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A porcine model of hypertensive cardiomyopathy: implications for heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFPEF) evolves with the accumulation of risk factors. Relevant animal models to identify potential therapeutic targets and to test novel therapies for HFPEF are missing. We induced hypertension and hyperlipidemia in landrace pigs \((n = 8)\) by deoxycorticosteroneacetate (DOCA, 100 mg/kg, 90-day-release subcutaneous depot) and a Western diet (WD) containing high amounts of salt, fat, cholesterol, and sugar for 12 wk. Compared with weight-matched controls \((n = 8)\), DOCA/WD-treated pigs showed left ventricular (LV) concentric hypertrophy and left atrial dilatation in the absence of significant changes in LV ejection fraction or symptoms of heart failure at rest. The LV end-diastolic pressure-volume relationship was markedly shifted leftward. During simultaneous right atrial pacing and dobutamine infusion, cardiac output reserve and LV peak inflow velocities were lower in DOCA/WD-treated pigs at higher LV end-diastolic pressures. In LV biopsies, we observed myocyte hypertrophy, a shift toward the stiffer titin isoform N2B, and reduced total titin phosphorylation. LV superoxide production was increased, in part attributable to nitric oxide synthase (NOS) uncoupling, whereas AKT and NOS isoform expression and phosphorylation were unchanged. In conclusion, we developed a large-animal model in which loss of LV capacitance was associated with a titin isoform shift and dysfunctional NOS, in the presence of preserved LV ejection fraction. Our findings identify potential targets for the treatment of HFPEF in a relevant large-animal model.

NEW & NOTEWORTHY

We present a large-animal model of hypertensive heart disease that mimics the cardiac phenotype of heart failure with preserved ejection fraction (HFPEF) in vivo and shows molecular alterations compatible with current pathophysiological concepts of HFPEF. This animal model will serve to test acute and chronic pharmacological interventions in HFPEF.

Heart failure (HF) is defined as the inability of the left ventricle (LV) to generate an adequate cardiac output at physiological filling pressures. When HF starts from myocardial damage and loss of cardiomyocytes, the LV will undergo dilative remodeling with a progressive reduction of ejection fraction (HFREF) and subsequent systemic neuro-humoral activation. However, almost 50% of clinical HF cases present with a preserved LV ejection fraction (HFPEF). Pharmacological treatment with angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and β-blockers, the cornerstone of HFREF therapy, has only minimal effects in HFPEF although aldosterone receptor antagonists showed some promise (10). This illustrates the different and still unclear pathophysiology underlying HFPEF compared with HFREF.

To develop pharmacological treatment strategies for HFPEF, preclinical animal models are essential. HFREF can be induced reliably by a cardiomyocyte insult, for example, myocardial infarction, ventricular tachypacing, or pressure overload by aortic banding, in virtually all animal species (18). HFPEF in turn is understood to evolve, not from a single trigger, but from the accumulation of cardiovascular risk factors over time, such as ageing, hypertension, obesity, diabetes,
renal dysfunction, and physical inactivity (31). Via mechanisms that are still not completely understood, a specific LV remodeling process is induced that ultimately leads to a loss of LV compliance and increased LV filling pressures, i.e., a leftward shift of the LV end-diastolic pressure-volume relationship (EDPVR). Such a shift of the EDPVR is a hallmark of LV dysfunction in patients with HFPEF (1, 8, 23, 39, 40, 43), which is further aggravated during exercise (7) and predicts clinical outcome (8).

A leftward shift of the LV EDPVR has been described in a variety of hypertensive and metabolic rodent models (17, 28, 36). In particular, the obese and hypertensive ZSF-1 rat strain mimics several molecular alterations observed in biopsies from human HFPEF hearts (14). However, rodent models rely on specific genetic backgrounds and do not allow for detailed hemodynamic assessment, including right heart catheterization or pacing, for instance. Thus large-animal models are required for the reliable preclinical evaluation of pharmacological interventions. Dr. Redfield’s group proposed aged dogs (>10 yr) with renal wrap-induced hypertension as a model of HFPEF, however, these animals do not show a pronounced shift of the LV EDPVR or clinical HF and are available only at limited numbers (13, 29). Pigs with aortic banding (27) or angiotensin-induced hypertension (34) have been proposed as models of HFPEF; however, LV remodeling in patients with aortic stenosis is distinct from HFPEF (38), and angiotensin does not seem to play a major causative role in HFPEF (10).

