	Total (n=341)		Male (n=176)		Female (n=165)	
	coefficient	p value	coefficient	p value	coefficient	p value
Cardiovascular Risk Factors						
Age	0.009	0.625	0.023	0.347	0.001	0.962
BMI	-0.057	0.164	-0.106	0.055	-0.009	0.879
SBP	-0.022	0.085	-0.025	0.236	-0.023	0.170
PP	-0.034	0.064	-0.023	0.398	-0.050	0.050
TG	0.000	0.999	0.000	0.921	0.001	0.768
HDL-C	0.000	0.978	0.017	0.382	-0.020	0.254
LDL-C	0.007	0.264	0.005	0.555	0.006	0.501
UA	0.222	0.195	0.464	0.053	0.174	0.525
FPG	-0.008	0.182	-0.018	**0.037**	0.000	0.978
HbA1c	-0.464	**0.031**	-0.773	**0.019**	-0.292	0.308
Kidney Function Parameters						
Cr	0.849	0.260	1.869	0.066	1.196	0.417
eGFR	-0.035	<**0.001**	-0.059	<**0.001**	-0.023	0.068
Proteinuria Score	-1.007	**0.014**	-0.835	0.128	-1.130	0.065
Smoking Status and Comorbidities						
Current Smoker	-0.143	0.772	0.099	0.864	0.299	0.820
Hypertension	-0.279	0.538	0.202	0.745	-0.691	0.299
Dyslipidemia	0.086	0.850	0.209	0.726	-0.136	0.848
Diabetes Mellitus	-0.259	0.553	-0.492	-0.489	0.415	0.931
Vascular Examinations						
FMD	0.241	**0.001**	0.102	0.393	0.307	**0.001**
baPWV	0.000	0.734	0.000	0.865	0.000	0.799
Medications Used						
ARB or ACEi	-0.466	0.266	0.159	0.776	-1.091	0.081
CCB	0.116	0.784	-0.025	0.965	0.321	0.618
β Blocker	0.101	0.877	0.478	0.572	-0.310	0.765
MR Antagonist	0.052	0.961	1.683	0.239	-1.602	0.124
Statin	-0.264	0.529	-1.060	0.263	-0.721	0.255
Ezetimibe	0.935	0.206	-0.063	0.960	1.332	0.175
Other Lipid-lowering Drugs	0.794	0.222	0.652	0.416	1.197	0.273
Antiplatelets	0.201	0.708	0.151	0.818	0.539	0.560
SU or Glinide	-0.352	0.639	-1.451	0.150	0.718	0.553
DPP-4i	-0.301	0.475	-0.511	0.365	-0.034	0.957
Metformin	-0.408	0.383	-0.003	0.997	-0.835	0.236
αGI	0.630	0.268	0.496	0.479	1.059	0.271
Pioglitazone	0.839	0.427	0.291	0.810	-0.470	0.842
SGLT2i	0.822	0.233	0.794	0.389	0.850	0.411
Insulin	0.272	0.655	-0.222	0.795	1.010	0.274
GLP-1RA	0.535	0.539	-0.132	0.917	0.962	0.426

(n=318) at 36M; R^2=0.7288, p<0.0001 (n=244) at 48M; R^2=0.6337, p<0.0001 (n=170) at 60M). Hence, we confirmed that the eGFR slope generated by data for eGFR obtained during a period of 24 months was a reliable clinical surrogate marker in this study.

Associations between FMD Values and eGFR Slope without Adjustment of Confounding Factors in Total Subjects, Male Subjects and Female Subjects

Firstly, we evaluated the statistical association of each variable with eGFR slope by simple linear regression analysis (Table 2). We found that HbA1c, eGFR, and proteinuria score were negatively correlated with eGFR slope and that FMD values were

