Left Atrial Remodeling and Atrioventricular Coupling in a Canine Model of Early Heart Failure With Preserved Ejection Fraction

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Background—Left atrial (LA) compliance and contractility influence left ventricular stroke volume. We hypothesized that diminished LA compliance and contractile function occur early during the development of heart failure with preserved ejection fraction (HFpEF) and impair overall cardiac performance.

Methods and Results—Cardiac magnetic resonance imaging, echocardiography, left ventricular and LA pressure-volume studies, and tissue analyses were performed in a model of early HFpEF (elderly dogs, renal wrap-induced hypertension, exogenous aldosterone; n=9) and young control dogs (sham surgery; n=13). Early HFpEF was associated with LA enlargement, cardiomyocyte hypertrophy, and enhanced LA contractile function (median active emptying fraction 16% [95% confidence interval, 13–24]% versus 12 [10–14]%, P=0.008; end-systolic pressure-volume relationship slope 2.4 [1.9–3.2]mm Hg/mL HFpEF versus 1.5 [1.2–2.2]mm Hg/mL controls, P=0.01). However, atrioventricular coupling was impaired and the curvilinear LA end-reservoir pressure-volume relationship was shifted upward/leftward in HFpEF (LA stiffness constant [β La] 0.16 [0.11–0.18]mm Hg/mL versus 0.06 [0.04–0.10]mm Hg/mL controls; P=0.002), indicating reduced LA compliance. Impaired atrioventricular coupling and lower LA compliance correlated with lower left ventricular stroke volume. Total fibrosis and titin isoform composition were similar between groups; however, titin was hyperphosphorylated in HFpEF and correlated with β La. LA microvascular reactivity was diminished in HFpEF versus controls. LA microvascular density tended to be lower in HFpEF and inversely correlated with β La.

Conclusions—In early-stage hypertensive HFpEF, LA cardiomyocyte hypertrophy, titin hyperphosphorylation, and microvascular dysfunction occur in association with increased systolic and diastolic LA chamber stiffness, impaired atrioventricular coupling, and decreased left ventricular stroke volume. These data indicate that maladaptive LA remodeling occurs early during HFpEF development, supporting a concept of global myocardial remodeling.

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Key Words: atrial remodeling ■ diastolic heart failure ■ hypertension ■ left atrium

See Clinical Perspective

Heart failure (HF) with preserved ejection fraction (HFpEF) commonly presents with LV diastolic dysfunction and LA chamber enlargement. Recent human studies have also proposed a role for LA dysfunction in HF development and uniquely in HFpEF pathophysiology, although corroborative invasive and histological assessment of LA myocardial properties has been lacking. Moreover, although LA function is recognized to decline early in the course of some dilated cardiomyopathies, consistent with a primary atrial myopathy, LA remodeling and dysfunction in HFpEF have traditionally been considered a late sequela of hypertension, LV diastolic dysfunction, and chronic LA pressure overload. By contrast, Paulus and Tschöpe have proposed a novel paradigm for HFpEF pathophysiology implicating coronary microvascular endothelial inflammation and altered intramyocardial...
signaling as seminal mechanisms. These are inherently not chamber-specific.

On the basis of this discord, we proposed a hypothesis that maladaptive alterations in LA structure and function could, in fact, be identified at an early stage of HFpEF development and would impair overall cardiac performance. To test this hypothesis, LA and LV properties and left atrio-ventricular coupling were defined using echocardiography, cardiac magnetic resonance imaging (MRI), and invasive pressure-volume loop analyses in a canine model of early-stage hypertensive HFpEF induced by aging, renal wrap-induced hypertension, and proinflammatory aldosterone excess. Chamber-specific fibrosis, myocyte hypertrophy, titin phosphorylation, and microvascular structure and endothelial function were assessed to elucidate pathophysiological mechanisms.

Methods

Animal Model

Nine elderly dogs (8–13 years; 25.5 [19.5–27.6] kg; 2 males; 7 females) underwent bilateral renal wrapping, which is an established model of experimental hypertension. Young control dogs (n=13; age = 1 year; 23.0 [22.5–25.3] kg; 7 males; 6 females) underwent sham surgery alone. On judicious review of published data, young and elderly dogs without hypertension exhibit comparable LV dimensions, indexed mass, and fibrosis. Young control dogs (n=13; age = 1 year; 23.0 [22.5–25.3] kg; 7 males; 6 females) underwent sham surgery alone. On judicious review of published data, young and elderly dogs without hypertension exhibit comparable LV dimensions, indexed mass, and fibrosis.

On the basis of the restricted availability of aged dogs, young dogs were, therefore, selected to undergo sham surgery, acknowledging the potential limitation. All animals were maintained in accordance with National Institutes of Health guidelines for the Care and Use of Laboratory Animals. The study protocol was approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Noninvasive Imaging

All dogs underwent 2D transthoracic echocardiography (Philips, 3.5 MHz transducer) in the conscious (standing) state before surgery and after 8 weeks (Methods II in the Data Supplement).

MRI (1.5-T scanner; GE Healthcare) was used to measure LA and LV chamber volumes and function at week 8 (Methods III in the Data Supplement). LA volume-time curves were constructed to determine LA maximum, minimum, mid-diastolic (end of rapid emptying), and preatrial contraction volumes. LA reservoir, stroke, and conduit volumes, and LA function parameters were calculated according to standard equations (Table I in the Data Supplement).

In Vivo Hemodynamic Study

Open chest, open pericardium hemodynamic studies were performed at week 8 under anesthesia (IV propofol; 2–6 mg/kg, followed by inhaled isoflurane, 0.5%–2.5%). Mid-ascending aortic pressure and LV and LA pressure-volume loops were measured using 7F manometer-tipped pressure and 7F (LV) or 5F (LA) ADVantage admittance catheters (Scisense, Ontario, Canada). Hydraulic occluders were placed around the superior and inferior vena cava to allow acute preload reduction. Intravenous dextran was administered before hemodynamic measurements to achieve a LV end-diastolic pressure =20 mm Hg. All data were acquired with ventilation suspended at end-expiration and atrial pacing at 10 to 15 bpm above the sinus rate (Methods IV in the Data Supplement).

Pressure-Volume Analysis

LA end-systolic volume (LA\textsubscript{VES}) was taken as the minimum LA volume at end-atrial contraction. Its corresponding pressure defined LA end-systolic pressure (LA\textsubscript{PES}). LA end-reservoir volume (LA\textsubscript{VER}) and pressure (LA\textsubscript{PVER}) were taken as the maximum LA volume and pressure immediately before mitral valve opening.

LA myocardial contractility was evaluated using a time-varying elastance model. LA\textsubscript{ES} and LA\textsubscript{VES} data from variably loaded pressure-volume loops were fit to the linear regression equation: LA\textsubscript{PES}=E_{La} (LA\textsubscript{VES}−V_{0s}), where the slope of the end-systolic pressure-volume relation represents LA systolic elastance (E\textsubscript{La}) and V\textsubscript{0s} is the extrapolated volume intercept of the relation. The coefficient of determination, $R^2$, was used to describe goodness-of-fit for pressure-volume data to the linear model. Curvilinearity over the measured range of LA pressure and volumes yielded lower $R^2$ values.

LA compliance was assessed from LA end-reservoir data during vena cava occlusion. LAP\textsubscript{VER} and LA\textsubscript{VER} were fit to the monoequation: LAP\textsubscript{VER}=E_{La} (LA\textsubscript{VER}−V_{0s}), where α reflects the y intercept (curve-fitting constant), e is the base of the natural logarithm, and β represents the dynamic stiffness constant (modulus of chamber stiffness). Data goodness-of-fit were assessed using $R^2$. LV pressure-volume loops were analyzed as previously described (Methods IV in the Data Supplement).