We therefore aimed to establish a relevant, risk factor-based porcine model of HFPEF by implanting a subcutaneous depot of deoxycorticosteroneacetate (DOCA, an aldosterone analog) combined with a Western diet (WD) containing high amounts of salt, fat, cholesterol, and sugar for 12 wk. We characterized LV remodeling and function by echocardiography and LV pressure-volume analysis and analyzed LV histological and molecular changes.

METHODS

The experimental protocol was approved by the local bioethics committee of Vienna, Austria (BMWF-66.010/0108-II/3b/2010), and it conforms with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Experimental model. Female landrace pigs (n = 8, 22 ± 1 kg) were sedated with 20 mg/kg ketamine, 0.4 mg/kg midazolam, 0.5 mg/kg azaperone, and 0.1 mg/kg butorphanol to implant a subcutaneous DOCA depot (100 mg/kg, 90-day release pellets; Innovative Research of America, Sarasota, FL) into the inguinal region. The animals were then fed a chow supplemented with high amounts of salt (4%), cholesterol (4%), crude fat (25%), cholate (0.5%), and sugar (26%). The animals had free access to water. Eight female, healthy, untreated pigs on a regular diet served as controls.

Echocardiography and invasive hemodynamics. After 12 wk of treatment, the animals were sedated, systolic blood pressure was measured noninvasively (tail cuff), and blood samples were drawn. Transthoracic echocardiography (Vivid I; GE Healthcare, Vienna, Austria) was next performed to record parasternal long- and short-axis 2D views. We did not succeed in obtaining reliable apical views in animals >40 kg. Anesthesia was then induced by 30–60 mg of propofol (Propofol Fresenius 1% emulsion; Fresenius Kabi, Graz, Austria) to allow for intubation with an endotracheal tube. Anesthesia was maintained with 0.5–1.0% isoflurane, 35 μg/kg fentanyl per hour, 1 mg/kg midazolam per hour, and 0.2 mg/kg pancuronium per hour. A balanced crystalloid infusion (Elo-Mel Isoton, Fresenius Kabi) was administered at a fixed rate of 10 ml/kg per hour throughout the protocol. A body temperature of 38–39°C was maintained by either surface cooling or a warming blanket. Instrumentation with Swann-Ganz and conductance catheters was done as described before (35).

Experimental protocol. All animals were allowed to stabilize for at least 30 min after instrumentation. Steady-state hemodynamics were acquired over three respiratory cycles at spontaneous heart rate. The aortic balloon catheter was inflated briefly three times to vary loading conditions and to obtain pressure-volume relationships. The tidal volume was temporarily decreased to the respirator’s minimum (50 ml) during these measurements to minimize respiration-induced changes of intrathoracic pressure. Heart rate was then increased to multiples of 20 beats/min by right atrial pacing as long as stimulation was followed by a regular and steady LV contraction. Steady-state recordings were repeated at each heart rate. Afterward, dobutamine was infused at 2.5 μg/kg per min. All measurements outlined above were repeated, and the dobutamine infusion was stopped. The animals were then allowed to return to baseline values for 60 min.

Finally, a thoracotomy was performed, and two to three transmural LV biopsies were taken from the beating heart, immediately divided into the subepicardial and subendocardial layer, rinsed carefully, and separately frozen into liquid nitrogen. A bolus injection of 100 mM potassium was given to euthanize the animals. Further biopsies were taken, rinsed carefully, and placed in 10% formaldehyde for histological analyses.

The left kidney was excised and weighed, and the left kidney/body weight ratio was calculated. A sample of the left kidney was preserved in 10% formaldehyde. The descending thoracic aorta was excised 10 cm proximal to the diaphragmal hiatus, and a cross-sectional sample was preserved in 10% formaldehyde.

Echocardiographic data analysis. LV end-diastolic wall thickness and diameter were measured in the basal region both in short- and long-axis views and averaged over three subsequent beats. Left atrial (LA) area was measured in a long-axis view at the time point before mitral valve opening.

Hemodynamic data analysis. Volumetric conductance data were calibrated before baseline and before dobutamine infusion by cardiac output and hypertonic saline injection (parallel conductance). Hemodynamic and conductance data were analyzed offline using a custom-made software (CircLab by P. Steendijk) as described before (35). One animal in the DOCA/WD group died during the instrumentation period because of sustained ventricular fibrillation; thus hemodynamic data from only seven pigs were analyzed.

