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Original Article

Pulmonary Hypertension Is Related to Peripheral Endothelial Dysfunction in Heart Failure With Preserved Ejection Fraction

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Background—Pulmonary hypertension (PH) and collagen metabolism abnormalities are prevalent in patients with heart failure with preserved ejection fraction (HFpEF). Peripheral endothelial dysfunction (PED) has been described in HF and in pulmonary arterial hypertension. Our aim is to determine whether PH is associated with PED and impaired collagen metabolism in patients with HFpEF.;

Methods and Results—Flow-mediated dilation of the brachial artery, matrix metalloproteinase-2 and matrix metalloproteinase-9, tissue metalloproteinase inhibitor 1, and C-terminal propeptide of type I procollagen were determined in 28 patients with HFpEF and 42 hypertensive controls. Patients with systolic pulmonary artery pressure >35 mm Hg on echocardiogram underwent a right heart catheterization. Patients with HFpEF had more severe PED than controls: flow-mediated dilation 1.95% (−0.81 to 4.92) versus 5.02% (3.90 to 10.12), P=0.002. Twenty patients with PH underwent right heart catheterization: mean pulmonary artery pressure 38 (27–52) mm Hg, wedge capillary pressure 18 (16–22) mm Hg, pulmonary vascular resistance 362 (235–603) dyn s cm^{-5}. There was a significant inverse correlation between flow-mediated dilation and pulmonary vascular resistance in patients with HFpEF and PH (r=−0.679; P=0.002). Patients with HFpEF showed higher matrix metalloproteinase-2 and C-terminal propeptide of type I procollagen values than hypertensive controls. Patients with HFpEF and higher C-terminal propeptide of type I procollagen values also had higher mean pulmonary artery pressure (r=0.553; P=0.014), transpulmonary gradient (r=0.560; P=0.013), and pulmonary vascular resistance (r=0.626; P=0.004).

Conclusions—In patients with HFpEF, there is a significant correlation between PED and pulmonary vascular resistance. Collagen metabolism was more impaired in patients with HFpEF and PH. PED and collagen metabolism assessment could be useful tools to identify patients with HFpEF at risk of developing PH. **(*Circ Heart Fail.* 2014;7:791-798.)**

Key Words: pulmonary circulation ■ vascular resistance

Heart failure with preserved ejection fraction (HFpEF) is an increasingly common diagnosis, accounting for 50% of HF cases.[1] Several studies have described the epidemiological characteristics of the HFpEF population: a predominance of older age, women, and history of hypertension and metabolic syndrome.[2] However, the precise mechanism underlying HFpEF is still not well known. The lack of understanding of the physiopathological pathways that lead to the disease may have contributed to the difficulty in finding specific treatments. Several therapies have been assayed with disappointing results, and to date there are no evidence-based therapeutic guidelines for this population.[3]

Clinical Perspective on p 798

Diastolic dysfunction and vascular stiffness have been described in this population and related to an imbalance in extracellular matrix collagen metabolism.[4] Peripheral endothelial dysfunction (PED) has been reported in patients with HF[5] and associated with poor outcomes.[6] Recent studies report a high prevalence of pulmonary hypertension (PH) in HFpEF, which is in turn related to a worse prognosis[7] although the mechanisms underlying the high prevalence of PH are unknown. Classical studies suggest that increased pulmonary vascular resistance (PVR) is related to abnormalities in smooth muscle tone caused by pulmonary endothelial function as a consequence of NO and endothelin-1 imbalances.[8,9] Those imbalances may also affect the peripheral vessels endothelium.

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Our aim was to analyze the association among PED, collagen metabolism, and PH in patients with HFpEF. Our working hypothesis was that patients with HFpEF have endothelial dysfunction that affects both the pulmonary and the peripheral vasculature. A PED assessment could reflect pulmonary endothelial dysfunction and, therefore, be related to the presence of PH. Abnormalities in endothelial function could account for a vasoreactive component in PH and HFpEF, in addition to the postcapillary contribution. Imbalanced collagen metabolism could be related to endothelial dysfunction and consequently, PH.

To test our hypothesis, we studied a group of patients with HFpEF and PH, assessing peripheral endothelial function, collagen metabolism, and invasive pulmonary hemodynamics, and compared the results with a group of asymptomatic controls with systemic hypertension.

Methods

Study Population

Consecutive adult patients with HFpEF referred to our clinic for HF or PH were prospectively enrolled. Inclusion criteria were ≥1 hospital admission for HF in the previous year, normal left ventricular (LV) systolic function (LVEF, ≥50%), and diagnosis of HFpEF according to current recommendations.[3] Exclusion criteria were untreated ischemic heart disease or valvular heart disease; constrictive parameters; restrictive cardiomyopathies; fibroproliferative systemic diseases including systemic sclerosis, renal failure (creatinine, ≥2.5 mg/dL), and lung fibrosis; and significant vascular or parenchymal lung disease: thromboembolic lung disease, pulmonary arterial hypertension, and obstructive or restrictive lung disease (first second forced expiratory volume, <55%; forced vital capacity, 60%; and total lung capacity, <60%).

A group of patients with systemic hypertension who had never presented symptoms or signs of HF were prospectively enrolled as controls. Inclusion criteria were asymptomatic adults with ≥5 years' history of systemic arterial hypertension. Exclusion criteria were the same as those for patients with HFpEF.

The institutional Ethics and Research committee of our hospital approved this study. All patients gave written informed consent.

Controls and patients underwent echocardiogram, endothelial function assessment, and blood collection on the same day, 1 month after discharge if they had been hospitalized. Studies were performed in a blind fashion. Patients with PH who consented, underwent right heart catheterization in the next 2 weeks after noninvasive evaluation. Images and sera were stored for blinded analysis in a second phase.

