Development of Heart Failure in Chronic Hypertensive Dahl Rats. Focus on Heart Failure With Preserved Ejection Fraction

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Development of Heart Failure in Chronic Hypertensive Dahl Rats

Focus on Heart Failure With Preserved Ejection Fraction

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Abstract—The impact of hypertension on left ventricular (LV) structure, pump function, and heart failure in Dahl salt-sensitive rats is poorly characterized but hypothesized to yield insights into the pathophysiology of heart failure with normal or preserved ejection fraction. Eighty Dahl salt-sensitive rats were fed either a high-salt (HS) or low-salt (LS, controls) diet starting at age 7 weeks. Ventricular properties were measured by echocardiography, hemodynamics and end-systolic and end-diastolic pressure-volume relationships (ESPVR and EDPVR, respectively). Compared with LS controls, HS rats developed severe hypertension and LV hypertrophy. At week 12, HS rats developed passive diastolic dysfunction (leftward/upward shifted EDPVR, increased chamber stiffness) with reductions in end-diastolic volume. However, the ESPVR also shifted upward (enhanced end-systolic function) so that overall pump function was enhanced compared with LS, and there was no change in end-diastolic pressure (EDP). At 16 and 20 weeks, HS hearts enlarged so that end-diastolic volumes and EDPVRs became similar to the respective age-matched LS controls. Concomitantly, the ESPVRs and overall pump function curves also moved toward controls, and ejection fraction declined. Despite normal or enhanced overall pump function at these times, EDP and wet lung weight increased, indicative of development of heart failure. In the Dahl salt-sensitive rat, which pathophysiologically retains salt and water, the development of heart failure (increased EDP and wet lung weight) is dissociated from changes in passive diastolic and active systolic properties. These observations suggest that a volume overload sate plays an important pathophysiological role in development of heart failure despite preserved overall ventricular pump function in this model of chronic hypertension. **(*Hypertension*. 2006;47:1-11.)**

Key Words: remodeling ■ heart failure ■ diastole ■ hypertrophy ■ renal disease

Heart failure commonly occurs in patients with a normal or preserved ejection fraction (HFPEF).[1–3] It was advocated previously that HFPEF is primarily because of diastolic dysfunction and, thus, termed "diastolic heart failure" (DHF).[4,5] However, data from our recent clinical and animal studies suggest that HFPEF can occur without diastolic dysfunction.[6–10] We found that HFPEF in the setting of idiopathic hypertrophic cardiomyopathy and infiltrative cardiac diseases is indeed because of diastolic dysfunction, defined by a reduced chamber capacitance in the absence of systolic dysfunction.[10] However, in the setting of long standing hypertension, HFPEF may not always be because of diastolic dysfunction.[10] We proposed other, extracardiac factors leading to a volume-overloaded state as the underlying pathology, such as might occur with salt and water handling impairments encountered in the setting of renal dysfunction.

Two potential limitations of prior studies contribute to the controversy. First, barring a few notable exceptions,[7,11–13] studies of HFPEF have evaluated diastolic properties without any serious consideration of systolic properties, although overall ventricular pump function [ie, the ability to generate pressure and stroke volume (SV)] depends on both,[13] and both must be thoroughly evaluated to make conclusions about mechanisms of hemodynamic abnormalities. Second, because it is impossible to predict which hypertensive patient will ultimately develop HFPEF, longitudinal studies of the changes in ventricular structure and function occurring during the development of HFPEF have been limited.[14]

The Dahl salt-sensitive rat has been used as a model for investigating the characteristics of myocardial hypertrophy, the transition to heart failure, and has been proposed as a model for studying DHF,[15,16] because it has been established that early in the course of hypertension heart failure occurs, whereas ejection fraction (EF) remains preserved. However, with regard to changes in ventricular pump properties (systole and diastole) occurring over time, this model remains incompletely characterized. Accordingly, the purpose of this study was to evaluate the pathophysiological mechanisms in the development of HFPEF by characterizing hemodynamics, mitral inflow dynamics, ventricular structure, and ventricular

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systolic and diastolic properties during the transition from hypertension to hypertension with HFPEF in salt-fed Dahl rats.

Methods

Animal Model

Low-salt (LS) laboratory chow containing 0.3% NaCl was fed to 80 weaning male Dahl salt-sensitive rats (Charles River Laboratories, Wilmington, MA) until the diet was switched to high salt (HS, 8% NaCl) in approximately half of the rats at 7 weeks of age.[15,16] The rats were divided into 8 groups according to diet and duration: those given HS for 8 weeks (HS-8, n=10), 12 weeks (HS-12, n=12), 16 weeks (HS-16, n=12), and 20 weeks (HS-20, n=14) or LS for 8 weeks (LS-8, n=8), 12 weeks (LS-12, n=8), 16 weeks (LS-16, n=8), and 20 weeks (LS-20, n=8). All of the rats were housed under identical conditions in a 12-hour light/dark cycle and given food and water ad libitum. The study conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health, and the protocol was approved by the Institutional Animal Care and Use Committee of Columbia University.

Hemodynamic Studies

Hemodynamic studies were performed as terminal procedures at each time point in both groups. After induction of anesthesia (2% isoflurane) and intubation, the right carotid artery was cannulated with a conductance catheter (1.4 Fr.; Millar Instruments) with pressure transducer that was advanced across the aortic valve into the left ventricle. Left ventricular (LV) end-systolic and EDPs and, after withdrawal of the catheter into the aorta, phasic and mean arterial pressures were measured. LV end-diastolic volume (EDV) and end-systolic volume (ESV) were measured using the conductance catheter technique, with volume calibrations performed as described elsewhere, including use of ex vivo phantoms of precisely known volumes and hypertonic saline injections in vivo.[17] All of the data were collected digitally with commercially available hardware and software (MacLab) at a sampling rate of 500 Hz. EF, SV, cardiac output, and stroke work were computed offline using a pressure-volume analysis program (PVAN 3.2, Millar Instruments). Data for determination of LV end-diastolic and end-systolic pressure-volume relations (EDPVR and ESPVR, respectively) were obtained by temporary inferior vena caval occlusion. End-systolic pressure-volume data were subjected to linear regression analysis to arrive at the slope, E_{es}, and volume axis intercept, V_0. EDPVR data were fit to a nonlinear equation [$P=\beta\times e^{\alpha(V/Vm)}$]. The time constant of LV isovolumetric pressure decline, τ, was calculated using the logarithmic method according to Weiss et al.[18]

Echocardiographic Studies

Transthoracic echocardiographic studies (Sonos 5500, 12-MHz transducer) were performed on anesthetized animals (2% isoflurane) in a left lateral decubitus position by an observer blinded to study group. Blinding was also maintained during all of the analyses. From the cardiac short axis (papillary level), an M-mode trace of the LV was obtained, and LV end-diastolic and end-systolic diameters (LVEDD and LVESD) and posterior and septal diastolic wall thickness (PWT and SWT) were measured. LV mass (LVM) was calculated according to:

$$LVM=1.04\times[(LVEDD+PWT+SWT)^3-LVEDD^3]$$[19,20]

Fractional shortening (FS) was calculated as:

$$FS=(LVEDD-LVESD)/LV\ end\text{-}systolic\ diameter$$

Mitral flow was recorded at the mitral valve tips from an apical view using pulse Doppler imaging. We measured maximal velocities of the E and A waves, E/A ratio, and deceleration time of the E wave as the interval between the peak early diastolic velocity and the point of steepest deceleration slope extrapolated to the baseline. From an apical view, propagation velocity was measured using a color M-mode Doppler echocardiographic image.[21] Myocardial velocities were recorded at the level of basal lateral and septal segment from the apical view using pulsed Doppler tissue imaging. Early (Em) and late diastolic waves were measured, and E/Em was calculated.

