

# Pulmonary haemodynamic effects of interatrial shunt in heart failure with preserved ejection fraction: a preclinical study



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## KEYWORDS

- chronic heart failure
- innovation
- preclinical research

## Abstract

**Aims:** The aim of this study was to evaluate the effect of the creation of a left-to-right interatrial shunt on pulmonary haemodynamics in rats with heart failure with preserved ejection fraction (HFPEF).

**Methods and results:** An interatrial communication (IAC) was created in 11 healthy rats (Lewis rats) and 11 rats which developed HFPEF (36-week-old spontaneously hypertensive rats [SHR]). Effects of the interatrial shunt were compared to 11 sham-operated Lewis and 11 sham-operated SHR. At 45 days post shunt, strain effect was observed in diastolic function (E/A ratio,  $p < 0.001$ ; isovolumetric relaxation time,  $p < 0.001$ ), left atrial volume ( $p = 0.005$ ) and pulmonary wall shear rate (WSR) ( $p = 0.02$ ) measured by Doppler echo. At sacrifice of the animals (60 days), a strain effect was also noted in elastin density ( $p = 0.003$ ) and eNOS protein expression ( $p = 0.001$ ). Interatrial shunt creation resulted in (i) an increase in pulmonary WSR ( $p = 0.04$ ) and a decrease in left atrial volume ( $p < 0.001$ ), (ii) an increase in elastin density ( $p < 0.005$ ), and (iii) an increase in eNOS protein expression ( $p = 0.03$ ).

**Conclusions:** Creation of a left-to-right atrial shunt in rats with HFPEF was effective in improving pulmonary haemodynamics. In addition, this study provides preliminary evidence of the potential risk of right volume overload and pulmonary hypertension due to atrial shunting.

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## Abbreviations

<b>ANP</b>	atrial natriuretic peptide
<b>CF</b>	cardiac frequency
<b>HFPEF</b>	heart failure with preserved ejection fraction
<b>IAC</b>	interatrial communication
<b>IVRT</b>	isovolumetric relaxation time
<b>LV</b>	left ventricle
<b>PA</b>	pulmonary artery
<b>SHR</b>	spontaneous hypertensive rat(s)
<b>VTI</b>	velocity time integral
<b>WSR</b>	wall shear rate

## Introduction

Heart failure is an increasingly common disease related to population ageing<sup>1</sup>. In clinical nosology, cardiac failure is identified either with a decrease in left ventricular ejection fraction (LVEF) or with a preserved ejection fraction (heart failure preserved ejection fraction [HFPEF]), always associated with an increase in tele-diastolic pressure<sup>2</sup> and left ventricular hypertrophy. The incidence of HFPEF is constantly increasing and represents up to 50% of patients with heart failure<sup>3</sup>. However, pharmacological treatment does not improve survival<sup>4</sup>, in contrast to what is observed in heart failure with decreased ejection fraction; alternative therapy is eagerly awaited.

Ben Driss et al studied the haemodynamic phenomena associated with HFPEF caused by a small left ventricular infarct in rats in an experimental model<sup>5</sup>. In this model, while there was no change in cardiac output or peripheral arterial circulation, Doppler ultrasound demonstrated an impact on the pulmonary circulation. Increase in pulmonary pressure is responsible for passive dilation of the pulmonary arteries (PA) and veins, because of the high capacitance of these low-pressure pulmonary arterial and venous circulations<sup>6</sup>. As a result, endothelial wall shear rate (WSR) is reduced in the pulmonary circulation. Decrease in WSR alters endothelial function in the pulmonary circulation, including decreasing NO synthase expression and a tendency to increase endothelin<sup>7</sup> and IL-6<sup>8</sup> expression and secretion. If the pressure stimulus persists, functional and structural remodelling occurs in the pulmonary arterial circulation, leading to pulmonary hypertension and right heart failure<sup>9</sup>.

Due to this pulmonary impact related to the increase in diastolic pressure above the left ventricle (LV), the creation of a shunt between the left and right atria in the clinical setting was recently proposed<sup>10</sup>. In the context of heart failure, this shunt decreases left atrial and pulmonary arterial pressures, at rest as well as during exercise, which improves symptoms during exercise (dyspnoea on exertion). The effect of an interatrial shunt on pulmonary haemodynamics has not been studied in experimental HFPEF to date.

The aim of this study was to develop a new model of interatrial shunt in rats, and to evaluate the effect of this left-to-right shunt on pulmonary haemodynamics in HFPEF. For this purpose, a left-to-right shunt was created (interatrial communication [IAC]) in normotensive healthy inbred Lewis rats and spontaneous hypertensive

rats (SHR). SHR develop compensated heart failure (HFPEF) in the first year of life, before dying at two years with decompensated cardiac heart failure<sup>11</sup>. The impacts of rat strain and of the interatrial shunt were evaluated using Doppler echocardiography and biological analyses at sacrifice.

## Materials and methods

### EXPERIMENTAL DESIGN

Four groups of animals were studied according to an experimental protocol of Latin square design - two groups of 11 inbred Lewis rats 11 weeks old weighing 400±25 gr (group 1 was sham-operated whereas IAC was created in group 2), and two groups of 11 inbred SHR 36 weeks old, of identical weight (all from Envigo, Gannat, France) (group 3 was sham-operated whereas IAC was created in group 4).

Thirty-six-week-old SHR are hypertensive rats which develop HFPEF without systolic dysfunction<sup>12</sup>. In addition, the SHR strain was derived from the Wistar rat strain<sup>13</sup>.