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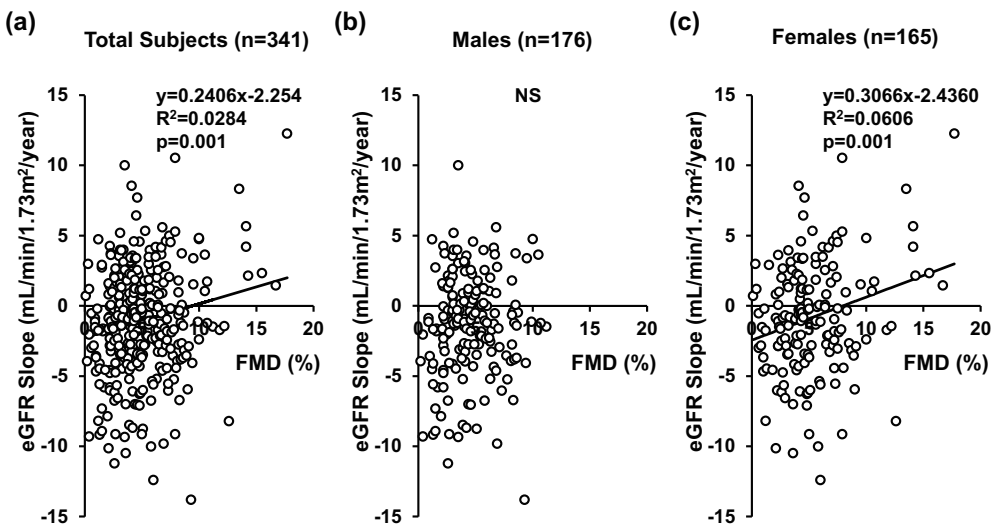

Fig. 1. Associations between FMD values and eGFR slope in total subjects, male subjects and female subjects with cardiovascular risk factors

(a) Scatterplot between FMD values and eGFR slope in total subjects
(b) Scatterplot between FMD values and eGFR slope in male subjects
(c) Scatterplot between FMD values and eGFR slope in female subjects

significantly and positively correlated with eGFR slope (**Table 2**, R^2=0.0284, p=0.001; **Fig. 1(a) and Supplemental Fig. 2(a)**). Because there was a gender difference in the values of FMD, we divided the subjects into a group of male subjects and a group of female subjects. There was a significant positive relationship between FMD values and eGFR slope in female subjects (**Table 2**, R^2=0.0606, p=0.001; **Fig. 1(c) and Supplemental Fig. 3(c)**) but not in male subjects **(Table 2, Fig. 1(b) and Supplemental Fig. 3(b))**. On the other hand, the value of baPWV was not associated with eGFR slope in patients with cardiovascular risk factors regardless of sex **(Table 2, Supplemental Fig. 4(a-c))**.

Baseline Characteristics and Associations between FMD Values and eGFR Slope in Male Subjects with and Those without a Current Smoking Habit

Since cigarette smoking has been shown to be an important risk factor for the development of CKD and endothelial dysfunction[19, 20], we divided the male subjects into a group of current smokers and a group of non-current smokers **(Table 3)**. There were no significant differences in age, BMI, SBP, PP, TG, LDL-C, HDL-C, HbA1c, Cr, baseline eGFR, proteinuria score, baPWV values and FMD values between the smoking and non-smoking male groups **(Table 3)**. In contrast, the value of UA and administration rate of a Ca-channel blocker (CCB) were significantly higher in smoking males than in non-smoking males **(Table 3)**. In this sub-analysis, we found that there was a significant positive association between the value of FMD and eGFR slope in non-smoking males (R^2=0.0336, p=0.033; **Fig. 2(b) and Supplemental Fig. 5(b)**) but not in smoking males **(Fig. 2(a) and Supplemental Fig. 5(a))**. Moreover, ANCOVA showed that there was no difference in the trends of associations between FMD and eGFR slope in non-current smoker males and females **(Supplemental Fig. 6)**.