At the end of the study, full-thickness LV and LA myocardial biopsies were obtained from the beating heart and flash-frozen in liquid nitrogen. Dogs were euthanized with intravenous potassium chloride under deep anesthesia, consistent with guidelines of the Panel on Euthanasia of the American Veterinary Medical Association.

Immunostaining and Fluorescence Microscopy

Cardiomyocyte cross-sectional area was determined by manual planimetry (ImageJ, National Institutes of Health) from 4-μm thick sections of paraffin-embedded LA and LV myocardial tissue stained with fluorescein isothiocyanate-conjugated wheat germ agglutinin (Vector Laboratories, Burlingame, CA), to delineate cell membranes (Methods VI in the Data Supplement). Minimum dimension was calculated as perpendicular to the delineated contour of the cell. A minimum of 100 cardiomyocytes were measured per section.

In Vitro Microvascular Function

LA microvessels were isolated from freshly harvested tissue and subjected to physiological levels of shear stress, as described previously, in the presence or absence of intact endothelium, the endothelial nitric oxide synthase inhibitor N(o)-nitro-1-arginine methyl ester (L-NAME), and iberiotoxin, an inhibitor of large conductance

Fibrosis Assessment

Transmural 4-μm thick cross sections of LA and LV tissue were stained with picrosirius red to demonstrate extracellular matrix (Methods V in the Data Supplement) and scanned by whole-field digital microscopy. Interstitial fibrosis was expressed histomorphometrically as a percentage of total tissue area, excluding endocardial, epicardial, and perivascular regions (ImageJ, National Institutes of Health, Bethesda, MD) in the LA anterior free wall (LAFW), LA appendage (LAA), and LA posterior wall adjacent to the pulmonary veins (LAPW). Myocardial collagen content was determined using the hydroxyproline assay (Methods V in the Data Supplement).

Titin Isoform Expression and Phosphorylation Status

Titin isoform expression was determined by SDS-PAGE and densitometry. Total phosphoprotein staining (Pro-Q Diamond) was used to assess total titin phosphorylation (Methods VII in the Data Supplement).

In Vitro Microvascular Function

LA microvessels were isolated from freshly harvested tissue and subjected to physiological levels of shear stress, as described previously, in the presence or absence of intact endothelium, the endothelial nitric oxide synthase inhibitor N(o)-nitro-1-arginine methyl ester (L-NAME), and iberiotoxin, an inhibitor of large conductance
Ca²⁺-activated K⁺ (BK) channels involved in nitric oxide–mediated shear stress–induced vasodilation (SSD; Methods VIII in the Data Supplement).

Microvascular Density
Paraffin-embedded myocardial sections were stained with the biotinylated endothelial cell-specific marker isolectin B4 (Vector Laboratories, Burlington, VT). Microvascular density was quantified on whole-field digital micrographs using an automated color-detection algorithm (Definiens Tissue Studio 3.5; Definiens, Germany; Methods IX in the Data Supplement). Microvessels (small precapillary arterioles) were defined by a cross-sectional area of 78.5 to 314 μm² and average luminal diameter of 10 to 20 μm.

Statistics
Continuous variables are presented as median (25th–75th percentile). On the basis of the sample size, the Wilcoxon rank-sum (nonparametric) test was used to compare characteristics between HFpEF and control dogs. The Wilcoxon signed-rank test was used for within-group comparisons of echocardiographic data at baseline (prior to surgery) versus week 8. Linear correlations between 2 variables were assessed using the Pearson correlation coefficient, \( r \). Goodness of fit/variance for pressure-volume data to regression models was assessed

Table 1. LV and LA Structure and Function by Magnetic Resonance Imaging (Week 8)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=10)</th>
<th>HFpEF (n=7)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV structure and function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>100 (91–100)</td>
<td>101 (90–115)</td>
<td>0.88</td>
</tr>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>46.6 (44.1–52.3)</td>
<td>34.3 (33.3–49.9)</td>
<td>0.077</td>
</tr>
<tr>
<td>LV end-systolic volume, mL</td>
<td>21.9 (18.2–24.4)</td>
<td>19.6 (11.5–24.3)</td>
<td>0.30</td>
</tr>
<tr>
<td>LV stroke volume, mL</td>
<td>25.5 (23.4–28.4)</td>
<td>21.1 (15.2–28.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>86.3 (78.8–92.3)</td>
<td>99.2 (89.5–129.6)</td>
<td>0.015</td>
</tr>
<tr>
<td>LV mass/end-diastolic volume, g/mL</td>
<td>1.9 (1.6–2.1)</td>
<td>2.3 (2.2–3.4)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>2.6 (2.2–3.0)</td>
<td>1.9 (1.5–3.2)</td>
<td>0.22</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>55.0 (52.1–58.8)</td>
<td>61.0 (40.3–66.0)</td>
<td>0.92</td>
</tr>
<tr>
<td>LA structure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum volume, mL</td>
<td>22.9 (19.7–25.4)</td>
<td>27.0 (24.5–28.6)</td>
<td>0.019</td>
</tr>
<tr>
<td>Preatrial contraction volume, mL</td>
<td>18.1 (15.8–20.7)</td>
<td>22.8 (21.3–23.7)</td>
<td>0.0018</td>
</tr>
<tr>
<td>Minimum volume, mL</td>
<td>15.9 (14.3–17.6)</td>
<td>18.5 (16.5–19.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>Reservoir volume, mL</td>
<td>7.4 (5.7–7.9)</td>
<td>8.4 (5.9–8.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Passive emptying volume, mL</td>
<td>4.8 (3.9–5.6)</td>
<td>4.2 (2.9–4.9)</td>
<td>0.24</td>
</tr>
<tr>
<td>LA stroke volume, mL</td>
<td>2.1 (1.8–2.9)</td>
<td>3.9 (3.4–5.2)</td>
<td>0.0029</td>
</tr>
<tr>
<td>LA contribution to LV stroke volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reservoir, %</td>
<td>21.2 (16.7–24.4)</td>
<td>25.3 (19.4–37.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Active, %</td>
<td>6.8 (5.8–8.0)</td>
<td>13.1 (8.9–22.3)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Conduit, %</td>
<td>78.8 (75.6–83.3)</td>
<td>74.7 (62.8–80.6)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Data presented as median (25th–75th percentile). HFpEF indicates heart failure with preserved ejection fraction; HR, heart rate; LA, left atrium; and LV, left ventricle.
by the coefficient of determination, \( R^2 \); \( P < 0.05 \) (2-sided) was considered statistically significant. Analyses were performed using JMP, version 9.0 (SAS Institute Inc, Cary, NC).

**Results**

**Model Development**

Weekly postoperative blood pressures were higher in HFpEF dogs than controls (Figure 1). Compared with baseline echocardiography (prior to surgery), HFpEF dogs at week 8 maintained a normal ejection fraction (≥50%); however, relative wall thickness and indexed LV mass increased and the LV diastolic dimension was diminished, indicating the development of concentric LV hypertrophy (Table I in the Data Supplement). LA area also increased (baseline, 74 [61–87] mm² versus week 8, 90 [81–102] mm²; \( P = 0.008 \)). Control dogs did not exhibit significant echocardiographic changes between baseline and week 8.

