Ventricular Structure and Function in Aged Dogs With Renal Hypertension
A Model of Experimental Diastolic Heart Failure

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Background—Heart failure (HF) with normal ejection fraction (diastolic HF [DHF]) usually occurs in elderly patients with hypertension. The presence and significance of altered systolic and diastolic ventricular function in DHF is increasingly controversial. Our objective was to develop a clinically relevant large-animal model to better understand the pathophysiology of DHF.

Methods and Results—Ventricular structure and function were characterized in young control (YC group; n=6), old control (OC group; n=7), and old dogs made hypertensive by renal wrapping (experimental DHF [ExDHF] group; n=8). The ExDHF group was associated with normal left ventricular (LV) volume, increased LV mass, and myocardial fibrosis. LV relaxation was impaired in ExDHF (τ=53±6 ms) compared with OC (τ=35±3 ms; P<0.05) and YC (τ=33±6 ms; P<0.05) dogs. The percent diastole at which relaxation is complete was increased in ExDHF (116±33 ms) compared with OC (69±8 ms; P<0.05) and YC (35±5 ms; P<0.05) dogs. The coefficient of LV diastolic stiffness was similar in OC, YC, and ExDHF dogs. Diastolic pressures increased dramatically in response to increases in blood pressure. End-systolic LV stiffness was enhanced in ExDHF dogs and after load enhancement of myocardial performance was maintained. Arterial stiffness was increased in ExDHF dogs.

Conclusions—Aged dogs with chronic hypertension exhibit LV hypertrophy and fibrosis with impaired LV relaxation but no increase in the coefficient of LV diastolic stiffness. LV systolic and arterial stiffness are increased, which may exacerbate load-dependent impairment of relaxation and contribute to increased filling pressures with hypertensive episodes. This model mimics many of the structural and functional characteristics described in the limited studies of human DHF and provides insight into the pathogenesis of DHF. (Circulation. 2005;111:1128-1135.)

Key Words: diastole • heart failure • hypertension • kidney • physiology

Several epidemiological studies have established that heart failure (HF) with normal ejection fraction (EF), termed diastolic HF (DHF), is common, particularly in elderly patients with hypertension. It has been assumed that DHF patients have intrinsic diastolic dysfunction. Only limited hemodynamic data in DHF patients exist to support this assumption. Recently, the concept that intrinsic diastolic dysfunction accounts for most cases of DHF has been challenged. A study in a small number of DHF patients found that neither ventricular relaxation nor passive diastolic stiffness was abnormal. Rather, increases in left ventricular (LV) systolic stiffness and arterial stiffness were observed. Others have suggested that LV volumes are increased and that upward shifts along a normal end-diastolic pressure-volume relationship explain elevated filling pressures in DHF. Still other studies suggest that systolic function is reduced in patients with DHF. Thus, the presence and significance of altered ventricular function in DHF are increasingly controversial.

Our understanding of systolic HF has been greatly facilitated by the presence of clinically relevant large-animal models. Although a useful rodent model of DHF has been described, a large-animal model of DHF would facilitate understanding of the pathophysiology of and therapeutic approach to DHF. Our objective was to characterize ventricular structure and function and arterial function in aged dogs with experimental hypertension. Recognizing the similar clinical profile of this model to human DHF, we hypothesized that aged, chronically hypertensive dogs would display diastolic dysfunction. Ventricular systolic and arterial stiffness were also assessed because clinical studies indicate that these indices are abnormal in DHF. Studies were performed in young control (YC group) and old control (OC group) dogs and old dogs with chronic hypertension produced by bilateral renal wrapping (experimental DHF [ExDHF group]).

Methods
The Mayo Institutional Animal Care and Use Committee approved the study.

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Study Design
Young (n=6; aged 0.5 to 1.0 years) and old (n=15; aged 7 to 10 years) dogs were studied. Eight old dogs underwent a midline abdominal incision (isoflurane anesthetic) with wrapping of both kidneys as previously described (ExDHF group). An indwelling aortic catheter was placed via the femoral artery for blood pressure (BP) measurement as previously described. Control dogs underwent a similar procedure without renal wrapping.