Ex Vivo EDPVR and Stiffness Calculation

To provide additional assessment of ventricular passive properties independent of the conductance catheter, the ex vivo passive LV pressure-volume relationship was measured with the heart arrested in diastole by retrograde coronary perfusion with high-concentration KCl solution and rapid excision.[22] The left atrium was opened, and a thin latex balloon attached to a stiff polyethylene tubing was advanced into the LV and held in place by a purse string suture around the mitral annulus. LV pressures were measured using a 5-F Millar micromanometer as volume was infused into the balloon in 0.025-mL increments until reaching an LV pressure of 40 mm Hg.

Chamber stiffness constant (α) was calculated according to the equation described by Mirsky and Pasipoularides[23]:

$$P=\beta\times e^{[\alpha\times(V/Vm)]} \quad (1)$$

where V_m is LV myocardial volume (=0.943×LV weight). α is a chamber stiffness constant, and β a scaling constant.

Myocardial stiffness (κ) is the slope of the stress (σ)-strain (ϵ) relationship. For this analysis, a spherical geometry of the heart was assumed. Midwall circumferential stress (σ_m) and Lagrangian strain (ϵ_m) were calculated with the use of following equations[23]:

$$\sigma_m=[1.36\times P\times V^{2/3}]/[(V+V_m)^{2/3}-V^{2/3}] \quad (2)$$

$$\epsilon_m=\{[V^{1/3}+(V+V_m)^{1/3}]/[V_0^{1/3}+(V_0+V_m)^{1/3}]\}-1 \quad (3)$$

where V_0 is the unstressed LV volume measured in the ex vivo preparation.

Ventricular Pump Function Curves

Zile et al[13] noted limitations of using the ESPVR alone to quantify pump function in the presence of abnormalities of diastolic performance. To overcome this limitation, it has been proposed that the area between the EDPVR and the ESPVR measured as a function of either EDP or EDV be used to index overall pump function.[24,25] This specific area, called the isovolumic pressure-volume area (PVA_{iso}), is independent of afterload and can be calculated analytically following curve fitting of the EDPVR and the ESPVR:

$$PVA_{iso}(V)=\int[P_{es}(V)-P_{ed}(V)]dV=0.5E_{es}(V-V_o)^2-V_m(\beta/\alpha)\,e^{\alpha\times(V/Vm)}$$

where $P_{es}(V)$ and $P_{ed}(V)$ are the end-systolic and EDPs, respectively, as a function of volume.

Statistical Analysis

Results are expressed as mean±SD. Analyses were performed with commercially available software (SPSS 11.5). Differences between groups were assessed using 1-way ANOVA. Pressure-volume relations were analyzed by analysis of covariance with Bonferroni correction for multiple comparisons. Differences in chamber stiffness constants were tested by linearizing the EDPVRs by logarithmic transformation and then applying analysis of covariance at each time point to test for differences between LS and HS groups. $P<0.05$ was considered statistically significant.

Results

Hemodynamics

The main hemodynamic parameters are summarized in Figure 1. LVESP was increased in HS compared with LS rats at all of the time points (Figure 1A). Whereas LVESP did not change over time in LS, it increased in HS

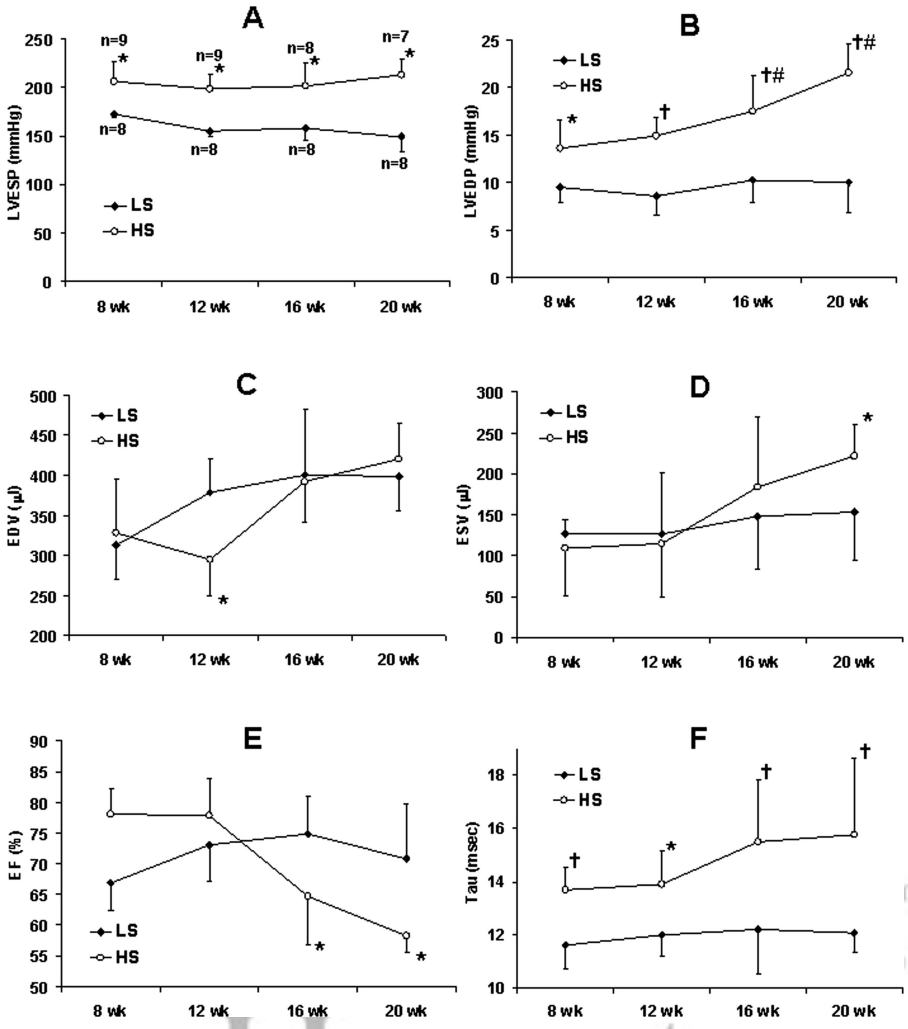

Figure 1. Hemodynamic parameter after HS (○) and LS (▲) diet over the course of the study. (A) LVESP. (B) LVEDP. (C) LVEDV. (D) LVESV. (E) EF. (F) The time constant of isovolumic relaxation (τ). n indicates the number of animals at each time point and are the same for each panel. *$P<0.05$ and $P<0.01$ vs age-matched control; #$P<0.05$ vs baseline (HS-8).