**Left Ventricular Structure and Function**

Cardiac MRI (at week 8) revealed greater LV mass and LV mass to end-diastolic volume ratio in HFpEF compared with controls, whereas ejection fraction and cardiac output were similar between groups (Table 1). In vivo hemodynamic assessment revealed higher LV systolic elastance (Ees) and LV diastolic elastance (\( \beta_{LA} \)) in HFpEF versus controls and a trend toward higher arterial elastance (\( E_a = 0.057 \)) (Results I in the Data Supplement, Table III in the Data Supplement).

\[ \text{LA booster pump function and systolic atrioventricular coupling} \]

By MRI, at equivalent heart rate, LA volumes were greater in HFpEF than controls (Figure 2A and 2B) and positively correlated with the LV mass to end-diastolic volume ratio (r=0.62 to 0.70; \( P < 0.01 \) for all). LA reservoir volume was similar between HFpEF and controls (Table 1). LA stroke volume was significantly greater in HFpEF, whereas LA passive emptying volume was numerically, but not significantly, lower in HFpEF than controls. Accordingly, total LA emptying fraction was similar between groups because of increased LA active but reduced passive emptying fractions in HFpEF (Table 1). In both groups, LA conduit volume contributed the most to LV filling. Estimated LA kinetic energy expenditure \( ^2 \) and heart rate–corrected mean LA ejection rate were higher in HFpEF, suggesting greater LA stroke work and inotropy (Table 1). These findings indicate augmented LA active (booster pump) function in early-stage HFpEF without a concomitant increase in LA reservoir function.

**LA Booster Pump Function and Systolic Atrioventricular Coupling**

Representative LA pressure-volume loops are shown in Figures 3A and 3B. LA pressure-volume data during vena cava occlusion demonstrated a linear LA end systolic pressure-volume relationship (\( R^2=0.96–0.99 \); Figure 3C). At matched heart rate and mean LA pressure, the LA end systolic pressure-volume relationship slope (\( E_{LA} \)) was steeper and the volume axis intercept (\( V_{LA} \)) was greater in HFpEF dogs compared with controls (Figure 3D; Table 2). When normalized for preload (ie, \( \text{LAV}_{ER} \)), \( E_{LA} \) remained significantly greater in HFpEF, confirming increased LA systolic elastance (Table 2).

\[ \text{LA compliance and atrioventricular coupling} \]

End-reservoir LA pressure-volume data displayed a curvilinear relationship over the measured range (Figure 3C). Compared with controls, the LA end reservoir pressure-volume relationship was shifted leftward and upward in HFpEF (Figure 3D), that is, the LA chamber stiffness constant (\( \beta_{LA} \)) was greater (Table 2), consistent with reduced LA compliance. Furthermore, \( \beta_{LA} \) was inversely correlated with LV stroke volume (r=−0.46; \( P = 0.04 \)), suggesting that increased LA stiffness limits LV performance in HFpEF. Notably, \( \beta_{LA} \) was not significantly correlated with maximum (\( P = 0.24 \)), preatrial contraction (\( P = 0.10 \)), or minimum (\( P = 0.09 \)) LA volume by MRI.

As the LA faces LV diastolic stiffness during atrial contraction, physiological left atrioventricular coupling may be
depicted as the ratio of LV diastolic elastance ($\beta_{LV}$) to ELA. $\beta_{LV}/\text{ELA}$ was numerically higher ($P=0.076$) in HFpEF than controls (Table 2). A higher $\beta_{LV}/\text{ELA}$ ratio was significantly associated with lower LV stroke volume (Figure 3F).

**LA and Ventricular Hypertrophy and Fibrosis**

Autopsy LV mass index was numerically greater but not significantly different in HFpEF dogs compared with controls (Figure 4A). However, the ratio of LV mass (autopsy) to LV end-diastolic volume (MRI; Figure 4B) and LV cardiomyocyte cross-sectional area (Figure 4C and 4D) were significantly increased, indicating concentric LV hypertrophy.

Autopsy LA mass index (Figure 4A) and LA cardiomyocyte cross-sectional area (Figure 4C and 4D) were greater in HFpEF dogs compared with controls. The ratio of LA mass (autopsy) to $\text{LAV}_{ER}$ (MRI; Figure 4B) was conserved between groups, indicating eccentric LA hypertrophy.

Figure 3. In vivo hemodynamic assessment. A–D, representative left atrial (LA) pressure-volume loops obtained from control and heart failure and preserved ejection fraction (HFpEF) dogs at matched mean LA pressure: A and B, steady state; C and D, during acute preload reduction (see text for definition of terms). E, correlation between LA systolic elastance ($E_{s}$), left ventricular (LV) systolic elastance ($E_{es}$), and effective arterial elastance ($E_{a}$). F, correlation between left ventricular stroke volume (LVSV), LA-LV physiological coupling ($\beta_{LV}/\text{ELA, left graph}$), and LA-LV contractile coupling ($E_{es}/\text{ELA, right graph}$). See text for further explanation. $\text{LAP}_{ER}$ indicates LA end-reservoir pressure; $\text{LAV}_{ER}$, LA end-reservoir volume; and $\text{LAV}_{ES}$, LA end-systolic volume.

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In the LAFW, LA appendage, and LV, there were no significant differences in interstitial fibrosis or collagen content, between HFpEF and controls (Figure 5A–5C). However, greater regional fibrosis was evident in the LA posterior wall of HFpEF dogs versus controls (Figure 5A and 5C). LA fibrosis exceeded LV fibrosis in all LA regions assessed. There was no correlation between LA or LV percentage fibrosis and LA or LV chamber stiffness constants ($P > 0.35$ for all).

**Titin Isoform Expression and Phosphorylation Status**

Titin isoform expression was similar between groups in the LAFW, LA appendage, and LV (Figure 6A and 6B). In the LAFW, both N2B (stiffer) and N2BA (compliant) titin isoforms were hyperphosphorylated in HFpEF versus controls (Figure 6C) and the extent of phosphorylation correlated with LA chamber stiffness ($\beta_{LA}$ versus N2B %phosphorylation, $r = 0.54$, $P = 0.03$; $\beta_{LA}$ versus N2BA %phosphorylation, $r = 0.49$, $P = 0.05$). Conversely, in the LA appendage, isoform N2B was hypophosphorylated in HFpEF and N2BA similar between groups (Figure 6C). Neither status correlated with LA chamber stiffness.

In the LV, both N2B and N2BA titin isoforms were hyperphosphorylated in HFpEF versus controls (Figure 6C) and the degree of phosphorylation correlated with LV chamber stiffness ($\beta_{LV}$ versus N2B %phosphorylation, $r = 0.48$, $P = 0.04$; $\beta_{LV}$ versus N2BA %phosphorylation, $r = 0.52$, $P = 0.03$).

**LA Microvessel Endothelial Function**

Physiological shear stress produced graded dilatation of LA microvessels from both groups; however, SSD was significantly attenuated in microvessels from HFpEF dogs compared with controls (Figure 7A), suggesting reduced shear stress–mediated vasodilator generation in HFpEF. Removal of the endothelium markedly attenuated SSD in both groups (Figure 7A). Inhibition of endothelial nitric oxide synthase with L-NAME virtually abolished SSD in control dogs but only partially inhibited the vasodilator response in HFpEF vessels (Figure 7B), indicating impaired NO signaling in HFpEF. Blockade of BK channels with iberiotoxin, almost completely abolished SSD in control vessels but had only a marginal effect in microvessels from HFpEF dogs (Figure 7C), suggesting that SSD in HFpEF microvessels is not BK channel dependent.

**Microvessel Density**

LA microvessel density was lower in HFpEF dogs than controls (2294 [1590–2712] vessels/mm$^2$ HFpEF versus 2694 [2245–2919] vessels/mm$^2$ controls; $P = 0.068$) and inversely correlated with the LA chamber stiffness constant, $\beta_{LA}$ (Figure 7D). LV microvessel density was also lower in HFpEF than controls (2678 [2485–2957] vessels/mm$^2$ HFpEF versus 3220 [3027–3399] vessels/mm$^2$ controls; $P = 0.0094$) but did not significantly correlate with the LV chamber stiffness constant, $\beta_{LV}$.