Echocardiogram

Controls and patients underwent echocardiography evaluation using a commercially available ultrasound system (IE33; Philips Medical Systems, Andover, MA). All parameters were measured in 3 cardiac cycles (5 cycles in subjects with atrial fibrillation [AF]) and averaged. Right and LV dimensions, left atrium diameter and area, and right ventricular function estimated by tricuspid annular plane systolic excursion were reported. LVEF was assessed by the Simpson method from 2-dimensional (2D) apical 2- and 4-chamber views. Preserved systolic function was defined as EF≥50%. LV diastolic function was assessed with mitral inflow velocities (E, A) and average of septal and lateral mitral annulus early diastolic velocity by tissue Doppler (e′). E/A and E/e′ ratios were reported. Systolic pulmonary artery pressure (PAP) was estimated by Doppler echocardiography from the systolic right ventricular to right atrial pressure gradient, applying the Bernoulli equation to tricuspid regurgitant wave velocity. Right atrial pressure was estimated using the inferior vena cava diameter and inspiratory oscillations (range, 5–20 mm Hg), which was added to the calculated gradient to estimate systolic PAP. None of the participants had significant right ventricular outflow tract obstruction.

Right Heart Catheterization

Patients with HFpEF showing systolic PAP≥35 mm Hg on echocardiogram were proposed to undergo a right heart catheterization. The patient was placed in the supine position, in a fasting state, without premedication.

A 7F thermodilution balloon-tipped catheter (Baxter 139F75) was inserted percutaneously into the brachial, jugular, or femoral vein and advanced under fluoroscopy through the right heart cavities into the pulmonary artery. The pulmonary capillary wedge position was confirmed by the change from the typical pulmonary artery waveform to the typical pulmonary artery wedge pressure waveform on inflation of the balloon catheter. Pressure transducers were balanced against atmospheric pressure, and the zero reference level was 5 cm below the sternal angle. The following measurements were recorded as the mean of 3 consecutive beats in patients on sinus rhythm (5 beats in AF): right atrial pressure; systolic, diastolic, and mean PAP; pulmonary artery wedge pressure at end-expiration; and cardiac output as determined by the average of 3 thermal dilution curves. Cardiac cycles with fusion of 2 consecutive diastolic wavers, as a consequence of a short relative risk interval, were excluded from analysis. The following parameters were calculated: cardiac index as cardiac output divided by corporal surface area, transpulmonary gradient (TPG) as mean PAP minus pulmonary artery wedge pressure, and PVR as TPG divided by cardiac output.

Peripheral Endothelial Function

Controls and patients underwent peripheral endothelial function evaluation using a commercially available ultrasound system (Sonos 5500; Agilent Technologies, Andover, MA). The method has been previously described.[10] Briefly, all participants fasted and avoided exercise, stimulants, and medications for ≥6 hours before the test. They were placed in a quiet, darkened, temperature-controlled room, and all measurements were taken at a similar time of day. Their right arm rested comfortably in a cradle support of the imaged artery for ≥10 minutes before the measurements. A pressure cuff was placed 2 cm distal to the elbow crease. A stereotactic adjustable prop holder was used to achieve a steady image throughout the study, and the sample volume of the pulsed wave Doppler was placed in the middle of the artery as a reference marker. Longitudinal images were obtained by high-resolution ultrasound.

The standard 4-step protocol was used:

1. First baseline scan was recorded.
2. Endothelium-dependent vasodilation was assessed: pressure cuff was inflated ≤300 mm Hg for 5 minutes and released, leading to reactive hyperemia. Pulsed wave Doppler signal of brachial artery flow and 2D images were scanned 55 to 65 s after cuff release.
3. Second baseline scan was obtained after 10 minutes rest to allow vessel recovery.
4. Endothelium-independent vasodilation was assessed: 400 μg of sublingual nitroglycerin was administered and a fourth scan was obtained 3 minutes later.

Images were analyzed by 2 independent observers and averaged. Arterial diameters were determined in an end-diastole frame with dedicated software (QLab; Philips Healthcare, Eindhoven, The Netherlands), placing calipers from the trailing edge of the anterior wall interface to the leading edge of the posterior wall interface and averaging 5 cardiac cycles in patients with sinus rhythm and 10 in patients with AF. Peak brachial artery flow velocity was determined with pulse-Doppler sampling volume in the vessel lumen midline with software correction for the incident angle, at rest and for the first 15 s after forearm cuff release. Shear rate was calculated as 4×peak flow velocity/arterial diameter.

Flow-mediated vasodilation (FMD) was used as an index of endothelium-dependent dilation and was calculated as the maximal absolute and percentage change in brachial artery diameter after reactive hyperemia divided by that obtained from the first baseline scan (steps 1 and 2). Normal threshold for our laboratory was considered FMD>5%. Nitroglycerin-mediated dilation was used as an

index of endothelium-independent vasodilation and was calculated as the maximal absolute and percentage change in brachial artery diameter after nitroglycerin administration (steps 3 and 4). Using this methodology and a nested ANOVA, interobserver and intraobserver variance for brachial artery diameter measurement has been reported as 0.00012 (0.02% of total variability) and 0.00075 (0.13% of total variability), respectively.[11]

Collection of Blood Samples and Analysis of Extracellular Matrix Proteins

Circulating matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9), tissue metalloproteinase inhibitor 1 (TIMP-1) and C-terminal propeptide of type I procollagen (CICP) levels were measured when patients were stable, at least a month after hospital discharge. Blood was withdrawn from an antecubital vein into non-heparinized tubes. It was kept at room temperature for ≥20 minutes to allow clot formation and then centrifuged at 3000 rpm for 15 minutes at 4°C. Immediately after centrifugation, serum samples were aliquoted and stored at −80°C until assay. Commercially available ELISA kits were used for serum quantification, and their minimum analytic detection limit (DL) was as listed: DMP2F0 for MMP-2 DL=0.047 ng/mL, DMP900 for MMP-9 DL=0.156 ng/mL, DTM100 for TIMP-1 DL=0.08ng/mL (R&D Systems, Inc, Minneapolis, MN), and Microvue 8033 for CICP, DL=0.2ng/mL (Quidel Corporation, San Diego, CA).