throughout the study. Similarly, LVEDP was also higher than HS in comparison to LS. Whereas EDP remained constant over time in LS, it increased over baseline at 16 and 20 weeks in HS, reaching values of ≈25 mm Hg at 20 weeks (Figure 1B). Changes in EDV and ESV (conductance method of LV volume measurement) are shown in Figure 1C and 1D. The trends in ESV were similar to those of EDP, with ESV almost doubling at 20 weeks in the HS group. However, EDV decreased significantly at 12 weeks in HS rats, increasing thereafter to reach the same values as in the LS group. This initial reduction in EDV at 12 weeks is suggestive of the development of passive diastolic dysfunction, as revealed more explicitly by the pressure-volume analysis (discussed in detail below). On balance, these changes in ESV and EDV resulted in no significant changes in EF in LS rats (Figure 1E). In comparison, EF was increased in HS at weeks 8 and 12 and declined significantly thereafter ($P<0.05$). The time constant of LV isovolumetric relaxation, τ, exhibited the same trends as EDP with regard to differences between groups and changes over time (Figure 1F); however, the changes were relatively small, amounting to ≈2 to 4 ms between groups or over time.

Body, Organ Weights, Renal Function, and Mortality

Table 1 summarizes the effects of LS and HS diet on body weight, LV, lung and kidney weights (all measured ex vivo), and mortality. All of the parameters except body weight were significantly increased in the HS group compared with the LS controls throughout the follow-up period. By 20 weeks, LV weight was >30% greater in HS than LS rats. Standard histological analysis with trichrome staining (results not shown) revealed cellular hypertrophy and interstitial fibrosis as reported in prior studies.[26,27]

Body weight showed less of an increase with age in the HS group compared with the LS group from 8 to 16 weeks but reached values similar to the controls at 20 weeks. The lung/body weight increased significantly in the HS group at weeks 16 and 20, suggestive of pulmonary edema, which correlated with the increase in EDP and development of edema, both consistent with a transition to a heart failure state. Kidney/body weight also showed a significant increase in the HS group compared with the control group. Renal function, as determined by plasma concentrations of urea nitrogen and creatinine, increased in the HS group but remained normal in the LS group.

TABLE 1. Effect of Dietary Salt on Body, Lung, and Kidney Weight and Mortality (mean±SD)

Group	No.	BW (g)	MAP (mm Hg)	LVW (g)	LV/BW (g/kg)	Lung/BW (g/kg)	Kidney/BW (g/kg)	BUN (mg/dL)	Creatinine (mg/dL)	Mortality (%)
LS-8	8	388±23	138±3	0.93±0.05	2.40±0.14	5.86±0.58	8.70±1.35	19.8±2.3	0.52±0.12	0
LS-12	8	386±23	123±7	1.02±0.08	2.63±0.15	4.89±0.22	8.15±0.73	17.8±2.0	0.64±0.15	0
LS-16	8	427±26*	134±21	1.05±0.13	2.45±0.30	4.80±0.24	7.96±0.67	18.9±2.7	0.43±0.20	0
LS-20	8	427±31*	120±14	0.92±0.04	2.16±0.17	4.53±0.24	6.91±2.05	18.2±2.5	0.46±0.25	0
HS-8	9	340±54†	160±11‡	1.08±0.14†	3.03±0.55‡	6.14±1.09	10.63±2.46†	27.3±3.9‡	0.52±0.20	10%
HS-12	9	352±17†	167±16‡	1.10±0.04†	3.08±0.24‡	5.11±0.20	10.33±1.06‡	22.4±2.6‡	0.43±0.19	25%†
HS-16	8	371±39†	171±25‡	1.11±0.05†	2.94±0.41‡	5.65±2.09†	10.59±1.17‡	24.2±6.0‡	0.51±0.26	35%†
HS-20	7	406±26*	182±15‡	1.26±0.21*‡	3.10±0.33‡	6.20±2.26†	13.09±1.67*‡	28.8±9.8‡	0.77±0.21*†	50%‡

BW indicates body weight; LVW, LV weight; LV/BW, left ventricular mass normalized to body weight; BUN, blood urea nitrogen.
*$P<0.05$ vs baseline (LS-8 or HS-8); †$P<0.05$ vs LS age-matched control; ‡$P<0.01$ vs LS age-matched control.

None of the LS rats died during the study, but HS rats showed increased mortality over time. The cause of death in these animals was not investigated.

Echocardiographic Studies

Compared with the LS rats, HS rats exhibited significant increases in SWT and PWT over the 20-week study (Figure 2A; only SWT shown) consistent with the marked increase in LVM in this group (Table 1). LVEDD (Figure 2B) increased in both groups with statistically nonsignificant trends for this parameter to be decreased at 12 weeks and increased at 20 weeks. LVESD increased by the same amounts from baseline to 12 weeks (Figure 2C), after which it increased more significantly in HS rats. Concomitantly, FS was preserved in both groups through 12 weeks but decreased significantly thereafter in the HS group (Figure 2D). Thus, based on standard clinically used echocardiographic parameters, marked hypertrophy developed in the HS group, and EF was normal through 12 weeks but declined thereafter.

Concomitantly, there was a nonsignificant increase in the E/A ratio, a nonsignificant decrease in deceleration time of the E wave, and a significant decrease in propagation velocity consistent with a more restrictive LV filling pattern (Figure 3). The E/Em ratio was not significantly different between groups or between study time points.

Pressure-Volume Analysis

Figure 4 shows representative steady-state pressure-volume loops at each time point. At baseline, EDVs and EDPs are similar in both groups, but end-systolic pressure is markedly elevated in HS compared with LS rats. At 12 weeks, the more marked hypertension persists, but the pressure-volume curve shows some elevation of EDP and reduction in LVEDV (Figure 1B, consistent with passive diastolic dysfunction). At weeks 16 and 20, the pressure-volume loops of the HS groups shifted rightward so that EDVs again became similar to those of the LS group although, as noted above (Figure 1B), EDP remained significantly higher.

Average results of the ESPVR analysis are summarized in Table 2. The slopes of the ESPVR did not vary significantly between groups or between time points ($P>0.05$ by ANCOVA). However, statistical analysis did indicate that the curves in the HS group were shifted upward at weeks 8 and 12, as indicated by the significantly reduced V_0 values in the HS group at those time points.