**Discussion**

The objective of this study was to determine whether mal-adaptive LA structural and functional remodeling was present in a canine model reflecting early-stage HFpEF comprising
advanced age, renal-induced hypertension, and proinflammatory aldosterone excess. Compared with controls, dogs with early hypertensive HFpEF demonstrated LA chamber and cardiomyocyte hypertrophy, increased LA booster pump function, and greater LA contractility. However, there was no attendant increase in LA reservoir function in HFpEF, as may be expected to compensate for the degree of LV concentric remodeling and diastolic dysfunction. Furthermore, LA compliance was reduced in HFpEF versus controls and atrioventricular coupling was impaired, in association with lower LV stroke volume. Thus, we have demonstrated that LA remodeling occurs early in HFpEF development and is maladaptive. Moreover, we provide novel evidence of LA myocyte hypertrophy, titin hyperphosphorylation, and endothelium-dependent LA microvascular dysfunction occurring in early HFpEF, concomitant with LV remodeling. Collectively, these data support the hypothesis that common pathophysiological processes drive global (all-chamber) myocardial remodeling in HFpEF.\textsuperscript{11}
Presence of an Atrial Myopathy and Impaired Left Atrioventricular Coupling in Early-Stage HFpEF

LA enlargement is common in HFpEF and correlates with the severity and duration of LV diastolic dysfunction. Accordingly, LA remodeling in HFpEF has been considered a late and adaptive response to increased LA afterload, facilitating greater LA preload and augmented LA active emptying fraction, as per an atrial Frank-Starling mechanism. In overt HFpEF, as in some dilated and hypertrophic cardiomyopathies, this compensatory LA contractile response is blunted. In the present study of early-stage HFpEF, however, we did not find evidence of LA contractile failure. Rather, LA hypertrophy was accompanied by augmented booster pump function and inotropy relative to controls, including when normalized for LA volume. Even so, the enhanced LA inotropy was insufficient to match increases in LV end-systolic elastance or adequately compensate for increased LV diastolic stiffness in HFpEF, thereby giving rise to impaired systolic (higher Ees/ELA) and physiological (higher P1V/E1LA) atrioventricular coupling. That both parameters of atrioventricular coupling inversely correlated with LV stroke volume suggest a key role for efficient mechanical matching between the LA and the LV in early-stage HFpEF. Similar abnormal atrioventricular coupling has been observed in patients with a recent myocardial infarction and in patients with HF and reduced ejection fraction; this study represents the first invasive assessment in HFpEF.

Recent human HFpEF studies have also reported a reduction in LA compliance elicited on strain echocardiography or, in a single study, from steady-state LA pressure-volume co-ordinates (maximal v wave height minus a wave nadir divided by echocardiography-derived LA reservoir volume). Herein, we provide verification of an upward and leftward shift in the LA end reservoir pressure-volume relationship, elicited during preload reduction in experimental HFpEF (ie, greater chamber stiffness or reduced compliance) versus controls, which negatively affected overall cardiac performance. This finding contrasts previous reports of LA adaptation in mild diastolic dysfunction, essential hypertension without HF, and LA volume overload in chronic mitral regurgitation, where LA compliance and reservoir function increase to preserve LV filling and buffer elevated LA pressures.

Potential mechanisms for LA stiffening in HFpEF include greater fibrosis, increased cardiomyocyte stiffness (principally related to the sarcomeric protein titin), or LA myocardial ischemia. We did not observe an increase in LAFW or LV fibrosis in this model, consistent with other reports of LA fibrosis as a late finding. The observed increase in localized peripulmonary vein (LA posterior wall) fibrosis may signify the onset of a fibrotic response and substrate for AF genesis, which is frequent in HFpEF. Similarly, LA and LV titin isoform

![Figure 6. Titin status. A, Representative gels comparing titin isoform expression and phosphorylation status for control and heart failure with preserved ejection fraction (HFpEF) left ventricular myocardium. B, Titin isoform expression ratio (box plot shows median, 25th to 75th percentile). C, Titin phosphorylation status for N2B and N2BA titin isoforms. LAA indicates left atrial appendage; LAFW, left atrial free wall; and LV, left ventricle.](http://circheartfailure.ahajournals.org/Downloaded from)
composition were similar between HFpEF and controls; however, LA and LV total titin phosphorylation was increased in HFpEF, and N2B phosphorylation (the shorter stiffer isoform) modestly correlated with LA and LV chamber stiffness coefficients. Although site-specific titin phosphorylation and passive cardiomyocyte tension were not directly assessed, protein kinase Cα-mediated hyperphosphorylation of the titin proline, glutamate, valine, and lysine site is recognized to increase passive cardiomyocyte stiffness34–36 and is a possible mechanism for this finding. Our corroborative demonstration of impaired NO signaling in HFpEF renders titin hyperphosphorylation less likely to result from cGMP-mediated mechanisms. Because LA and LV myocardium exhibited the same pattern, a chamber-specific response is also excluded and supports a process of global (all-chamber) adverse myocardial remodeling in HFpEF.

Lastly, attenuation of microcirculatory reserve has been associated with reduced LA compliance in experimental HF and reduced ejection fraction.37 LA microvascular dysfunction, with impaired NO signaling, and microvascular rarefaction, as seen in this study, may contribute to LA diastolic dysfunction in early-stage HFpEF. Importantly, the inverse correlation between LA microvessel density and LA chamber stiffness, which was not observed in the LV, suggests that the LA is both vulnerable and potentially more sensitive than the LV to the effects of microvascular ischemia.

**Inflammation and Endothelial Dysfunction in Early-Stage HFpEF**

It is hypothesized that comorbidity-driven systemic and coronary microvascular inflammation results in impaired NO-cGMP signaling as the impetus to myocardial fibrosis and cardiomyocyte stiffening in HFpEF.11 Our experimental model incorporates renal wrap–induced perinephritis and tubulointerstitial inflammation,38 along with mineralocorticoid excess, which is proinflammatory in cardiac tissue.13 In this setting, we demonstrated impaired endothelium-dependent LA microvascular vasodilator capacity. Because L-NAME did not completely block SSD in HFpEF dogs, we cannot rule out concomitant upregulation of an alternative endothelium-derived vasodilating factor, and therefore, further studies are required to elucidate the precise mechanism of SSD in HFpEF, to include age-matched controls. The additional presence of microvascular rarefaction, however, supports a role for microvascular inflammation in the development of HFpEF.

**Limitations**

Our experimental model incorporates well-established features of human HFpEF, including advanced age, hypertension, and proinflammatory mineralocorticoid excess; thus, our findings are relevant for a hypertensive HFpEF phenotype. Additional studies are required to evaluate LA parameters.

![Figure 7. Microvessel in vitro reactivity and rarefaction.](http://circheartfailure.ahajournals.org/Downloaded from)
in HFpEF subjects without LV concentric remodeling. We recognize the limitation of not studying an elderly control group; however, our previous examination of this model exhibited similar characteristics between young and elderly control dogs with respect to LV and vascular remodeling and diastolic function, and distinct from dogs with experimental HF. Thus, we believe our findings represent a significant effect of experimental HF, over and above that of aging alone. Importantly, elderly dogs available for research are frequently retired breeders, and therefore our sample is predominantly female. Published data demonstrate that sex differences in LA size are almost completely accounted for by body size, however, further examination of sex differences in LA function is warranted.