Histological analysis. LV and LA myocardial tissue samples were stained with picrosirius red (PSR) and with hematoxylin and eosin (HE). The percentage of area of positive PSR staining as well as the cross-sectional area (CSA) from transversally sectioned cardiomyocytes in HE staining were calculated using the ImageJ software (National Institutes of Health, Bethesda, MD). Renal sections were stained with Schiff reagent (PAS). In all cases, a minimum of 50 equatorial glomerular cross sections was evaluated. A semiquantitative staining score was assigned to each glomerulus based on the extent of PAS-positive deposits (0: no deposits; 1: <1/3; 2: 1/3-2/3; 3: >2/3 of glomerular cross section with PAS-positive deposits).

Titin isoforms and phosphorylation. Titin isoforms were separated as described (15). Tissue samples were solubilized in 50 mM Tris-SDS buffer (pH 6.8) containing 8 μg/ml leupeptin (Peptin Institute, Osaka, Japan) and phosphatase inhibitor cocktail (P2880, 10 μl/ml; Sigma, St. Louis, MO). Samples were heat-activated for 3 min at 96°C and centrifuged. Samples (20 μg: equal concentration checked by spectrophotometric methods) were then separated by agarose-strengthened 1.8% SDS-PAGE. Gels were run at 5-mA constant current for 16 h. To determine total titin phosphorylation and expression, titin gels were stained for 1 h with Pro-Q Diamond phosphoprotein stain. Fixation, washing, and destaining were performed according to the manufacturer’s guidelines (Molecular Probes, Eugene, OR). To assess total protein content, gels were stained overnight with SYPRO Ruby (Molecular Probes). Staining was visualized using the LAS-4000.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>DOCA/WD (n = 8)</th>
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<tbody>
<tr>
<td>Body weight, kg</td>
<td>67 ± 1</td>
<td>74 ± 2*</td>
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<tr>
<td>Tail-cuff systolic blood pressure, mmHg</td>
<td>97 ± 6</td>
<td>139 ± 11*</td>
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<tr>
<td>Total cholesterol, mg/dl</td>
<td>69 ± 3</td>
<td>553 ± 44*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>25 ± 2</td>
<td>331 ± 35*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>38 ± 1</td>
<td>209 ± 14*</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>30 ± 7</td>
<td>66 ± 10*</td>
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Echocardiographic parameters

<table>
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<th></th>
<th>Control (n = 8)</th>
<th>DOCA/WD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV septal wall thickness, mm</td>
<td>12.7 ± 0.5</td>
<td>20.3 ± 0.6*</td>
</tr>
<tr>
<td>LV posterior wall thickness, mm</td>
<td>10.0 ± 0.4</td>
<td>14.5 ± 0.6*</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>44.5 ± 1.1</td>
<td>42.3 ± 1.3</td>
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<tr>
<td>LV relative wall thickness</td>
<td>0.51 ± 0.02</td>
<td>0.83 ± 0.03*</td>
</tr>
<tr>
<td>LA area, cm²</td>
<td>9.7 ± 0.7</td>
<td>12.4 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Left ventricular (LV) relative wall thickness was defined as (LV septal wall thickness + LV posterior wall thickness)/LV end-diastolic diameter. LA, left atrial; DOCA/WD, deoxycorticosteroneacetate/Western diet-fed group. *P < 0.05 vs. control.

RESULTS

On the day of death, DOCA/WD-treated animals weighed slightly more than controls (Table 1). No pig developed signs and symptoms of HF at rest throughout the whole observation period.

Noninvasive blood pressure and blood samples. During sedation, noninvasively assessed systolic blood pressure was higher in DOCA/WD vs. control (Table 1). DOCA/WD treatment resulted in higher plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides (Table 1). Plasma creatinine, urea, and glucose of all animals remained in the normal range.

Echocardiography. DOCA/WD-treated animals developed concentric LV hypertrophy with higher end-diastolic septal and posterior wall thickness at similar LV end-diastolic diameter (i.e., a higher relative wall thickness, Table 1). LA area was significantly larger in DOCA/WD (Table 1).