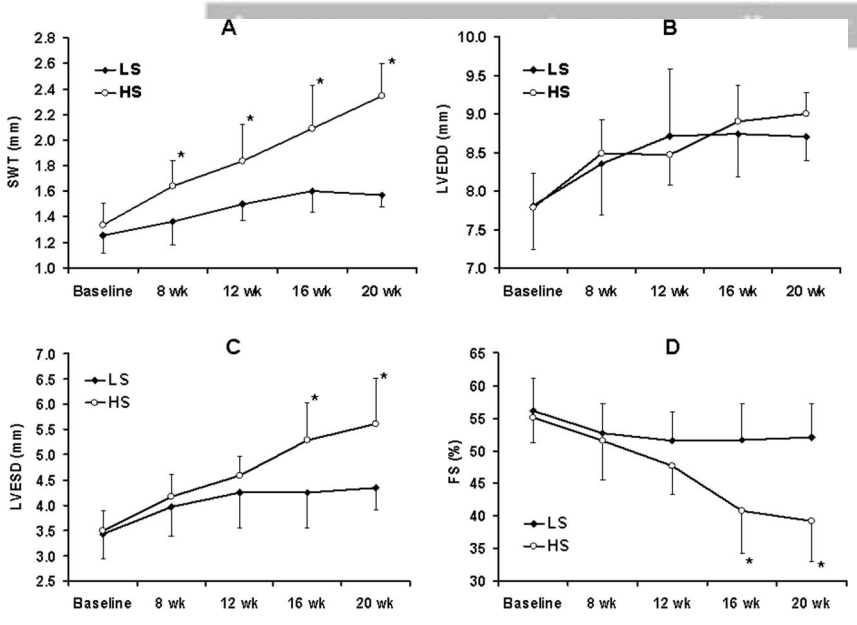

Figure 2. Echocardiographic parameter in HS (○) and LS (▲) rats. (A) Thickness of the interventricular septum (SWT) increased significantly in HS rats over the time period of 20 weeks from similar baseline values, signifying significant cardiac hypertrophy because of systolic hypertension. (B) LVEDD was reduced at 12 weeks in HS rats and increased thereafter. (C) LVESD showed a significant increase in HS rats compared with LS rats at the 16 and 20-week time points. (D) FS was preserved in HS rats up to 12 weeks of diet and started to deteriorate at the later time points. *$P<0.05$ vs LS group. Number of animals as detailed in Figure 1.

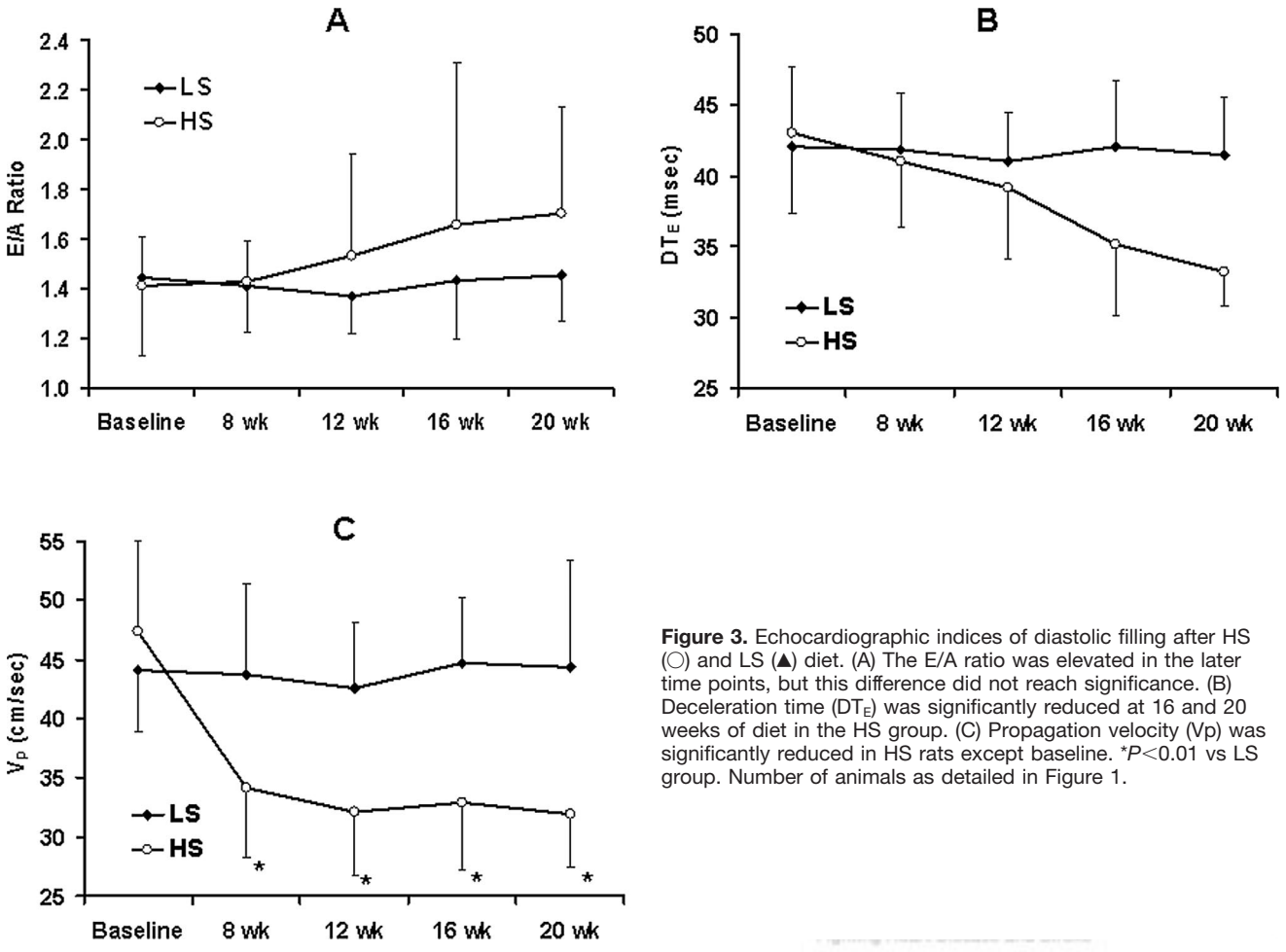

Figure 3. Echocardiographic indices of diastolic filling after HS (○) and LS (▲) diet. (A) The E/A ratio was elevated in the later time points, but this difference did not reach significance. (B) Deceleration time (DT_E) was significantly reduced at 16 and 20 weeks of diet in the HS group. (C) Propagation velocity (Vp) was significantly reduced in HS rats except baseline. *$P<0.01$ vs LS group. Number of animals as detailed in Figure 1.