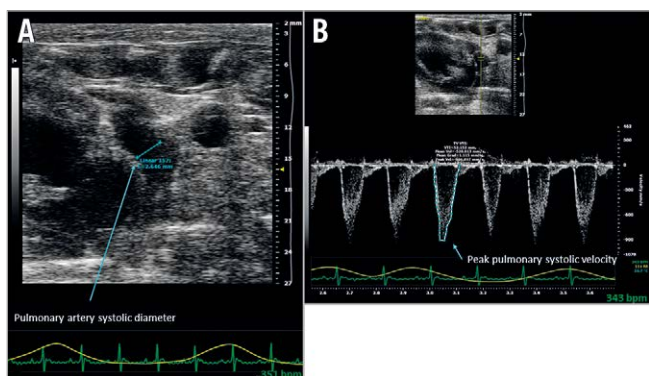
### EXPERIMENTAL RAT MODEL OF IAC

The rats were anaesthetised with isoflurane 4%, then intubated and ventilated. Anaesthesia was maintained by isoflurane 2%. After right thoracotomy and pericardiectomy, a purse-string suture was performed on the right atrium using an 8/0 polypropylene wire. A micro-balloon catheter (Mini Trek<sup>®</sup>, 2 mm in diameter, 8 mm in length; Abbott Vascular, Santa Clara, CA, USA) was introduced through this purse string to perforate the interatrial septum. Then, the purse string was tightened simultaneously to catheter removal. Haemostasis was completed using SURGICEL<sup>®</sup> (Cordis, Cardinal Health, Milpitas, CA, USA) and simple stitches of 8/0 polypropylene when necessary. At the end of the intervention, the rats were subjected to volume expansion by receiving 10 mL of NaCl 0.9% subcutaneously together with an injection of analgesic (buprenorphine 0.05 mg/kg subcutaneously). The sham-operated rats underwent a similar procedure without creation of an IAC.

The project was evaluated by the Animal Experiments Ethics Committee N°121 (French Ministry of Higher Education, Research and Innovation) and received a favourable decision. The project authorisation reference number was APAFIS#12184-20171115151515174156 v6.

### DOPPLER ECHOCARDIOGRAPHIC STUDY 45 DAYS AFTER OPERATION

Forty-five days after surgery, the rats were anaesthetised with 2% isoflurane in order to perform Doppler echocardiography (Vevo<sup>®</sup> 2100, probe MS-200; VisualSonics, Toronto, ON, Canada). In the parasternal and apical sections we measured the following: (i) diameter and maximal systolic velocity of the PA, as measured 10 mm downstream of the pulmonary valve, allowing calculation of WSR in the PA (in s<sup>-1</sup>) (**Figure 1**), the formula used to calculate endothelial WSR in the PA being  $\tau = (4x \text{ max. systolic velocity}) / (\text{PA systolic diameter})^{14}$ ; (ii) diastolic function evaluated by measurement of the transmitral Doppler E/A ratio and by measurement



**Figure 1.** Doppler ultrasound wall shear rate measurement in the pulmonary artery. ( $\tau = [4 \times \text{max. systolic velocity}] / [\text{pulmonary artery systolic diameter}]$ ) at 45 days. A) Measurement of pulmonary artery systolic diameter, as measured 10 mm downstream of the pulmonary valve. B) Measurement of maximum systolic velocity in the pulmonary artery, as measured 10 mm downstream of the pulmonary valve.

of isovolumetric relaxation time (IVRT in ms)<sup>15</sup>; (iii) ejection fraction obtained by calculating the Teichholz index; (iv) systemic cardiac output (in mL/min) calculated from cardiac frequency (CF), velocity time integral (VTI) and diameter of outflow tract of the LV (d) using the formula  $\Pi d^2/4 \times \text{ITV} \times \text{CF}$ ; and (v) anteroposterior diameter of the left atrium (D), as measured next to the aortic valve, permitting calculation of the left atrial volume (in mm<sup>3</sup>) using the formula  $\Pi/6 \times D^3$ .

### MORPHOLOGICAL STUDY

Sixty days after the operation, the animals were weighed and then sacrificed under anaesthesia (pentobarbital 0.2 mg/kg intraperitoneal). Hearts and lungs were harvested and weighed separately. Patency of IAC was verified visually after opening the left and right atria. Rats presenting a non-patent IAC were excluded from the study. PA, LV, right ventricle and right and left atria were dissected out and weighed. The PA was removed and fixed in 10% formaldehyde, then embedded in paraffin, and sections of 5  $\mu\text{m}$  thickness were used for histological analysis. After staining with orcein, a morphometric study of the PA was performed, including quantification of media thickness and elastin. Elastin density (%) was obtained by calculating the ratio between elastin density and media thickness.

### ATRIAL NATRIURETIC PEPTIDE (ANP)

A blood sample was collected on ethylenediamine tetraacetic acid (EDTA) during sacrifice to measure the plasma ANP concentration, using an ELISA kit (RayBiotech, Peachtree Corners, GA, USA) according to the manufacturer's instructions.

### WESTERN BLOT OF eNOS

eNOS is a marker of endothelial WSR<sup>16</sup>. At sacrifice, a lung fragment was directly frozen and then pulverised using liquid nitrogen with mechanical stirring. On this sample, eNOS protein expression was quantified in a western blot analysis (**Supplementary Appendix 1**).

### STATISTICAL ANALYSIS

Continuous variables were expressed as means  $\pm$  standard deviation. Normal distribution was verified using the Kolmogorov-Smirnov test, and homogeneity of variance was tested by Levene's test. Two-factor analysis of variance (interatrial shunt/strain factor, two-way ANOVA) was performed for testing the effect of strain and the effect of the shunt. Distribution of PA systolic diameter followed a normal distribution; therefore, inter-group comparison for this parameter was made using the Student's t-test. A p-value of  $<0.05$  was considered significant. All calculations were made using SPSS software, Version 20.0.0.0 (IBM Corp., Armonk, NY, USA). Figures were created using GraphPad Prism v7.0 (GraphPad Software, La Jolla, CA, USA).

### Results

#### RAT CHARACTERISTICS (Figure 2, Supplementary Table 1)

Forty-six rats were operated; two Lewis rats had a non-permeable IAC and were excluded from the investigation. In total, 11 rats were included in each group. A strain effect was noted for the weights normalised to body weight for lungs (SHR  $>$  Lewis,  $F=27.5$ ,  $p<0.001$ ), whole heart (SHR  $>$  Lewis,  $F=52.3$ ,  $p<0.001$ ), LV (SHR  $>$  Lewis,  $F=641.1$ ,  $p<0.001$ ), right ventricle (SHR  $>$  Lewis,  $F=23.2$ ,  $p<0.001$ ) and the right and left atria (SHR  $>$  Lewis,  $F=14.0$ ,  $p=0.001$ ). Creation of IAC did not change body, heart or lung weights (IAC vs sham). The weight of Lewis and SHR was similar 60 days after intervention ( $430 \pm 52$  versus  $412 \pm 28$  gr,  $p=0.145$ ).