Invasive hemodynamics. Baseline cardiac output was not different between groups; however, the maximum cardiac output achieved by pacing and by pacing plus dobutamine infusion was lower in DOCA/WD vs. control (Fig. 1A). LV end-diastolic pressure remained unchanged in control animals,
Cardiomyocytes was larger in DOCA/WD (LV: 738 m²) compared with control (LV: 456 m², both *P* < 0.05 vs. control; †*P* < 0.05, Fig. 4). Whereas it was significantly increased in DOCA/WD during pacing and dobutamine infusion (Fig. 1B), LV ejection fraction was preserved in DOCA/WD-treated animals (Table 2), and dobutamine infusion increased mean arterial pressure and LV dP/dt max by the same magnitude in both groups (Table 2).

The LV end-systolic PVR (ESPVR) and EDPVR were shifted leftward in DOCA/WD vs. control (Fig. 2A). During dobutamine infusion, the ESPVR remained unchanged in control animals (Fig. 2B) but was shifted rightward in DOCA/WD (Fig. 2C). The ESPVR was shifted leftward by dobutamine in both groups (Fig. 2, B and C).

The time constant of LV isovolumic relaxation, τ, was not different under baseline conditions and decreased during pacing in both groups (Fig. 3A). Dobutamine further decreased τ only in control animals (Fig. 3, B and C). The peak inflow velocity (maximum dV/dt) was lower in DOCA/WD at any heart rate (Fig. 3D) and was increased in response to dobutamine infusion in both groups (Fig. 3, E and F). During pacing, LV end-diastolic pressure (LVPed) was higher (Fig. 3G), and LV end-diastolic volume (LVVed) was lower at any heart rate in DOCA/WD (Fig. 3H), such that the ratio of LVPed/LVVed was always higher in DOCA/WD (Fig. 3I). This ratio decreased during dobutamine infusion at higher heart rates in DOCA/WD but not in controls (Fig. 3, N and O).

**Histological analyses.** The area of positive picrosirius red staining was lower in DOCA/WD vs. control. The CSA of cardiomyocytes was larger in DOCA/WD (LV: 738 ± 84 μm², LA: 172 ± 7 μm²) compared with control (LV: 456 ± 39 μm², LA: 114 ± 9 μm², both *P* < 0.05, Fig. 4).

**Titin isoforms and phosphorylation.** Titin isoform composition was similar in subepicardial LV muscle layers but shifted toward the stiffer N2B isoform in subendocardial LV muscle layers of DOCA/WD hearts (Fig. 5A). Total titin phosphorylation was lower in DOCA/WD animals in all samples, reaching statistical significance for subendocardial N2B phosphorylation and subepicardial N2BA phosphorylation (Fig. 5B).

**Superoxide production.** In the presence of n-NAME (negative control), LV superoxide release (assessed by the tiron-inhibitable fraction of lucigenin-enhanced chemiluminescence) was higher in DOCA/WD pigs compared with controls. LV superoxide release remained unchanged in the presence of the NOS inhibitor, L-NAME, in controls but decreased significantly in DOCA/WD pigs, indicating that, under these conditions, NOS produced reactive oxygen species rather than NO (Fig. 5C).

**Expression and phosphorylation of AKT and NOS.** Data on expression and phosphorylation of AKT and NOS are presented in Figs. 6 and 7. There was no systematic difference between groups.

**Plasma NT-pro-BNP levels.** Neither the porcine-specific ELISA (2 separate runs) nor the human-specific ELISA was able to detect NT-pro-BNP in the present study. All plasma samples (including the positive control samples obtained in pigs with up to 3-fold dilated atria) were below the lower detection limit. There was no evidence for a technical error, as the standard curves were suitable.

**Renovascular morphometrics and histopathology.** Data on renovascular morphometrics and histopathology are presented.

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**Table 2. Invasive hemodynamics at baseline and during dobutamine infusion**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Dobutamine</th>
<th>DOCA/WD (n = 7)</th>
<th>Dobutamine</th>
</tr>
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<tr>
<td></td>
<td>Baseline</td>
<td>DObutamine</td>
<td>Baseline</td>
<td>DObutamine</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>51 ± 3</td>
<td>58 ± 2</td>
<td>68 ± 3*</td>
<td>74 ± 4*</td>
</tr>
<tr>
<td>LV maximum pressure, mmHg</td>
<td>101 ± 3</td>
<td>115 ± 5†</td>
<td>107 ± 4</td>
<td>127 ± 6†</td>
</tr>
<tr>
<td>LV maximum dP/dt, mmHg/s</td>
<td>1387 ± 63</td>
<td>2981 ± 154†</td>
<td>1554 ± 124</td>
<td>3401 ± 175†</td>
</tr>
<tr>
<td>LV minimum dP/dt, mmHg/s</td>
<td>−1842 ± 31</td>
<td>−2445 ± 111†</td>
<td>−1850 ± 107</td>
<td>−2285 ± 245†</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>85 ± 3</td>
<td>96 ± 5†</td>
<td>86 ± 5</td>
<td>102 ± 7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P* < 0.05 vs. control; †*P* < 0.05 vs. baseline.

