Echocardiography and invasive hemodynamics during stress testing for diagnosis of heart failure with preserved ejection fraction: an experimental study

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Leite S, Oliveira-Pinto J, Tavares-Silva M, Abdellatif M, Fontoura D, Falcão-Pires I, Leite-Moreira AF, Lourenço AP. Echocardiography and invasive hemodynamics during stress testing for diagnosis of heart failure with preserved ejection fraction: an experimental study. Am J Physiol Heart Circ Physiol 308: H1556–H1563, 2015. First published April 11, 2015; doi:10.1152/ajpheart.00076.2015.—Inclusion of exercise testing in diagnostic guidelines for heart failure with preserved ejection fraction (HFpEF) has been advocated, but the target population, technical challenges, and underlying pathophysiologic complexity raise difficulties to implementation. Hemodynamic stress tests may be feasible alternatives. Our aim was to test Trendelenburg positioning, phenylephrine, and dobutamine in the ZSF1 obese rat model to find echocardiographic surrogates for end-diastolic pressure (EDP) elevation and HFpEF. Seventeen-week-old Wistar-Kyoto, ZSF1 lean, and obese rats (n = 7 each) randomly and sequentially underwent (crossover) Trendelenburg (30°), 5 μg·Kg⁻¹·min⁻¹ dobutamine, and 7.5 μg·Kg⁻¹·min⁻¹ phenylephrine with simultaneous left ventricular (LV) pressure-volume loop and echocardiography evaluation under halogenate anesthesia. Effort testing with maximum O₂ consumption (VO₂ max) determination was performed 1 wk later. Obese ZSF1 showed lower effort tolerance and VO₂ max along with higher resting EDP. Both Trendelenburg and phenylephrine increased EDP, whereas dobutamine decreased it. Significant correlations were found between EDP and 1) peak early filling Doppler velocity of transmitral flow (E) to corresponding myocardial tissue Doppler velocity (E′) ratio, 2) E to E-wave deceleration time (EDT) ratio, and 3) left atrial area (LAA). Diagnostic efficiency of E/DT dLAA by receiver-operating characteristic curve analysis for elevation of EDP above a cut-off of 13 mmHg during hemodynamic stress was high (area under curve, AUC = 0.95) but not higher than that of E/E′ (AUC = 0.77, P = 0.15). Results in ZSF1 obese rats suggest that noninvasive echocardiography after hemodynamic stress induced by phenylephrine or Trendelenburg can enhance diagnosis of stable HFpEF and constitute an alternative to effort testing.

HFpEF is increasingly recognized as a major health issue (17), and, although it is the leading cause of hospitalization in the Western world, effective therapies are lacking (19). Exercise intolerance and dyspnea on exertion are its cardinal features, and the underlying mechanisms have been recently revised (1). Diagnostic criteria for HFpEF are controversial and still evolving. Many patients fail to meet diagnostic criteria under resting conditions and may still show impairment during effort testing; thus the inclusion of effort testing in diagnostic guidelines is warranted (26). Nevertheless, establishing these criteria is problematic because of the complex hemodynamic and systemic influences elicited by exercise and because of the target population of predominantly elderly patients with extensive comorbidities and multiple causes for exercise intolerance (27).

We have recently characterized an experimental rat model of HFpEF associated with the metabolic syndrome. Twenty-week-old hypertensive, obese, and diabetic ZSF1 obese rats show high filling pressures and lung congestion and increased cardiomyocyte stiffness with preserved systolic and renal function (5). Our aim was to document effort impairment in the ZSF1 obese rat model of HFpEF and to characterize its diastolic response to increased afterload, preload, and HR achieved by phenylephrine infusion, Trendelenburg positioning, and dobutamine infusion, respectively, and correlate hemodynamic stress changes with simultaneously acquired echocardiographic indexes in vivo. Finally, we tried to identify the best noninvasive surrogate indexes for EDP.