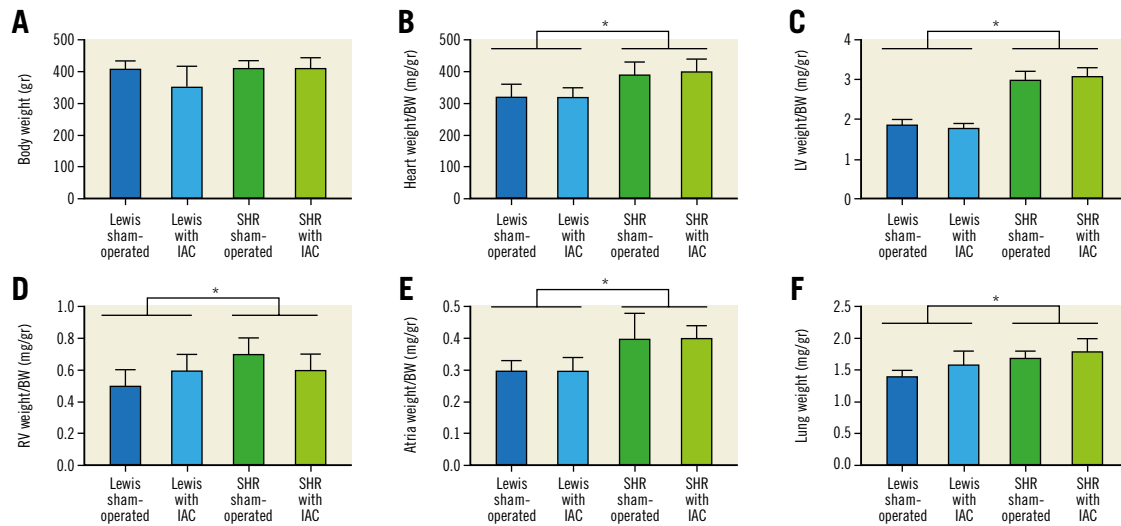
#### DOPPLER ECHOCARDIOGRAPHY (Figure 3, Supplementary Table 2)

A strain effect was observed in heart rate (SHR  $<$  Lewis,  $F=33.5$ ,  $p<0.001$ ) and in diastolic function: E/A ratio was greater ( $F=17.3$ ,  $p<0.001$ ) and IVRT was longer ( $F=36.4$ ,  $p<0.001$ ) in SHR compared to Lewis rats. Furthermore, in SHR, atrial volume ( $F=4.5$ ,  $p=0.041$ ) and LVEF ( $F=23.7$ ,  $p<0.001$ ) were higher than in Lewis rats. Finally, a strain effect was noted in PA WSR (SHR  $>$  Lewis,  $F=5.8$ ,  $p=0.02$ ). Creation of an IAC resulted in a significant increase in PA WSR ( $F=4.3$ ,  $p=0.04$ ) in both SHR and Lewis rats. IAC also tended to reduce IVRT ( $F=3.6$ ,  $p=0.07$ ) (**Figure 4**) and decreased left atrial volume ( $F=18.8$ ,  $p<0.001$ ), but did not change heart rate.

A statistical interaction occurred between rat strain and IAC showing that the left atrial volume decreased more in SHR than in Lewis rats ( $F=5.1$ ,  $p=0.03$ ). PA systolic diameter increased in the Lewis strain ( $2.9 \pm 0.2$  versus  $3.1 \pm 0.3$ ,  $p=0.04$ ) but decreased in SHR ( $2.9 \pm 0.3$  versus  $2.6 \pm 0.1$ ,  $p=0.001$ ) after IAC creation.

#### PLASMA ANP CONCENTRATION

ANP concentration was  $7.1 \pm 5.5$   $\mu\text{g/mL}$  in sham-operated Lewis rats,  $11.3 \pm 10.1$   $\mu\text{g/mL}$  in Lewis rats with IAC,  $5.6 \pm 4.9$   $\mu\text{g/mL}$  in sham-operated SHR, and  $8.6 \pm 3.3$   $\mu\text{g/mL}$  in SHR with IAC. There was no significant effect of strain ( $p=0.29$ ) or shunt ( $p=0.08$ ) on ANP concentration.