With regard to EDPVRs, complimentary, confirmatory data were obtained from in vivo and ex vivo data; in the ex vivo setting, it was possible to acquire pressure-volume data over a broader range of values, independent of group and time. Average ex vivo EDPVRs are shown in Figure 5. As shown in Figure 5A, there is no detectible difference between the EDPVRs at 8 weeks. At 12 weeks, however, the EDPVR of LS rats shifts toward larger volumes, a consequence of normal growth, whereas that of the HS group shifts toward smaller volumes, indicative of passive diastolic dysfunction. At 16 weeks, the EDPVR shifts toward larger volumes in both groups, with HS becoming indistinguishable from the LS group. At 20 weeks, there is no further enlargement (ie, growth) noted in the LS group, whereas the HS group shows a trend toward additional enlargement. The unitless chamber stiffness constant is increased in the HS group at 12 weeks but did not differ from baseline or the LS group at other time points (Table 2). Similarly, V_{30}, the volume at a pressure of 30 mm Hg, is also the same between groups except at 12 weeks, when V_{30} is significantly lower in HS compared with LS. Interestingly, the myocardial stiffness constant (κ) is not significantly different between the groups at any time point.

Overall Pump Function

Changes in systolic, diastolic, and overall pump function are summarized in Figure 6. The left panel of each row shows average ESPVRs and EDPVRs for the LS (solid lines) and HS (dashed lines) groups derived from the average parameter values in Table 2; corresponding PVA_{iso}-EDP and PVA_{iso}-EDV relationships are shown on the right. At 8 weeks, the EDPVRs are indistinguishable between groups, whereas the ESPVR and PVA_{iso}-EDP and PVA_{iso}-EDV relationships are slightly increased in HS rats, indicating superior overall pump function. At 12 weeks, prominent leftward/upward shifted EDPVR is present in HS rats. The ESPVR is also shifted leftward/upward, even more so than the EDPVR. Consequently, the PVA_{iso}-EDP and PVA_{iso}-EDV relationships are also shifted upward, indicating enhanced overall pump function at that time point. Therefore, at this time point in particular, the upward shifted EDPVR by itself is not enough to conclude the presence of pump dysfunction. At later times, the EDPVRs and ESPVRs of the 2 groups converge, as do the PVA_{iso}-EDP and PVA_{iso}-EDV relationships; the overall pump function curve and ESPVR of the HS group never falls below that of the LS group despite the reduction of EF.

Discussion

Dahl rats fed an HS diet develop severe hypertension that leads to progressive hypertrophy and, ultimately, heart failure. At 12 weeks, early in the course of the development of hypertrophy, EF remains within the normal range, but there is a reduction in EDV and a leftward/upward shift

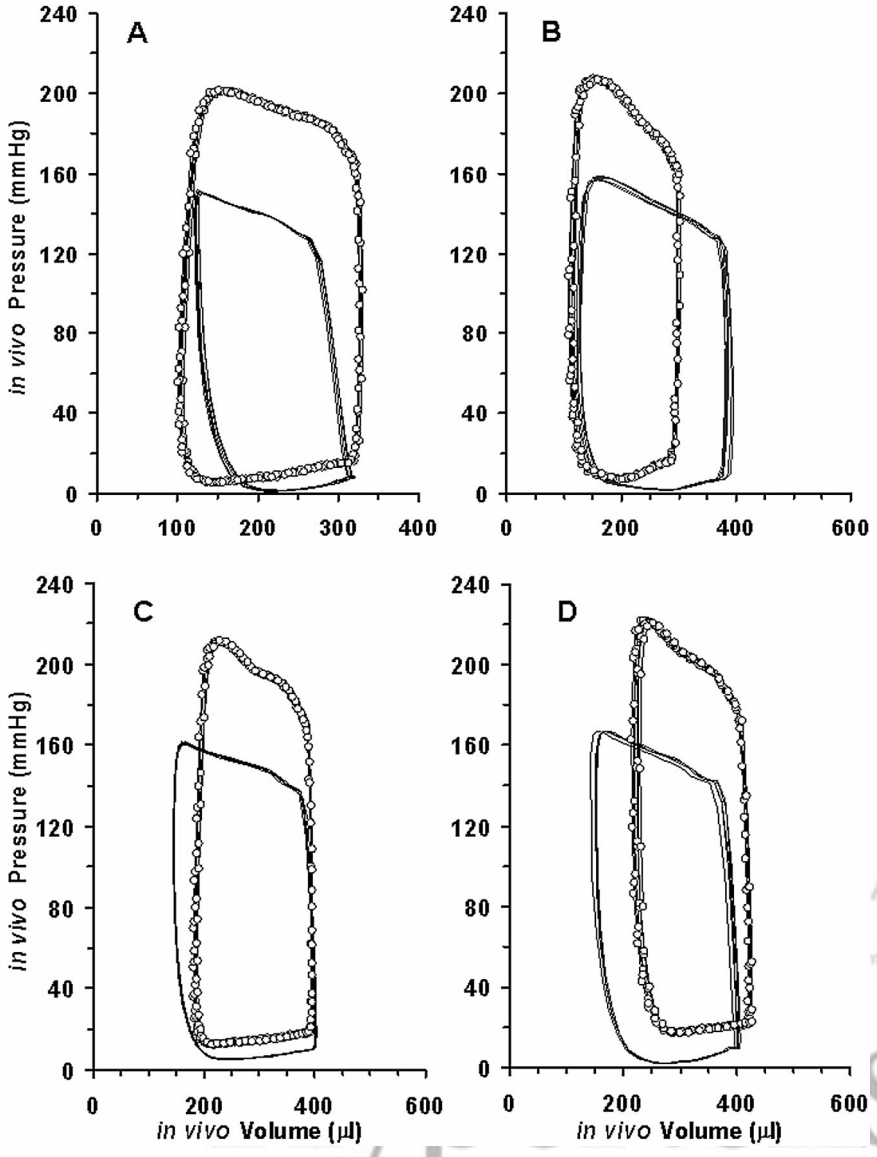

Figure 4. Representative pressure-volume loops from individual HS (○) and LS rats at 8 (A), 12 (B), 16 (C), and 20 (D) weeks of diet. The shape of the P-V loops in LS rats showed no dramatic changes over the time period of 20 weeks except an age-dependent small shift to larger EDVs. In contrast, the loops from the HS rats show a shift toward smaller volumes at week 12. Subsequently, the loop shifted rightward and upward by week 20.

of the EDPVR. These changes, if considered as isolated findings, would be considered to be indicative of passive diastolic dysfunction, as defined originally by Grossman.[28] At this time point, however, EDP was minimally elevated compared with controls (2 mm Hg), lung weight was not abnormal, and Doppler parameters showed no indication of abnormal filling patterns, indicating no evidence of heart failure. Over the next 4 weeks, hypertrophy continues to develop, but the heart dilates so that the EDPVR becomes essentially indistinguishable from those of age-matched controls, and there is normalization of chamber stiffness constant (no indication of passive diastolic dysfunction). From this point onward, EF and SV decline, EDP and wet lung weight increase, and abnormalities of Doppler parameters of diastolic filling are observed. Heart failure (increased filling pressure, fluid accumulation, and decline of SV) becomes increasingly more severe, but passive diastolic dysfunction is not present, although there is a ≈4-ms increase in the time constant of relaxation. The concordance between in vivo and ex vivo EDPVRs suggest that this change in τ did not contribute to diastolic dysfunction in the form of incomplete relaxation. Examination of the EDPVR and other indexes of diastolic properties in isolation, therefore, portray a time sequence of pathophysiological changes that is not tightly linked to development of heart failure.