Statistical Analysis

Participant characteristics are presented as percentage for qualitative variables and as median and quartiles for quantitative variables. Nonparametric tests were used for comparisons throughout the study: Fisher exact test was used to compare qualitative variables, and Mann–Whitney *U* test was used to compare quantitative variables. Correlation between plasma biomarkers or FMD and pulmonary hemodynamics was evaluated by linear regression analysis. Because there were extreme values for PVR and TPG, a sensitivity analysis for these variables was performed. All statistical analyses were performed using IBM SPSS 18 software (IBM Corporation, Armonk, NY). Statistical significance was set at 2-sided $P<0.05$.

Results

Population Characteristics

Twenty-eight patients with HFpEF were compared with 42 systemic hypertensive controls. Their demographic characteristics are shown in Table 1. As expected, patients with HFpEF had a much higher prevalence of AF (81% versus 2%; $P<0.001$) and were more often treated with β-blockers, aldosterone-receptor blockers, diuretics, insulin, vitamin K antagonists, and digoxin. The brain natriuretic peptide levels were higher in patients with HFpEF than in controls.

Echo Parameters

As shown in Table 2, there were no differences between patients with HFpEF and hypertensive controls in LV dimensions. When compared with hypertensive controls, patients with HFpEF had a larger right ventricle end-diastolic diameter, a worse right ventricular function (assessed by tricuspid annular plane systolic excursion), and signs of a more impaired diastolic function: increased right atrial size, higher E wave velocity, higher E/A ratio, and higher E/e' ratio.

The PAP estimated from tricuspid regurgitation jets could be analyzed in 33% of hypertensive controls and in 89% of the patients with HFpEF. None of the hypertensive controls and 22 (78%) of the patients with HFpEF showed an estimated systolic PAP>35 mm Hg.

Pulmonary Hemodynamics

Twenty patients with HFpEF and PH as determined by echocardiography consented to undergo a right heart catheterization. Their mean PAP was 38 (27–52) mm Hg, wedge capillary pressure was 18 (16–22) mm Hg, cardiac output 4.3 (3.1–5.4) L/min, and PVR 362 (235–603) dyn s cm^{-5} (Table 3).

Peripheral Endothelial Function

Baseline brachial artery diameter did not differ between hypertensive controls and patients with HFpEF. There was significantly less FMD in the HFpEF group when compared with hypertensive controls, both in absolute and percentage change from baseline diameter (β-coefficient, −0.18 mm [−0.28, −0.07]; $P=0.001$ and β-coefficient −4.41% [−7.17, −1.65];

Table 1. Population Characteristics of HTN and Patients With HFpEF

	HTN Controls (n=42)	HFpEF (n=28)	*P* Value
Age, y	68 (61–77)	71 (64–78)	0.283
Women, %	50	82	0.011
Height, cm	1.62 (1.58–1.70)	1.58 (1.55–1.65)	0.027
Weight, kg	75 (69–80)	73 (60–84)	0.290
Body mass index, kg/m²	27 (25–29)	27 (24–31)	0.858
Dyslipidemia, %	38	44	0.624
Diabetes mellitus,%	28	41	0.310
Atrial fibrillation, %	2	81	<0.001
Smoking, %	16	9	0.142
S-AP, mm Hg	134 (120–148)	125 (110–147)	0.151
D-AP, mm Hg	74 (68–83)	64 (56–71)	0.001
Pulse pressure, mm Hg	58 (51–71)	66 (46–77)	0.605
Heart rate, bpm	69 (59–79)	70 (58–80)	0.704
BNP, pg/mL	44 (18–60)	147 (82–294)	<0.001
Creatinine, mg/dL	0.83 (0.70–0.95)	1.12 (0.82–1.32)	0.016
GRF, mL/min per square meter	36 (31–38)	29 (25–36)	0.060
Na^+, mmol/L	141 (138–142)	141 (139–143)	0.750
Hemoglobin, g/dL	131 (116–137)	124 (105–133)	0.260
Treatment, %			
β-blocker, %	12	52	0.001
Calcium antagonist	19	26	0.562
ACE-inhibitor/ARB	83	74	0.546
Diuretic	36	85	<0.001
Insulin	0	22	0.003
Oral hypoglycemic drugs	24	30	0.78
Statins	37	44	0.615
Vitamin K antagonists	2	70	<0.001
Digoxin	0	41	<0.001
Nitrates	0	22	0.003
Hydralazine	0	4	0.397

Values are given as median and quartiles. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; D-SAP, diastolic systemic arterial pressure; GFR, glomerular filtration rate; HFpEF, heart failure and preserved ejection fraction; HTN, hypertension; and S-SAP, systolic systemic arterial pressure.

Table 2. Echocardiographic Findings of HTN and Patients With HFpEF

	HTN Controls (n=42)	HFpEF (n=28)	*P* Value
Ejection fraction, %	60 (60–65)	58 (55–62)	0.016
LVEDD, mm	48 (45–53)	50 (47–54)	0.203
LVESD, mm	30 (27–33)	32 (28–35)	0.163
RVEDD, mm	34 (30–36)	40 (37–44)	<0.001
IVS, mm	12 (11–13)	12 (12–14)	0.071
LPW, mm	11 (11,12)	12 (11–13)	0.093
Left atrium, mm	38 (35–41)	50 (42–57)	<0.001
Left atrium area, cm^2	19 (16–21)	26 (23–33)	<0.001
E wave velocity, cm/s	61 (53–72)	117 (80–149)	<0.001
Mitral E/A ratio	0.70 (0.59–0.81)	1.9 (0.8–3.5)	<0.001
Tissue Doppler e′ velocity, cm/s	8.3 (6.9–10.5)	9.4 (8–11.8)	0.089
Mitral E/e′ ratio	7.1 (5.6–9.7)	12.8 (9.4–17.8)	<0.001
TAPSE, mm	23 (20–26)	16 (13–21)	<0.001
Valid TR jet, %	33	89	<0.001
Mean estimated S-PAP, mm Hg	32 (28–34)	62 (55–88)	<0.001

Values are given as median and quartiles. HFpEF indicates heart failure and preserved ejection fraction; HTN, hypertension; IVS, interventricular septum; LPW, left ventricle posterior wall; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; RVEDD, right ventricle end-diastolic diameter; S-PAP, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion; and TR, tricuspid regurgitation.