Glossary

- A Peak Doppler velocity of late filling wave (A-wave) in transmitral flow
- AT Anaerobic threshold
- AUC Area under curve
- BW Body weight
- CI Cardiac index
- CL Cycle length
- CO Cardiac output
- dP/dt maximum Maximum rate of pressure rise
- DT Deceleration time of the E-wave and deceleration time normalized for cycle length
- E Peak Doppler velocity of early filling wave (E-wave) in transmitral flow
- E′ Peak mitral annulus tissue Doppler velocity in early diastole
- EDP End-diastolic pressure

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\( \dot{V}_O_2 \) as a function of WL in peak effort testing (A) and endurance effort testing (B) and analysis of the AT by the V-slope method (C). Values from WKY (open circles), ZSF1 Ln (light gray square), and ZSF1 Ob (dark gray triangles) are represented. Data were stacked for simplicity sake. The \( \dot{V}_O_2 \)-WL relationship was well described by a 2-parameter natural logarithmic fit curve (A and B). In C, AT (large symbols) as established by the intersection of 2 linear regression lines (V-slope method) is represented along with a percentage of \( \dot{V}_O_{2\text{max}} \). *\( P < 0.01 \) vs. WKY and †\( P < 0.05 \) vs. ZSF1 Ln for \( \dot{V}_O_{2\text{max}} \), AT, and maximum WL, respectively; ‡\( P < 0.001 \) vs. WKY and ZSF1 Ln in covariance analysis of the fit-curve parameters. For definition of terms, please see Glossary.
H1558

STRESS ECHO IN EXPERIMENTAL HFpEF

ZSF1

Ln ZSF1 lean rats
ZSF1

Ob ZSF1 obese rats

τ Time constant of isovolumic relaxation

METHODS

Animal model. Seventeen-week-old male ZSF1 Ln (n = 7), ZSF1 Ob (n = 7), and WKY (n = 7) (Charles River Laboratories) randomly underwent hemodynamic stress with Trendelenburg positioning (30° head-down tilt) and intravenous infusion of dobutamine and phenylephrine at 5 μg·kg\(^{-1}\)·min\(^{-1}\) and 7.5 μg·kg\(^{-1}\)·min\(^{-1}\), respectively, with washout and stabilization periods between interventions (cross-over). Continuous pressure-volume and stable LV echocardiography recordings were obtained. One week later, animals randomly underwent effort testing. Animals were kept in individually ventilated chambers, in groups of two per cage under controlled environment with a 12-h:12-h-light/dark cycle at 22°C room temperature. All animals received humane care. Experimental procedures were approved by the Faculty of Medicine of Porto and complied with the Faculty of Medicine of Porto guidelines and were performed in accordance with Portuguese law on animal welfare, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 2011).

Peak effort, \(\hat{V}O_2\) max, AT, and endurance capacity. Animals were evaluated on a close-chamber treadmill coupled to a gas analyzer at a treadmill inclination of 15°. Peak effort and endurance tests were separated by 24 h. After an initial adaption period at 15 cm/s in peak effort testing, velocity was changed to 30 cm/s and then stepped up by 5 cm/s every 60 s to assess \(\hat{V}O_2\) max, whereas, in endurance testing, velocity was also stepped up by 5 cm/s every 15 min to assess fatigue. AT was obtained by systematically varying an intersection point splitting the \(\hat{V}O_2\) to \(\hat{V}O_2\) relationship into two linear regressions until the error of regression was minimized (V-slope method).