Simultaneous evaluation of systolic and diastolic properties, yielding an assessment of overall pump function (Figure 6), reveals that the PVA_{iso}-EDP and PVA_{iso}-EDV relationships are shifted upward in the HS group. This indicates that at any given filling pressure, the heart can do more work. At 12 weeks, when the EDPVR has shifted upward (indicative of passive diastolic dysfunction), the ESPVR has also exhibited a further, rather significant upward shift. In fact, the magnitude of the upward shift of the average ESPVR was greater than the upward shift of the EDPVR. Thus, despite smaller filling volumes, the heart exhibited the capability to do more work at any given filling pressure. As already noted, in this state, at rest, there is no physiological evidence of heart failure. As the process continues over time, the EDPVR shifts

TABLE 2. Stiffness and Contractility Parameters (mean±SD, No. as in Table 1)

In Vivo ESPVR				Ex Vivo EDPVR			
Group	Heart Rate (min^{-1})	E_{es} (mm Hg/mL)	V_0 (mL)	a (unitless)	β (mm Hg)	V_{30} (mL)	κ (unitless)
LS-8	347±25	247.1±107	−0.412±0.12	18.07±3.56	0.012±0.013	0.446±0.06	44.52±7.65
LS-12	316±19	255.9±110	−0.343±0.12	18.45±6.75	0.008±0.009	0.488±0.07	48.37±9.99
LS-16	334±18	238.3±114	−0.410±0.10	16.79±3.72	0.012±0.012	0.510±0.06	42.43±6.39
LS-20	320±22	245.0±19.1	−0.306±0.10	16.70±2.77	0.022±0.015	0.546±0.04	35.71±5.94
HS-8	306±21	262.9±107	−0.593±0.07*	21.63±4.70	0.008±0.015	0.457±0.06	48.98±8.32
HS-12	309±19	285.2±161	−0.736±0.14*	23.84±6.35*	0.011±0.008	0.382±0.06*	46.76±7.45
HS-16	319±34	233.8±77.1	−0.611±0.11	16.68±2.75	0.012±0.013	0.521±0.05	42.50±6.17
HS-20	308±36	193.6±65.7	−0.410±0.07	14.72±2.34	0.011±0.010	0.594±0.06	42.67±4.64

E_{es} indicates slope of the ESPVR; V_0, volume at the pressure of 0 mm Hg; α, chamber stiffness constant; β, scaling constant; V_{30}, volume at the ex vivo pressure of 30 mm Hg (a measurement of LV capacitance); κ, myocardial stiffness.

*$P<0.05$ vs LS at same week.

back to that of the age-matched controls and so too does the ESPVR, so that by 20 weeks, the ESPVRs and EDPVRs of the 2 groups are nearly superimposed and so too are the overall pump function curves. Thus, despite the fact that, at these later time points, the pressure-volume–based measures of pump function (ESPVR, EDPVR, and overall pump function curves) never indicate worse function, and EF decreases in the HS group compared with the LS control group, only the HS animals develop findings compatible with heart failure.

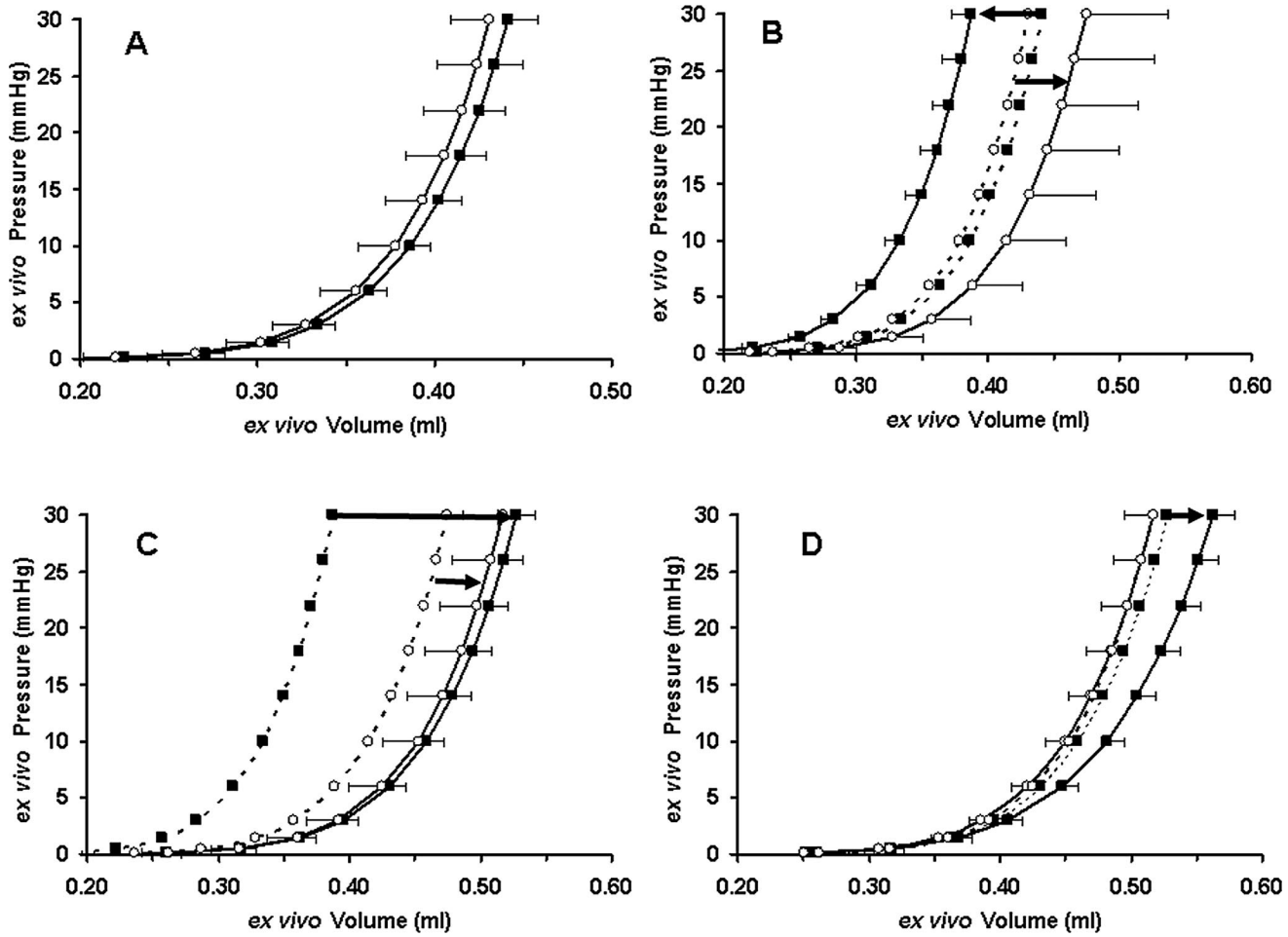

Figure 5. The ex vivo EDPVR in HS (■) and LS (○) rats at 8 (A), 12 (B), 16 (C), and 20 (D) weeks. Dashed lines represent EDPVRs from the respective previous time point. Compared with 8 weeks, at 12 weeks the HS EDPVR shifted to the left toward smaller volumes (filled arrow), whereas the LS EDPVR showed a small shift to the right (open arrow). At 16 weeks, both EDPVRs shifted to the right with the largest volume in HS rats at 20 weeks. Number of animals as detailed in Figure 1.