P=0.002, respectively). After adjusting for age, sex, and nitrate use, differences remained significant (absolute FMD, *P*=0.001; β-coefficient 0.2 mm [0.08–0.32] and percentage FMD, *P*=0.001; β-coefficient, 5.18% [2.14–8.22]). Nitroglycerin-mediated dilation was similar in both groups (Table 4).

Among the 20 patients with HFpEF and PH who underwent right heart catheterization, subsequent analysis of the association between absolute and percentage FMD and PVR disclosed an inverse correlation (r=−0.679; *P*=0.002 and r=−0.623; *P*=0.006, respectively); in other words, the less

Table 3. Pulmonary Hemodynamics of Patients With Heart Failure and Preserved Ejection Fraction and Pulmonary Hypertension (n=20)

RAP, mm Hg	12 (8–13)
S-PAP, mm Hg	66 (59–92)
D-PAP, mm Hg	23 (17–32)
M-PAP, mm Hg	38 (27–52)
PAWP, mm Hg	18 (16–22)
CO, L/min	4.3 (3.1–5.4)
CI, L/min per square meter	2.3 (1.9–3)
TPG, mm Hg	18.5 (13–30.7)
PVR, dyn s cm^{-5}	362 (235–603)

Values are given as median and quartiles. CI indicates cardiac index; CO, cardiac output; D-PAP, diastolic pulmonary artery pressure; M-PAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; S-PAP, systolic pulmonary arterial pressure; and TPG, transpulmonary gradient.

Table 4. Peripheral Endothelial Function of HTN and Patients With HFpEF

	HTN Controls (n=42)	HFpEF (n=28)	*P* Value
Baseline brachial artery diameter, mm	4.22 (3.91–4.86)	4.11 (3.63–4.60)	0.634
Baseline PBFV, cm/s	113 (87–123)	108 (89–133)	0.763
Baseline shear rate, s^{-1}	993 (788–1208)	1020 (796–1430)	0.441
After cuff occlusion PBFV, cm/s	141 (127–170)	136 (117–171)	0.769
After cuff occlusion shear rate, s^{-1}	1235 (979–1590)	136 (117–171)	0.470
Absolute FMD, mm	0.21 (0.15–0.40)	0.10 (−0.03–0.19)	0.001
Percentage FMD, %	5.02 (3.90–10.12)	1.95 (−0.81–4.92)	0.002
Absolute NMD, mm	0.58 (0.29–0.67)	0.31 (0.19–0.55)	0.066
Percentage NMD, %	12.52 (6.63–15.74)	7.03 (3.89–14.23)	0.103

Values are given as median and quartiles. FMD indicates flow-mediated dilation; HFpEF, heart failure with preserved ejection fraction; HTN, hypertension; NMD, nitroglycerin-mediated dilation; and PBFV, peak blood flow velocity.

the brachial artery dilated in response to flow, the higher the PVR (Figure 1A).

Absolute and percentage FMD also showed a significant correlation with systolic PAP (r=−0.585; *P*=0.011 and r=−0.503; *P*=0.033, respectively), diastolic PAP (r=−0.573; *P*=0.013 and r=−0.514; *P*=0.029, respectively), mean PAP (r=−0.599; *P*=0.009 and r=−0.521; *P*=0.027, respectively; Figure 1B), cardiac output (r=0.520; *P*=0.037 and *P*=0.479; *P*=0.044, respectively), and TPG (r=−0.523; *P*=0.026 and r=−0.456; *P*=0.057, respectively). One patient showed high PVR and TPG and was considered an outlier. After excluding this patient from analysis, the correlation with PVR remained significant (absolute FMD, r=−0.586; *P*=0.013 and percentage FMD, r=−0.493; *P*=0.044) but not the correlation with TPG. No association was found with pulmonary artery wedge pressure.

Extracellular Matrix Proteins

Patients with HFpEF had higher MMP-2 and CICP values than hypertensive controls (β-coefficient, 36.09 ng/mL [12.26–59.93]; *P*=0.004 and β-coefficient, 18.36 ng/mL [6.15–30.57]; *P*=0.004, respectively; Figure 2). After adjusting for age and sex, differences remained significant (MMP-2 β-coefficient, 38.14 ng/mL [12.83–63.45]; *P*=0.004 and CICP β-coefficient, 20.43 ng/mL [6.68–34.17]; *P*=0.004). There were no differences in MMP-9 (β-coefficient, −87.26 ng/mL [−296.44 to 121.92]; *P*=0.404) or TIMP-1 values (β-coefficient, 21.17 ng/mL [−15.55 to 57.90]; *P*=0.254) between groups.

In the 20 patients who underwent a right heart catheterization, CICP values showed a positive linear correlation with mean PAP (r=0.513; *P*=0.029), TPG (r=0.522; *P*=0.026), and PVR (r=0.597; *P*=0.009; Figure 3). MMP-2, MMP-9, TIMP-1, and brain natriuretic peptide levels were not significantly correlated with pulmonary hemodynamic parameters. One patient showed high PVR and TPG and was considered an outlier. After excluding this patient from analysis, the correlation with PVR and TPG was not statistically significant.