Hemodynamic and echocardiography evaluation. Rats were anesthetized by inhalation of sevoflurane (8% for induction and 2–2.5% for maintenance), orotracheally intubated (14 G), and mechanically ventilated in pressure support ventilation mode (MouseVent; Kent Scientific) with pressure support set at 10 cmH\(_2\)O above 5 cmH\(_2\)O positive end-expiratory pressure. Homeostasis was maintained by anesthetic monitoring (MouseSTAT, pulse oximeter and heart rate monitor; CapnoScan, end-tidal CO\(_2\) monitor; RightTemp, temperature monitor and homeothermic controller; Physiosuite; Kent Scientific). Fluid replacement with warm Ringer’s lactate solution at 32 ml·kg\(^{-1}\)·h\(^{-1}\) was instituted through the right jugular vein, which was catheterized (24 G) under surgical microscopy. A pressure volume catheter (SPR-847; Millar Instruments) was inserted through the right common carotid artery and advanced into the LV (see supplemental videos; supplemental material for this article is available online at the American Journal of Physiology Heart and Circulatory Physiology website). Signals were continuously acquired (MPVS 300; Millar Instruments), digitized at 1,000 Hz (ML880 PowerLab16/30; ADInstruments), and analyzed offline (PVAN 3.5; Millar Instruments). Parallel conductance was determined by 40 μl of 10% hypertonic saline injection, and slope factor \(\alpha\) was derived by simultaneous measurement of CO with echocardiography. Echocardiography was performed sequentially at baseline, interventions, and after each washout period using a 15-MHz linear probe and an echocardiography system (Acuson Sequoia CS12; Siemens) as previously described (5). Stroke volume was determined by PW Doppler aortic flow velocity curve integration in the apical five-chamber view, and aortic root dimensions were estimated on long-axis parasternal M-mode. CO was derived from stroke volume and HR as assessed by the RR interval. LV volumes and EF were calculated according to Teichholz formula based on parasternal short-axis M-mode dimensions obtained at the level just above the papillary muscles. LV filling was assessed by PW Doppler transmural flow velocity tracings obtained just above the tip of the mitral leaflets. Peak early (E) and late (A) wave velocities as well as E-wave DT were measured. Myocardial S’ and E’ were measured with TD imaging at the lateral mitral annulus. LAA was measured at atrial end-diastole by 2D echocardiography in the four-chamber view. At baseline, LV endocardial and epicardial short-axis areas and parasternal long-axis dimension were recorded to derive LVM by the area-length method. At least three stable cardiac cycles were averaged for all measurements. Volumes and masses were indexed for body surface area, as defined by 9.1 × BW\(^{2/3}\). To account for varying HR, time intervals were normalized for CL.

Statistical analysis. Normality of variables was checked by Shapiro–Wilks test. Nonnormally distributed \(\hat{V}O_2\) and WL were compared with Kruskal–Wallis. Joint analysis of \(\hat{V}O_2\) and WL was performed by analysis of covariance after fitting plots to a two-parameter logarithmic function. BW and LVM were compared with one-way ANOVA. Two-way repeated-measures ANOVA was used to compare all other variables. Homogeneity of variances and the assumption of sphericity were checked with Levene’s and Mauchly’s tests, respectively. Post hoc comparisons were performed using Tukey’s test. To account for joint changes in EDV and EDP, we used repeated-measures multiple comparisons.