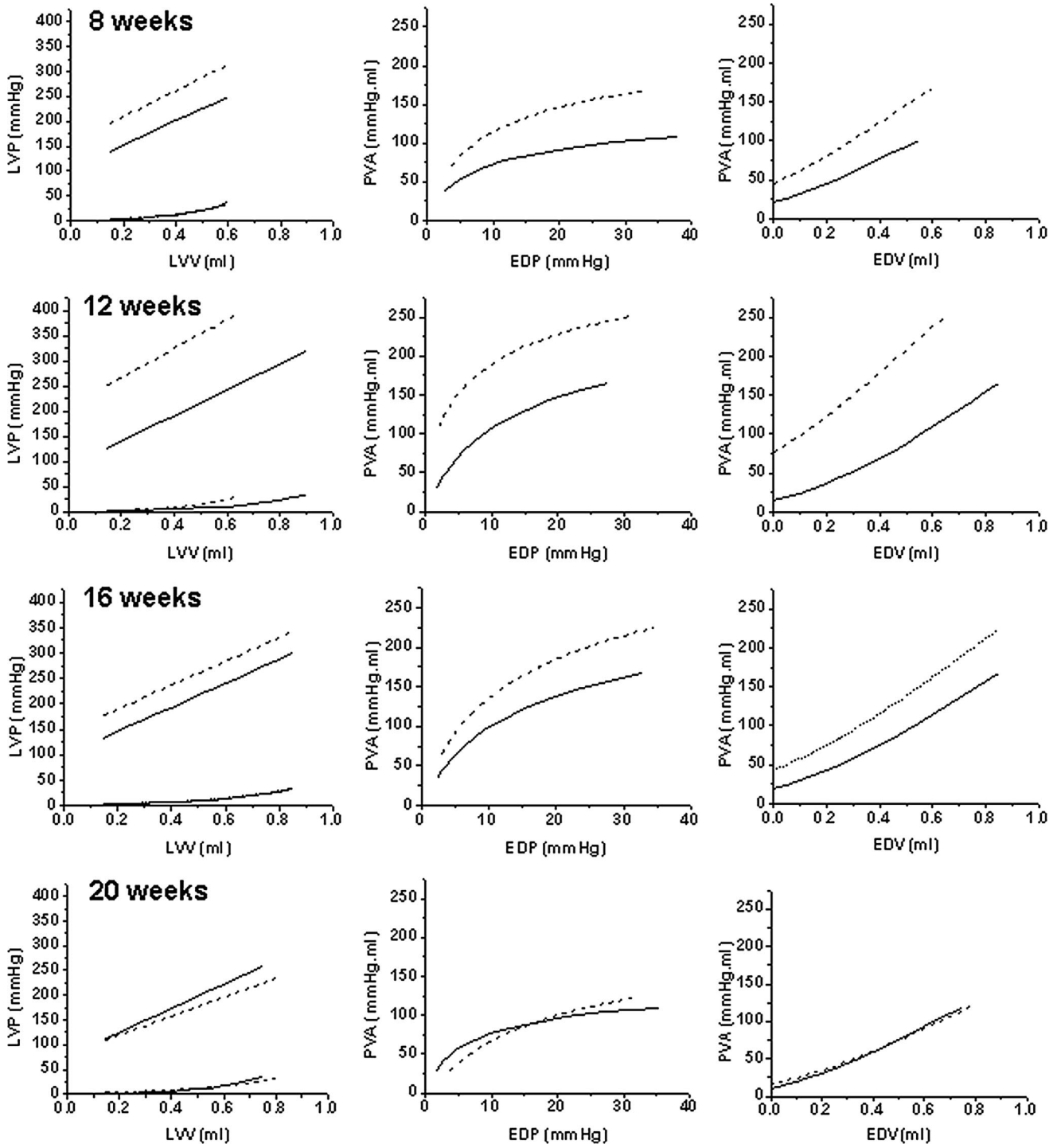

Figure 6. At each time point, the average ESPVR and EDPVR (left) and resultant overall pump function curve indexed by the relationship between isovolumic pressure-volume area (PVA_{iso}) and EDP and EDV. Baseline data are shown in the solid lines and the relevant data from the representative time point in the dashed line. Details are provided in the text. Note that at 12 weeks, prominent leftward/upward shifted EDPVR is present in HS rats. The ESPVR is also shifted leftward/upward, even more so than the EDPVR. Consequently, the PVA_{iso}-EDP and PVA_{iso}-EDV relationships are also shifted upward, indicating enhanced overall pump function at that time point. Therefore, at this time point in particular, the upward shifted EDPVR by itself is not enough to conclude the presence of pump dysfunction. At later times, the EDPVRs and ESPVRs of the 2 groups converge, as do the PVA_{iso}-EDP and PVA_{iso}-EDV relationships; the overall pump function curve and ESPVR of the HS group never falls below that of the LS group. LVP indicates LV pressure; LVV, LV volume.

Although the observations in this study appears to be contrary to traditional teachings about heart failure, they are consistent with more recently proposed concepts of why pulmonary venous pressure increases.[29] Detailed analysis supported by results of animal studies suggests that heart failure can occur as a result of fluid accumulation even if heart function is normal. This is of particular relevance to the current findings obtained in an animal model in which the primary dysfunction is

in the kidney[30,31] and in which the development of heart failure parallels the deterioration of renal function, not of diastolic, systolic, or overall pump dysfunction.[32,33]

It is also important that a strict distinction be made between pump dysfunction and myocardial dysfunction. With regard to systole, myocardium and muscle cells isolated from this model have been shown to have abnormal contractile force on the basis of abnormal excitation-contraction coupling.[34,35] Measurement of endocardial shortening (Figure 2) and even estimates of midwall shortening fraction[36,37] (a more sensitive measure of myocardial systolic function) were all normal at 12 weeks but were reduced compared with controls by ≈30% at 20 weeks of HS diet, consistent with other studies of pressure overload hypertrophy.[38–40] However, pump function depends not only on muscle function, but also on muscle mass, chamber geometry, and activation sequence.[39,41] Thus, despite abnormalities of muscle contractile properties, which progress over time in this model, other factors, such as increased muscle mass, render overall systolic pump function as indexed by the ESPVR and the PVA_{iso}-EDP and PVA_{iso}-EDV relationships remain normal or enhanced, at least through 20 weeks of HS diet. Similarly, with regard to diastole, the effects of hypertrophy and interstitial fibrosis affect myocardial properties, but these effects can be offset by changes in geometry. The EDPVR may be shifted upward/leftward as was observed early after introduction of the HS diet, can be similar to the controls as in the intermediate time points, or can be downward/rightward shifted as was trending at 20 weeks. Based on our observations, however, the unitless myocardial stiffness constant (κ, which is determined by taking into account changes in mass and chamber size) did not vary significantly between groups or between different times of observation (Table 2). This conclusion is identical to that reached by Munagala et al[42] in a canine model of hypertension and Cingolani et al[43] in spontaneously hypertensive rats in which hypertrophy and substantial interstitial fibrosis were observed without any change in myocardial stiffness constant.