Associations between FMD Values and eGFR Slope with Adjustment of Confounding Factors in Total Subjects, Total Male Subjects, Male Subjects with and Those without a Current Smoking Habit, and Female Subjects

In multiple regression analysis targeting the eGFR slope in total subjects, we found that baseline eGFR and proteinuria score were negatively and independently associated with eGFR slope (t value: -1.721, p<0.001 and t value: -4.402, p=0.004, respectively) **(Table 4)**. In contrast, an independent and positive association of FMD value with eGFR slope were observed (t value: 2.928, p<0.001) **(Table 4)**. In subgroup analysis including total male subjects, male subjects with and those without a current smoking habit, and female subjects, the variables identified in analysis of total subjects (HbA1c, baseline eGFR, proteinuria score and FMD) were used in each multiple regression analysis. As a result, FMD value was a common and independent protective factor against decline in eGFR slope in non-smoking males

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Table 3. Baseline Clinical Characteristics of Male Subjects with and Those without a Current Smoking Habit

Male Subjects	Current Smoker (n=70)	Non-Current Smoker (n=106)	p value
Cardiovascular Risk Factors			
Age (years)	64.9 ± 11.4	65.7 ± 11.3	0.744
BMI (kg/m^2)	24.4 ± 4.6	25.7 ± 5.2	0.098
SBP (mmHg)	128.3 ± 12.4	131.6 ± 14.0	0.110
PP (mmHg)	52.5 ± 10.2	53.7 ± 10.0	0.483
TG (mg/dL)	123.4 ± 73.7	133.7 ± 89.0	0.444
HDL-C (mg/dL)	53.1 ± 13.3	54.9 ± 14.9	0.411
LDL-C (mg/dL)	94.9 ± 27.9	100.4 ± 32.2	0.217
UA (mg/dL)	5.9 ± 1.3	5.5 ± 1.0	**0.048**
FPG (mg/dL)	122.9 ± 37.2	121.0 ± 28.8	0.681
HbA1c (%)	6.4 ± 0.9	6.4 ± 0.8	0.580
Kidney Function Parameters			
Cr (mg/dL)	0.92 ± 0.29	0.93 ± 0.26	0.819
eGFR (ml/min/1.73m^2)	69.6 ± 18.2	68.5 ± 19.3	0.745
eGFR Slope (mL/min/1.73m^2/year)	-1.28 ± 4.08	-1.36 ± 3.40	0.989
Proteinuria Score	0.31 ± 0.63	0.21 ± 0.40	0.254
Comorbidities			
Hypertension (n, (%))	54 (77)	72 (68)	0.184
Dyslipidemia (n, (%))	46 (66)	72 (68)	0.760
Diabetes Mellitus (n, (%))	50 (71)	70 (66)	0.452
Vascular Examinations			
FMD (%)	4.72 ± 2.23	4.39 ± 2.41	0.292
baPWV (cm/sec)	1643.6 ± 369.3	1725.6 ± 358.3	0.168
Medications Used			
ARB or ACEi (n, (%))	40 (57)	53 (50)	0.353
CCB (n, (%))	37 (53)	40 (38)	**0.048**
β Blocker (n, (%))	10 (14)	12 (11)	0.560
MR Antagonist (n, (%))	4 (6)	3 (3)	0.338
Statin (n, (%))	33 (47)	52 (49)	0.804
Ezetimibe (n, (%))	5 (7)	6 (6)	0.691
Other Lipid-lowering Drugs (n, (%))	12 (17)	13 (12)	0.364
Antiplatelets (n, (%))	22 (31)	20 (19)	0.056
SU or Glinide (n, (%))	6 (9)	11 (10)	0.691
DPP-4i (n, (%))	33 (47)	43 (41)	0.389
Metformin (n, (%))	25 (36)	25 (24)	0.081
αGI (n, (%))	18 (26)	17 (16)	0.115
Pioglitazone (n, (%))	4 (6)	6 (6)	0.988
SGLT2i (n, (%))	7 (10)	11 (10)	0.936
Insulin (n, (%))	7 (10)	18 (17)	0.194
GLP-1RA (n, (%))	3 (4)	6 (6)	0.685

and females (t value: 2.031, p=0.045 and t value: 3.393, p=0.001, respectively) (Table 4). However, there was no significant association between FMD value and eGFR slope in the total males or smoking males (Table 4). Values of variance inflation factor (VIF) indicated no multicollinearity problem in each multiple regression analysis (Table 4). Thus, the results obtained suggest that cigarette smoking directly or indirectly abolished the interaction between endothelial function of the brachial artery and renal prognosis in males with cardiovascular risk factors (Fig. 3).