Conscious Studies
Two-dimensional and M-mode echocardiography for EF assessment and BP measurement was performed in conscious dogs before acute hemodynamic study at 6 to 8 weeks after surgery.

Acute Hemodynamic Study
Dogs were anesthetized with intravenous fentanyl 0.25 mg/kg followed by 0.18 mg/kg per hour and midazolam 0.75 mg/kg followed by 0.59 mg/kg per hour and ventilated with supplemental oxygen. The pericardium was resected, and dogs were instrumented with a LV micromanometer catheter (Millar), piezoelectric crystals (Sonometrics Corp), a pneumonic inferior vena cava occluder, and an atrial pacing wire and paced at 10 to 20 bpm above sinus rate. Steady-state data were collected before and after adrenergic blockade (propranolol 2 mg/kg IV and atropine 0.5 to 1.25 mg). A 500-mL bolus of warmed saline was given before inferior vena cava occlusion. Steady-state data after volume expansion were collected in OC and ExDHF dogs. In OC and ExDHF dogs, phenylephrine (5 μg/min, increasing as needed) was given to increase BP to conscious levels. Dogs were euthanized by intravenous KCl, consistent with guidelines of the Panel on Euthanasia of the American Veterinary Medical Association. The heart was harvested, and LV mass was measured. Formalin-fixed sections were stained (picrosirius red). Collagen volume fraction was measured with a computer-assisted image analysis system (Ziess Vision).

Results
End-systolic elastance, the diastolic chamber stiffness constant β, and the curve-fitting constant α were derived. Arterial elastance was calculated as end-systolic pressure/stroke volume. Preload recruitable stroke work was calculated as previously described in OC and ExDHF dogs. Statistical Methods
Data are reported as mean±SEM. Between- and within-group comparisons used the Student t test with significance set at P<0.05. To determine the relationship between end-systolic elastance and LV structure indices, standard least-squares regression was preformed, and the relative strength of the associations was expressed by the standardized coefficient.

Blood Pressure
In ExDHF dogs, conscious BP increased over time (Figure 1 inset). At 6 to 8 weeks after surgery, conscious BP (Figure 1) was not different in YC and OC dogs but was increased in ExDHF dogs. Under anesthesia, LV peak systolic pressure was similar in YC (113±4 mm Hg), OC (105±5 mm Hg), and ExDHF (119±5 mm Hg) groups. Phenylephrine infusion restored systolic pressure toward conscious levels in OC1BP (151±3 mm Hg) and ExDHF1BP (203±5 mm Hg) dogs.

Ventricular Structure
LV end-diastolic volume was similar in YC, OC, and ExDHF dogs (Figure 2). End-diastolic volume/body weight was also similar in the 3 groups (data not shown). In conscious dogs, the LV end-diastolic dimension/body weight was similar in YC (0.172±0.003 cm/kg), OC (0.152±0.004 cm/kg), and ExDHF (0.172±0.009 cm/kg) dogs (P>0.05). Indexed LV mass and percent LV fibrosis were similar in YC and OC dogs but were increased in ExDHF dogs (Figure 2). The LV mass to LV end-diastolic dimension tended to be higher in ExDHF than in YC (P=0.11) or OC (P=0.40) dogs and was significantly higher in ExDHF (35.2±2.6 g/cm) than YC dogs (27.6±1.1 g/cm; P=0.05) and tended to be higher than that in OC dogs (31.1±1.5 g/cm; P=0.18) when 2 ExDHF dogs with less robust hypertension (conscious mean BP=138 mm Hg [n=2] versus 187 mm Hg [n=6]) were excluded.
Diastolic Function

We found that $\tau$ was increased in ExDHF compared with YC and OC dogs (Figure 3). When BP was increased in ExDHF dogs by phenylephrine infusion (ExDHF$_{1\text{BP}}$), $\tau$ increased further ($P<0.05$ versus ExDHF with normal BP [ExDHF$_{\text{norm}}$]). $\tau$ was slightly higher in OC than YC dogs.