Participants in the highest tertile of MMP-2 levels had significantly less absolute FMD than those in the first tertile (0.13

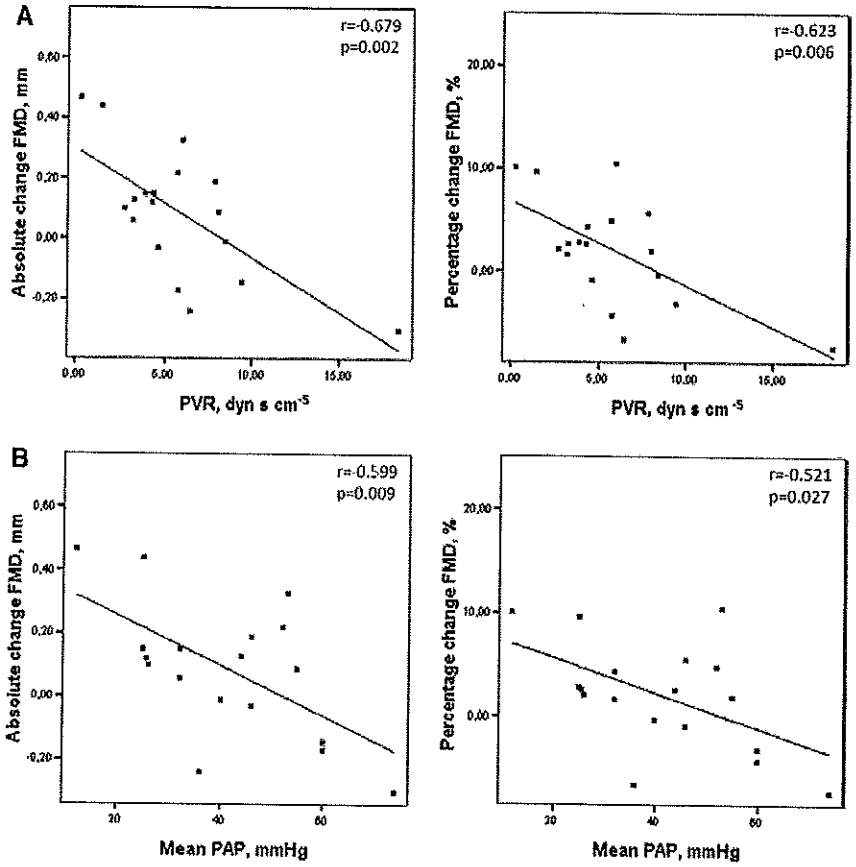

Figure 1. Relationship between peripheral endothelial dysfunction and pulmonary hemodynamics. Percentage and absolute changes in flow-mediated dilation (FMD) disclosed an inverse correlation with pulmonary vascular resistance (PVR; **A**) and mean PAP (**B**), showing that the less the brachial artery dilation in response to flow, the higher the PVR and mean pulmonary artery pressure (PAP).

[−0.01 to 0.23] mm versus 0.22 [0.16 to 0.37] mm; P=0.025). There was a moderate inverse correlation between MMP-2 or CICP values and lower FMD (r=−0.276; P=0.034 and r=−0.306; P=0.018, respectively). No association was found between FMD and MMP-9 or TIMP-1 values.

Discussion

This study shows that patients with HFpEF have impaired peripheral endothelial function when compared with patients with systemic hypertension and diastolic dysfunction who had never presented HF symptoms. In patients with HFpEF and PH, invasive pulmonary hemodynamics disclosed a remarkable precapillary component (shown by increased TPG and PVR). In these patients, we described for the first time an inverse correlation between PVR and peripheral endothelial function. Patients with increased collagen metabolism proteins showed higher PAP, TPG, and PVR and worse PED.

HFpEF is an increasingly prevalent pathology whose underlying mechanisms are not yet understood. The development of PH in a patient with hypertension and diastolic dysfunction might be related to the development of HF symptoms. Some studies have reported a surprisingly high prevalence of PH among patients with HFpEF at baseline[7] or during exercise[12] and described an important vasoreactive component.[7,13–15] Moreover, the presence of PH has been associated with an increased mortality in this population.[16–19] For these reasons, some authors have studied the potential benefit of pulmonary vasodilators in HFpEF, with controversial results, mainly because of different inclusion criteria related to the presence of PH.[14,20]

Peripheral Endothelial Dysfunction

Studies of patients with HF have described PED in the presence of preserved[5] and reduced[21] EF, as well as pulmonary arterial hypertension.[22] The presence of PED has been identified as an independent predictor of cardiovascular events and mortality in the population with HF, HFpEF, and reduced EF,[5,23] but the mechanism that mediates this association is poorly understood.

Using invasive measurements, we identified an important precapillary component in PH secondary to HFpEF in addition to the postcapillary contribution of pulmonary venous congestion. Our findings indicate a relationship between an impaired peripheral endothelial function and the presence and degree of PH in this subset of patients. This relationship has been described in idiopathic pulmonary arterial hypertension,[24] in PH associated with congenital heart disease[25] and in connective tissue diseases, such as scleroderma,[26] but has never before been reported in HFpEF. The presence of PED may be associated with impaired pulmonary endothelial function and could account for the precapillary component of the PH that has been described in these patients. The worse prognosis of patients with HFpEF and PED compared with patients without PED could be at least partly related to the association between PED and PH.