Conclusions

In the setting of hypertensive HFpEF, LA remodeling occurs early and exhibits maladaptive alterations in LA compliance and left atrioventricular coupling, which compromise overall cardiac performance and may exacerbate increases in LA and pulmonary pressures. Early development of LA noncompliance and atrioventricular mismatch may underlie reported associations between LA dysfunction and future HF in populations with preserved LV ejection fraction. Pathophysiological mechanisms, including myocardial hypertrophy, titin hyperphosphorylation, and microvascular rarefaction, affect LA and LV myocardium concomitantly, even in the absence of prolonged atrial hypertension or marked fibrosis, thus supporting a paradigm of global (all-chamber) adverse myocardial remodeling in HFpEF. Novel therapeutic strategies targeting these mechanisms may be able to lessen or reverse LA and LV remodeling in HFpEF and warrant investigation in human studies.

Acknowledgments

We sincerely thank Lorna Bowen and Jimmy Storlie for their technical assistance in the care and conduct of the animal studies and Marion von Freiling-Salewsky for help with the titin gels. Further thanks to Drs Michael Jeroch-Herold, Philip Glockner, Ravi Shah, Philip Haines, and Philip Araoz for their assistance in setting up the magnetic resonance imaging protocol.

Sources of Funding

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Disclosures

None.

References

LA Remodeling in Experimental HFpEF


**CLINICAL PERSPECTIVE**

Left atrial (LA) compliance and contractility are important determinants of left ventricular (LV) stroke volume. Preliminary human studies have proposed LA dysfunction in heart failure with preserved ejection fraction; however, corroborative invasive and histological data have been lacking. Furthermore, it is not known whether LA dysfunction represents a late sequela of LV diastolic dysfunction or early feature of global myocardial remodeling in heart failure with preserved ejection fraction. In a canine model reflecting early-stage heart failure with preserved ejection fraction comprising advanced age, renal-induced hypertension, and aldosterone excess, we observed LA chamber and myocyte hypertrophy and enhanced LA remodeling compared with controls. However, left atrioventricular coupling was impaired (in sinus rhythm) and LA compliance was reduced, in association with lower LV stroke volume. Pathophysiological alterations, including myocyte hypertrophy, titin hyperphosphorylation, and microvascular rarefaction, were congruous between LA and LV myocardium, suggesting early and global (ie, nonchamber-specific) maladaptive remodeling. In heart failure with preserved ejection fraction, development of LA noncompliance may contribute to increases in LA and pulmonary pressures, predisposing to dyspnea, pulmonary hypertension, and right ventricular failure. Novel therapeutic strategies targeting early global remodeling mechanisms may modify LA and LV properties and warrant investigation in human studies.
Left Atrial Remodeling and Atrioventricular Coupling in a Canine Model of Early Heart Failure With Preserved Ejection Fraction
Rosita Zakeri, Gilles Moulay, Qiang Chai, Ozgur Ogut, Saad Hussain, Hiroyuki Takahama, Tong Lu, Xiao-Li Wang, Wolfgang A. Linke, Hon-Chi Lee and Margaret M. Redfield

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SUPPLEMENTAL MATERIALS

SUPPLEMENTAL METHODS

i. Renal wrap model

Dogs were anesthetized with ketamine (10mg/kg), diazepam (0.5mg/kg) and isoflurane (0.5-2.5% maintenance). Each kidney was accessed separately via an oblique flank incision, and wrapped in silk, carefully avoiding constriction of the renal vessels. A femoral artery catheter with subcutaneous port was placed to facilitate weekly measurement of arterial blood pressure (BP). Control dogs underwent a sham surgical procedure with aortic catheter placement only.

ii. Echocardiography

Left ventricular (LV) dimensions and wall thickness were measured by M-mode echocardiography. LV mass (LVM; Devereux)\(^1,2\), relative wall thickness (RWT)\(^3\), and LV ejection fraction (EF; Teichholz)\(^4\) were calculated using standard formulae. Peak early (E) and late (A) Doppler mitral inflow velocities and their ratio (E/A) were recorded. Tissue Doppler was used to measure peak mitral annular velocity (e’) at the lateral wall and a ratio of peak early mitral inflow velocity to peak mitral annular velocity (E/e’) calculated. All Doppler spectra were recorded for five to seven cardiac cycles at a sweep speed of 200 mm/s. Left atrial (LA) maximum (anteroposterior) dimension and 2-dimensional cross-sectional area were obtained from the apical 4-chamber view.

iii. Cardiac magnetic resonance imaging

Image acquisition

Dogs were anesthetized with propofol (induction; 2-6mg/kg) and isoflurane (5% induction, 1.5-2% maintenance) and positioned supine within a 1.5T scanner with front and back surface coils (GE Healthcare, USA). All images were acquired using ECG (preferred) or pulse oximetry gating.
For LA volumes and function, steady-state free precession (SSFP) cine sequences were acquired in the short-axis plane from the atroioventricular junction (mitral annulus) to the base of the heart. Typically 5-7 contiguous slices (no gap) with a slice thickness of 6mm were required for full coverage of the LA and LA appendage (LAA). For LV volumes and function, SSFP short-axis cine images were acquired from the atroioventricular junction to the LV apex. Typically 10-12 contiguous slices (no gap) with a slice thickness of 6mm were required. Long-axis 4-, 3-, 2-chamber and LV outflow tract cine views were also obtained. Scan parameters included a field of view of (34cm), repetition time 8.2ms and echo time 4.5ms, resulting in a phase duration (temporal resolution) of 21ms. The in-plane spatial resolution (acquisition matrix size) was 256×192 (1.5-2.0mm in-plane), slice thickness 6mm with no gap. A flip angle of 30° was used in the long axis studies, and 60° in the short-axis imaging. All cine images were acquired during a single breath-hold in mid-expiration.

**Image analysis**

Epicardial (LV) and endocardial (LV and LA) contours were manually traced in every phase of the cardiac cycle. For LA volume assessment, 2- and 4-chamber views were used to confirm the position of the atrioventricular junction and LA base. A straight line drawn between the leading edges of the mitral valve annulus was taken to represent the atrioventricular junction\(^5\). LA volume was calculated using Simpson’s slice summation method (summation of outlined areas×slice thickness). The LAA was included in LA volume measurement; pulmonary veins were carefully excluded at their ostia.

LA volume-time curves were constructed by plotting each instantaneous LA volume against the time after the electrocardiogram R-wave at which the acquisition was performed (Figure 1A)\(^5\). LA maximum, minimum, mid-diastolic (end of rapid emptying), and pre-atrial contraction (pre-A) volumes were determined from the volume-time curve as shown in Figure 1A. LA reservoir, stroke, and conduit volumes, and LA function parameters were calculated according to recognized equations (Supplemental Table 1). Notably, in some studies LA reservoir or conduit volume is considered to be the volume unloaded to the LV during early diastole\(^5\). We defined LA reservoir volume as the total increase in LA volume during LV systole, when the LA is acting as a reservoir for
pulmonary venous return. Additionally, we defined LA conduit volume as the difference between LV stroke volume and LA reservoir volume, i.e. the volume of pulmonary venous return entering the LV, which is not derived from the LA reservoir. LA ejection rate during atrial contraction was calculated as the active LA emptying fraction divided by LA ejection time (time from pre-A to minimum LA volume), and reported corrected for heart rate.  

LV end-diastolic and end-systolic volumes were similarly calculated using Simpson’s slice summation method. LV ejection fraction and LV myocardial mass were computed according to established algorithms. LV mass-volume ratio was calculated by dividing LV end-diastolic mass by LV end-diastolic volume. Papillary muscles were excluded from LV volume and included in LV mass measurements.

iv. Pressure-volume data collection

The chest was opened via midline sternotomy and the pericardium widely excised to form a pericardial cradle. The LV admittance catheter was placed via an apical stab incision. The LA admittance catheter was placed through the LV apical incision and mitral valve into the LA body immediately after completion of LV pressure-volume measurements. All catheters were pre-soaked in saline and calibrated before use; zero pressure was set at the level of the right atrium. Stroke volume from cardiac MRI was used for volume calibration. Catheter placement was visualized and confirmed using echocardiography.