Table 1. Hemodynamic stress changes in WKY, ZSF1 Ln, and ZSF1 Ob

<table>
<thead>
<tr>
<th>Variable Group</th>
<th>Before</th>
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<td>MAP, mmHg</td>
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<tr>
<td>WKY</td>
<td>99 ± 9</td>
<td>125 ± 8</td>
<td>100 ± 11</td>
<td>109 ± 9</td>
<td>112 ± 8</td>
<td>85 ± 9</td>
<td>P &lt; 0.01,</td>
<td>P &lt; 0.001,</td>
<td>NS</td>
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<td>ZSF1 Ln</td>
<td>125 ± 6</td>
<td>150 ± 2</td>
<td>118 ± 6</td>
<td>135 ± 9</td>
<td>116 ± 8</td>
<td>105 ± 9</td>
<td>*P &lt; 0.01,</td>
<td>*P &lt; 0.001,</td>
<td>*P &lt; 0.001</td>
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<td>ZSF1 Ob</td>
<td>116 ± 6</td>
<td>154 ± 8</td>
<td>127 ± 5</td>
<td>155 ± 6</td>
<td>112 ± 6</td>
<td>115 ± 3</td>
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<td>HR, beats/min</td>
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<tr>
<td>WKY</td>
<td>317 ± 14</td>
<td>310 ± 13</td>
<td>310 ± 14</td>
<td>315 ± 12</td>
<td>321 ± 12</td>
<td>391 ± 16</td>
<td>P &lt; 0.001,</td>
<td>P &lt; 0.001,</td>
<td>NS</td>
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<td>ZSF1 Ln</td>
<td>365 ± 11</td>
<td>341 ± 12</td>
<td>347 ± 5</td>
<td>366 ± 9</td>
<td>350 ± 7</td>
<td>423 ± 5</td>
<td>*P &lt; 0.01,</td>
<td>*P &lt; 0.001,</td>
<td>*P &lt; 0.001</td>
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<td>ZSF1 Ob</td>
<td>310 ± 11</td>
<td>319 ± 11</td>
<td>297 ± 8</td>
<td>325 ± 10</td>
<td>300 ± 8</td>
<td>384 ± 10</td>
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<td>NS</td>
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<td>EF, %</td>
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<tr>
<td>WKY</td>
<td>79 ± 3</td>
<td>81 ± 5</td>
<td>77 ± 2</td>
<td>73 ± 1</td>
<td>81 ± 3</td>
<td>89 ± 3</td>
<td>NS</td>
<td>&lt; 0.01,</td>
<td>NS</td>
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<td>ZSF1 Ln</td>
<td>81 ± 1</td>
<td>83 ± 1</td>
<td>81 ± 1</td>
<td>81 ± 1</td>
<td>79 ± 3</td>
<td>90 ± 2</td>
<td>*P &lt; 0.001,</td>
<td>*P &lt; 0.001,</td>
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<tr>
<td>ZSF1 Ob</td>
<td>80 ± 3</td>
<td>83 ± 2</td>
<td>80 ± 2</td>
<td>76 ± 1</td>
<td>77 ± 2</td>
<td>89 ± 2</td>
<td>NS</td>
<td>&lt; 0.05,</td>
<td>NS</td>
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<td>EDV, μl/cm(^2)</td>
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<tr>
<td>WKY</td>
<td>1.05 ± 0.09</td>
<td>1.33 ± 0.24</td>
<td>0.89 ± 0.07</td>
<td>1.87 ± 0.37</td>
<td>1.07 ± 0.21</td>
<td>1.07 ± 0.18</td>
<td>NS</td>
<td>&lt; 0.05,</td>
<td>NS</td>
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<tr>
<td>ZSF1 Ln</td>
<td>1.17 ± 0.08</td>
<td>1.39 ± 0.22</td>
<td>1.23 ± 0.11</td>
<td>1.76 ± 0.32</td>
<td>1.05 ± 0.09</td>
<td>1.06 ± 0.10</td>
<td>NS</td>
<td>&lt; 0.05,</td>
<td>NS</td>
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<tr>
<td>ZSF1 Ob</td>
<td>1.51 ± 0.12</td>
<td>1.65 ± 0.25</td>
<td>1.52 ± 0.12</td>
<td>1.88 ± 0.47</td>
<td>1.44 ± 0.18</td>
<td>1.39 ± 0.10</td>
<td>NS</td>
<td>&lt; 0.05,</td>
<td>NS</td>
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Values are means ± SE; n = 7 per group. EDV, is given by 9.1 × (BW\(^{2/3}\)), where BW is in grams. Statistically significant for main effects of group (G) and interactions between intervention and time (I X T) and group, intervention, and time (G X I X T), as well as planned comparisons and post hoc tests are given in the rightmost columns. ZSF1 Ln vs. WKY; ZSF1 Ob vs. WKY; ZSF1 Ob vs. ZSF1 Ln; dobutamine vs. both phenylephrine and Trendelenburg; phenylephrine vs. Trendelenburg. NS, nonsignificant. For other definitions, please refer to Glossary.
ANOVA on EDP and EDV variations from baseline followed by multiple ANOVA tests and post hoc comparisons to establish the source of interaction (significance level was adjusted to 0.004 to correct for multiple testing). Correlations between variables were assessed by Pearson’s coefficient after logarithmic transformation for nonnormally distributed variables. To account for repeated observations in correlation, mixed-linear models with animal and group as random effects were also undertaken (24). ROC curve analysis based on the nonparametric approach developed for data obtained from the same individuals (3) was performed for echocardiography parameters for an EDP cutoff of 13 mmHg. Data are means ± SE. Significance was set at two-tailed \( P < 0.05 \).