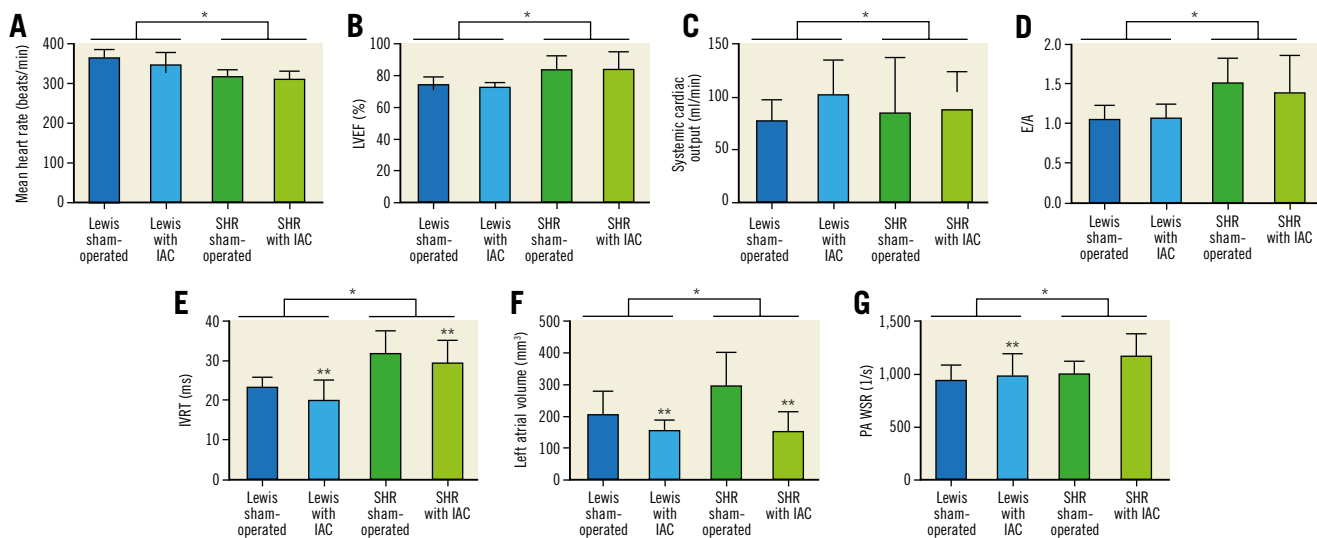


**Figure 2.** Weights of body, heart and lungs. A) Body weight, gr. strain effect:  $F=2.4$ ,  $p=NS$ . IAC effect:  $F=3.6$ ,  $p=NS$ . B) Heart weight/body weight, mg/gr. strain effect:  $F=52.3$ ,  $*p<0.001$ . IAC effect:  $F=0.2$ ,  $p=NS$ . C) Left ventricle weight/body weight, mg/gr. strain effect:  $F=6.41$ ,  $*p<0.001$ . IAC effect:  $F=0.2$ ,  $p=NS$ . D) Right ventricle weight/body weight, mg/gr. strain effect:  $F=23.2$ ,  $*p<0.001$ . IAC effect:  $F=0.1$ ,  $p=NS$ . E) Atria weight/body weight, mg/gr. strain effect:  $F=14.0$ ,  $*p<0.001$ . IAC effect:  $F=0.9$ ,  $p=NS$ . F) Lung weight, gr. strain effect:  $F=27.5$ ,  $*p<0.001$ . IAC effect:  $F=3.5$ ,  $p=NS$ . BW: body weight; IAC: interatrial communication; LV: left ventricle; NS: non-significant; RV: right ventricle; SHR: spontaneous hypertensive rats

#### MEASUREMENT OF PA MEDIA THICKNESS AND ELASTIN DENSITY (ELASTIN/MEDIA THICKNESS RATIO) BY QUANTITATIVE HISTOMORPHOMETRY

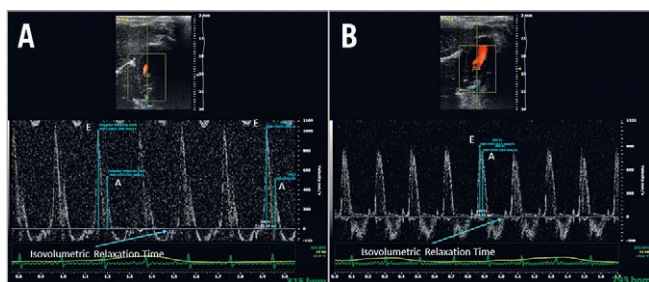
A strain effect was noted in elastin density (SHR > Lewis,  $F=10.1$ ,  $p=0.003$ ). Moreover, elastin density increased after creation of the

IAC ( $F=49.5$ ,  $p<0.001$ ). A statistical interaction occurred between strain and IAC for elastin density ( $F=6.2$ ,  $p=0.017$ ), showing that PA elastin density increased more in SHR than in Lewis rats. Examples of orcein staining and results of quantitative histomorphometry are presented in **Figure 5** and **Table 1**.

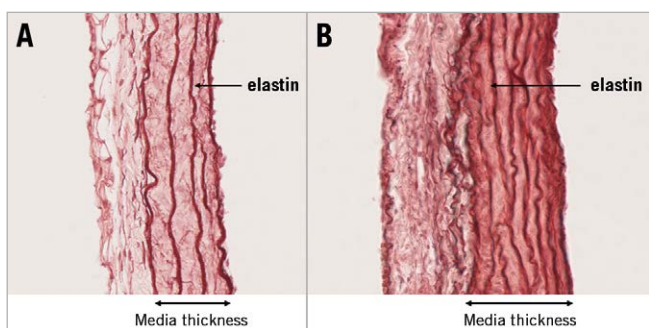


**Figure 3.** Doppler echocardiography results on systolic function, diastolic function and pulmonary artery wall shear rate (two-way ANOVA). A) Mean heart rate (beats/min). Strain effect:  $F=33.5$ ,  $*p<0.001$ . IAC effect:  $F=2.7$ ,  $p=NS$ . B) LVEF (%). Strain effect:  $F=23.7$ ,  $*p<0.001$ . IAC effect:  $F=0.1$ ,  $p=NS$ . C) Systemic cardiac output (ml/min). Strain effect:  $F=0.8$ ,  $p=NS$ . IAC effect:  $F=0.5$ ,  $p=NS$ . D) E/A. Strain effect:  $F=17.3$ ,  $*p<0.001$ . IAC effect:  $F=0.2$ ,  $p=NS$ . E) IVRT (ms). Strain effect:  $F=36.4$ ,  $*p<0.001$ . IAC effect:  $F=3.6$ ,  $p=NS$ . F) Left atrial volume (mm<sup>3</sup>). Strain effect:  $F=4.5$ ,  $*p=0.04$ . IAC effect:  $F=18.8$ ,  $**p<0.001$ . G) PA WSR (1/s). Strain effect:  $F=5.8$ ,  $*p=0.02$ . IAC effect:  $F=4.3$ ,  $**p=0.04$ . IAC: interatrial communication; IVRT: isovolumetric relaxation time; LVEF: left ventricular ejection fraction; NS: non-significant; PA: pulmonary artery; SHR: spontaneous hypertensive rats; WSR: wall shear rate





**Figure 4.** Doppler ultrasound measurement of diastolic function at 45 days (E/A ratio and isovolumetric relaxation time). A) Sham-operated SHR. B) SHR with interatrial communication.



**Figure 5.** Section of pulmonary artery after staining with orcein (elastin). Media thickness and elastin density were increased in SHR with interatrial communication. A) Sham-operated SHR. B) SHR with interatrial communication.

