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Endothelial dysfunction and abnormal vascular structure are simultaneously present in patients with heart failure with preserved ejection fraction☆

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ABSTRACT

Background: Endothelial dysfunction and abnormal vascular structure may be involved in the pathogenesis of chronic heart failure (HF). The purpose of this study was to evaluate simultaneously vascular function and vascular structure in patients with heart failure with preserved ejection fraction (HFpEF).
Methods: We measured flow-mediated vasodilatation (FMD) and nitroglycerine-induced vasodilation as indices of vascular function and intima-media thickness (IMT) as an index of vascular structure of the brachial artery in 41 patients with HFpEF (23 men and 18 women; mean age, 66 ± 12 yr) and 165 patients without HF (95 men and 70 women; mean age, 54 ± 16 yr).
Results: FMD was significantly smaller in patients with HFpEF than in patients without HF (2.9 ± 2.1% versus 4.6 ± 2.7%, $P = 0.0002$). Nitroglycerine-induced vasodilation was significantly smaller in patients with HFpEF than in patients without HF (9.3 ± 4.1% versus 12.9 ± 4.9%, $P < 0.0001$). Brachial artery IMT was significantly larger in patients with HFpEF than in patients without HF (0.35 ± 0.06 mm versus 0.31 ± 0.07 mm, $P = 0.0002$). After adjustment for age, sex, hypertension, dyslipidemia, and diabetes mellitus, the associations remained significant between HFpEF and FMD (odds ratio, 0.79; 95% confidence interval, 0.66–0.92; $P = 0.0032$), nitroglycerine-induced vasodilation (odds ratio, 0.88; 95% confidence interval, 0.80–0.96; $P = 0.0039$), and brachial artery IMT (odds ratio, 1.08; 95% confidence interval, 1.01–1.17; $P = 0.033$).
Conclusions: These findings suggest that both endothelial dysfunction and abnormal vascular structure may contribute to the pathogenesis and maintenance of HFpEF. Endothelial function and vascular structure may be potential therapeutic targets for HFpEF.

1. Introduction

The prevalence of heart failure (HF) patients with preserved ejection fraction (HFpEF) is increasing, and patients with HFpEF have higher mortality rates than those in age- and sex-matched subjects without HF [1]. An effective treatment for HFpEF has not been established since the etiology and pathophysiology of HFpEF are still unclear. It has been reported that global cardiovascular reserve function, including endothelial function, is impaired in patients with HFpEF [2]. Recently, Akiyama et al. [3] demonstrated that peripheral endothelial dysfunction determined by using reactive hyperemia-peripheral arterial tonometry was an independent predictor of future cardiovascular events in patients who have HF with normal left ventricular ejection fraction (LVEF). In addition, it has been shown that carotid arterial stiffness is increased in patients with HFpEF [4]. It is likely that HFpEF is closely related to vascular function and structure.

Measurement of flow-mediated vasodilation (FMD) as an index of endothelium-dependent vasodilation and nitroglycerine-induced

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vasodilation as an index of endothelium-independent vasodilation in the brachial artery using high-resolution ultrasound has been widely used as a method for assessing vascular function [5,6]. Endothelial function is initially impaired in the pathogenesis of atherosclerosis [7,8]. Measurement of FMD is noninvasive and reflects nitric oxide (NO) production. In addition, growing evidence has shown that endothelial function assessed by FMD can serve as an independent predictor of cardiovascular events [9,10].

Findings concerning the relationship between intima-media thickness (IMT) and HFpEF and the relationship between FMD and HFpEF have been controversial [2–4,11]. In previous studies, different vascular beds were used for the assessment, i.e., common carotid artery for IMT and brachial artery for FMD. Measurements of IMT, FMD and nitroglycerine-induced vasodilation in the brachial artery offer the opportunity to investigate the interrelation between morphologic and functional parameters within the same artery by a single examination. There is no information on vascular function and structure in the same artery in patients with HFpEF. Therefore, the purpose of this study was to evaluate simultaneously vascular function and vascular structure in patients with HFpEF.

2. Methods

2.1. Subjects

Between June 2007 and July 2015, we studied 261 patients with symptoms or signs of HF who underwent FMD and echocardiography and 200 patients without symptoms or signs of HF who underwent FMD and echocardiography. We enrolled 261 consecutive patients with HF and 200 consecutive patients without HF who agreed to participate in this study during the study period. Two hundred and twenty of the 261 patients who had symptoms or signs of HF, including 42 patients with reduced LVEF, 37 patients with valvular disease, 29 patients with atrial fibrillation, 15 patients with cardiomyopathy, 4 patients with severe renal dysfunction, 31 patients with the presence of regional wall motion abnormality, 7 patients with neoplasms, and 55 patients who did not meet diagnostic criteria, were excluded. Finally, 41 patients who had symptoms or signs of HF were enrolled from Hiroshima University Hospital. We defined patients with no symptoms, no signs of HF and either normal NT-proBNP or normal echocardiography on the basis of the diagnostic criteria of the European Working Group for HF as patients without HF [12]. Thirty-five of the 200 patients without HF who did not meet the diagnostic criteria were excluded. Finally, 165 patients without symptoms or signs of HF were enrolled from Hiroshima University Hospital.

Patients with HFpEF: patients presented with symptoms or signs of HF and were diagnosed with HFpEF at the Hiroshima University Hospital. HFpEF was defined according to the diagnostic criteria of the European Working Group for HFpEF [12]. Exclusion criteria were significant valvular disease, atrial fibrillation, cardiomyopathy, severe renal dysfunction, LVEF <50% and the presence of regional wall motion. Severe renal dysfunction was defined as estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2.