Discussion

In this study, baseline FMD values were positively correlated with eGFR slope in female and non-smoking male patients with cardiovascular risk

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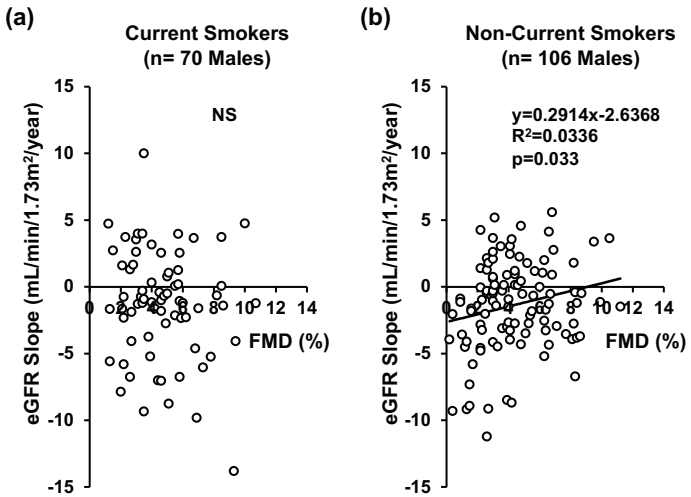

Fig. 2. Associations between FMD values and eGFR slope in smoking male subjects and non-smoking male subjects with cardiovascular risk factors

(a) Scatterplot between FMD values and eGFR slope in male subjects with a current smoking habit
(b) Scatterplot between FMD values and eGFR slope in male subjects without a current smoking habit

Table 4. Multiple Regression Analysis for Determinants of eGFR Slope in Total Subjects, Male Subjects, Male Subjects with and Those without a Current Smoking Habit, and Female Subjects

	Total (n=341)			Male									Female (n=165)		
				Total (n=176)			Current Smoker (n=70)			Non-Current Smoker (n=106)					
	t value	VIF	p value	t value	VIF	p value	t value	VIF	p value	t value	VIF	p value	t value	VIF	p value
HbA1c	-2.061	1.029	0.086	-1.949	1.024	0.053	-0.589	1.074	0.558	-1.867	1.018	0.065	-0.242	1.108	0.809
Baseline eGFR	-1.721	1.025	**<0.001**	-4.090	1.031	**<0.001**	-2.060	1.052	**0.043**	-3.890	1.022	**0.001**	-2.544	1.053	**0.012**
Proteinuria Score	-4.402	1.048	**0.004**	-1.849	1.014	0.066	-1.640	1.030	0.106	-1.089	1.019	0.279	-2.376	1.101	**0.019**
FMD	2.928	1.005	**0.001**	0.658	1.016	0.511	1.320	1.079	0.191	2.031	1.008	**0.045**	3.393	1.036	**0.001**

VIF: Variance Inflation Factor

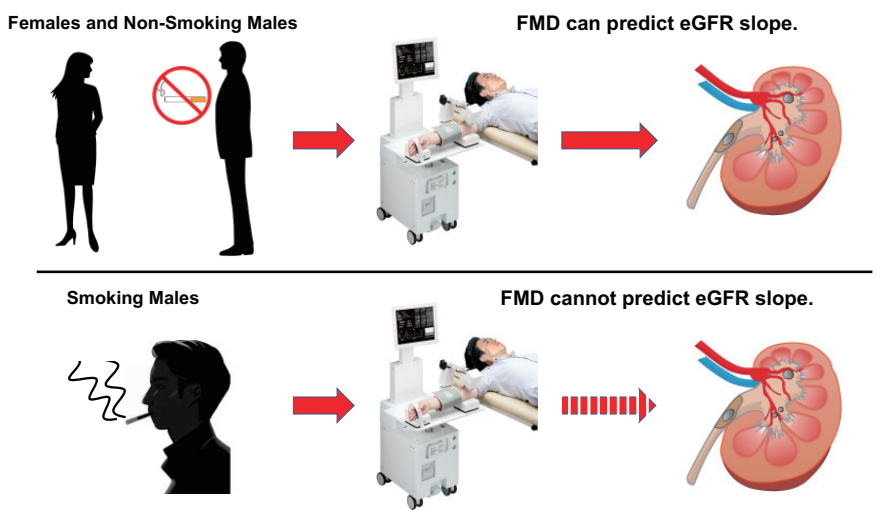

Fig. 3. Graphical Summary

We demonstrated that in individuals with cardiovascular risk factors, FMD can predict eGFR slope in females and non-smoking males but not in smoking males.