RESULTS

ZSF1 Ob showed higher \( (P < 0.01) \) BW (569 ± 10 g) and LVMi (1.28 ± 0.05 g/cm²) than both ZSF1 Ln (451 ± 14 g and 1.06 ± 0.05 g/cm², respectively) and WKY (348 ± 7 g and 0.93 ± 0.04 g/cm², respectively). None of the animals died or developed acute lung edema during testing. ZSF1 Ob reached lower \( \dot{V}O_2 \) \(_{\text{max}} \) and maximum WL in both peak effort and endurance tests compared with ZSF1 Ln and WKY (Fig. 1, A and B, respectively). The \( \dot{V}O_2 \) and WL relationship was also downward shifted in ZSF1 Ob, revealing lower achieved \( \dot{V}O_2 \) at similar WLs during peak effort (Fig. 1B). Although the AT as a percentage of \( \dot{V}O_2 \) \(_{\text{max}} \) did not differ between groups in peak effort, its absolute value was higher in ZSF1 Ln compared with WKY and lower in ZSF1 Ob (Fig. 1C).

Both ZSF1 groups showed higher MAP compared with WKY despite similar EDVi and EF (Table 1). \( dP/dt_{\text{max}} \) and S’ (Fig. 2) were increased \( (P < 0.001) \) in both ZSF1 Ob (9,810 ± 160 mmHg/s and 9.6 ± 0.4 cm/s, respectively) and ZSF1 Ln (10,300 ± 260 mmHg/s and 9.0 ± 0.2 cm/s, respectively) compared with WKY (8,480 ± 350 mmHg/s and 7.1 ± 0.4 cm/s, respectively). ZSF1 Ln also showed increased HR (Table 1). Representative hemodynamic and Doppler tracings are given in Fig. 2. At baseline, ZSF1 Ob presented elevated EDVI and prolonged \( \tau \) (Fig. 3), denoting high LV filling pressures and delayed relaxation, as well as disturbed transmural flow pattern characterized by higher E (120 ± 2 vs. 89 ± 2 and 86 ± 3 cm/s in ZSF1 Ln and WKY, respectively; \( P < 0.001 \); Fig. 2) and echocardiographic indexes, suggesting impaired diastolic function, such as increased ratio of E to DT normalized for CL (E/DTn), ratio of E to E’, and LAAi (Fig. 3). Nevertheless, E’ was increased \( (P < 0.001) \) in ZSF1 Ob (7.8 ± 0.2 cm/s) compared with both ZSF1 Ln (6.2 ± 0.1 cm/s) and WKY (6.6 ± 0.2 cm/s).

All hemodynamic stresses increased CI (321 ± 99 vs. 259 ± 101 \( \mu l \cdot \text{min}^{-1} \cdot \text{cm}^{-2} \) at baseline; main effects \( P < 0.001 \)) with no difference between groups. Dobutamine increased HR, EF, and contractility indexes \( dP/dt_{\text{max}} \) (17,700 ± 750 vs. 9,800 ± 270 mmHg/s; \( P < 0.001 \)) and S’ (11.5 ± 0.7 vs. 8.4 ± 0.5 cm/s; \( P < 0.001 \)) but slightly decreased MAP and did not alter EDVi (Table 1). These effects were significantly different from phenylephrine and Trendelenburg, which had no remarkable actions on EF and raised MAP and EDVi, respectively (Table 1 and representative tracings in Fig. 2). Moreover, Trendelenburg elicited slight tachycardia that was not observed with phenylephrine (Table 1), whereas the latter significantly raised \( dP/dt_{\text{max}} \) (12,600 ± 380 vs. 9,590 ± 320 mmHg/s; \( P < 0.01 \)).