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Fig. 2. Both LV end-systolic and LV end-diastolic pressure-volume relationships (ESPVR and EDPVR) were shifted leftward in DOCA/WD animals (A). Dobutamine shifted the EDPVR rightwards in DOCA/WD (C) but not in control (B).
Fig. 3. Parameters of LV diastolic function during right atrial pacing at increasing heart rates. **Left**: baseline condition. **Middle**: effect of dobutamine in control animals. **Right**: effect of dobutamine in DOCA/WD. The isovolumic relaxation constant, $\tau$, was unchanged in DOCA/WD at baseline (A). Dobutamine further accelerated $\tau$ in control (B) but not in DOCA/WD (C). The peak inflow velocity (maximum $dV/dt$) was lower in DOCA/WD both at baseline and during dobutamine infusion (D–F). LV end-diastolic pressure (LVPed) was higher and end-diastolic volume (LVVed) was lower in DOCA/WD (G–L), such that their ratio (LVPed/LVVed) was higher in DOCA/WD (M). LVPed/LVVed decreased during dobutamine infusion in DOCA/WD (O) but not in control (N).
in Figs. 8 and 9. DOCA/WD-treated animals showed signs of hypertensive kidney disease and had a thicker aortic tunica media.

**DISCUSSION**

We found that pigs rendered hypertensive by DOCA administration and fed a WD developed LV concentric hypertrophy together with a marked leftward shift of the LV EDPVR. Decreased LV compliance was associated with a reduction in total titin phosphorylation, a shift toward the stiffer titin N2B isoform, and uncoupled NOS activity.

**Effect of risk factors.** The main risk factors for the development of HFPEF are aging, hypertension, diabetes, and obesity (31). Zhu et al. (42) reported that a 12-wk high-lipid diet per se causes LV diastolic abnormalities in pigs, mediated by oxidative stress. Clinical data imply that aldosterone receptors rather than angiotensin II or sympathetic activation are causally involved in the evolution of HFPEF (10). We therefore reasoned that the aldosterone analog, DOCA, together with a high-salt and high-fat diet may be a promising approach to induce hypertension and possibly HFPEF in pigs. As shown in Table 1, DOCA/WD animals developed hypertension and hyperlipidemia. This was associated with pronounced LV hypertrophy, concentric cardiac remodeling, and atrial enlargement, as well as thickening of the aortic wall and histologic signs of hypertensive kidney disease. Risk factor exposure in the present study therefore induced a cardiac and systemic phenotype of hypertensive end-organ damage. However, none of the animals showed clinical signs and symptoms of HF at rest. The model presented here therefore corresponds to stage B HF according to the ACC/AHA guidelines (19).

**Invasive hemodynamics.** Invasive hemodynamics did not indicate major differences in LV end-diastolic pressure, cardiac output, or heart rate at rest (Fig. 1, A and B) in keeping with the absence of clinically manifest HF in these animals. In humans, the clinical syndrome of LV dysfunction in HFPEF develops during exercise rather than in the sedentary state (7, 37, 39). We therefore challenged LV function by stepwise right atrial pacing and dobutamine infusion as a surrogate of exercise during anesthesia. As shown in Fig. 1, DOCA/WD-treated
animals had a lower cardiac output at higher LV end-diastolic pressures, indicating a reduced cardiac reserve during simulated exercise. Parameters of LV systolic function, i.e., ejection fraction, dP/dt\text{max}, and the ESPVR, did not indicate a deficit of LV contractility in DOCA/WD pigs, both at baseline and during dobutamine infusion (Table 2 and Fig. 2). In contrast, the LV EDPVR at rest was markedly shifted leftward (Fig. 2A); for example, the LV end-diastolic volume at an end-diastolic pressure of 10 mmHg was 157 ± 13 ml in controls but only 94 ± 8 ml in DOCA/WD. This ~40% loss of LV capacitance at a representative LV end-diastolic pressure is in keeping with values reported in patients with HFPEF [~65% in Zile et al. (43), ~40% in Andersen et al. (1), ~10% in Lam et al. (23)].