Patients without HF: patients had normal LVEF, did not present with symptoms of HF, and either had normal NT-proBNP or normal echocardiography, and had never been diagnosed with or treated for HF. The patients also met the exclusion criteria of the European Working Group for HFpEF.

Hypertension was defined as systolic blood pressure of >140 mmHg or diastolic blood pressure of >90 mmHg, in a sitting position, on at least three different occasions. Normotension was defined as systolic blood pressure of <140 mmHg and diastolic blood pressure of <90 mmHg. Diabetes was defined according to the American Diabetes Association or a previous diagnosis of diabetes [13]. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program [14]. The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all of the subjects.

2.2. Study protocol

We measured vascular responses to reactive hyperemia and IMT in the brachial artery and echocardiograms in all subjects. Subjects fasted the previous night for at least 12 h prior to the measurements. The study began at 8:30 AM. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22 °C–25 °C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the supine position, brachial-ankle pulse wave velocity (baPWV), FMD, IMT, and nitroglycerine-induced vasodilation were measured. The observers were blind to the form of examination.

Thirty minutes after remaining in the supine position, Rho-associated kinase (ROCK) activity and fasting serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, blood urea nitrogen, creatinine, glucose, and N-terminal pro-brain natriuretic peptide (NT-proBNP) were measured.

2.3. Measurement of FMD and nitroglycerine-induced vasodilation

Vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co., Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter [15]. Please see the online data supplement for additional details.

2.4. Measurement of brachial IMT

Before FMD measurement, baseline longitudinal ultrasonographic images of the brachial artery, obtained at the end of diastole from each of 10 cardiac cycles, were automatically stored on a hard disk for off-line assessment of IMT with a linear, phased-array high-frequency (10-MHz) transducer using an UNEXEF18G ultrasound unit (UNEX Co.) [16]. Please see the online data supplement for additional details.

2.5. Measurement of baPWV

Aortic compliance was assessed noninvasively on the basis of Doppler ultrasound measurements of PWV along the descending thoracoabdominal aorta, as previously published and validated [17]. Please see the online data supplement for additional details.

2.6. Echocardiography

Echocardiograms were obtained by using a Philips iE33 (Philips Co. Ltd., Bothell, WA, USA) with a 1.0 to 5.0 MHz transducer (S5-1). Please see the online data supplement for additional details.

2.7. Measurement of ROCK activity

ROCK activity was assayed in peripheral blood leukocytes as the amount of phospho-Thr853 in the myosin-binding subunit of myosin light chain phosphatase (MLCPh), because the myosin-binding subunit on MLCPh is one of the downstream targets of ROCK. Please see the online data supplement for additional details.

2.8. Statistical analysis

Results are presented as means ± SD for continuous variables and as percentages for categorical variables. Statistical significance was set at a level of $P < 0.05$. Continuous variables were compared by using ANOVA for multiple groups. Categorical variables were compared by means of the χ^2 test. Relationships between variables were determined by Pearson correlation coefficients analysis. Multivariable regression analysis was performed to assess the association between vascular function and HFpEF in risk factors. In addition, we created matched pairs (1 patient with HFpHF to 1 control) in the supplemental data. Paired *t*-test was used for comparison of mean values of continuous variables between the 2 groups. Correlations between continuous variables were estimated with the use of Pearson correlation coefficients. The data were processed using the software package Stata, version 9 (Stata Co., College Station, TX).

3. Results

3.1. Baseline clinical characteristics

The baseline clinical characteristics of the 165 patients without HF and 41 patients with HFpEF are summarized in Table 1. Age, systolic blood pressure, NT-proBNP, and prevalence of hypertension were significantly higher in patients with HFpEF than in patients without HF. eGFR was significantly lower in patients with HFpEF than in patients without HF. There was no significant difference in other parameters between patients without HF and patients with HFpEF.

3.2. Echocardiographic parameters

Echocardiographic parameters of the 165 patients without HF and 41 patients with HFpEF are summarized in Table 2. Left atrial (LA) diameter, interventricular septal thickness, posterior LV wall thickness, late (A) diastolic mitral inflow velocity, deceleration time of E velocity, early (E) diastolic mitral inflow velocity to late diastolic mitral annulus velocity (E/E′) ratio, LA volume index, and LV mass index were significantly higher in patients with HFpEF than in patients without HF. The ratio of early to late peak velocities (E/A), E′ velocity of septal and lateral wall, and mean E′ velocity of septal and lateral wall were significantly lower in patients with HFpEF than in patients without HF. There was

no significant difference in other parameters between patients without HF and patients with HFpEF.

3.3. Vascular function and structure

FMD was significantly lower in patients with HFpEF than in patients without HF (2.9 ± 2.1% versus 4.6 ± 2.7%, $P = 0.0002$) (Fig. 1A). Nitroglycerine-induced vasodilation was significantly lower in patients with HFpEF than in patients without HF (9.3 ± 4.1% versus 12.9 ± 4.9%, $P < 0.0001$) (Fig. 1B). Brachial artery IMT was significantly higher in patients with HFpEF than in patients without HF (0.35 ± 0.06 mm versus 0.31 ± 0.07 mm, $P = 0.0002$) (Fig. 1C). baPWV was significantly higher in patients with HFpEF than in patients without HF (1948 ± 434 cm/s versus 1632 ± 367 cm/s, $P < 0.0001$) (Fig. 1D).