Disturbances induced on diastolic function were most evident with Trendelenburg and phenylephrine. Both raised EDP and prolonged \( \tau \), whereas dobutamine had opposite effects (Fig. 3, A and B, respectively). Multivariate analysis of EDP and EDVi corroborated these findings; changes in EDVi were independent of EDVi and opposite between dobutamine that decreased EDP and Trendelenburg and phenylephrine, which increased it. Moreover, reduction of EDP by dobutamine was significantly more pronounced in ZSF1 Ob. Despite the visible trend for higher EDP elevation by phenylephrine and lower EDVi elevation by Trendelenburg in ZSF1 Ob, these were not statistically significant (Fig. 4). Simultaneous echocardiographic evaluation showed no significant increase in E/DTn and decreased E/E’ and LAAi after dobutamine distinctly from phenylephrine or Trendelenburg (Fig. 3, C–E, respectively). Both the latter raised E/DTn, whereas Trendelenburg increased predominantly LAAi, and phenylephrine mostly raised E/E’ (Fig. 3, D and E, respectively).

Significant correlations were found between EDP and E/DTn, E/E’, and LAAi (Fig. 5). The strongest correlation was observed for E/DTn. Results were confirmed with mixed lin-
ear-model analysis including the random effects of animal and group ($P < 0.001$). Contrastingly, no correlation was observed between $E/E'$ and $LVM_i$, whereas a strong correlation was found for $E/D_{Tn}$. ROC curve analysis for a cutoff $EDP$ of 13 mmHg, which was observed only in ZSF1 Ob rats after either Trendelenburg or phenylephrine (reference line in Fig. 3), revealed good AUC for all echocardiography-derived parameters with a slight nonsignificant advantage of $E/D_{Tn}$ and $LAA_i$ (Fig. 6).

### DISCUSSION

The lack of suitable animal models has partly limited the research on HFpEF. We have recently characterized a new model of HFpEF based on cardiometabolic risk, the ZSF1 Ob (5). With the present work, we establish that ZSF1 Ob show impaired effort tolerance, reinforcing the potentiality of this model for HFpEF pathophysiology and therapy research. Additionally, we assessed acute hemodynamic stress responses to increased afterload, preload, and HR achieved by phenylephrine, Trendelenburg, and dobutamine, respectively, and echocardiography parameters as a means to identify early diastolic dysfunction and noninvasive surrogates.

ZSF1 Ob showed decreased $V_{O2\ max}$ and endured lower WL both in peak and endurance effort tests, denoting effort intolerance, the cardinal manifestation of HFpEF. Additionally, they also showed lower $V_{O2}$ at any WL and a lower AT in peak effort testing. Lower submaximal performance and effort-independent AT values indicate more reliance on anaerobic metabolism and lower aerobic reserve. Patients with HFrEF show similar behavior, which has been attributed to lower skeletal muscle oxidative metabolism and should not be viewed as improved mechanical efficiency because, despite the lower $V_{O2}$ at each WL, the excessive reliance on anaerobic metabolism and excessive $O_2$ debt must be compensated for during recovery (8). To our knowledge, this is the first report in experimental HFpEF and validates this preclinical model.