The percent diastole at which relaxation is complete was increased in OC versus YC dogs and in ExDHF compared with YC and OC dogs (Figure 4) and was influenced both by the slower rate of relaxation (higher $\tau$) in ExDHF and by higher heart rate (ExDHF = 116±6 bpm versus OC = 99±5 bpm versus YC = 93±5 bpm) and longer duration of systole (ExDHF = 343±19 ms versus OC = 343±18 ms versus YC = 298±9 ms), both factors that shorten diastole. At baseline, after anesthesia and surgical preparation and before administration of propranolol or any fluid, LV end-diastolic pressures were not significantly different in the 3 groups (ExDHF, 8.2±0.5 mm Hg; OC, 9.2±0.9 mm Hg; YC, 7.0±0.3 mm Hg). In ExDHF dogs, increases in systolic pressure produced by phenylephrine were associated with elevated minimum (17.2±0.5 mm Hg), end-diastolic (20.0±0.6 mm Hg), and mean LV diastolic pressure (19.8±1.8 mm Hg).

The coefficient of passive LV diastolic stiffness ($\beta$) was similar in OC, YC, and ExDHF, whereas the curve-fitting constant ($\alpha$) was higher in OC than YC (Figure 5). Preload reduction during phenylephrine yielded values for $\beta$ similar to those obtained at baseline in ExDHF (0.044±0.003 mm Hg/mL in ExDHF$_{1\text{BP}}$ versus 0.042±0.004 mm Hg/mL in ExDHF with normal BP [ExDHF$_{\text{norm}}$]) and OC dogs (0.032±0.002 mm Hg/mL in OC$_{1\text{BP}}$ versus 0.031±0.002 mm Hg/mL in OC$_{\text{norm}}$); $\alpha$ was also similar before and after phenylephrine infusion in OC and ExDHF dogs.

Figure 2. LV end-diastolic volume (LVEDV), LV mass to body weight ratio, collagen volume fraction (% fibrosis), and representative picrosirius red stained–LV sections in YC, OC, and ExDHF dogs.

Figure 3. $\tau$ calculated assuming a zero (left) or non-zero (right) asymptote in YC and OC dogs and in ExDHF dogs before (ExDHF$_{\text{norm}}$) and after (ExDHF$_{1\text{BP}}$) phenylephrine.
Systolic Function
In the conscious state, EF was similar in YC (58±4%) and OC (55±4%) but was lower in ExDHF (49±4%) than YC (P<0.05) dogs. During EF measurement in ExDHF dogs, BP was 230±11/135±9 mm Hg. However, end-systolic elastance was increased in ExDHF compared with both YC and OC dogs (Figure 6A and 6D). End-systolic elastance indexed to the LV mass index (Figure 6B) was higher in ExDHF than YC or OC dogs. Preload recruitable stroke work tended to be higher in ExDHF than OC dogs (Figure 6C). When afterload was increased by phenylephrine infusion, the end-systolic pressure-volume relationship was consistently shifted leftward (Figure 7) without a significant increase in end-systolic elastance. Increases in end-systolic elastance were closely related to the degree of LV fibrosis (collagen volume fraction; Figure 6E) and to the degree of hypertrophy (LV mass index; Figure 6F). By multivariate analysis controlling for the degree of LV hypertrophy, end-systolic elastance was more closely related to the collagen volume fraction (standardized coefficient=0.634, P=0.0002) than to LV mass index (standardized coefficient=0.350, P=0.02).

Vascular Function
Arterial systolic stiffness (arterial elastance) was increased in ExDHF compared with YC dogs (Figure 8). After volume expansion, arterial elastance was increased compared with OC dogs.