3.4. Relationships between vascular function, vascular structure, and various parameters

Univariate regression analysis revealed that FMD was significantly correlated with age, systolic blood pressure, diastolic blood pressure, eGFR, aortic root diameter, LA diameter, interventricular septal thickness at end-diastole, posterior LV wall thickness at end-diastole, E/A, E′ velocities of septal and lateral, mean E′ septal and lateral wall, E/E′ ratio, LA volume index, nitroglycerine-induced vasodilation, brachial artery IMT, and baPWV.

Univariate regression analysis also revealed that nitroglycerine-induced vasodilation was significantly correlated with age, systolic blood pressure, heart rate, eGFR, aortic root diameter, LA diameter, interventricular septal thickness at end-diastole, posterior LV wall thickness at end-diastole, A velocity, E/A, E′ velocity of septal and lateral, E/E′ ratio, LA volume index, LV mass index, brachial artery IMT, and baPWV.

Furthermore, univariate regression analysis revealed that brachial IMT was significantly correlated with age, systolic blood pressure, diastolic blood pressure, eGFR, NT-proBNP, aortic root diameter, LA diameter, interventricular septal thickness at end-diastole, posterior LV wall thickness at end-diastole, E velocity, A velocity, E/A, deceleration time, E′ velocity of septal and lateral, E/E′ ratio, mean E′ septal and lateral wall, LA volume index, LV mass index and baPWV.

Univariate regression analysis also revealed that baPWV was significantly correlated with age, body mass index, systolic blood pressure, diastolic blood pressure, eGFR, NT-proBNP, LV end-diastolic dimension, LV end-systolic dimension, E velocity, A velocity, E/A, deceleration time, E′ velocity of septal and lateral, E/E′ ratio, mean E′ septal and lateral wall, and LV end-diastolic volume index (supplemental Table S1).

After adjustment for age, sex, hypertension, dyslipidemia, and diabetes mellitus, the associations of HFpEF with FMD (odds ratio, 0.79; 95% confidence interval, 0.66–0.92; $P = 0.0029$), nitroglycerine-induced vasodilation (odds ratio, 0.88; 95% confidence interval, 0.80–0.96; $P = 0.0038$), and brachial artery IMT (odds ratio, 1.08; 95% confidence interval, 1.01–1.17; $P = 0.036$) remained significant, but the association with baPWV was not significant (odds ratio, 1.00; 95% confidence interval, 1.00–1.00; $P = 0.051$) (Table 3).

Table 1
Clinical characteristics of the patients without HF and patients with HFpEF.

Variables	Patients without HF ($n = 165$)	Patients with HFpEF ($n = 41$)	*P* value
Age, yr	54 ± 16	66 ± 12	<0.0001
Sex, men/women	95/70	23/18	0.86
Body mass index, kg/m^2	24.3 ± 4.7	24.3 ± 4.7	0.93
Body surface area, m^2	1.64 ± 0.21	1.62 ± 0.25	0.48
Systolic blood pressure, mmHg	134 ± 19	146 ± 25	0.0012
Diastolic blood pressure, mmHg	81 ± 12	85 ± 16	0.066
Heart rate, bpm	71 ± 12	68 ± 11	0.14
Total cholesterol, mmol/L	4.97 ± 1.01	5.07 ± 0.88	0.58
Triglycerides, mmol/L	1.65 ± 1.22	1.39 ± 0.68	0.19
HDL cholesterol, mmol/L	1.53 ± 0.54	1.60 ± 0.47	0.37
LDL cholesterol, mmol/L	2.87 ± 0.88	2.97 ± 0.88	0.44
Glucose, mmol/L	6.05 ± 1.50	6.27 ± 1.78	0.47
BUN, mmol/L	5.0 ± 1.3	6.0 ± 1.6	<0.0001
Creatinine, mmol/L	66.3 ± 15.9	69.8 ± 16.8	0.16
eGFR, mL/min/1.73 m^2	69 ± 22	59 ± 16	0.0076
NT-proBNP, pg/mL	55 ± 52	380 ± 214	<0.0001
ROCK activity	1.01 ± 0.62	1.09 ± 0.67	0.57
Medical history, n (%)			
Hypertension	125 (75.8)	39 (95.1)	0.0019
Dyslipidemia	96 (58.2)	26 (63.4)	0.54
Diabetes mellitus	39 (23.6)	10 (24.4)	0.92
Previous coronary heart disease	24 (14.6)	4 (9.8)	0.41
Previous stroke	8 (4.9)	0 (0.0)	0.057
Smoker, n (%)	22 (13.3)	10 (24.4)	0.095
Medication, n (%)			
Antiplatelets	39 (23.6)	9 (22.0)	0.82
Calcium channel blockers	76 (46.1)	18 (43.9)	0.80
RAS inhibitors	37 (22.4)	14 (34.2)	0.13
β-blockers	12 (7.3)	6 (14.6)	0.16
Diuretics	8 (4.6)	3 (7.3)	0.54
Statins	47 (28.5)	10 (24.4)	0.60
Nitrates	0 (0)	0 (0)	N/A
Medically treated diabetes mellitus			
Any	22 (13.3)	3 (7.3)	0.27
Insulin dependent	3 (1.8)	2 (4.9)	0.30

Results are presented as means ± SD for continuous variables and percentages for categorical variables.
BUN = blood urea nitrogen; eGFR = estimated-glomerular filtration rate; HDL = high-density lipoprotein; HF = heart failure; HFpEF = heart failure with preserved ejection fraction; LDL = low-density lipoprotein; NT-proBNP = N-terminal pro-brain natriuretic peptide; RAS = renin angiotensin system; ROCK = Rho-associated kinase.