Most authors would agree that abnormalities in LV relaxation and stiffness underlie HFpEF (31). Present diagnostic criteria rely mostly on data obtained by noninvasive evaluation.
under resting condition in patients admitted to the hospital with acute HF decompensation (18). Echocardiography data may not mirror the stable outpatient that, despite pathophysiological features of HFpEF, may remain asymptomatic. Not surprisingly, when outpatients with unexplained chronic dyspnea are assessed by invasive methods and effort testing, present guidelines fail to reach adequate sensitivity and specificity (20); thus many experts suggest an integration of exercise evaluation into the algorithm for evaluation of HFpEF (26). Disturbances in effort testing in HFrEF have been well characterized, and $\dot{V}O_2_{max}$ is a key prognostic factor in the management of severe HFrEF (12). Much less is known regarding exercise response in HFpEF (1, 22), but undoubtedly HFpEF is characterized by a rigid LV and impaired preload reserve. Indeed, invasively assessed LV chamber stiffness seems to be one of the main pathophysiological mechanisms underlying exertional dyspnea (23).

Besides the technical challenge of exercise echocardiography, several factors make exercise testing and its interpretation complex in HFpEF. Exercise capacity has multifactorial determinants. Patients with HFpEF are typically elderly. Peak exercise declines ~10% per decade because of multiple mechanisms (6, 27). Additionally, comorbidities may preclude effort testing in patients with HFpEF. Although less strenuous protocols may be used, the range of elderly patients with HF that is actually able to endure effort testing in studies ranges from 20 to 70% (10). Finally, the group of HFpEF is a heterogeneous group with variable responses during effort testing (11).

Given the limitations of exercise testing in HFpEF, an alternative approach might be to perform hemodynamic stress tests by manipulating load conditions and HR. Because we have previously documented HFpEF in 20-wk-old ZSF1 Ob (5), we carried out our experimental assessment in 17-wk-old rats presuming that they would be at risk of HFpEF and tried to isolate the effects of these hemodynamic stresses by Trendelenburg, phenylephrine, and dobutamine. Both phenylephrine and dobutamine can be administered by a peripheral intravenous line without major foreseeable side effects in a medically controlled environment although careful selection of patients is warranted. Trendelenburg increased preload as intended, with a slight increase in HR. Reflex changes in systemic arterial pressure likely took place (21). Preload elevation increased EDP and prolonged $\tau$. The role of preload stress echocardiography in HF prognosis stratification has been recently advocated (29). With $\alpha$-adrenergic drug phenylephrine infusion, we observed higher MAP without significant changes in EDVi or HR and therefore selectively increased afterload. We have recently demonstrated that even acute elevations of afterload can upwardly shift the EDP-EDV relationship (7). Another hypothetical alternative to exercise testing could be dobutamine, which is a cornerstone to echocardiography stress testing (25). Indeed, many view dobutamine and exercise stress testing as interchangeable. Nevertheless, compared with exercise, dobutamine induces smaller increases in CO and systolic wall tension while decreasing EDV, peripheral venous tone, central venous pressure, and pulmonary capillary wedge pressure (13). We found decreased EDP and curtailed $\tau$, suggesting improved diastolic performance. Also, in patients with concentric hypertrophy, a pattern similar to ZSF1 Ob, the wall stress at peak effort is reduced by dobutamine that paradoxically reduces myocardial oxygen consumption, partly explaining the higher rate of false negatives in stress testing for coronary artery disease (30).