Extracellular Collagen Metabolism

Previous studies have shown a progressive increase in extracellular matrix protein circulating levels in healthy controls, patients with hypertension, and patients with HFpEF.[27,28] An

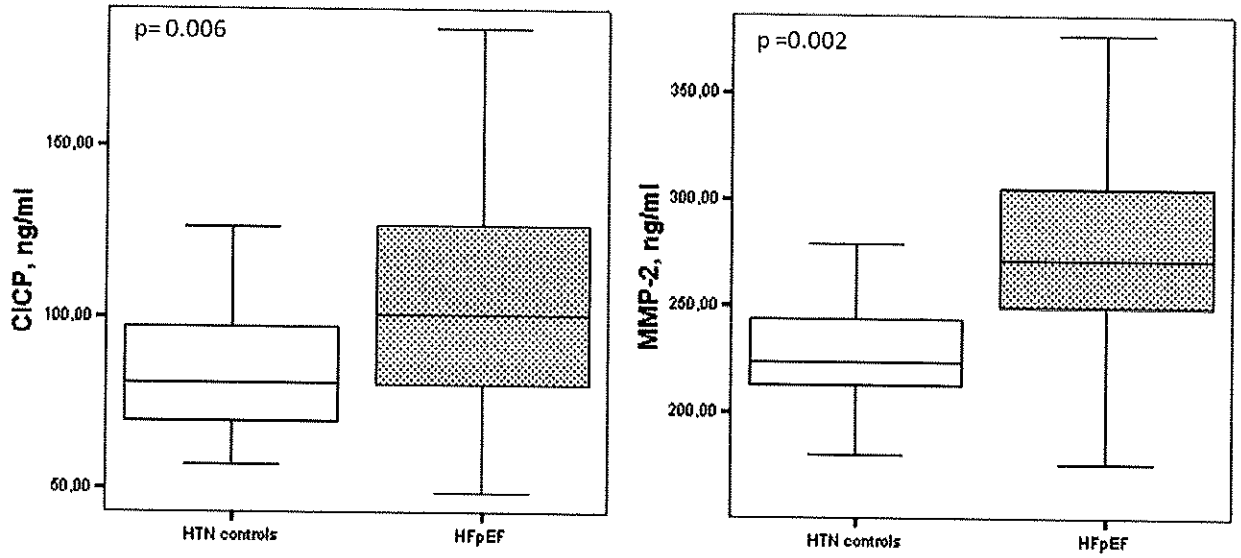

Figure 2. Comparison of serum extracellular matrix protein levels. Patients with heart failure with preserved ejection fraction (HFpEF) showed significantly higher circulating levels of C-terminal propeptide of type I procollagen (CICP) and matrix metalloproteinase (MMP)-2 when compared with hypertensive (HTN) controls.

increased collagen turnover has been linked to a more severe diastolic dysfunction[1] and arterial stiffness[29]; therefore, it has been proposed as an etiopathogenic mechanism for HFpEF. Also, high levels of circulating extracellular matrix proteins have been related to the presence of severe pulmonary arterial hypertension.[30] Consistent with previous reports, patients with HFpEF in our study showed significantly higher levels of MMP-2 and CICP compared with systemic hypertensive controls. We could also establish a statistical relationship between increased circulating extracellular matrix protein levels, higher PH invasively determined parameters, and a more impaired peripheral endothelial function. More studies are needed to assess whether collagen metabolism may play a role in the development of endothelial dysfunction and PH, or whether it is just a nonspecific marker of overall HFpEF severity.

Limitations

First, we were able to demonstrate an association between FMD and PH, but the observational design of the study does not allow us to suggest a causal relationship.

Second, the small sample size of our study is because of its invasive nature. However, in contrast to most of the previous studies where indirect measurements are shown, we provide more reliable data. Moreover, our results are consistent with previous literature on HFpEF or PH. Third, there were some differences in baseline characteristics between HFpEF and controls about sex, medical treatment, and AF prevalence. Although peripheral endothelial function measurements were made after 6 hours of medication washout, hypertensive controls, and patients with HFpEF differed in their baseline pharmacological treatment. Spirinolactone, β-blockers, calcium blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blocker have been reported to improve endothelial function[31–34] and were in fact more common in the HFpEF group; consequently, differences between the groups in peripheral endothelial function could have been underestimated. Fourth, our population had higher PAP and PVR values when compared with previous studies,[13] which may indicate a selection bias related to the high complexity of our center and the referral of patients with more severe HF. Prevalence of

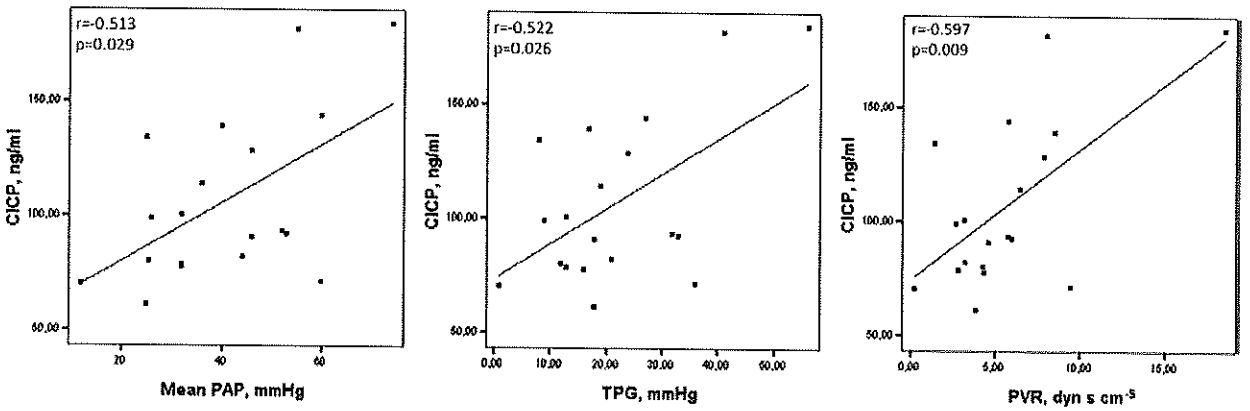

Figure 3. Correlation between serum extracellular matrix proteins and pulmonary hemodynamics. In patients with heart failure and preserved ejection fraction who had pulmonary hypertension, C-terminal propeptide of type I (CICP) levels showed a significant positive correlation with mean pulmonary artery pressure (PAP), transpulmonary gradient (TPG), and pulmonary vascular resistance (PVR) values.