Discussion
We describe a model of hypertensive heart disease in elderly canines characterized by normal LV volumes with LV hypertrophy and fibrosis. Diastolic function is abnormal with slowed relaxation such that relaxation was not complete until late diastole. Despite the presence of hypertrophy and fibrosis in ExDHF, β was not increased. Although EF measured in the conscious state at average systolic BP of 235 mm Hg (Figure 1) was slightly lower in ExDHF dogs than in controls, other
indices of systolic function including end-systolic elastance, end-systolic elastance/LV mass, and preload recruitable stroke work were increased in ExDHF dogs, and afterload enhancement of systolic performance was maintained. Increases in end-systolic elastance correlated with increases in fibrosis and hypertrophy, suggesting that structural changes rather than enhanced inotropic state may be responsible for increased end-systolic elastance. Arterial elastance was increased. These findings were qualitatively similar but more dramatic than those previously observed in young hypertensive dogs.14

Figure 5. Coefficient of passive LV diastolic stiffness (β; top) and curve-fitting constant (α; middle) in YC, OC, and ExDHF dogs. Bottom, Representative end-diastolic pressure-volume relationships in YC (black), OC (blue), and ExDHF (red) dogs.

LV Structure
Hypertension in elderly dogs was associated with increases in LV mass and increased LV fibrosis in the absence of LV dilation. Most studies in humans with DHF report normal LV volumes and variable increases in LV mass, with approximately 40% of DHF patients meeting echo criteria for LV hypertrophy.16,17 The degree of hypertrophy observed here is consistent with reports in humans that also report increases in LV mass/LV cavity size, suggesting concentric hypertrophy.17 Although studies of LV fibrosis in human DHF have not been extensive, increased LV fibrosis in hypertensives has been described.18 Thus, the structural characteristics of the LV in this model are consistent with those observed in DHF.

LV Relaxation
In ExDHF dogs, we observed increases in τ analogous to those observed in humans with DHF.3 However, another study in human DHF did not report increased τ.5 Assuming that relaxation may not be complete until 3.5τ,19 we demonstrate that relaxation could potentially influence LV filling pressures throughout most of diastole in ExDHF dogs, whereas the influence of relaxation is limited to early diastole in control dogs. It should be acknowledged that this calculation assumes that relaxation continues to follow a monoexponential course after filling starts, an assumption that may not be true. In normal dogs, the importance of LV relaxation in maintaining normal filling pressures during exercise has been demonstrated.20 Furthermore, in DHF patients, handgrip exercise was associated with increases in systolic pressure, τ, and filling pressures.3 Similarly, we observed further increases in τ and in diastolic pressures in response to increased systolic pressure in ExDHF dogs. In dogs with systolic dysfunction, enhancement of LV relaxation with exercise is lost.21 Whether LV relaxation or conscious LV filling pressures worsen with exercise in ExDHF is unclear, but the findings here suggest that impaired relaxation could be an important mediator of increased filling pressures with hemodynamic stress. If so, therapeutic approaches to enhancing lusitropy may well prove beneficial in improving symptoms in DHF. Brain natriuretic peptide,22 novel regulators of calcium reuptake,23 or gene therapy to increase expression of SERCA in the myocardium24 represent potential lusitropic therapies.

LV Diastolic Stiffness
We did not find increases in β or α despite the presence of LV hypertrophy and fibrosis in ExDHF, a finding similar to that reported recently in patients with DHF5 but in contrast to other studies in human hypertensive heart disease25,26 and a recent study in human DHF.4 There are several potential explanations for this observation. One may speculate that the duration of hypertension or degree of structural changes may be insufficient to produce the increases in passive stiffness that might be present in human DHF. Alternatively, it may be that increases in passive diastolic stiffness are not key factors in the pathogenesis of DHF, as recently suggested.6 However, inherent difficulties in characterizing diastolic stiffness by a single parameter may limit the ability to demonstrate in-
creased diastolic stiffness in human DHF or in the current model. The end-diastolic pressure and volume points are fit to a monoexponential relationship \( P = \alpha \exp(\beta V) \) or \( P = \alpha \exp(\beta V + \beta_0) \), and the shape and position of the curve will be characterized not just by \( \beta \) (the rate at which LV stiffness changes with changes in volume) but also by \( \alpha \) and by the pressure intercept \( (P_0) \). Thus, each curve may be fit best with divergent changes in \( \alpha \), \( \beta \), and \( P_0 \). This is a limitation induced in part by trying to fit data that are not perfectly monoexponential to this relationship. Relatively small changes in \( \alpha \) or \( \beta \) will have dramatic effects on filling pressures, and such small changes may require large numbers of subjects to detect. Caution in overreliance on a single derived parameter when characterizing the stiffness of the ventricle has been recommended.\(^{27,28}\)