The EDPVR can be shifted leftward by changes in a number of LV diastolic parameters. The LV isovolumic relaxation constant, \(\tau\), decreased with pacing both in controls and DOCA/WD pigs in keeping with faster relaxation at higher heart rates. During dobutamine infusion, controls showed a further slight decrease in \(\tau\) that was absent in DOCA/WD. However, the differences were rather minor, as was the case for the ratio of diastolic time intervals and \(\tau\) (see Fig. 10). Incomplete LV relaxation therefore did not play an important role in the leftward shift of the EDPVR in our model. Isovolumic...

Fig. 6. Representative Western blots for nitric oxide synthase 1 (NOS1), NOS3, phosphorylated NOS3 (p-NOS3-S1177 and p-NOS3-Thr495), and GAPDH (A). NOS1 expression was not different between groups (B). In subendocardial tissue samples, NOS3 expression was higher in DOCA/WD; however, there was no systematic difference in NOS3 phosphorylation between groups (B).

Fig. 7. Representative Western blots for AKT phosphorylation (p-AKT), total AKT, and GAPDH (A). There was no significant difference in total AKT expression and AKT phosphorylation in subepicardial and subendocardial tissue samples between both groups (B).
relaxation is mainly governed by myofilament calcium unbinding and ATP-dependent resequestration into the sarcoplasmic reticulum and thereby is a sensitive indicator of myocardial ischemia (26). The absence of a difference in isovolumic relaxation between groups argues against a coronary blood flow deficit in the DOCA/WD group, as could possibly result from coronary microvascular dysfunction in hypertrophied myocardium. In patients with HFPEF, τ is typically prolonged (1, 23, 32, 39, 40, 43), particularly during exercise, and has been associated with cardiomyocyte energy deficiency, as can result from myocardial hyperperfusion (33).

In line with the absence of major differences in LV isovolumic relaxation, there was no difference in the time to peak LV inflow velocity between groups (see Fig. 10). Peak LV filling velocities, however, were markedly lower in DOCA/WD under any condition tested (Fig. 3, D—F). Because LV stroke volumes and filling volumes, respectively, were only borderline lower in the DOCA/WD group (see Fig. 10), and isovolumic relaxation was not different between groups, LV suction and/or atrial contractility were probably reduced in the hypertrophied DOCA/WD hearts. Diastolic LV suction, i.e., an intraventricular mitral-to-apical pressure gradient during early diastole (9), is mediated by LV untwisting. Such untwisting is indeed reduced in patients with HFPEF, in particular during exercise (37). Also, LA dilatation and dysfunction are characteristics of HFPEF (6), and LA dilatation was observed in the present model.

We observed substantially lower LV end-diastolic volumes in DOCA/WD together with a much steeper increase of LVPed during pacing or dobutamine, as observed in patients with HFPEF during exercise (7). It would have been worthwhile to assess the EDPRV at each pacing step; however, this elicited severe arrhythmia (e.g., ventricular fibrillation) and distorted signals (see ESPVR in Fig. 1), probably because of mechanical contact between the pressure-volume catheter and the LV endocardium in the small lumen of DOCA/WD ventricles. We therefore calculated the ratio of LVPed and LVVed during steady-state conditions. This end-diastolic pressure/volume quotient (eDPVQ) was recently validated as an estimate of the LV EDPRV at spontaneous heart rate in DOCA/WD pigs, suggesting that β-adrenergic stimulation may improve LV compliance under these conditions. Taken together, these findings provide in vivo evidence for a role of β-adrenoceptor signaling as an acute modulator of myocardial stiffness, specifically in diseased animals, extending in vitro findings obtained in cardiac tissues from a canine model of HFPEF (13).