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factors.

Sex differences in endothelial function have a considerable clinical impact on vascular heath and overall risks of CVDs and CKD across the lifespan[21-23]. Because the increase in CVD risk coincides with menopause, it is well known that estrogens in females are cardiovascular protective hormones having the capability for preservation of endothelial function[23]. On the other hand, cardioprotective roles of androgens in males are very complicated. The only certain knowledge about androgens in the cardiovascular system is that both an excess and a lack of androgens are exacerbation factors for the development of CVDs. Aside from the pivotal roles of sex hormones and their receptors in the control of endothelial function in males and females, sex differences exist in other mechanisms that influence endothelial biology and function. As candidate mechanisms, it has been documented that there are sex-dependent differences in vascular responsiveness to endothelin-1 (ET-1) and the renin angiotensin system (RAS)[21]. Since cigarette smoking has been shown to affect plasma ET-1 concentration[24] and alter the homeostasis of the RAS by upregulating the detrimental angiotensin-converting enzyme/angiotensin II type 1 receptor axis[25], there is a possibility that the higher prevalence of cigarette smoking in males than in females exerted a sex difference in the association between FMD and eGFR slope.

Furthermore, Hashimoto *et al.* demonstrated that FMD decreases with an increase in the number of smoking pack-years[20] and cigarette smoking has been recognized as a crucial exacerbation factor for eGFR decline[26-28]. Although the detailed mechanism is largely unknown, the harmful effects of smoking have been presumed to be mediated by endothelial dysfunction with accumulation of advanced glycation end products that are present in cigarettes and by increased vascular permeability and insulin resistance[29]. Therefore, previous studies clearly demonstrated that smoking cessation or never smoking is pivotal for preventing CKD as well as CVD. However, in the present study, we could not find any significant difference in the eGFR slope or FMD value between male subjects with and those without a current smoking habit. A comparison of baseline characteristics of male subjects with and those without a current smoking habit showed that male subjects with a current smoking habit had a significantly higher frequency for CCB use and a tendency of higher frequency of antiplatelet agent use.

In addition to hypertension, visit-to-visit BP variability (BPV) has been shown to be associated with macro- and microvascular outcomes and organ damage[30, 31]. Jeffers and Zhou demonstrated that patients with declining renal function tended to have higher systolic BPV values than those in subjects with preserved renal function even after adjustment of confounding factors and they showed that amlodipine-based treatment efficiently reduced BPV compared to the effects of other antihypertensive drugs, regardless of eGFR levels[32]. In the present study, as mentioned above, a higher percentage of male current smokers used a CCB than did male non-current smokers.

A sub-analysis of the FMD-J study showed that endothelial dysfunction assessed by FMD was not associated with SBP levels in individuals receiving hypotensive drugs[33]. Therefore, we evaluated the influence of a CCB with other hypotensive drugs on endothelial function in the subjects of the present study. As shown in **Supplemental Table 1**, CCB showed an inverse relationship with FMD values as was found in our previous study[8]. Since it has been shown that baseline vascular diameter of the brachial artery was a major negative determinant of FMD value[34, 35], we speculate that CCB administration dilated the baseline vascular diameter of the brachial artery resulting FMD reduction. On the other hand, CCB administration reduced systemic SBP with visit-to-visit BPV resulting in preservation of renal function.

In addition, the mean total number of hypotensive drugs taken by male current smokers were significantly larger than that taken by male non-current smokers (male current smokers vs. male non-current smokers; 1.30 ± 1.00 drugs vs. 1.02 ± 0.90 drugs, $p=0.042$ (Mann–Whitney U test, one-tailed)). Taken together, the results of the present study suggest that intensive hypotensive pharmaceutical treatment, including CCB treatment disrupts the relationship between FMD value and eGFR slope in male current smokers.