Although no single parameter of diastolic performance fully assesses the complex interactions that underlie diastole, and a
stepwise and clinically integrated approach to diagnosis is proclaimed by expert consensus (18), the attempt to identify simple indexes that may reliably predict EDP elevation during effort is ongoing. In our experimental setting including a range of healthy, hypertensive, and HFpEF animals undergoing extensive hemodynamic stress testing, we found stronger correlations between invasively measured EDP and E/DTn and LAAv, than between EDP and E/E′. ROC curve analysis for a cutoff EDP of 13 mmHg, however, only showed a trend for improved prediction of the joint estimator combining E/DTn and LAAv compared with E/E′ although our sample size may not ensure enough power to draw a definite conclusion. Burgess et al. (2) reported good correlation between LV EDP and E/E′ during submaximal single-leg supine cycle ergometry exercise in 37 patients. Nevertheless, reliance on E′ as a parameter of LV relaxation kinetic may be flawed by sudden changes of cavity size and loading conditions (15, 25) during effort testing or hemodynamic stress echocardiography. Shortening of DT of the E-wave has long been recognized as a noninvasive surrogate for LV chamber stiffness (9). DT, however, is also lengthened by impaired relaxation; thus its interpretation is not linear. Also, by definition, DT is longer for higher E-wave velocities; thus normalization by E usually yields better estimates (14). Compared with E/E′, the E/DT can be obtained from a single Doppler acquisition while probing a large part of early filling; therefore, it reduces variability and is not a snapshot index. Our results are in accordance with the recent findings of Nuyen et al. (16), who found stronger correlations between E/DT and lung and right ventricular remodeling in aortic banded rats when compared with E/E′ (16) and suggest that alternative indexes may be helpful in stress echocardiography.

Rats have entirely distinct mechanisms governing myocardial relaxation and end-diastolic stiffness, as denoted by distinct HR, responses to load, and myofilament composition. The ZSF1 Ob presents an extreme and untreated metabolic syndrome phenotype at a young age and therefore does not account for the effects of aging and HFpEF therapy. All of the above mentioned reasons limit generalization of findings to the clinical scenario. A puzzling finding that is at clear contradiction to what is observed in patients with HFpEF (28) is the baseline increase in E′ in ZSF1 Ob, which could be explained by higher restoring forces (25). In this preclinical model, it was not feasible to study ventilatory and hemodynamic responses during the effort tests, which would have been more informative on the response to exercise. The small sample size and different timings of evaluation preclude attempts to correlate effort testing data with invasive hemodynamics and echocardiography. During hemodynamic evaluation under stress, it was not possible to perform inferior vena cava occlusions because surgical manipulation would not have been compatible with simultaneous echocardiography. We did not perform concomitant assessment of right ventricular hemodynamics although our data in this experimental model revealed no pulmonary hypertension (5). Because the study was designed as a crossover study with short drug interventions, we are unable to assess potential acute molecular mechanisms by which drugs may have influenced LV function. E′ was analyzed at the lateral annulus, which is less reproducible in the clinics but not in animal experiments with linear probes. Although some selectivity was achieved in hemodynamic interventions, the in vivo experimental setting makes it impossible to control for reflex changes. Moreover, the surgical procedure was not possible without minimal sedation, which required assisted breathing with pressure support ventilation, which preserves effective breathing and tends to minimize cardiovascular effects compared with controlled ventilation, but the influence of anesthetics and mechanical ventilation must be taken under consideration. Echocardiographic evaluation did not comprise state-of-the-art evaluation of strain by speckle-tracking methods that are independent of angle, segmental motion disturbances, and tethering to adjoining structures. Strain-derived indexes may fare better than conventional Doppler in the early detection of HFpEF during stress echocardiography (4). Nonetheless, strain analysis is less straightforwardly applicable to routine clinical practice and requires lower HR and good echocardiographic window.

In conclusion, we have documented decreased effort tolerance, Vo2 max, and AT in the ZSF1 Ob model of metabolic syndrome and HFpEF, reinforcing the role of this preclinical model in therapeutic and pathophysiological research. Hemodynamic stress with dobutamine did not disturb relaxation or elevate EDP despite tachycardia, whereas increased afterload and preload induced by phenylephrine and Trendelenburg did. These results support the use of hemodynamic challenges such as phenylephrine infusion or Trendelenburg positioning in stress echocardiography for early detection of HFpEF as an alternative to exercise testing. Among the echocardiography parameters, E/DTn and LAAv tended to be better predictors of EDP elevation than E/E′. Several simple hemodynamic indexes can track acute changes in EDP and should be reconsidered as alternatives to E/E′.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS

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