Table 2
Echocardiographic parameters of the patients without HF and patients with HFpEF.

Variables	Patients without HF ($n = 165$)	Patients with HFpEF ($n = 41$)	*P* value
Ejection fraction, %	64.6 ± 4.4	63.5 ± 4.8	0.16
LA diameter, mm	34.7 ± 5.2	37.8 ± 4.8	0.0006
LA diameter index, mm/m^2	21.2 ± 3.0	23.9 ± 3.9	<0.0001
LA volume index, mL/m^2	30.3 ± 7.2	37.4 ± 11.7	<0.0001
Interventricular septal thickness , mm	8.9 ± 1.3	10.8 ± 2.3	<0.0001
Posterior LV wall thickness, mm	9.0 ± 1.3	10.6 ± 2.0	<0.0001
LV end-diastolic dimension, mm	46.9 ± 4.6	46.2 ± 5.1	0.39
LV end-systolic dimension, mm	29.8 ± 3.8	29.9 ± 4.1	0.89
LV end-diastolic dimension index, mm/m^2	28.8 ± 3.0	28.9 ± 3.8	0.72
LV end-systolic dimension index, mm/m^2	18.3 ± 2.4	18.7 ± 2.8	0.28
E velocity, cm/s	66.5 ± 16.7	63.5 ± 13.2	0.29
A velocity, cm/s	64.8 ± 18.1	71.0 ± 17.8	0.049
E/A	1.11 ± 0.41	0.95 ± 0.31	0.027
Deceleration time, ms	216 ± 47	237 ± 47	0.013
Septal A′ velocity, cm/s	9.4 ± 1.8	8.9 ± 1.9	0.068
Septal E′ velocity, cm/s	7.7 ± 2.7	5.4 ± 1.3	<0.0001
Lateral E′ velocity, cm/s	10.0 ± 3.1	7.3 ± 1.9	<0.0001
Mean E′ septal and lateral wall, cm/s	8.8 ± 2.7	6.3 ± 1.4	<0.0001
E/E′ ratio	8.0 ± 2.3	10.5 ± 2.9	<0.0001
LV mass index (penn), g/m^2	100.4 ± 24.7	129.6 ± 38.2	<0.0001
LV end-diastolic volume index, mL/m^2	62.9 ± 11.4	62.1 ± 13.4	0.70
LV end-systolic volume index, mL/m^2	21.3 ± 6.1	22.0 ± 6.4	0.46
LV stroke volume index, mL/m^2	41.6 ± 7.7	40.0 ± 8.5	0.25

A = late diastolic flow velocity; A′ = late diastolic annular velocity; E = early diastolic flow velocity; E′ = early diastolic annular velocity; E/E′ ratio = averaging septal and lateral E′ velocity; HF = heart failure; HFpEF = heart failure with preserved ejection fraction; LA = left atrial; LV = left ventricular.