Systolic Function
Whether systolic function is normal, reduced, or enhanced in patients with DHF remains unclear.\(^{29}\) DHF patients presenting with pulmonary edema and marked hypertension did not have abnormal EF.\(^{29}\) However, other studies report reduced velocity of myocardial shortening or stress-corrected midwall shortening in patients with DHF despite normal EF,\(^{8-12}\) suggesting that impaired contractile function may contribute to the pathogenesis of DHF. However, Kawaguchi et al\(^{5}\) found that end-systolic elastance was increased in DHF. Traditionally, end-systolic elastance is considered a load-independent index of contractility. In ExDHF dogs, we too found increases in end-systolic elastance. Afterload enhancement of systolic performance was maintained, a characteristic that is lost early in the progression of systolic dysfunction.\(^{30}\) However, structural factors may influence systolic stiffness, and we found a close relationship between the degree of fibrosis and hypertrophy and end-systolic elastance, with
end-systolic elastance most strongly related to the degree of fibrosis. Thus, increases in end-systolic elastance do not necessarily suggest enhanced inotropic state. Although these findings suggest maintenance of systolic performance, increases in end-systolic elastance may have adverse effects, exacerbating load-induced impairment in relaxation.5

Vascular Function
Arterial load influences systolic and diastolic LV performance.31,32 Increases in arterial force and pressure increase the mechanical performance of the LV.30,32 This leftward shift of the end-systolic pressure-volume relationship with increases in arterial load is attenuated early in the progression of systolic dysfunction.30,33 Increases in arterial load may also prolong relaxation.33 Afterload-induced prolongation of contraction and relaxation shortens the diastolic filling period. The relationship between vascular and ventricular function has been well defined in systolic HF, but less is known of these relationships in DHF. Arterial load is characterized here by arterial elastance, which incorporates resistance, total vascular compliance (primarily determined by aortic compliance), characteristic impedance, and systolic and diastolic time intervals. Changes in aortic compliance are (inversely) coupled to changes in resistance in a complex fashion.34 Clinical studies indicate that arterial stiffness is increased in clinical DHF,5,35 and we observed increases in arterial elastance in ExDHF dogs. Increases in arterial elastance may be due to increased resistance with secondary decreases in compliance, or there may be primary decreases in aortic compliance as well. Further studies are needed to determine the mechanism whereby arterial elastance is increased in this model.

Limitations
Phenylephrine was used to restore BP to conscious levels but does not necessarily reproduce all aspects of conscious hemodynamics. Although this model is referred to as a model of experimental DHF, symptoms are difficult to assess in the canine. End-diastolic pressures were measured in the anesthetized, open-chest, and extensively instrumented state, which has dramatic effects on BP and potentially diastolic pressures. Although end-diastolic pressure was not significantly increased in ExDHF compared with YC or OC dogs, there were highly significant differences in preload-insensitive indices of diastolic function, systolic function, arterial function, and ventricular structure, all consistent with findings described in human DHF. Whether this represents preclinical (stage B) DHF or overt (stage C) DHF (with increased conscious diastolic pressures) is difficult to say, and exercise studies in conscious, chronically instrumented animals may be needed to address this point.

Conclusions
Recent clinical studies underscore the controversies regarding the pathogenesis of DHF. The clinically relevant large-animal model characterized here provides insight into the pathogenesis of DHF and may prove useful in the development of novel therapies for DHF.

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