Limitations

There are several limitations in the current study. First, this study was a retrospective observational study, and causation of the observed relationships therefore cannot be confirmed. Second, the number of subjects with lifestyle-related diseases who were enrolled in this study was too small to clarify the detailed mechanisms underlying the sex-associated and cigarette smoking-associated phenotypic differences. Third, data for smoking status did not include data for pack-years, and there is therefore a possibility that smoking status was underestimated or

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overestimated. Fourth, because of the small number of current smokers in female subjects, it is unclear whether there is a gender difference in the influence of smoking status on the relationship between baseline FMD value and eGFR slope. Fifth, other critical comorbidities such as coronary artery disease, stroke and peripheral arterial diseases were not noted and not analyzed in this study. Sixth, the results obtained in this study cannot be extended to the general population because we studied only patients with cardiovascular risk factors. Seventh, in the present study population, logistic regression analysis for the determinants of rapid decline in eGFR (equal to or less than -5ml/min/1.73m^2/year) did not show any statistically significant contribution of the FMD value in total subjects, non-smoking males and females (data not shown). Although the sample size in the present study was sufficient for showing associations between continuous variables, including FMD value and eGFR slope, we think that the sample size was too small for identifying the rapid eGFR decliners in the logistic regression analysis. Eighth, it has remained unclear whether interventions for amelioration of FMD improve renal prognosis in clinical practice. Therefore, a large-scale investigation is required to assess and clarify the sex-dependent prognostic value of FMD in renal prognosis in subjects with cardiovascular risk factors.

Conclusion and Perspectives

In individuals with cardiovascular risk factors, FMD value was found to be positively associated with eGFR slope in females and non-smoking males, and the results of the present study suggested that vascular endothelial function is a more accurate prognostic factor than proteinuria for the development of CKD in female and non-smoking patients with lifestyle-related diseases. Therefore, in individuals with a low FMD value, early comprehensive intervention for improving vascular endothelial function should be performed to protect renal function.

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Conflict of Interest

The authors declare no competing interest.