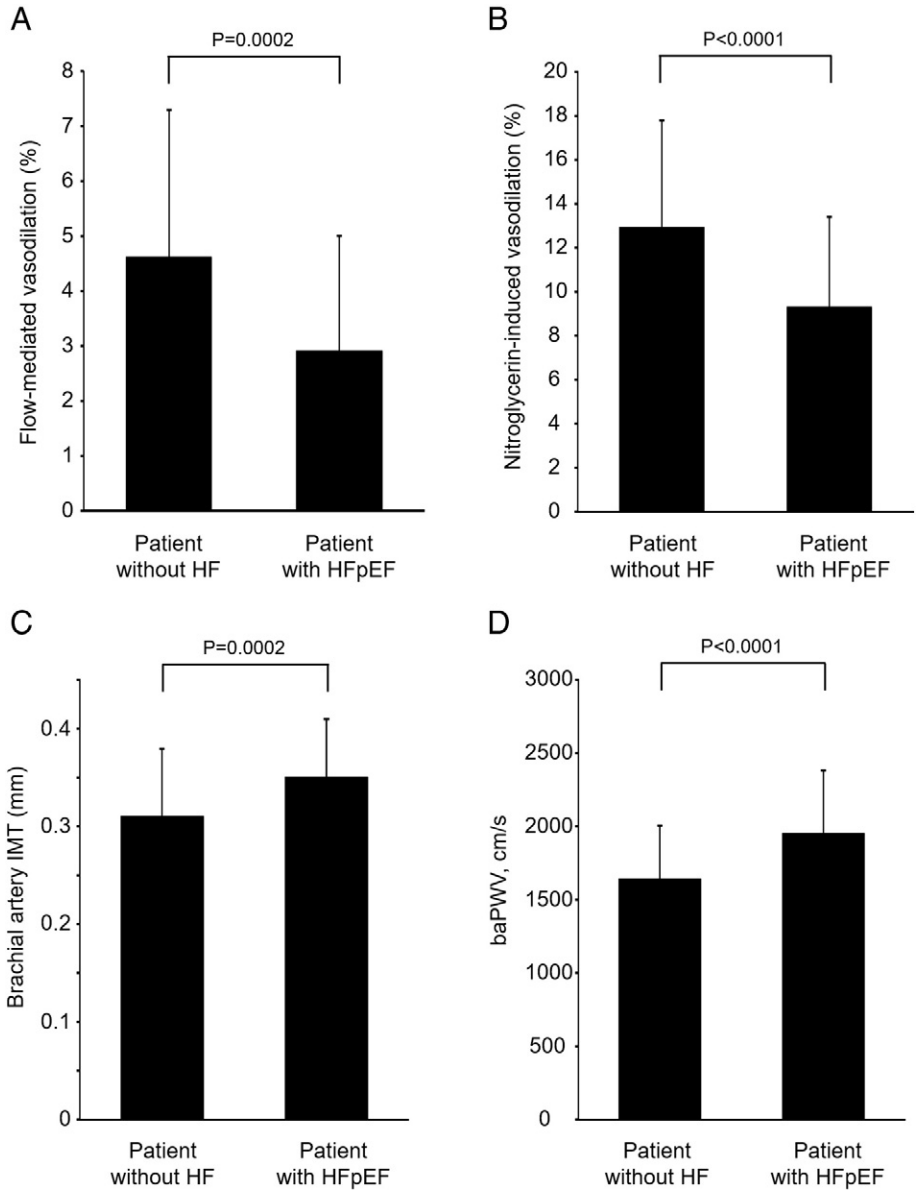

Fig. 1. Bar graphs show flow-mediated vasodilation (A), nitroglycerine-induced vasodilation (B), intima-media thickness (IMT) (C) and brachial-ankle pulse wave velocity (baPWV) (D) in patients without heart failure (HF) and patients with heart failure with preserved ejection fraction (HFpEF).

In addition, we evaluated vascular function and vascular structure in patients with HFpEF and control subjects using created matched pairs (1 patient with HFpHF to 1 control). The baseline clinical characteristics and echocardiographic parameters of the 41 patients without HF and 41 patients with HFpEF are summarized in supplemental Tables S1 and S2. FMD was significantly lower in patients with HFpEF than in patients without HF (2.9 ± 2.1% versus 4.8 ± 2.5%, $P = 0.0003$) (supplemental Fig. S1A), and nitroglycerine-induced vasodilation was significantly lower in patients with HFpEF than in patients without HF (9.3 ± 4.1% versus 12.6 ± 4.7%, $P = 0.0016$) (supplemental Fig. S1B). Brachial artery IMT was significantly higher in patients with HFpEF than in patients without HF (0.35 ± 0.06 mm versus 0.31 ± 0.05 mm, $P = 0.0024$) (supplemental Fig. S2A). There was no significant difference in baPWV between patients with HFpEF and patients without HF (1948 ± 434 cm/s versus 1829 ± 397 cm/s, $P = 0.22$) (supplemental Fig. S2B). Results of univariate regression analysis of relationships among FMD, nitroglycerine-induced vasodilation, brachial IMT, baPWV, and variables are shown in supplemental Table S4.

4. Discussion

In the present study, we demonstrated that FMD and nitroglycerine-induced vasodilation were significantly smaller in patients with HFpEF than in patients without HF and that brachial artery IMT and baPWV were significantly larger in patients with HFpEF than in patients without HF. Furthermore, we showed that the significant associations of HFpEF with FMD, nitroglycerine-induced vasodilation and brachial artery IMT, but not the association with baPWV, remained after adjustment for cardiovascular risk factors. It was shown for the first time that both endothelial dysfunction assessed by FMD and abnormal vascular structure assessed by IMT in the same artery are related to HFpEF.

4.1. Endothelial dysfunction and abnormal vascular structure with HFpEF

Previous studies have clearly shown that predisposing conditions for HFpEF are advanced age and hypertension [18,19]. Indeed, in the present study, patients with HFpEF were older and had higher prevalences of hypertension and renal dysfunction. Interestingly, risk factors

Table 3
Multivariate analysis of relationships between vascular function and HFpEF.

Variables	Unadjusted OR (95% CI) *P* value	Adjusted[a] OR (95% CI) *P* value	Adjusted[b] OR (95% CI) *P* value
FMD (%)	0.75 (0.63–0.87) 0.0003	0.78 (0.65–0.91) 0.0014	0.79 (0.66–0.92) 0.0029
Nitroglycerine-induced vasodilation (%)	0.85 (0.77–0.92) <0.0001	0.87 (0.80–0.95) 0.0019	0.88 (0.80–0.96) 0.0038
Brachial artery IMT (mm)	1.11 (1.05–1.19) 0.0002	1.09 (1.01–1.17) 0.019	1.08 (1.01–1.17) 0.036
baPWV (cm/s)	1.002 (1.001–1.003) <0.0001	1.001 (1.0002–1.002) 0.019	1.00 (1.00–1.00) 0.051

baPWV = brachial-ankle pulse wave velocity; CI = confidence interval; FMD = flow-mediated vasodilation; HFpEF = heart failure with preserved ejection fraction; IMT = intima-media thickness; OR = odds ratio.
[a] Adjusted: age, sex.
[b] Adjusted: age, sex, hypertension, dyslipidemia, diabetes mellitus.

of HFpEF are similar to those of endothelial dysfunction and abnormal vascular structure.