Left ventricular pressure-volume analysis

The LV end-systolic pressure-volume relationship (ESPVR) was defined as ESP=Ees*(ESV-V0), where ESP is LV end-systolic pressure, ESV is LV end systolic volume, Ees (LV end-systolic elastance) is the slope of the ESPVR, and V0 is the volume axis intercept. The LV end-diastolic pressure volume relationship (EDVPR) points were fit to a monoexponential equation: EDP=αe^β*EDV using least-squares nonlinear regression, where EDP is LV end diastolic pressure, EDV is LV end diastolic volume, α is the elastic constant and β is the chamber stiffness coefficient.

Ees was normalized to preload (Ees*LVEDV). ESV at an ESP of 200mmHg (ESV_{200}=[200/Ees]+V0) was calculated to reflect systolic capacitance, and EDV at an EDP
of 20mmHg (EDV$_{20}$=\[ln(20/\alpha)/\beta\]) was used to characterize diastolic capacitance$^7$. The linear relationship between $dP/dt_{\text{max}}$ and EDV was assessed to corroborate Ees as a measure of ventricular systolic function$^7$.

v. Fibrosis assessment

**Histomorphometry**

Transmural cross-sections of tissue were obtained from the LA free wall (LAFW), LA posterior wall myocardium adjacent to the pulmonary veins (LAPW), LA appendage (LAA), and LV (mid-ventricle level), fixed in 10% formalin and embedded in paraffin. Sections 4μm thick were stained with Picrosirius Red to demonstrate extracellular matrix. Whole-slide photomicrographs were obtained at x20 magnification using a NanoZoomer 2.0HT Digital Pathology system (Hamamatsu) and manually edited in Adobe Photoshop® to produce a reference myocardial tissue area excluding endocardial, epicardial and border artefact regions. A second reference area was defined to additionally exclude perivascular regions (vessels and perivascular fibrosis). Fibrosis was quantified using a color discrimination algorithm, calibrated on 6 representative myocardial segments from different dogs (3 control and 3 HFpEF) to accurately identify extracellular matrix on the basis of its red color (Image J software, National Institutes of Health, Bethesda, MD). Repeated measures of the same sample yielded <5% variability.

**Hydroxyproline assay**

Collagen content of myocardial tissue was determined using the hydroxyproline assay$^8$. Briefly, tissue samples (250mg) were homogenized, and hydrolysed with 12M HCl for 24 hours at 100°C. Hydroxyproline content was measured using a colorimetric chloramine T assay and a standard curve of 0 to 5μg of hydroxyproline. Data were expressed as micrograms of collagen per milligram of tissue, assuming that collagen contains an average of 12.5% hydroxyproline$^8$.

vi. Assessment of cardiomyocyte size

**Immunostaining and fluorescence microscopy**
Cardiomyocyte size was determined on 4μm thick sections of paraffin-embedded myocardial tissue stained with fluorescein isothiocyanate-conjugated wheat germ agglutinin (FITC-WGA; Vector Laboratories, Burlingame, CA), to delineate the cell membranes⁹. Coverslips were mounted with DAPI-containing Vectashield (1.5μg/mL DAPI, Vector Labs) to allow visualization of the nuclei. Systematic sampling at fixed x- and y-direction increments⁹ was used to obtain 20-25 photomicrographs from each section (x40 magnification), ensuring representative sampling of the whole slide (Axiovert 200M; AxioVision Rel. 4.8.1; Zeiss). Regions with mostly parallel-running (longitudinally oriented) cardiomyocytes were excluded.

**Image analysis**

Photomicrographs were opened in Image J (National Institutes of Health). Cell cross-sectional area (CSA), and maximum and minimum transverse dimensions were used as indices of cardiomyocyte size. Since these parameters vary along the length of a cell, in order to increase the probability of consistent and true cross-sectional sampling only cardiomyocytes meeting the following criteria were accepted for measurement: (i) cellular cross-sectional profile, (ii) visible, round, and approximately centrally located nuclei present, and (iii) intact cellular basement membrane. Cardiomyocyte CSA was determined by manual planimetry; maximum and minimum dimensions were then calculated by the Image J software as perpendicular to the outer delineated contour of the cell. A minimum of 100 cardiomyocytes were measured per section. Shrinkage due to fixation and paraffin embedding¹⁰ was assumed to affect control and HFpEF specimens comparably.

vii. **Titin isoform expression and phosphorylation status**

**Titin isoform separation**

Frozen tissue samples were homogenized in 50mM Tris-sodium dodecyl sulphate (SDS) buffer (pH 6.8) containing 8μg/mL leupeptin (Peptin Institute, Japan) and phosphatase inhibitor cocktails (PIC I [P2850] and PIC II [P5726], 10μL/mL from each; Sigma), heated, centrifuged, and separated on agarose-strengthened 2% sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE). Samples were loaded in duplicate and gels run at 5mA for 16 hours. Gels were washed and stained with SYPRO Ruby (Molecular Probes,
Eugene, Ore) according to the manufacturer’s instructions. The optical volume of protein bands (integrated optical density) was determined using a LAS-4000 Image Reader (Fuji Science Imaging Systems) and ImageQuant TL software. Titin isoform (N2BA, N2B) composition was expressed relative to total titin expression (where N2BA+N2B=100%).

**All-titin phosphorylation**

Tissue samples were handled as for titin isoform separation (2% SDS-PAGE; 20μg dry weight/lane). Gels were stained with Pro-Q Diamond phosphoprotein stain (Molecular Probes) for 1 hour, washed and subsequently stained with SYPRO Ruby (Molecular Probes) overnight. Phosphorylation signals for titin isoforms (Pro-Q diamond signals) were expressed relative to the SYPRO Ruby-stained total protein signals to correct for differences in sample loading.

**viii. In vitro microvascular vasoreactivity**

**Preparation of left atrial microvessels**

Isolated LA microvessels were mounted between two micropipettes in a vessel chamber containing Krebs’ solution, and maintained at a constant intraluminal pressure of 80mmHg using a syringe microinjection pump and pressure-servo controller (Living Systems, Burlington, VT) as previously described. Vessels were equilibrated for 60 min in oxygenated (21% O₂, 5%CO₂, balanced with N₂, 37°C) Krebs’ solution. Endothelin-1 (ET-1, 10⁻⁹ to 10⁻⁸M) was applied to pre-constrict vessels to approximately 50% of their passive diameter. The effects of ET-1 were sustained for the duration of the shear stress experiments (≈ 30 min).

**Shear stress experiments**

Incremental levels of shear stress (1, 5, 10, 15, 20, 25 dynes/cm²) were applied to each vessel via the microinjection pump, and flow rates calculated by the following equation:

\[ Q = \pi D^3 / 32\eta \tau \]
where \( Q \) is the flow rate, \( D \) is the vessel diameter, \( \tau \) is the shear stress, and \( \eta \) is the viscosity of fluid. Continuous recording of vessel diameter was achieved using a real-time edge detection system (V94 Living Systems Instrumentation; Burlington, VT). Signal output was acquired by a 16-bit data acquisition system (DIGI-DATA 1321A, Axon Instruments, Foster City, CA) with a sampling frequency of 2kHz (no filter), and was recorded real-time with Axoscope software (Axon). Flow rates were adjusted to achieve pre-specified incremental levels of shear stress based on the vessel diameter attained.