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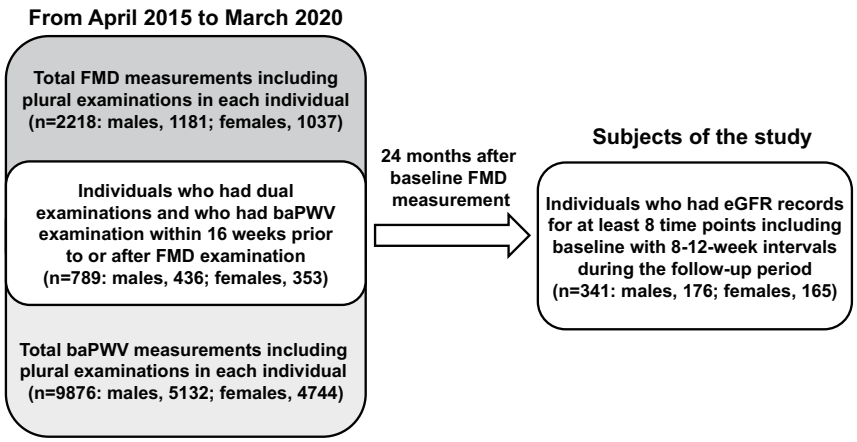
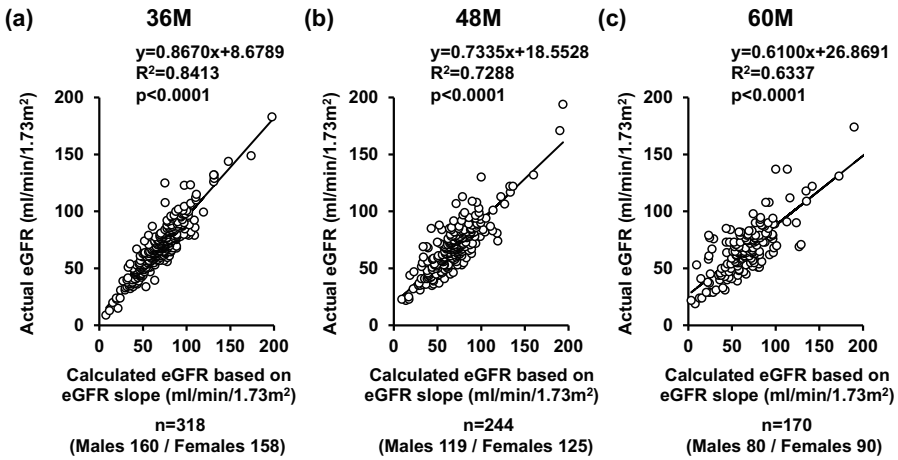
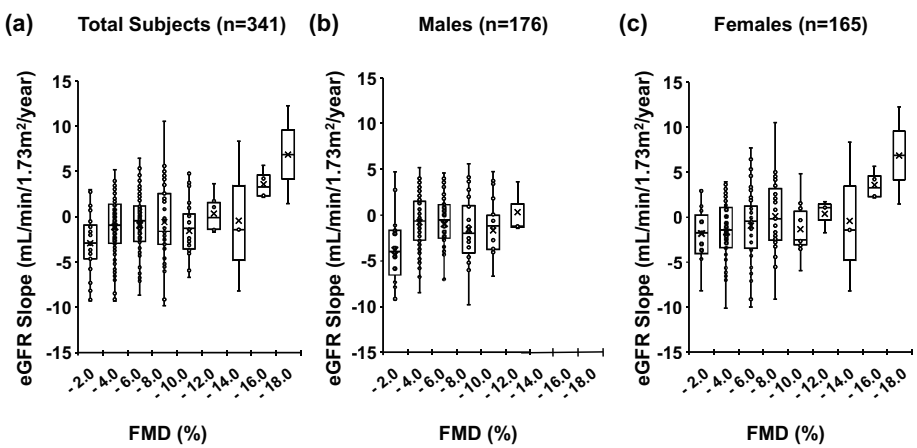


Supplemental Fig. 1. Diagram showing the selection of study subjects



Supplemental Fig. 2. Associations between actual eGFR values and calculated eGFR values based on the eGFR slope

Scatterplots between actual eGFR values and calculated eGFR values based on the eGFR slope at (a) 36 months, (b) 48 months and (c) 60 months after the baseline examination.



Supplemental Fig. 3. Associations between FMD values and eGFR slope in total subjects, male subjects and female subjects with cardiovascular risk factors

(a) Boxplot between FMD values and eGFR slope in total subjects
(b) Boxplot between FMD values and eGFR slope in male subjects
(c) Boxplot between FMD values and eGFR slope in female subjects

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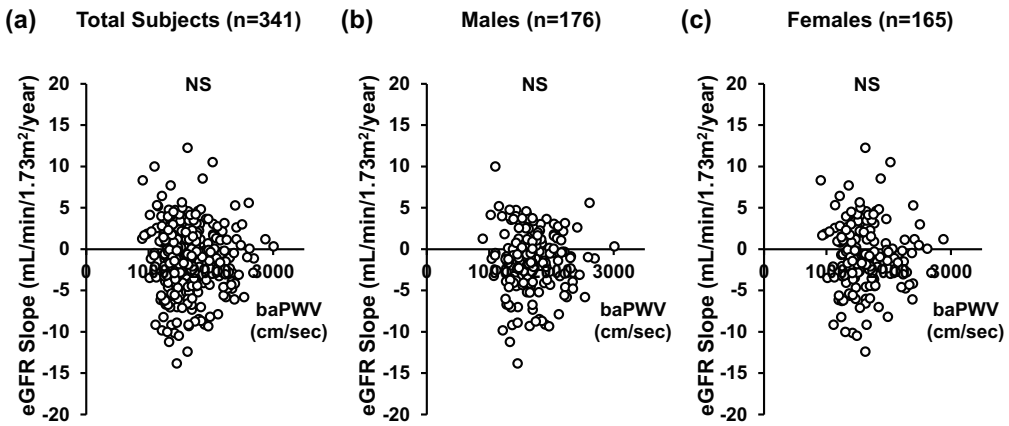
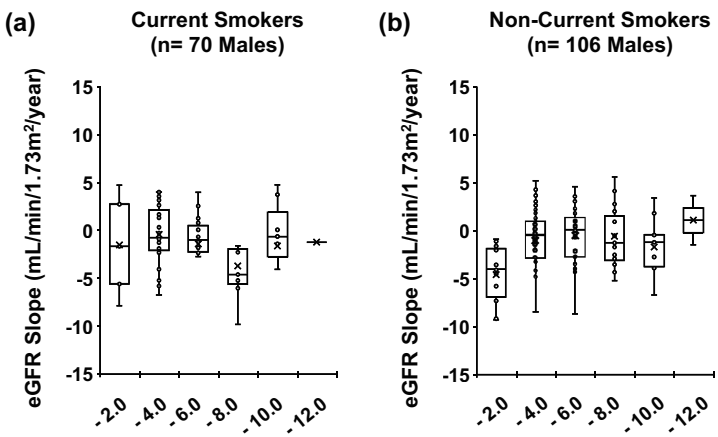