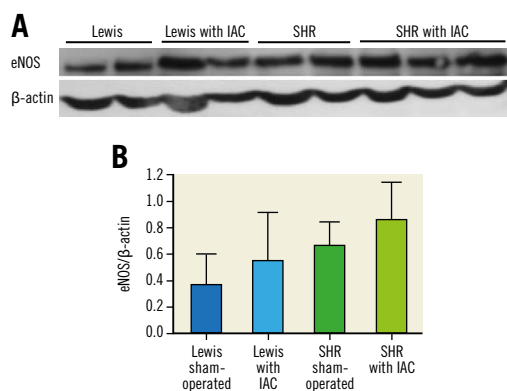
**Table 1. Quantitative histomorphometry of media thickness and elastin density performed on orcein-stained pulmonary artery sections (two-way ANOVA).**

	IAC		ANOVA		
	-	+	Strain effect	IAC effect	Interaction
Media thickness ( $\mu\text{m}$ )					
Lewis	26.1 $\pm$ 11.2	42.0 $\pm$ 12.6	F=1.1	F=3.3	F=1.5
SHR	38.0 $\pm$ 18.3	41.2 $\pm$ 23.2	$p$ =NS	$p$ =NS	$p$ =NS
Elastin density (%)					
Lewis	31.2 $\pm$ 12	42.1 $\pm$ 5.2	F=10.1	F=49.5	F=6.2
SHR	17.5 $\pm$ 2.7	40.5 $\pm$ 7.0	$p$ =0.003	$p$ <0.001	$p$ =0.017

Values are expressed as means $\pm$ standard deviation. IAC: interatrial communication; NS: non-significant; SHR: spontaneous hypertensive rat

#### EXPRESSION OF eNOS PROTEIN BY WESTERN BLOT

eNOS/ $\beta$ -actin ratio was 0.38 $\pm$ 0.23 in sham-operated Lewis rats, 0.56 $\pm$ 0.36 in Lewis rats with IAC (+47% increase), 0.67 $\pm$ 0.17 in sham-operated SHR and 0.87 $\pm$ 0.28 (+30% increase) in SHR with IAC. A strain effect (F=12.7,  $p$ =0.001) and a shunt effect (F=4.9,  $p$ =0.03) were observed, without significant interaction. Results of western blot are illustrated in **Figure 6** and **Supplementary Figure 1**.



**Figure 6.** Measurement of eNOS protein expression by western blot in lung fragments. A) Gel representing eNOS protein expression (anti-eNOS and anti- $\beta$ -actin antibodies). B) Quantification of eNOS normalised to  $\beta$ -actin (ImageJ<sup>®</sup> software). Interatrial creation increased eNOS/ $\beta$ -actin ratio. A strain effect (F=12.7,  $p$ =0.001) and a shunt effect (F=4.9,  $p$ =0.03) were observed, without significant interaction.

#### Discussion

This study showed a strain effect (hypertensive versus normotensive) on heart and lung weights, diastolic function, left atrial volume and pulmonary WSR measured by Doppler ultrasound, elastin density evaluated by histomorphometry and eNOS protein expression. Creation of an IAC in both SHR and Lewis rats decreased left atrial volume and increased pulmonary WSR measured by Doppler ultrasound, providing evidence for the functionality of the shunt. It also tended to improve diastolic function (IVRT), increased media thickness and elastin density of the PA and expression of eNOS protein in the lungs. Wall shear stress is proportional to both viscosity (considered as constant in this case) and velocity and is inversely proportional to the PA dimensions (radius).

In this experiment, changes in the PA diameter associated with interatrial shunting were opposite (interaction,  $p$ <0.001) in SHR and Lewis rats. PA diameter increased with shunting in Lewis rats ( $p$ =0.05) potentially due to active dilation, whereas it decreased in SHR ( $p$ =0.001) potentially due to the pressure decrease. These results suggest that creation of a left-to-right interatrial shunt may be effective in reducing pulmonary capillary pressure in SHR, which may transfer into improvement in HFPEF symptoms, but created a right volume overload in healthy conditions. It is well known that spontaneous atrial septal defects in humans are often benign, but could evolve with time towards right heart failure and pulmonary hypertension<sup>17</sup>.

There was also a trend towards decreased IVRT (without change in heart rate), suggesting improved diastolic function with IAC in SHR, although this result was not significant. One would have expected a lower ANP concentration in SHR with IAC in keeping with lower left atrial volume, as atrial dilation is the stimulus for ANP secretion<sup>18</sup>. However, no such difference was found. One hypothesis is that the creation of a left-to-right atrial shunt would increase right atrial volume and pressure (not measured), which would explain the absence of any decrease in ANP concentration.

Several models of left-to-right atrial shunt have been developed in large animals<sup>19</sup> but, to our knowledge, no such model has been described in small animals to study HFPEF. Our initial technique consisted of creating a shunt by interposing a syngeneic aortic graft between left and right atrial appendages. This difficult and time-consuming procedure was modified for this study by using a micro-balloon catheter. The systematic study of IAC patency during animal sacrifice confirmed the effectiveness of the procedure (two failures out of 24 IACs created).

Distally ligating the anterior interventricular artery in Lewis rats is a good model for HFPEF<sup>20</sup>. However, performing this procedure followed by creation of an interatrial shunt resulted in a high death rate (ethical issue). For this reason, we preferred to use SHR with diastolic dysfunction without decreased systolic function (compensated heart failure, HFPEF)<sup>21</sup>. Thirty-six-week-old SHR develop diastolic dysfunction before the appearance of hypertrophy/hypertension and decrease of LVEF, in contrast to what is observed later in life<sup>12</sup>. Beyond this age, LV systolic dysfunction appears and rats are likely to die of congestive heart failure<sup>11</sup>. As normotensive controls, we chose to use inbred Lewis rats, since the usual control rats, Wistar Kyoto, are not an inbred strain (out-bred) and body weights are quite different.

The safety and feasibility of creating a left-to-right artificial shunt by performing a percutaneous IAC have been studied in humans<sup>22</sup>. In a multicentre non-controlled phase I study<sup>23</sup>, 68 patients over 40 years of age with heart failure and LVEF >40% were included. The interventional procedure did not lead to any major cardiovascular or cerebral complications. The percutaneous device was successfully implanted in 64 patients (97%). The first results were encouraging since most patients presented an improvement in NYHA class, dyspnoea, quality of life, exercise capacity, cardiac output and pulmonary arterial oxygen saturation at six months. However, these encouraging clinical benefits were limited<sup>24</sup>. Indeed, NYHA functional class changed from III to II, and the average distance for the six-minute walk test increased from 313 to 346 metres. A multicentre sham-controlled phase two randomised study<sup>25</sup> showed that shunting was associated with a decrease in pulmonary capillary pressure during exercise, providing physiopathological arguments and demonstrating the effectiveness of the procedure. Nevertheless, one month after the procedure, NYHA functional class and exercise duration were similar in the IAC group and the control group. This suggests a potential placebo effect in the phase I study. In addition, the creation of a left-to-right atrial shunt may lead to late right ventricular dysfunction<sup>26</sup>. Finally, the creation of an artificial left-to-right shunt may improve symptoms of HFPEF, but it is not curative. Thus, it is also essential to find pharmacological and interventional solutions to improve diastolic dysfunction<sup>27</sup>.