*Molecular changes.* The cardiomyocyte cytoskeletal protein, titin, stores potential energy during systolic sarcomere compression and releases it during diastole by elastic recoil of its molecular spring segment. Thereby, the titin springs dominate cardiomyocyte passive stiffness and contribute greatly to overall diastolic myocardial stiffness (25). Cardiomyocyte stiffness at physiological sarcomere lengths is modulated by alternative splicing and by posttranslational modifications of the titin spring segment (25). Titin isoform shift and hypophosphorylation of titin were reported in human HFPEF (4) and in experimental models (3, 13, 14), and our data are in line with these findings (Fig. 5). Titin can be phosphorylated by PKA, PKG, PKC-α, ERK-II, and CaMK-IIδ (25). PK-α phosphorylation increases myocardial stiffness, whereas all other kinases decrease it. Diastolic dysfunction in unilaterally nephrectomized and DOCA-treated mice was attributed to NOS uncoupling (a condition by which, in the presence of an oxidative environment, NOS produces superoxide rather than NO) (24, 36). In the present study, we found no effect on expression and phosphorylation of AKT, endothelial NOS, or neuronal NOS but demonstrated increased superoxide radical production in DOCA/WD LV myocardium that was partially blunted in the presence of L-NAME, as expected in the presence of NOS uncoupling (31). A dysfunctional NO-cGMP axis may result in decreased PKG-mediated titin phosphorylation and increased ectopic activity due to the altered NOS activity under these conditions.
Nitrosative stress, decreased cGMP levels, and PKG activity were demonstrated in human HFPEF samples (38), and PKG administration in turn decreased human HFPEF cardiomyocyte stiffness ex vivo. Increased titin phosphorylation and reduced cardiomyocyte passive stiffness were also reported in dog hearts following in vivo treatment with sildenafil and BNP (3), both of which are expected to increase cGMP signaling in the myocardium.

**Limitations.** We assessed blood pressure and hemodynamics during sedation and anesthesia and hence measured cardiac function at only a minor degree of hypertension in the present study.

As landrace pigs grow fast, we were restricted to a 12-wk induction period. We measured an approximately twofold higher cardiomyocyte CSA in control animals than what is considered normal [180–250 μm², (12)]. Morphometric analyses are subject to many potential sources of error, including tissue harvesting, tissue fixation, cutting, staining, and the cross-section selection (12). The high absolute CSA may therefore result from, to some extent, suboptimal methodology. However, the 60% relative increase of CSA after DOCA/WD treatment is very well in line with our echocardiographic data (concentric hypertrophy) and thus represents a plausible finding.

Because of anatomical limitations, we were not able to obtain reliable apical views during echocardiography and do not report on E/A ratio, E/E’, or atrial volumes. We could not detect plasma NT-pro-BNP in DOCA/WD animals using...
commercially available ELISA kits. We included plasma samples obtained from pigs with induced atrial fibrillation and pronounced atrial dilatation as a positive porcine control and reasoned that these animals should have elevated plasma natriuretic peptides. However, all samples were below the lower detection limit as well, while standard curves with controls provided by the manufacturer did not indicate a technical mistake. We conclude that the ELISA kits used in the present study did not work in our hands. Thus we cannot report on plasma NT-pro-BNP values in the present study.

Clinical implications. We observed hemodynamic and molecular changes typically seen in HFPEF but did not observe signs and symptoms of HF in the DOCA/WD animals. The lack of prolonged LV relaxation in the DOCA/WD animals might indicate that either fibrosis or microvascular dysfunction, or both, with subsequent subendocardial ischemia is missing to render cardiac dysfunction into failure. Alternatively, it can be speculated that a loss of LV capacitance is a prerequisite but by itself is not sufficient to induce HFPEF and that comorbidities have to add to LV dysfunction. Indeed, organ dysfunction in HFPEF has been recognized to extend beyond the LV, comprising LA dilatation and dysfunction, pulmonary hypertension, right ventricular impairment, arterial stiffening, and skeletal muscle abnormalities (6). In line with that, spironolactone treatment improved parameters of LV diastolic function but not exercise capacity in patients with HFPEF (10). Exercise training increased exercise capacity in patients with HFPEF in several studies (11, 22), but some studies reported effects rather on skeletal muscles than on cardiac function (16, 30). On the other hand, cardiac adaptation during 1 yr of high-intensity endurance training clearly involved a rightward shift of the LV EDPVR (2). We therefore believe that to improve LV compliance is a reasonable treatment target in HFPEF and that our model represents a relevant platform to test acute and chronic interventions aiming to reshift the LV EDPVR rightward.

Conclusion. In summary, we established a porcine model of hypertensive heart disease with a loss of LV capacitance...
similar to that reported in patients with HFPEF. LV remodeling reduced cardiac reserve but did not induce the clinical condition of HF at rest. This model identified titin and NOS uncoupling as potential treatment targets and represents a relevant platform to test acute and chronic interventions aiming to shift the LV EDPVR rightward.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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