Supplemental Fig. 4. Associations between baPWV values and eGFR slope in total subjects, male subjects and female subjects with cardiovascular risk factors

(a) Scatterplot between baPWV values and eGFR slope in total subjects
(b) Scatterplot between baPWV values and eGFR slope in male subjects
(c) Scatterplot between baPWV values and eGFR slope in female subjects



Supplemental Fig. 5. Associations between FMD values and eGFR slope in smoking male subjects and non-smoking male subjects with cardiovascular risk factors

(a) Boxplot between FMD values and eGFR slope in male subjects with a current smoking habit
(b) Boxplot between FMD values and eGFR slope in male subjects without a current smoking habit

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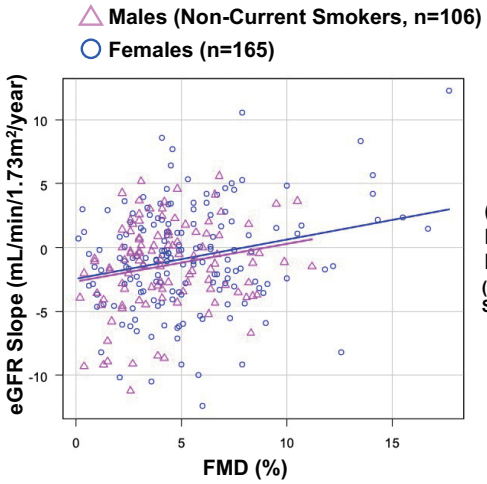

	Estimate	Std. Error	t value	p value
(Intercept)	-2.4141	0.4988	-4.840	<0.001
FMD	0.3026	0.0767	3.947	<0.001
Male (Non-Current Smokers)	-0.2717	0.4656	-0.584	0.560

Supplemental Fig. 6. ANCOVA analysis to compare the association trend of FMD value and eGFR slope between non-smoking males and females

Supplemental Table 1. Multiple Regression Analysis of Associated Medications for Determinants of FMD

Variables	t value	VIF	*p* value
Male	-1.971	1.083	0.049
Age	-5.050	1.215	<0.001
ARB/ACEi	0.686	1.336	0.493
CCB	-2.288	1.298	0.023
β blocker	-0.123	1.133	0.902
MR antagonist	0.170	1.133	0.865
Statin	-0.089	1.172	0.929
Ezetimibe	-0.264	1.068	0.792
Other lipid-lowering drugs	1.573	1.138	0.117
Antiplatelets	0.410	1.326	0.682
SU or Glinide	-0.765	1.132	0.445
DPP-4i	-1.341	1.506	0.181
Metformin	0.179	1.612	0.858
αGI	-0.883	1.203	0.378
Pioglitazone	0.231	1.078	0.818
SGLT2i	0.342	1.276	0.733
Insulin	1.421	1.029	0.156
GLP-1RA	-1.006	1.395	0.315

VIF: Variance Inflation Factor

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