## Limitations

This experimental design had some limitations. First, typically age-matched normotensive Wistar Kyoto rats are the controls for SHR<sup>28</sup>. However, rats were matched on weight rather than age

for reproducibility technical reasons. Furthermore, Lewis rats are a pure strain while Wistar Kyoto rats are not. Second, when the IAC was created, the atrial septum was perforated “blindly”. Indeed, the catheter path could be anticipated but the atrial septum was perforated through the right atrium, and it was impossible to verify the reality of the IAC. IAC patency was thus checked during dissection of the heart at sacrifice (day 60), and two Lewis rats with non-patent IAC were excluded. Third, Doppler echocardiography is operator-dependent and requires learning time, and only one Doppler echo measurement was taken for each parameter. Fourth, in SHR, although E/A ratio was greater and IVRT was longer, the mean heart rate was lower as compared to Lewis rats, making the comparison between the two strains difficult. Fifth, even though quantitative histomorphometry provided the expected results (lower elastin density), a limitation of this technique is represented by random sampling. Finally, it was demonstrated that exercise training improved delayed deterioration of cardiac function and was an effective treatment in HFPEF rats<sup>29</sup> and humans<sup>30</sup>. Therefore, it will be important to test the effect of atrial shunting on exercise tolerance in our experimental model. This could be done by using a forced swimming test in rats<sup>31</sup>. This study was limited to haemodynamic, biochemical and histomorphometric studies of the animals, and therefore improvement in symptoms, exercise tolerance or mortality was not evaluated. This should be the next step, now that we have been able to validate this new animal model.

## Conclusions

Creating an IAC with this new model effectively produces a left-to-right atrial shunt in SHR. This technique appears to be effective in improving pulmonary haemodynamics in experimental HFPEF. In addition, our experimental study provides preliminary evidence of the potential risk of right volume overload and pulmonary hypertension due to atrial shunting in healthy rats. Finally, this new preclinical model should be carefully studied.

## Impact on daily practice

Creating a left-to-right interatrial shunt in heart failure with preserved ejection fraction seems to be effective in improving pulmonary haemodynamics. This study could justify and corroborate the further development of a left-to-right interatrial shunt in humans with heart failure with preserved ejection fraction, even though the clinical effects of this shunt remain uncertain.

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## Conflict of interest statement

The authors have no conflicts of interest to declare.