AF was also higher than previously reported for patients with HFpEF, perhaps accounting for the severity[35] of the disease in our cohort. The potential influence of AF on our results must be acknowledged because pulse irregularity has been reported to be a risk factor for PED independently of HF phenotype.[36] It is difficult to discern how much of the differences in FMD are specific to the presence of HFpEF or related to AF. The relationship between FMD and PVR in our patients supports the idea that PED may be related to PH in HFpEF, but the influence of AF in this finding is unclear and should be addressed in future studies.

Conclusions

This study provides evidence that patients with HFpEF have an impaired peripheral endothelial function when compared with hypertensive controls, and that it is associated with the presence of PH and high PVR. Extracellular collagen metabolism abnormalities can be detected in this population. Routine assessment of peripheral endothelial function and extracellular collagen metabolism could help us to identify a subgroup of patients with HFpEF at higher risk for the development of PH and provide a rationale for treating this selected group with pulmonary vasodilators.

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Disclosures

None.

References

1. Bursi F, Weston SA, Redfield MM, Jacobsen SJ, Pakhomov S, Nkomo VT, Meverden RA, Roger VL. Systolic and diastolic heart failure in the community. *JAMA*. 2006;296:2209–2216.
2. Klapholz M, Maurer M, Lowe AM, Messineo F, Meisner JS, Mitchell J, Kalman J, Phillips RA, Steingart R, Brown EJ Jr, Berkowitz R, Moskowitz R, Soni A, Mancini D, Bijou R, Sehhat K, Varshneya N, Kukin M, Katz SD, Sleeper LA, Le Jemtel TH; New York Heart Failure Consortium. 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*. 2004;43:1432–1438.
3. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Køber L, Lip GY, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Rønnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A, Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S, McDonagh T, Sechtem U, Bonet LA, Avraamides P, Ben Lamin HA, Brignole M, Coca A, Cowburn P, Dargie H, Elliott P, Flachskampf FA, Guida GF, Hardman S, Iung B, Merkely B, Mueller C, Nanas JN, Nielsen OW, Orn S, Parissis JT, Ponikowski P; Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology; ESC Committee for Practice Guidelines. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2012;14:803–869.
4. Martos R, Baugh J, Ledwidge M, O'Loughlin C, Conlon C, Patle A, Donnelly SC, McDonald K. Diastolic heart failure: evidence of increased myocardial collagen turnover linked to diastolic dysfunction. *Circulation*. 2007;115:888–895.
5. Borlaug BA, Olson TP, Lam CS, Flood KS, Lerman A, Johnson BD, Redfield MM. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2010;56:845–854.
6. Akiyama E, Sugiyama S, Matsuzawa Y, Konishi M, Suzuki H, Nozaki T, Ohba K, Matsubara J, Maeda H, Horibata Y, Sakamoto K, Sugamura K, Yamamuro M, Sumida H, Kaikita K, Iwashita S, Matsui K, Kimura K, Umemura S, Ogawa H. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. *J Am Coll Cardiol*. 2012;60:1778–1786.
7. Lam CS, Roger VL, Rodeheffer RJ, Borlaug BA, Enders FT, Redfield MM. Pulmonary hypertension in heart failure with preserved ejection fraction: a community-based study. *J Am Coll Cardiol*. 2009;53:1119–1126.
8. Cooper CJ, Landzberg MJ, Anderson TJ, Charbonneau F, Creager MA, Ganz P, Selwyn AP. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. *Circulation*. 1996;93:266–271.
9. Cooper CJ, Jevnikar FW, Walsh T, Dickinson J, Mouhaffel A, Selwyn AP. The influence of basal nitric oxide activity on pulmonary vascular resistance in patients with congestive heart failure. *Am J Cardiol*. 1998;82:609–614.
10. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Lüscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation*. 2012;126:753–767.
11. Sitges M, Heras M, Roig E, Durán M, Masotti M, Zurbano MJ, Roqué M, Sanz G. Acute and mid-term combined hormone replacement therapy improves endothelial function in post-menopausal women with angina and angiographically normal coronary arteries. *Eur Heart J*. 2001;22:2116–2124.
12. Borlaug BA, Nishimura RA, Sorajja P, Lam CS, Redfield MM. Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. *Circ Heart Fail*. 2010;3:588–595.
13. Afshar M, Collado F, Doukky R. Pulmonary hypertension in elderly patients with diastolic dysfunction and preserved ejection fraction. *Open Cardiovasc Med J*. 2012;6:1–8.
14. Guazzi M, Vicenzi M, Arena R, Guazzi MD. Pulmonary hypertension in heart failure with preserved ejection fraction: a target of phosphodiesterase-5 inhibition in a 1-year study. *Circulation*. 2011;124:164–174.
15. Hill NS, Preston I, Roberts K. Defining the phenotypes for pulmonary hypertension associated with diastolic heart failure. *Circ Heart Fail*. 2011;4:238–240.
16. Kjaergaard J, Akkan D, Iversen KK, Kjoller E, Køber L, Torp-Pedersen C, Hassager C. Prognostic importance of pulmonary hypertension in patients with heart failure. *Am J Cardiol*. 2007;99:1146–1150.
17. Ghio S, Gavazzi A, Campana C, Inserra C, Klersy C, Sebastiani R, Arbustini E, Recusani F, Tavazzi L. Independent and additive prognostic value of right ventricular systolic function and pulmonary artery pressure in patients with chronic heart failure. *J Am Coll Cardiol*. 2001;37:183–188.
18. Bursi F, McNallan SM, Redfield MM, Nkomo VT, Lam CS, Weston SA, Jiang R, Roger VL. Pulmonary pressures and death in heart failure: a community study. *J Am Coll Cardiol*. 2012;59:222–231.
19. Carrasco-Sánchez FJ, Ortiz-López E, Galisteo-Almeda L, Camacho-Vázquez C, Ruiz-Frutos C, Pujol-De La Llave E. Prognostic importance of pulmonary hypertension in heart failure with preserved ejection fraction. *Rev Clin Esp*. 2010;210:489–496.
20. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, Felker GM, Goldsmith SR, Bart BA, McNulty SE, Ibarra JC, Lin G, Oh JK, Patel MR, Kim RJ, Tracy RP, Velazquez EJ, Anstrom KJ, Hernandez AF, Mascette AM, Braunwald E; RELAX Trial. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA*. 2013;309:1268–1277.
21. Sitges M, Roig E, Morales M, Azqueta M, Pérez Villa F, Paré C, Orús J, Heras M, Sanz G. Impaired endothelium-dependent forearm vasodilation in idiopathic dilated cardiomyopathy is related to severe left ventricular dysfunction and elevated serum tumor necrosis factor levels. *Rev Esp Cardiol (Engl Ed)*. 2005;58:477–483.