A healthy endothelium maintains vascular tone and structure by regulating the balance between vasodilation and vasoconstriction, growth inhibition and growth promotion, anti-thrombosis and pro-thrombosis, anti-inflammation and pro-inflammation, and also anti-oxidation and pro-oxidation [7,8,20]. Therefore, endothelial dysfunction leads to vasoconstriction, abnormal angiogenesis, pro-thrombosis, pro-inflammation, and pro-oxidation states. In the present study, endothelial function was impaired in patients with HFpEF. FMD remained an independent predictor of HFpEF after adjustment of cardiovascular risk factors, including age, gender, diabetes mellitus, hypertension, and dyslipidemia. Although the precise mechanisms underlying the association between endothelial function and HFpEF are not known, it is likely that endothelial dysfunction in the conduit artery contributes to HFpEF. On the other hand, previous studies demonstrated that patients with HFpEF did not have conduit artery endothelial dysfunction as assessed by brachial artery FMD [11]. Although the precise reasons for the discrepant results regarding endothelial function in patients with HFpEF are unclear, different definitions of HFpEF might have caused the different findings. Since HFpEF was defined in previous studies as a previous history of clinical symptoms of HF and EF of over 50%, the possibility that the study population included healthy subjects cannot be ruled out. In the present study, we diagnosed HFpEF in accordance with a consensus statement on the diagnosis of HF with normal LVEF by 2016 European Society of Cardiology guidelines [12]. The use of criteria that include previous history of clinical symptoms and/or signs of HF, a preserved EF (EF ≥ 50%), an increase in NT-proBNP level, and objective evidence of other cardiac functional and structural alterations underlying HF enabled HFpEF to be defined more specifically.

Previous studies have shown that HFpEF is associated with carotid arterial stiffness [4,20]. We confirmed a significant relationship between HFpEF and IMT of the brachial artery. Measurement of IMT in the artery is established as an index of structural change of the artery. IMT is one of the manifestations of atherosclerosis and is usually assessed in the carotid and/or femoral arteries [21–23]. Several lines of evidence suggest that IMT, especially carotid IMT, is associated with cardiovascular risk factors and is a predictor of cardiovascular outcomes [24,25]. Recently, we have shown that IMT of the brachial artery is also increased in relation to cumulative cardiovascular risk factors and is significantly correlated with cardiovascular risk factors and that there is a significant relationship between brachial IMT and carotid IMT [16]. In this study, brachial artery IMTs of subjects with low, intermediate and high cardiovascular risk factors were 0.19 ± 0.33 mm, 0.28 ± 0.02 mm and 0.40 ± 0.07 mm, respectively. Brachial artery IMT in patients without HF was higher than that in the low cardiovascular risk factor group. In addition, brachial artery IMT was significantly higher in patients with HFpEF than in patients without HF. In addition, Frick et al. [26] reported that brachial IMT predicts late cardiovascular events in male patients admitted for evaluation of chest pain. However, there is no information on concomitant assessment of vascular function and vascular structure in patients with HFpEF. In the present study, brachial artery IMT as well as FMD was significantly associated with HFpEF after adjustment of cardiovascular risk factors. Although it is unclear whether endothelial dysfunction and abnormal vascular structure in the conduit artery are a cause or a consequence of HFpEF, endothelial dysfunction and abnormal vascular structure in the conduit artery would contribute to the pathogenesis and maintenance of HFpEF.

In the present study, we confirmed the simultaneous presence of endothelial dysfunction and abnormal vascular structure in HFpEF. Advancement of atherosclerosis leads to gradual impairment of vascular smooth muscle function and vascular structure. Indeed, several investigators, including us, have shown that endothelium-dependent vasodilation induced by NO synthase activators, as an index of endothelial function, is impaired in animal models of and patients with low-grade atherosclerosis. In addition, it has been demonstrated that endothelium-independent vasodilation induced by NO donors is maintained in the early to mild atherosclerosis stage, that endothelium-independent vasodilation is gradually impaired in relation to progression of atherosclerosis, and that vascular function and vascular structure are not improved by any interventions under the condition of severe atherosclerosis [27,28]. In the present study, both FMD and nitroglycerine-induced vasodilation were impaired in patients with HFpEF. It is thought that the condition of the vasculature in patients with HFpEF is the same as severe vascular failure in patients with advanced atherosclerosis.

Some possible mechanisms by which endothelial dysfunction and abnormal vascular structure might contribute to pathogenesis and maintenance of HFpEF are postulated. Several studies have shown changes in arterial and ventricular stiffening measured by LV end-systolic elastance and arterial elastance in patients with HFpEF [2,29,30]. A combination of alterations in arterial and ventricular stiffening increases systolic pressure sensitivity to cardiac loading. Vascular failure composed by endothelial dysfunction and abnormal vascular structure blunts vascular compliance and causes the variability and instability in blood pressure, leading to a vicious circle between advanced vascular failure and advanced HF in patients with HFpEF.

ROCK plays a pivotal role in the regulation of many cellular functions, including functions of endothelial cells and cardiomyocytes [21]. It has been shown, in vivo, that ROCK contributes to early atherosclerotic lesion formation, vascular remodeling, and cardiac hypertrophy [22]. ROCK activity is enhanced in animal models of HF, and inhibition of ROCK activity prevents the development of hypertrophic remodeling in these models. In a clinical setting also, ROCK activity is enhanced in patients with HF [23]. In addition, endothelial NO synthase (eNOS) expression is up-regulated by inhibition of ROCK activity via an increase of eNOS mRNA stability and eNOS phosphorylation in endothelial cells [31]. However, in the present study, ROCK activity levels were similar

in patients with and those without HFpEF. There was no significant relationship between ROCK activity and FMD. ROCK activity is unlikely to be involved in the endothelial dysfunction in patients with HFpEF and is unlikely to contribute to the pathogenesis of HFpEF.