## References

- Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, Gong Y, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med*. 2006;355:260-9.
- Little WC, Applegate RJ. Congestive heart failure: systolic and diastolic function. *J Cardiothorac Vasc Anesth*. 1993;7:2-5.
- Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251-9.
- Sharma K, Kass DA. Heart failure with preserved ejection fraction mechanisms, clinical features, and therapies. *Circ Res*. 2014;115:79-96.
- Ben Driss A, Devaux C, Henrion D, Duriez M, Thuillez C, Levy BI, Michel JB. Hemodynamic stresses induce endothelial dysfunction and remodeling of pulmonary artery in experimental compensated heart failure. *Circulation*. 2000;101:2764-70.
- Engelberg J, Dubois AB. Mechanics of pulmonary circulation in isolated rabbit lungs. *Am J Physiol*. 1959;196:401-14.
- Lepailleur-Enouf D, Egidy G, Philippe M, Louedec L, Henry JP, Mulder P, Michel JB. Pulmonary endothelinergic system in experimental congestive heart failure. *Cardiovasc Res*. 2001;49:330-9.
- Gaertner R, Lepailleur-Enouf D, Gonzalez W, Nicoletti A, Mandet C, Philippe M, Mercadier JJ, Michel JB. Pulmonary endothelium as a site of synthesis and storage of interleukin-6 in experimental congestive heart failure. *Eur J Heart Fail*. 2003;5:435-42.
- Torrent-Guaspa F, Buckberg GD, Clemente C, Cox JL, Coghlan HC, Gharib M. The structure and function of the helical heart and its buttress wrapping. I. The normal macroscopic structure of the heart. *Semin Thorac Cardiovasc Surg*. 2001;13:301-19.
- Kaye DM, Hasenfuß G, Neuzil P, Post MC, Doughty R, Trochu JN, Kolodziej A, Westenfeld R, Penicka M, Rosenberg M, Walton A, Muller D, Walters D, Hausleiter J, Raake P, Petrie MC, Bergmann M, Jondeau G, Feldman T, Veldhuisen DJ, Ponikowski P, Silvestry FE, Burkhoff D, Hayward C. One-Year Outcomes After Transcatheter Insertion of an Interatrial Shunt Device for the Management of Heart Failure With Preserved Ejection Fraction. *Circ Heart Fail*. 2016;9:e003662.
- Boluyt MO, Bing OH, Lakatta EG. The ageing spontaneously hypertensive rat as a model of the transition from stable compensated hypertrophy to heart failure. *Eur Heart J*. 1995;16 Suppl N:19-30.
- Slama M, Ahn J, Varagic J, Susic D, Frohlich ED. Long-term left ventricular echocardiographic follow-up of SHR and WKY rats: effects of hypertension and age. *Am J Physiol Heart Circ Physiol*. 2004;286:H181-5.
- Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27:282-93.
- Gnasso A, Carallo C, Irace C, Spagnuolo V, De Novara G, Mattioli PL, Pujia A. Association between intima-media thickness and wall shear stress in common carotid arteries in healthy male subjects. *Circulation*. 1996;94:3257-62.
- Prunier F, Gaertner R, Louedec L, Michel JB, Mercadier JJ, Escoubet B. Doppler echocardiographic estimation of left ventricular end-diastolic pressure after MI in rats. *Am J Physiol Heart Circ Physiol*. 2002;283:H346-52.
- Heeneman S, Smits JF, Leenders PJ, Schiffers PM, Daemen MJ. Effects of angiotensin II on cardiac function and peripheral vascular structure during compensated heart failure in the rat. *Arterioscler Thromb Vasc Biol*. 1997;17:1985-94.
- Nashat H, Montanaro C, Li W, Kempny A, Wort SJ, Dimopoulos K, Gatzoulis MA, Babu-Narayan SV. Atrial septal defects and pulmonary arterial hypertension. *J Thorac Dis*. 2018;10:S2953-65.
- Levin ER, Gardner DG, Samson WK. Natriuretic peptides. *N Engl J Med*. 1998;339:321-8.
- Eigler NL, del Rio CL, Verheye S, McConnell PI, Lilly SM, George R, Hamlin RL, Ueyama Y, Youngblood BL, Shkurovich S, Keren G, Abraham WT. Cardiac Unloading with an Implantable Interatrial Shunt in Heart Failure: Serial Observations in an Ovine Model of Ischemic Cardiomyopathy. *Structural Heart*. 2017;1:40-8.
- Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol*. 1978;90:57-70.
- Marzak H, Ayme-Dietrich E, Lawson R, Mokni W, Combe R, Becker J, Fertak LE, Champy MF, Monassier L. Old spontaneously hypertensive rats gather together typical features of human chronic left-ventricular dysfunction with preserved ejection fraction. *J Hypertens*. 2014;32:1307-16.
- Guimaraes L, Lindenfeld J, Sandoval J, Bayés-Genis A, Bernier M, Provencher S, Rodés-Cabau J. Interatrial shunting for heart failure: current evidence and future perspectives. *EuroIntervention*. 2019;15:164-71.
- Hasenfuß G, Hayward C, Burkhoff D, Silvestry FE, McKenzie S, Gustafsson F, Malek F, Van der Heyden J, Lang I, Petrie MC, Cleland JGF, Leon M, Kaye DM; REDUCE LAP-HF study investigators. A transcatheter intracardiac shunt device for heart failure with preserved ejection fraction (REDUCE LAP-HF): a multicentre, open-label, single-arm, phase I trial. *Lancet*. 2016;387:1298-304.
- Søndergaard L, Reddy V, Kaye D, Malek F, Walton A, Mates M, Franzen O, Neuzil P, Ihlemann N, Gustafsson F. Transcatheter treatment of heart failure with preserved or mildly reduced ejection fraction using a novel interatrial implant to lower left atrial pressure. *Eur J Heart Fail*. 2014;16:796-801.
- Feldman T, Mauri L, Kahwash R, Litwin S, Ricciardi MJ, van der Harst P, Penicka M, Fail PS, Kaye DM, Petrie MC, Basuray A, Hummel SL, Forde-McLean R, Nielsen CD, Lilly S, Massaro JM, Burkhoff D, Shah SJ, REDUCE LAP-HF I Investigators and Study Coordinators. Transcatheter Interatrial Shunt Device for the Treatment of Heart Failure With Preserved Ejection Fraction (REDUCE LAP-HF I [Reduce Elevated Left Atrial Pressure in Patients With Heart Failure]): A Phase 2, Randomized, Sham-Controlled Trial. *Circulation*. 2018;137:364-75.
- L'Epine Y, Drobinski G, Sotirov Y, Moussallem N, Voudris V, Komajda M, Grosogoeat Y. Right heart failure due to an inter-atrial shunt after percutaneous mitral balloon dilatation. *Eur Heart J*. 1989;10:285-7.
- Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J*. 2011;32:670-9.
- Lindesay G, Ragonnet C, Chimenti S, Villeneuve N, Vayssettes-Courchay C. Age and hypertension strongly induce aortic stiffening in rats at basal and matched blood pressure levels. *Physiol Rep*. 2016 May;4(10).
- Andrade DC, Arce-Alvarez A, Toledo C, Diaz HS, Lucero C, Schultz HD, Marcus NJ, Del Rio R. Exercise training improves cardiac autonomic control, cardiac function, and arrhythmogenesis in rats with preserved-ejection fraction heart failure. *J Appl Physiol (1985)*. 2017;123:567-77.
- Pandey A, Parashar A, Kumbhani D, Agarwal S, Garg J, Kitzman D, Levine B, Drazner M, Berry J. Exercise training in patients with heart failure and preserved ejection fraction; meta-analysis of randomized control trials. *Circ Heart Fail*. 2015;8:33-40.
- Commons KG, Cholanians AB, Babb JA, Ehlinger DG. The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior. *ACS Chem Neurosci*. 2017;8:955-60.

## Supplementary data

**Supplementary Appendix 1.** Description of western blot analysis.

**Supplementary Figure 1.** Densitometry results of eNOS protein expression.

**Supplementary Table 1.** Weights of body, heart and lungs.

**Supplementary Table 2.** Doppler echocardiography results on systolic function, diastolic function and pulmonary artery wall shear rate (two-way ANOVA).

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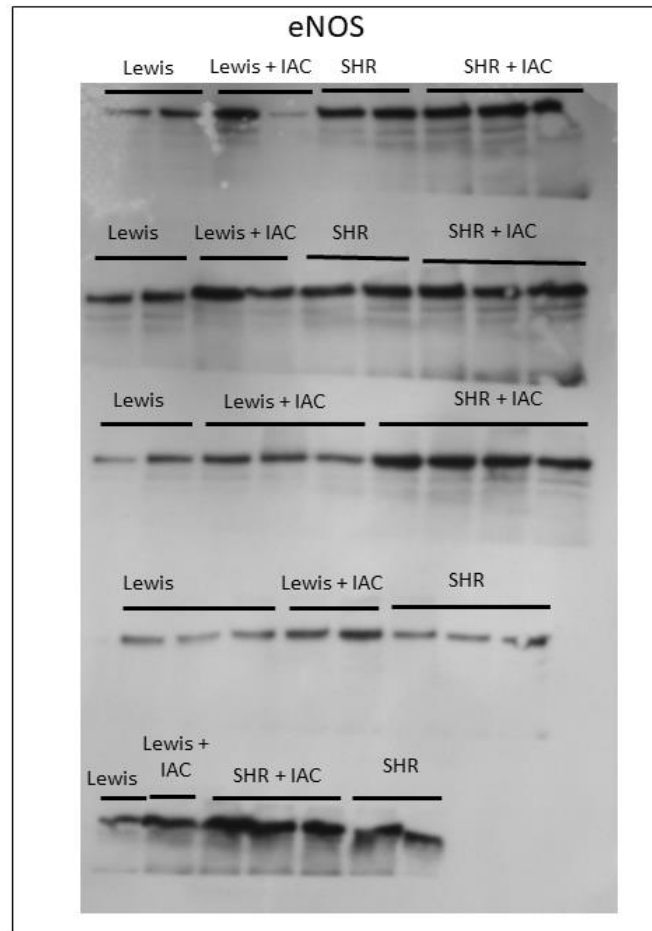