Furthermore, in a rat model of pressure overload induced by chronic aortic banding leading to hypertrophy (≈44% increase in LVM, similar in magnitude to what we observed), Norton et al[39] noted that some rats exhibited concentric chamber remodeling with preserved EF. Despite the development of significant hypertrophy and lack of significant chamber enlargement, these rats showed no significant change in myocardial stiffness or in the EDPVR. An upward shifted ESPVR was also observed in these animals. These findings are generally consistent with what we found in the HS-fed Dahl rats. In contrast to our findings, however, these rats did not develop heart failure. Although not measured, it is assumed that intrinsic renal function is normal in aortic banded rats. It is, therefore, intriguing that Dahl rats, which exhibit the same degree of hypertrophy but which have an intrinsic renal abnormality of salt metabolism, develop increased lung weight and high LVEDPs characteristic of heart failure. This supports the hypothesis that impairment of renal dysfunction (specifically salt metabolism) may play an important role in the pathogenesis of heart failure (discussed further below).[10]

Although often used in the clinical setting, assessment of Doppler parameters of ventricular filling dynamics to diagnose the presence of passive diastolic dysfunction has certain limitations.[9] The results of the present study, in which multiple Doppler parameters have been measured along with true measures of diastolic properties (ie, EDPVR and τ) and EDP highlights such limitations. Changes in E/A ratio and deceleration time more closely tracked EDP than changes in the EDPVR or τ. The same was true for indexes derived from tissue Doppler techniques (Table 2). These conclusions are consistent with those of recent clinical studies.[44] In addition, whereas 2D echocardiographic guided M-mode measurements of ventricular size based on chordal dimension are also used in the clinical setting, such measures have limitations. Specifically, it does not account for a relative increase in ventricular length that appears to occur in human hypertensive heart failure with a normal EF.[45] Furthermore, if the image plane from which the chordal dimension does not pass precisely through the middle of the ventricle, the measured dimension will be an underestimation of the true dimension.[46] We believe these methodologic limitations account for the apparent discrepancies between the 2D echocardiographic measures of ventricular width and the volumetric data derived from the conductance catheter that were observed at the 12-week time period. The lack of a significant change in LV width does not negate the volumetric findings as determined by the conductance catheter.

Doi et al[15] previously suggested the salt-sensitive Dahl rat as an animal model of DHF, and several studies have reinforced the utility of this model.[16,34] Measurements made in the current study that are common to these prior studies are confirmatory. Our study extends prior studies by focusing on a characterization of overall pump function, which accounts for both systole and diastole through detailed characterization of ESPVR, EDPVR, and PVA_{iso}-EDP and PVA_{iso}-EDV relationships. Early in the time course of the study (eg, 12-week), there is evidence of passive diastolic dysfunction (upward shifted EDPVR and reduced chamber capacitance) with enhanced systolic performance (upward shifted ESPVR), thereby maintaining a normal EF, FS, and high blood pressure. However, despite the presence of passive diastolic dysfunction, findings of heart failure (significantly increased EDP and lung weight) are absent. At later time points (16 and 20 weeks), concomitant with the development of heart failure, there is a return of pressure-volume–based indices of systolic and diastolic function to the control conditions, a decline in renal function, and reduction in EF and FS.

A recent study of relatively young men with HFNEF demonstrated the presence of passive diastolic dysfunction evidenced by an upward/leftward shifted end-diastolic pressure-volume point.[5] That study, however, did not report information about systolic function other than EF, so it is not known whether the ESPVR was normal or supernormal. In our recent study of predominantly older women with HFNEF,[10] we concluded that, on average, passive diastolic dysfunction was not present, and systolic function (ie, ESPVR) was normal. We measured increased plasma volume in these patients despite high-dose diuretics. Renal dysfunction (indexed by serum creatinine) was also significantly worse than controls in these

patients. It is tempting to speculate that the younger patients studied in the former study[5] correspond to rats in the early stage of the response to hypertension (ie, the 12-week group), and those of the latter study[10] correspond with rats in the later stage of the response to hypertension (ie, the 20-week group). The potential role of renal dysfunction and fluid accumulation as an important mechanism contributing to the development of high-filling pressures should be considered further.

Heart failure with a normal or preserved EF in the setting of hypertension is a heterogeneous clinical syndrome with various associated comorbid conditions[47] and a spectrum of clinical presentations ranging from effort or exercise intolerance[48] to elevated LV EDP with[49] or without[50] acute pulmonary edema. Recent studies have demonstrated that increased ventricular vascular stiffening[3,11,42] characterized by upward and leftward shifted ESPVR and EDPVR (eg, at the 12-week time period in the HS rats) can result in increased load lability[11] and is closely correlated with exercise duration and peak oxygen consumption.[51] Thus, whereas the 12-week HS rats did not manifest with increased EDP nor increased lung/body weight ratios indicating pulmonary edema, the pathophysiological characteristics at this time point are similar to those described in other animal models[42] and human[11] observations and are potential pathophysiological explanations for the reduced exercise capacity that is characteristic of HFPEF subjects. However, our focus was on the pathophysiological explanation for an elevated EDP at rest and resultant pulmonary edema, which has been observed in several investigations.[11,49,50] The data at the 16- and 20-week time points suggest that extracardiac factors, specifically renal dysfunction, play an important pathophysiological role in the genesis of this clinical aspect of this syndrome.

Perspectives

Dahl rats consuming HS diet initially develop an elevation of the EDPVR indicative of passive diastolic dysfunction, but this is compensated by greater changes in the ESPVR such that overall pump function is supernormal, and there is no physiological evidence of pump dysfunction; there is also no evidence of heart failure at that time. Over time, however, the changes in the EDPVR resolve as the heart dilates. Over the 20-week time period studied, the ESPVR, on average, does not drop below that of the control animals. Despite this, heart failure develops in the group receiving HS diet, evidenced by increases in wet lung weight and LV end-diastolic pressure. Furthermore, these parameters did not correlate with either systolic or diastolic pump dysfunction. It is evident that the Dahl rat, which retains salt and water, is an excellent model to study the pathogenesis of heart failure in the setting of hypertension, whereas overall pump function remains normal. These results are compatible with emerging data from the clinical setting showing that heart failure can occur without evidence of systolic or diastolic dysfunction.

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