4.2. Study limitations

Although there were significant associations between FMD, IMT and HFpEF, the number of subjects in this study was relatively small. In addition, the subjects were enrolled in a single medical center. Thus the results of this study need to be confirmed by a multicenter study in large clinical trials. We enrolled consecutive patients who agreed to participate and who underwent both FMD and echocardiography as the study population in this study. Therefore, we cannot deny the possibility that there is a selection bias. It is difficult to diagnose true HFpEF. We used diagnostic criteria of the European Working Group for HFpEF to define HFpEF in the present study. A median NT-proBNP value of 380 ± 214 is relatively low. However, we used NT-proBNP >125 pg/mL to define HFpEF in accordance with diagnostic criteria of the European Working Group for HFpEF. The median NT-proBNP value might have been low because almost all of the patients with HFpEF had already been treated for heart failure. It is well known that pharmacological therapy, such as treatment with renin angiotensin system inhibitors, beta-blockers and diuretics, improves endothelial function. We previously showed that carvedilol improves endothelium-dependent vasodilation evoked by acetylcholine in patients with dilated cardiomyopathy [32]. In the present study, there were significant associations of HFpEF with FMD and IMT after adjustment of use of drugs, while the use of beta-blockers and diuretics was more frequent in patients with HFpEF. However, we cannot rule out the possibility that differences in pharmacological treatment affect endothelial function. Finally, measurement of ROCK activity in endothelial cells, vascular smooth muscle cells or cardiomyocytes from patients with HFpEF would enable more specific conclusions concerning the role of ROCK activity in the pathogenesis and maintenance of HFpEF to be drawn. Previously, several investigators including us have reported that measurement of leukocyte ROCK activity is a useful method as is measurement of vascular ROCK activity [6,33,34]. In addition, it has been shown that leukocyte ROCK activity was related to Framingham risk score and was an independent predictor of cardiovascular events [35,36]. Therefore, we believe that leukocyte ROCK is an established biomarker for cardiovascular disease and cardiovascular events. However, it is unclear whether leukocyte ROCK activity is adaptive for the assessment of ROCK activities of endothelial cells, vascular smooth muscle cells or cardiomyocytes in patients with HFpHF and HF.

5. Conclusions

Endothelial dysfunction and abnormal vascular structure are simultaneously present in patients with HFpEF, suggesting that patients with HFpEF have advanced vascular failure. Vascular function and structure as well as myocardial function and structure may contribute to the pathogenesis and maintenance of HFpEF. Unfortunately, although optimal treatment for HFpEF remains unclear, endothelial function and vascular structure may be potential therapeutic targets for HFpEF.

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Disclosures

The authors report no relationships that could be construed as a conflict of interest.

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Appendix A. Supplementary data

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References

[1] R.S. Bhatia, J.V. Tu, D.S. Lee, P.C. Austin, J. Fang, A. Haouzi, et al., Outcome of heart failure with preserved ejection fraction in a population-based study, N. Engl. J. Med. 355 (2006) 260–269.
[2] B.A. Borlaug, T.P. Olson, C.S. Lam, K.S. Flood, A. Lerman, B.D. Johnson, et al., Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction, J. Am. Coll. Cardiol. 56 (2010) 845–854.
[3] E. Akiyama, S. Sugiyama, Y. Matsuzawa, M. Konishi, H. Suzuki, T. Nozaki, et al., Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction, J. Am. Coll. Cardiol. 60 (2012) 1778–1786.
[4] D.W. Kitzman, D.M. Herrington, P.H. Brubaker, J.B. Moore, J. Eggebeen, M.J. Haykowsky, Carotid arterial stiffness and its relationship to exercise intolerance in older patients with heart failure and preserved ejection fraction, Hypertension 61 (2013) 112–119.
[5] D.S. Celermajer, K.E. Sorensen, V.M. Gooch, D.J. Spiegelhalter, O.I. Miller, I.D. Sullivan, et al., Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, Lancet (Lond. Engl.) 340 (1992) 1111–1115.
[6] J. Soga, K. Noma, T. Hata, T. Hidaka, Y. Fujii, N. Idei, et al., Rho-associated kinase activity, endothelial function, and cardiovascular risk factors, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 2353–2359.
[7] R. Ross, Atherosclerosis—an inflammatory disease, N. Engl. J. Med. 340 (1999) 115–126.
[8] Y. Higashi, K. Noma, M. Yoshizumi, Y. Kihara, Endothelial function and oxidative stress in cardiovascular diseases, Circ. J. 73 (2009) 411–418.
[9] N. Gokce, J.F. Keaney Jr., L.M. Hunter, M.T. Watkins, J.O. Menzoian, J.A. Vita, Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study, Circulation 105 (2002) 1567–1572.
[10] A. Lerman, A.M. Zeiher, Endothelial function: cardiac events, Circulation 111 (2005) 363–368.
[11] M.J. Haykowsky, D.M. Herrington, P.H. Brubaker, T.M. Morgan, W.G. Hundley, D.W. Kitzman, Relationship of flow-mediated arterial dilation and exercise capacity in older patients with heart failure and preserved ejection fraction, J. Gerontol. A Biol. Sci. Med. Sci. 68 (2013) 161–167.
[12] P. Ponikowski, A.A. Voors, S.D. Anker, H. Bueno, J.G. Cleland, A.J. Coats, et al., 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC, Eur. J. Heart Fail. 18 (2016) 891–975.
[13] American Diabetes Association, Clinical practice recommendations 1999, Diabetes Care 22 (Suppl. 1) (1999) S1–114.
[14] Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), JAMA 285 (2001) 2486–2497.
[15] T. Maruhashi, J. Soga, N. Fujimura, N. Idei, S. Mikami, Y. Iwamoto, et al., Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1401–1408.
[16] Y. Iwamoto, T. Maruhashi, Y. Fujii, N. Idei, N. Fujimura, S. Mikami, et al., Intima-media thickness of brachial artery, vascular function, and cardiovascular risk factors, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 2295–2303.
[17] E. Kimoto, T. Shoji, K. Shinohara, M. Inaba, Y. Okuno, T. Miki, et al., Preferential stiffening of central over peripheral arteries in type 2 diabetes, Diabetes 52 (2003) 448–452.
[18] A.M. From, C.G. Scott, H.H. Chen, The development of heart failure in patients with diabetes mellitus and pre-clinical diastolic dysfunction a population-based study, J. Am. Coll. Cardiol. 55 (2010) 300–305.
[19] M. Klapholz, M. Maurer, A.M. Lowe, F. Messineo, J.S. Meisner, J. Mitchell, et al., Hospitalization for heart failure in the presence of a normal left ventricular ejection fraction: results of the New York Heart Failure Registry, J. Am. Coll. Cardiol. 43 (2004) 1432–1438.
[20] T. Weber, S. Wassertheurer, M.F. O'Rourke, A. Haiden, R. Zweiker, M. Rammer, et al., Pulsatile hemodynamics in patients with exertional dyspnea: potentially of value in