## Supplementary data

### Supplementary Appendix 1. Description of western blot analysis

200 mg of powder was homogenised at 4°C in RIPA buffer supplemented with a protease inhibitor cocktail. Homogenates were centrifuged at 13,000 g for 20 min at 4°C, and the supernatants were assayed. The protein concentration was determined using the BCA protein assay kit (ThermoFischer Scientific®). Samples were denatured in Laemmli buffer (125 mmol/l Tris HCl, 2% SDS [sodium dodecyl sulfate], 1% b-mercaptoethanol, 10% glycerol, 0.005% bromophenol blue, pH 6.8) and heated at 95°C for 5 min. Fifty micrograms of proteins per sample were separated on 10% polyacrylamide gel under constant current. Proteins were then transferred to polyvinylidene difluoride (0.45 m membrane; Millipore) in a transfer buffer, at 60 V for two hours. Membranes were then blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween 20 for 1 hr at room temperature and then immunoblotted with the desired primary antibody in 0.5% non-fat milk overnight at 4°C. The source and concentration of each antibody were as follows: anti-eNOS (Cell Signalling; 32027S; 1:1000), anti-β-actin (Sigma Aldrich; A3854; 1:25000). After several washes in Tris-buffered saline 0.1% Tween 20, membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibody (1:5,000) for 1 hr at room temperature before development using the enhanced chemiluminescence detection kit (VWR). Bands were visualised and quantified using ImageJ® software (National Institutes of Health, Maryland, USA). eNOS protein expression rates were normalised to endogenous β-actin expression.





**Supplementary Figure 1.** Densitometry results of eNOS protein expression.

**Supplementary Table 1. Weights of body, heart and lungs.**

	IAC		ANOVA		
	-	+	Strain effect	IAC effect	Interaction
<b>BW, gr</b>					
Lewis	409±25	353±64	F=2.4	F=3.6	F=3.3
SHR	412±22	413±33	<i>p</i> =NS	<i>p</i> =NS	<i>p</i> =NS
<b>Heart weight/BW, mg/gr</b>					
Lewis	3.2±0.4	3.2±0.3	F=52.3	F=0.2	F=0.4
SHR	3.9±0.4	4.0±0.4	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>LV weight/BW, mg/gr</b>					
Lewis	1.9±0.1	1.8±0.1	F=6.41	F=0.2	F=1.6
SHR	3.0±0.2	3.1±0.2	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>RV weight/BW, mg/gr</b>					
Lewis	0.5±0.1	0.6±0.1	F=23.2	F=0.1	F=2.5
SHR	0.7±0.1	0.6±0.1	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>Atria weight/BW, mg/gr</b>					
Lewis	0.3±0.03	0.3±0.04	F=14.0	F=0.9	F=0.002
SHR	0.4±0.08	0.4±0.04	<i>p</i> =0.001	<i>p</i> =NS	<i>p</i> =NS
<b>Lung weight, gr</b>					
Lewis	1.4±0.1	1.6±0.2	F=27.5	F=3.5	F=1.8
SHR	1.7±0.1	1.8±0.2	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS

Values are expressed as means±standard derivations.

BW: body weight; IAC: interatrial communication; LV: left ventricle; NS: non-significant; RV: right ventricle; SHR: spontaneous hypertensive rat

**Supplementary Table 2. Doppler echocardiography results on systolic function, diastolic function and pulmonary artery wall shear rate (two-way ANOVA).**

	IAC		ANOVA		
	-	+	Strain effect	IAC effect	Interaction
<b>Mean heart rate (beats/min)</b>					
Lewis	364±22	346±31	F=33.5	F=2.7	F=1.1
SHR	318±14	314±18	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>LVEF (%)</b>					
Lewis	74.4±4.8	73.4±2.5	F=23.7	F=0.1	F=0.03
SHR	84.5±7.9	84.3±10.6	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>Systemic cardiac output (ml/min)</b>					
Lewis	78.4±19.2	103±32.3	F=0.8	F=0.5	F=3.0
SHR	86.6±51.3	89.3±34.6	<i>p</i> =NS	<i>p</i> =NS	<i>p</i> =NS
<b>E/A</b>					
Lewis	1.06±0.17	1.09±0.15	F=17.3	F=0.2	F=0.6
SHR	1.51±0.31	1.4±0.46	<i>p</i> <0.001	<i>p</i> =NS	<i>p</i> =NS
<b>IVRT (ms)</b>					
Lewis	23.4±2.4	20±5	F=36.4	F=3.6	F=0.2
SHR	31.7±5.8	29.5±5.7	<i>p</i> <0.001	<i>p</i> =0.07	<i>p</i> =NS
<b>Left atrial volume (mm<sup>3</sup>)</b>					
Lewis	204±73	159±30	F=4.5	F=18.8	F=5.1
SHR	298±104	156±58	<i>p</i> =0.04	<i>p</i> <0.001	<i>p</i> =0.03
<b>PA systolic diameter (mm)</b>					
Lewis	2.9±0.2	3.1±0.3	F=10.9	F=0.6	F=17.1
SHR	2.9±0.3	2.6±0.1	<i>p</i> =0.002	<i>p</i> =NS	<i>p</i> <0.001
<b>Maximum PA systolic velocity (mm/s)</b>					
Lewis	676±104	745±162	F=1.0	F=1.5	F=0.2
SHR	736±130	767±133	<i>p</i> =NS	<i>p</i> =NS	<i>p</i> =NS
<b>PA WSR (1/s)</b>					
Lewis	952±135	985±208	F=5.8	F=4.3	F=2.0
SHR	1,002±114	1,179±196	<i>p</i> =0.02	<i>p</i> =0.04	<i>p</i> =NS

Values are expressed as means±standard deviation.

IAC: interatrial communication; IVRT: isovolumetric relaxation time; LVEF: left ventricular ejection fraction; NS: non-significant; PA: pulmonary artery; SHR: spontaneous hypertensive rat; WSR: wall shear rate