22. Wolff B, Lodziewski S, Bollmann T, Opitz CF, Ewert R. Impaired peripheral endothelial function in severe idiopathic pulmonary hypertension correlates with the pulmonary vascular response to inhaled iloprost. *Am Heart J*. 2007;153:1088.e1–1088.e7.
23. Katz SD, Hryniewicz K, Hriljac I, Balidemaj K, Dimayuga C, Hudaihed A, Yasskiy A. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. *Circulation*. 2005;111:310–314.
24. Peled N, Bendayan D, Shitrit D, Fox B, Yehoshua L, Kramer MR. Peripheral endothelial dysfunction in patients with pulmonary arterial hypertension. *Respir Med*. 2008;102:1791–1796.
25. Nakamura M, Yoshida H, Naganuma Y, Kon H, Sugawara S, Hiramori K. Peripheral vasodilatory dysfunction in adult patients with congenital heart disease and severely elevated pulmonary vascular resistance. *Angiology*. 2002;53:715–720.
26. Peled N, Shitrit D, Fox BD, Shlomi D, Amital A, Bendayan D, Kramer MR. Peripheral arterial stiffness and endothelial dysfunction in idiopathic and scleroderma associated pulmonary arterial hypertension. *J Rheumatol*. 2009;36:970–975.
27. González A, López B, Ravassa S, Beaumont J, Arias T, Hermida N, Zudaire A, Díez J. Biochemical markers of myocardial remodelling in hypertensive heart disease. *Cardiovasc Res*. 2009;81:509–518.
28. Zhou S, Feely J, Spiers JP, Mahmud A. Matrix metalloproteinase-9 polymorphism contributes to blood pressure and arterial stiffness in essential hypertension. *J Hum Hypertens*. 2007;21:861–867.
29. Yasmin SW, McEniery CM, Dakham Z, Pusalkar P, Maki-Petala K, Ashby MJ, Cockcroft JR, Wilkinson IB. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biology*. 2005;25:372–378.
30. Schumann C, Lepper PM, Frank H, Schneiderbauer R, Wibmer T, Kropf C, Stoiber KM, Rüdiger S, Kruska L, Krahn T, Kramer F. Circulating biomarkers of tissue remodelling in pulmonary hypertension. *Biomarkers*. 2010;15:523–532.
31. Farquharson CA, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation*. 2000;101:594–597.
32. Nishioka K, Nakagawa K, Umemura T, Jitsuiki D, Ueda K, Goto C, Chayama K, Yoshizumi M, Higashi Y. Carvedilol improves endothelium-dependent vasodilation in patients with dilated cardiomyopathy. *Heart*. 2007;93:247–248.
33. Ghiadoni L, Versari D, Magagna A, Kardasz I, Plantinga Y, Giannarelli C, Taddei S, Salvetti A. Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients. *J Hypertens*. 2007;25:361–366.
34. Koh KK, Han SH, Ahn JY, Chung WJ, Lee Y, Shin EK. Amlodipine improves endothelial function and metabolic parameters in patients with hypertension. *Int J Cardiol*. 2009;133:23–31.
35. Zakeri R, Chamberlain AM, Roger VL, Redfield MM. Temporal relationship and prognostic significance of atrial fibrillation in heart failure patients with preserved ejection fraction: a community-based study. *Circulation*. 2013;128:1085–1093.
36. Guazzi M, Arena R. Endothelial dysfunction and pathophysiological correlates in atrial fibrillation. *Heart*. 2009;95:102–106.

CLINICAL PERSPECTIVE

Heart failure with preserved ejection fraction (HFpEF) is an increasingly common diagnosis and is often associated with pulmonary hypertension. Peripheral endothelial dysfunction (PED) and collagen metabolism abnormalities have been described in this population. Our aim was to determine whether pulmonary hypertension is associated with PED and impaired collagen metabolism in patients with HFpEF. A group of 28 patients with HFpEF was compared with a group of 42 hypertensive controls: echocardiograms, PED studies, and analyses of collagen metabolism were performed. Patients with HFpEF who showed pulmonary artery pressure ≥35 mm Hg by echocardiogram also underwent right heart catheterization. Patients with HFpEF had impaired PED and collagen metabolism compared with hypertensive controls. Interestingly, we were able to describe for the first time a correlation between PED and pulmonary vascular resistance, assessed by invasive pulmonary hemodynamics, in patients with HFpEF and pulmonary hypertension. Weaker correlations were also found between collagen metabolism and pulmonary hemodynamics. Our results are important because pulmonary hypertension has important prognostic implications in heart failure. Therefore, noninvasive assessment of endothelial function and collagen metabolism could help us to identify a subgroup of patients with heart failure at higher risk for the development of out of proportion pulmonary hypertension and provide rationale for future treatment strategies in this selected group.