the diagnostic evaluation of suspected heart failure with preserved ejection fraction, J. Am. Coll. Cardiol. 61 (2013) 1874–1883.
[21] M. Uehata, T. Ishizaki, H. Satoh, T. Ono, T. Kawahara, T. Morishita, et al., Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension, Nature 389 (1997) 990–994.
[22] Z. Mallat, A. Gojova, V. Sauzeau, V. Brun, J.S. Silvestre, B. Esposito, et al., Rho-associated protein kinase contributes to early atherosclerotic lesion formation in mice, Circ. Res. 93 (2003) 884–888.
[23] T. Kishi, Y. Hirooka, A. Masumoto, K. Ito, Y. Kimura, K. Inokuchi, et al., Rho-kinase inhibitor improves increased vascular resistance and impaired vasodilation of the forearm in patients with heart failure, Circulation 111 (2005) 2741–2747.
[24] D.H. O'Leary, J.F. Polak, R.A. Kronmal, T.A. Manolio, G.L. Burke, S.K. Wolfson Jr., Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group, N. Engl. J. Med. 340 (1999) 14–22.
[25] M.L. Bots, A.W. Hoes, P.J. Koudstaal, A. Hofman, D.E. Grobbee, Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study, Circulation 96 (1997) 1432–1437.
[26] M. Frick, A. Suessenbacher, H.F. Alber, W. Dichtl, H. Ulmer, O. Pachinger, et al., Prognostic value of brachial artery endothelial function and wall thickness, J. Am. Coll. Cardiol. 46 (2005) 1006–1010.
[27] T.F. Luscher, Y. Dohi, M. Tschudi, Endothelium-dependent regulation of resistance arteries: alterations with aging and hypertension, J. Cardiovasc. Pharmacol. 19 (Suppl. 5) (1992) S34–S42.
[28] Y. Higashi, S. Sasaki, K. Nakagawa, M. Kimura, S. Sasaki, K. Noma, et al., Severity of hypertension affects improved resistance artery endothelial function by angiotensin-converting enzyme inhibition, J. Cardiovasc. Pharmacol. 39 (2002) 668–676.
[29] M. Kawaguchi, Combined ventricular systolic and arterial stiffening in patients with heart failure and preserved ejection fraction: implications for systolic and diastolic reserve limitations, Circulation 107 (2003) 714–720.
[30] C.H. Chen, M. Nakayama, E. Nevo, B.J. Fetics, W.L. Maughan, D.A. Kass, Coupled systolic-ventricular and vascular stiffening with age: implications for pressure regulation and cardiac reserve in the elderly, J. Am. Coll. Cardiol. 32 (1998) 1221–1227.
[31] S. Wolfrum, A. Dendorfer, Y. Rikitake, T.J. Stalker, Y. Gong, R. Scalia, et al., Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1842–1847.
[32] K. Nishioka, K. Nakagawa, T. Umemura, D. Jitsuiki, K. Ueda, C. Goto, et al., Carvedilol improves endothelium-dependent vasodilation in patients with dilated cardiomyopathy, Heart 93 (2007) 247–248.
[33] A. Nohria, M.E. Grunert, Y. Rikitake, K. Noma, A. Prsic, P. Ganz, et al., Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease, Circ. Res. 99 (2006) 1426–1432.
[34] P.Y. Liu, J.H. Chen, L.J. Lin, J.K. Liao, Increased Rho kinase activity in a Taiwanese population with metabolic syndrome, J. Am. Coll. Cardiol. 49 (2007) 1619–1624.
[35] M. Kajikawa, K. Noma, T. Maruhashi, S. Mikami, Y. Iwamoto, A. Iwamoto, et al., Rho-associated kinase is a predictor of cardiovascular outcomes, Hypertension 63 (2014) 856–864.
[36] M. Kajikawa, K. Noma, A. Nakashima, T. Maruhashi, Y. Iwamoto, T. Matsumoto, et al., Rho-associated kinases activity is an independent predictor of cardiovascular events in acute coronary syndrome, Hypertension 66 (2015) 892–899.