Shear stress induced vasodilation (SSD) in response to the increasing physiological levels of shear stress was compared between HFpEF and control vessels in the presence and absence of: 100μmol/L N(ω)-nitro-L-arginine methyl ester (L-NAME, endothelial nitric oxide synthase [eNOS] inhibitor) and separately 100nM iberiotoxin (IBTX, inhibitor of large conductance Ca\(^{2+}\)-activated K\(^+\) [BK] channels). In some vessels the endothelium was denuded by a slow injection of 3 to 5mL of air into the unpressurized vessel lumen. The effectiveness of endothelium denudation was verified by demonstrating: (i) failure of vasodilation to 1μmol/L acetylcholine; (ii) normal constriction to ET-1; and (iii) normal dilation to 100μmol/L sodium nitroprusside.

At the end of each experiment, vessels were constricted with 100mmol/L KCl and then maximally dilated with a Ca\(^{2+}\)-free Krebs’ solution (with 1mM EGTA added). The vasodilator response was calculated as a percentage of this maximum diameter, according to the following equation:

\[
\text{Vasodilation (}\%\text{maximum}) = \left( \frac{D_{\text{SS}} - D_{\text{ET}}}{D_{\text{MAX}} - D_{\text{ET}}} \right) \times 100\%
\]

where \( D_{\text{SS}} \) is the vessel diameter at a specific level of shear stress, \( D_{\text{ET}} \) is the vessel diameter after application of ET-1 in the absence of shear stress, and \( D_{\text{MAX}} \) is the vessel diameter in Ca\(^{2+}\)-free Krebs’ solution in the absence of shear stress but in the presence of ET-1. This is considered to be the most accurate representation of SSD.

Each vessel was used for a single experiment. Therefore different sets of vessels were used for determination of the effects of endothelial denudation versus pre-incubation with pharmacological agents. Vessels exhibiting leaks, failure to constrict by 50% to 10nM ET-1 or 100mM KCL, or failure to dilate to 50% of the maximal dilation in Ca2+-free solution, 100μM nitroprusside, or 1μM acetylcholine were excluded from the analysis.
ix. Microvascular density

Paraffin-embedded myocardial sections were stained with a lectin-binding endothelial cell-specific marker, biotinylated isolectin B4 (Vector Laboratories, Burlington, VT) according to the manufacturer’s instructions. Whole field digital micrographs were obtained and microvascular density was quantified using an automated color-detection algorithm (Definiens Tissue Studio 3.5, Definiens®, Germany). For each tissue section, regions of interest were manually selected to include vessels with a predominant circumferential orientation. The color detection threshold was adjusted to optimally discriminate between isolectin B4 (brown stain) and background. Microvessels (small pre-capillary arterioles) were defined by a cross sectional area of 78.5-314μm² and average luminal diameter between 10 and 20μm. Vessel count and tissue area were used to calculate microvessel density, i.e. number of vessels per mm² tissue area.
SUPPLEMENTAL RESULTS

i. *In vivo* left ventricular and vascular function

Under steady-state anesthetized conditions, at matched heart rate and LV end-diastolic pressure, invasively assessed LVEF was similar between groups, CO tended to be lower, while peak dP/dt tended to be higher in HFpEF versus controls (*Supplemental Table 3*). LV end-systolic elastance (Ees) was steeper and left-shifted in HFpEF, resulting in a greater rise in LV pressure for any given rise in end-systolic volume. Ees normalized for preload remained significantly higher and systolic capacitance (ESV<sub>200</sub>) was concordantly lower in HFpEF dogs compared with controls. The rate of isovolumic relaxation (tau) was slower in HFpEF than controls but did not achieve statistical significance. The mean diastolic stiffness coefficient, β<sub>LV</sub>, was higher in HFpEF and LV diastolic capacitance (EDV<sub>20</sub>) was significantly reduced, consistent with a leftward-shift of the EDPVR and reduced LV compliance, as previously reported in this model<sup>15-17</sup>.

Effective arterial elastance (Ea) was higher and SVR tended to be higher in HFpEF dogs versus controls. Arterial and LV end-systolic elastance values were significantly correlated (r=0.60, p=0.0038) in the steady-state, though vascular-ventricular coupling (Ea/Ees) was deranged in HFpEF (2.5±0.7 versus 1.5±0.9 for controls, p=0.027) with a proportionately greater rise in Ees. There was a significant negative correlation between LV afterload (Ea) and LV systolic (r=-0.66, p=0.001) but not diastolic (r=-0.40, p=0.10) capacitance. This hemodynamic profile mimics studies of HFpEF in humans where a discordant increase in LV elastance occurs in the presence of subtle LV systolic impairment<sup>18</sup>, enhanced passive LV stiffness<sup>19</sup>, and impaired ventricular-vascular coupling<sup>20</sup>. 
### Supplemental Table 1. Calculations for left atrial function parameters on magnetic resonance imaging.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volumes</strong></td>
<td></td>
</tr>
<tr>
<td>LA reservoir volume, mL</td>
<td>Maximum LAV – Minimum LAV</td>
</tr>
<tr>
<td>LA passive emptying volume, mL</td>
<td>Maximum LAV – Mid-diastolic LAV</td>
</tr>
<tr>
<td>LA stroke volume, mL</td>
<td>Pre-A wave LAV – Minimum LAV</td>
</tr>
<tr>
<td>LA conduit volume, mL</td>
<td>LV stroke volume – LA reservoir volume</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td></td>
</tr>
<tr>
<td>Total LA emptying fraction, %</td>
<td>(LA reservoir volume / Maximum LAV) *100</td>
</tr>
<tr>
<td>Passive LA emptying fraction, %</td>
<td>(LA passive emptying volume / Maximum LAV) *100</td>
</tr>
<tr>
<td>Active LA emptying fraction, %</td>
<td>(LA stroke volume / Pre-A wave LAV) *100</td>
</tr>
<tr>
<td>LA conduit function, %</td>
<td>(LA conduit volume / LV stroke volume) *100</td>
</tr>
<tr>
<td>LA reservoir contribution to LV stroke volume, %</td>
<td>(LA reservoir volume / LV stroke volume) *100</td>
</tr>
<tr>
<td>LA active contribution to LV stroke volume, %</td>
<td>(LA stroke volume / LV stroke volume) *100</td>
</tr>
<tr>
<td>LA expansion index, %</td>
<td>(LA reservoir volume / Minimum LA volume) *100</td>
</tr>
<tr>
<td>LA kinetic energy(^{21}), kdynes*cm</td>
<td>0.5 * 1.06 * LA stroke volume * [peak A velocity(^2)]</td>
</tr>
<tr>
<td><strong>Rates</strong></td>
<td></td>
</tr>
<tr>
<td>Mean LA filling rate, mL/s</td>
<td>LA reservoir volume / Total LA filling duration</td>
</tr>
<tr>
<td>Passive emptying rate, mL/s</td>
<td>LA passive emptying volume / Total LA passive emptying duration</td>
</tr>
<tr>
<td>Active LA ejection rate, mL/s</td>
<td>LA stroke volume / Total LA ejection duration</td>
</tr>
<tr>
<td>HR corrected LA ejection rate, %/s</td>
<td>Active LA emptying fraction / (LA ejection time/ √ R-R interval)</td>
</tr>
</tbody>
</table>

HR, heart rate.
**Supplemental Table 2. Model characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HFpEF</td>
<td>Control</td>
<td>HFpEF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=9)</td>
<td>(n=13)</td>
<td>(n=9)</td>
<td></td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>23.9 (22.5-25.3)</td>
<td>25.5 (19.5-27.6)</td>
<td>24.5 (23.6-26.5)‡</td>
<td>25.8 (20.9-28.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>147 (115-187)</td>
<td>113 (104-126)</td>
<td>120 (107-139)</td>
<td>131 (112-145)</td>
<td></td>
</tr>
<tr>
<td>Left atrial area, mm²</td>
<td>89 (78-93)</td>
<td>74 (61-87)</td>
<td>85 (81-93)</td>
<td>90 (81-102)‡</td>
<td></td>
</tr>
<tr>
<td>LV diastolic dimension, mm</td>
<td>41.4 (38.8-45.0)</td>
<td>44.2 (40.2-47.1)</td>
<td>40.3 (38.9-44.2)</td>
<td>42.5 (39.6-43.4)‡</td>
<td></td>
</tr>
<tr>
<td>Relative wall thickness, mm</td>
<td>0.42 (0.37-0.45)</td>
<td>0.41 (0.34-0.53)</td>
<td>0.42 (0.37-0.46)</td>
<td>0.49 (0.44-0.54)†‡</td>
<td></td>
</tr>
<tr>
<td>LV mass (diastole), g</td>
<td>105.5 (96.9-118.9)</td>
<td>124.1 (98.3-172.4)</td>
<td>113.5 (97.5-134.2)</td>
<td>148.9 (120.8-184.0)†‡</td>
<td></td>
</tr>
<tr>
<td>LV mass index, g/kg</td>
<td>4.5 (4.2-4.9)</td>
<td>5.1 (4.1-6.1)</td>
<td>4.8 (4.0-5.2)</td>
<td>6.8 (4.8-8.2)†‡</td>
<td></td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>68.0 (60.5-70.5)</td>
<td>64.0 (55.0-72.5)</td>
<td>66.0 (62.0-68.5)</td>
<td>59.0 (53.0-69.0)‡</td>
<td></td>
</tr>
</tbody>
</table>

Median (25th-75th percentile)

‡ p<0.05 vs. control at same time point; † p<0.05 vs. baseline

LV, left ventricular
**Supplemental Table 3.** Hemodynamic assessment of LV and vascular function.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=13)</th>
<th>HFpEF (n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>127 (122-138)</td>
<td>135 (129-153)</td>
<td>0.17</td>
</tr>
<tr>
<td>LVESP, mmHg</td>
<td>94.5 (83.3-101.6)</td>
<td>118.0 (78.0-133.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>20.8 (16.9-27.4)</td>
<td>21.9 (16.4-23.4)</td>
<td>0.76</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>45.1 (36.3-52.4)</td>
<td>54.7 (36.9-58.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>LVESV, mL</td>
<td>48.2 (39.4-58.8)</td>
<td>40.6 (27.6-50.6)</td>
<td>0.089</td>
</tr>
<tr>
<td>LVEDV, mL</td>
<td>91.6 (75.2-99.3)</td>
<td>66.6 (53.2-83.9)</td>
<td>0.030</td>
</tr>
<tr>
<td>LVSV, mL</td>
<td>37.2 (29.7-44.5)</td>
<td>28.2 (21.4-38.4)</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>LV systolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO, L/min</td>
<td>4.6 (3.9-6.0)</td>
<td>3.3 (3.0-5.1)</td>
<td>0.057</td>
</tr>
<tr>
<td>EF, %</td>
<td>47.1 (38.0-49.1)</td>
<td>42.7 (38.0-51.4)</td>
<td>0.53</td>
</tr>
<tr>
<td>SW, mmHg*mL</td>
<td>411.0 (286.8-468.0)</td>
<td>311.4 (254.7-437.8)</td>
<td>0.37</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mmHg/s</td>
<td>1596.8 (1504.7-1757.3)</td>
<td>2056.1 (1532.9-2231.1)</td>
<td>0.066</td>
</tr>
<tr>
<td>Ees, mmHg/mL</td>
<td>1.07 (0.83-1.20)</td>
<td>3.26 (2.35-4.19)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ees R value</td>
<td>0.98 (0.97-0.99)</td>
<td>0.98 (0.96-0.99)</td>
<td>0.72</td>
</tr>
<tr>
<td>V&lt;sub&gt;0&lt;/sub&gt;, mL</td>
<td>-51.6 (-63.6-30.0)</td>
<td>-1.9 (-13.0-3.2)</td>
<td>0.0018</td>
</tr>
<tr>
<td>ESV&lt;sub&gt;200&lt;/sub&gt;, mL</td>
<td>150.5 (131.6-180.9)</td>
<td>54.3 (44.5-86.3)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Normalized Ees, mmHg</td>
<td>89.7 (71.4-100.8)</td>
<td>203.3 (156.7-236.9)</td>
<td>0.0004</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;-EDV, mmHg/s/mL</td>
<td>10.5 (6.18-15.4)</td>
<td>23.4 (17.8-35.3)</td>
<td>0.0047</td>
</tr>
<tr>
<td><strong>LV diastolic function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;min&lt;/sub&gt;, mmHg/s</td>
<td>-1660.0 (-1898.9- -1222.6)</td>
<td>-1766.3 (-2381.8- -1266.1)</td>
<td>0.24</td>
</tr>
<tr>
<td>Tau (Glantz), ms</td>
<td>23.6 (21.1-40.7)</td>
<td>31.1 (30.3-50.7)</td>
<td>0.089</td>
</tr>
<tr>
<td>β&lt;sub&gt;LV&lt;/sub&gt;, mmHg/mL</td>
<td>0.015 (0.012-0.021)</td>
<td>0.044 (0.016-0.069)</td>
<td>0.011</td>
</tr>
<tr>
<td>β&lt;sub&gt;LV&lt;/sub&gt; R value</td>
<td>0.98 (0.97-0.99)</td>
<td>0.96 (0.95-0.98)</td>
<td>0.19</td>
</tr>
<tr>
<td>α</td>
<td>5.2 (2.8-8.1)</td>
<td>1.1 (0.4-6.0)</td>
<td>0.060</td>
</tr>
<tr>
<td>EDV&lt;sub&gt;20&lt;/sub&gt;, mL</td>
<td>85.0 (61.5-105.7)</td>
<td>59.1 (52.2-82.6)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Arterial function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ea, mmHg/mL</td>
<td>2.5 (1.9-3.1)</td>
<td>3.6 (2.4-6.7)</td>
<td>0.057</td>
</tr>
<tr>
<td>SVR, dyne<em>sec</em>cm&lt;sup&gt;5&lt;/sup&gt;</td>
<td>763.6 (593.5-814.4)</td>
<td>899.2 (690.3-1639.0)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Vascular-Ventricular coupling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ea/Ees</td>
<td>2.4 (1.9-3.0)</td>
<td>1.2 (0.9-2.0)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Median (25<sup>th</sup>-75<sup>th</sup> percentile)
α, elastic constant; β, LV diastolic stiffness coefficient; CO, cardiac output; Ea, effective arterial elastance; EF, ejection fraction; EDP, end diastolic pressure; EDV, end diastolic volume; EDV_{20}, LV diastolic capacitance; Ees, LV elastance; ESP, end systolic pressure; ESV, end systolic volume; ESV_{200}, LV systolic capacitance; LV, left ventricular; MAP, mean arterial pressure; Normalized Ees, Ees*LV end-diastolic volume; SV, stroke volume; SVR, systemic vascular resistance; SW, stroke work. V_0, volume axis intercept. See supplemental methods section iv for definitions.
SUPPLEMENTAL REFERENCES


3. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography 2005;18:1440-63.


10. Tracy RE, Sander GE. Histologically measured cardiomyocyte hypertrophy correlates with body height as strongly as with body mass index. Cardiology research and practice 2011;2011:658958.


